

*P*roceedings

American Academy of Forensic Sciences



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AMERICAN ACADEMY OF FORENSIC SCIENCES

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PROCEEDINGS

of the American Academy of Forensic Sciences 67th Annual Scientific Meeting

The Proceedings of the American Academy of Forensic Sciences is an official publication of the American Academy of Forensic Sciences (AAFS). It is devoted to the publication of the abstracts of technical oral papers and posters presented at the AAFS annual scientific meeting. These include various branches of the forensic sciences such as pathology, toxicology, anthropology, psychiatry, immunology, odontology, jurisprudence, criminalistics, questioned documents, digital evidence, and engineering. Similar submissions dealing with forensic oriented aspects of the social sciences are also included.

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S1 Past Presidents Future Science: Hot Leads in Contemporary Forensic Research

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After attending this presentation, attendees will: (1) describe many future directions of disciplines within the American Academy of Forensic Sciences; (2) recognize cutting-edge research and technologies in a variety of forensic science fields; (3) predict how law and forensics will be shaped by today's leaders in the coming decade; and, (4) discuss the future of forensic science with those in their own fields as well as other disciplines.

This presentation will impact the forensic science community by assembling visionaries in all 11 sections of the American Academy of Forensic Sciences who will discuss contemporary issues in their respective fields. Speakers will emphasize hot leads from the laboratory, theoretical advances, and emerging technologies.

The Past Presidents of the American Academy of Forensic Sciences (AAFS) represent a vast repository of forensic science knowledge, insight, and wisdom. As a group, they are unique in the world with regard to the scope of their collective influence and leadership vision in the forensic sciences. This year's Interdisciplinary Symposium will harness the energy from this eminent group of forensic scientists and focus it on the Academy's future. The Interdisciplinary Symposium will showcase Past Presidents and other representatives from each of the Academy's 11 sections. The speakers will share their vision for the future of forensic science in their respective disciplines, emphasizing hot leads from the laboratory, theoretical advances, and emerging technologies. The goal of this program is to envision where the forensic sciences will be a decade from now, the impact of these emerging advances on the law, and our place in it. This historic endeavor will be of significant interest to all Academy members and will provide a unique forum for learning from each other about the future of forensic science. Current AAFS President, Daniel A. Martell, PhD, will provide the Interdisciplinary Symposium's introductory remarks.

Anthropology

Douglas H. Ubelaker, PhD — AAFS Past President (2011-12): Dr. Ubelaker believes that predicting the future of any discipline can prove challenging, yet current trends and developments provide useful clues. Within forensic anthropology, Dr. Ubelaker sees these trends related to issues of certification, accreditation, the nature of case applications, employment, training, enhanced methodology, and new research.

Criminalistics

Ronald L. Singer, MS — AAFS Past President (2004-05): Mr. Singer says there is a familiar quote with regard to this topic: "May you live in interesting times." According to Mr. Singer, the future of criminalistics is indeed going to be interesting. Technological advances and the increased reliance on computer-driven systems and databases will reduce the time required for analytical processes, will make them more specific, and even allow many procedures to be handled at the crime scene as opposed to the laboratory. Using advanced DNA technologies, we will be able to predict physical features of unknown donors; probative information will be obtained from smaller and smaller samples; computer-assisted evaluation of the surfaces of fired bullets and cartridge cases will reduce the subjectivity involved in those analyses; and database searches will become faster and more accurate. With these advances come many challenges that will also have to be addressed. In some instances, criminalists will have to shift their focus from the bench to the preparation and monitoring of protocols for methods actually applied by non-scientists. In the laboratory, quality and accountability to our clients, the public, and the judiciary will continue to dominate our processes, but with considerably more oversight from outside



Special Session - 2015

bodies. This will inevitably impact our ability to deliver the services expected of us. As mandatory certification and accreditation become realities, education and training demands will increase, creating financial and staffing burdens on agencies, which in many cases will be met by a regionalization of some services, and an increased cooperation between laboratories. The Past Presidents from the Criminalistics Section are confident that these changes will lead to a vibrant future for the discipline.

Digital & Multimedia Sciences

Zeno J. Geradts, PhD — AAFS Director (2010-13): The Academy's newest section, Digital & Multimedia Sciences, is represented by Zeno J. Geradts, PhD. With regard to future expectations, Dr. Geradts, in conjunction with his section's working group, expects developments in big data analytics as well as multimedia analytics, due to the exponentially growing size of data. The use of strong encryption in data sources as well as communication channels is expected to rise. For that reason, forensic investigation of cloud computing, local storage media, silicon chip forensics, and mobile forensics will remain important. It is expected that many devices will be increasingly connected to the internet, such as robotic vacuum cleaners, other household appliances, as well as various medical devices; this is being increasingly described as the "internet of things." Forensic analysis of log files and location data of these and other tools will be more important in relation to crime scene investigation.

Engineering Sciences

Thomas L. Bohan, PhD, JD — AAFS Past President (2009-10): Having presided during the first year following the release of the 2009 National Academy of Sciences Report, *Strengthening Forensic Science in the United States: A Path Forward*, part of his presentation will deal with the effect — or to date — the "non-effect" of that report on forensic engineering sciences. Engineering science practitioners come from a diversity of disciplines practically as wide as that of the AAFS itself. They include "rocket scientists" (really, engineers), space physicists, medical device designers, transportation experts, materials testers and developers, fire investigators, and nearly everything except practicing physicians and attorneys, though some engineering scientists are physicians and attorneys as well. As such, they will be intimately involved in the complex activities shaping our society in the near and, it is hoped, distant future. It will be, to an even greater extent than at present, an electronically monitored society. This monitoring includes governmental surveillance and automotive "black boxes," but also includes the microprocessor-coupled systems that "observe" and control our airplanes, ventilation systems, and surgical procedures. This monitoring is data-based and it produces data; however, it occasionally fails in its assigned control tasks. Planes crash, surgical suites catch on fire, and building designs incorporate dangerous flaws. In the recipe for resolving the civil and criminal disputes created by such failures, practitioners from the forensic engineering sciences comprise the most common ingredient, often exceeding in number even the litigators. Dr. Bohan's presentation will explore this role of his colleagues and how it will be affected by forensic reform measures when they are finally applied to the forensic engineering sciences.

General

John E. Gerns, MFS — AAFS Secretary (2013-15): Diversity of the General Section will continue to play a pivotal role in the future horizons of the forensic sciences. In keeping up with the changes being driven by the National Institute of Science and Technology (NIST) Organization for Scientific Areas Committees (OSAC) and Congressional Legislative action, the General Section's Ad hoc Long Term Planning Committee is developing a long-term strategic plan which will serve as the General Section's "blueprint for our future." This is critical since many of our forensic disciplines fall under several of these new guidance committees. Discussion will focus on the potential challenges that face several of our forensic disciplines in the future, along with innovations in those disciplines which will enhance the application of the forensic sciences to the investigative mission.

Jurisprudence

Carol E. Henderson, JD — AAFS Past President (2008-09): Law and science have long been engaged in what has been called a reluctant embrace. The vast majority of civil and criminal cases involve scientific evidence that requires forensic scientists to comply with certain admissibility rules. While the legal system has usually reacted to emerging scientific discoveries, more recently it has been proactively striving to formulate a more robust legal/scientific framework for the introduction of scientific evidence into the courtroom. Topics to be explored in this presentation include trends regarding the admissibility of forensic evidence, the role of national and international organizations in shaping policies and guidelines to strengthen the foundation and acceptance of forensic science, strides made toward requiring more rigor in methodology of various fields of forensic science, judicial recognition of accreditation and certification, and current efforts to ensure lawyers and judges have the expertise necessary to comprehend and evaluate forensic evidence.

Odontology



Special Session - 2015

Robert E. Barsley, DDS, JD — AAFS Past President (2012-13): The future for the Odontology Section will include progress in four key areas in the field. First, in the identification of unknown human remains, the digitization and adoption of the electronic health record on a worldwide scale will greatly improve the capture and transferability of the biometric data underlying dental identification. In particular, the rapid adoption of digital radiographic imaging not only benefits traditional methods of dental comparison but has also impacted the second area undergoing rapid development — dental aging. This includes age at time of death for unidentified remains as well as the use of oral-facial development to determine the age of living individuals, a technique important in immigration status, employment status, and even in criminal matters in various jurisdictions. The adoption of digital imaging has also played a key role in renewed interest in dental markers for sexual dimorphism. The third field is the study of dental biometric analysis in disaster victim identification. The “richness” or “granularity” (the detail) of dental data captured and then used in the calculations that develop and rank-order antemortem and postmortem findings is an area enjoying rapid development. At the same time, odontologists are working across disciplines to develop unified identification software that allows those disciplines to share and use information as needed to complete identifications. Finally, the area of dental-related patterned injury (bitemark) analysis is under intense scrutiny. In addition to at least one recently completed National Institute of Justice (NIJ) -funded study, numerous ongoing and completed studies are focused on pattern comparison, tissue injury and response in living test subjects, and the area of cognitive bias. A project to provide a decision tree for analysis and comparison of human bitemarks that may be useful in suspected biter linking and/or exclusion is underway as well.

Pathology/Biology

Edmund R. Donoghue, MD — AAFS Past President (2005-06): Dr. Donoghue reports that, at present, the future of forensic pathology appears good. Easy gains can be accomplished by distributing currently existing radiology and computer-imaging technology to medical examiner offices. A number of situations could create difficulty for forensic pathology in the future. If the American Board of Pathology drops its current requirement for forensic pathology training, hospital residents would no longer join medical examiner offices and could not be recruited for training in forensic pathology. Maintenance of certification and re-certification examination requirements are burdensome and create the possibility that some forensic pathologists might become unemployed in the future. Standards for accreditation of medical examiner offices and autopsy standards are becoming increasingly detailed and rigorous. These standards may be obtainable with adequate staffing, infrastructure, and funding, but may become unreachable when resources are scarce. Forensic pathologists will need to be vigilant to avoid over-regulation by the federal government and other entities.

Psychiatry & Behavioral Science

Richard Rosner, MD — AAFS Past President (1996-97): Dr. Rosner states, “As one wit put it, ‘Prediction is always difficult, especially about the future.’” Given that caveat, the foreseeable future of forensic psychiatry and behavioral science will have at least two facets. On the one hand, the current effort to advance neuroscience (e.g., the United States government’s proposed \$100 million Brain Research Through Advancing Innovative Neurotechnologies (BRAIN) initiative is likely to advance our knowledge of the functioning of the brain and increase the scientific basis for our specialty). On the other hand, as noted by Thomas Nagel in his recent book, *Mind and Cosmos*, certain basic problems are likely to remain either unaddressed or inadequately addressed. These problems include the nature of consciousness, the relationship of the brain to the mind, and whether or not our subjective experience of “free will” is credible. Because our concept of personal responsibility (e.g., for criminal behaviors) is inextricably linked to those three issues, the impact of the anticipated scientific advances will be limited.

Questioned Documents

John L. Sang, MS — AAFS Past Vice President (2010-11): The future of questioned documents will likely be inclined toward digital signature examination and the use of automatic forensic handwriting analysis systems by Forensic Document Examiners (FDEs) to assist in case work. Certainly, handwriting examinations will become more data oriented and the field will see more measurement data and statistical analyses being conducted. Eventually, the field may get to the point where a machine can conduct a full examination, comparison, and analysis of handwriting; however, this is still a very long way down the road. In any event, a human examiner will still have to make the final decision. Clearly, the numbers of FDEs are declining and there will be very few government positions or a cadre of highly-skilled private FDEs as in Osborn’s days. There are a good number of jurisdictions that can’t afford to have FDEs, including state, local, and law enforcement agencies. The field is barraged with an influx of individuals, generally graphologists, who claim to have been trained as FDEs via internet instruction and training. These people do not come close to meeting the minimum standards required by the AAFS Questioned Documents Section; however, they are often allowed to testify as experts. Hopefully, the courts will stem this problem but, for now, that seems unlikely. It would make sense that the courts require FDEs to be certified before they can testify. The Questioned Documents Section has worked hard over the years to have a strong certification program through the American Board of Forensic Document Examiners (ABFDE) and a good network of professional organizations. A good base of standards has been developed by the American Society for Testing and Materials (ASTM)/the Scientific Working Group for Forensic Document Examination

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Special Session - 2015

(SWGDOC). The new National Institute of Standards and Technology (NIST) and its Law Enforcement Standards Office (OLES) will be discussed in the presentation. A high priority will be the continuing research into Terminology for Expressing Conclusions of FDEs, and it is expected that all standards will grow stronger every year moving forward. Improvements in printing technology and 3D printers will provide more challenges and opportunities for FDEs. More research will be conducted into data produced by devices used to create mechanically produced signatures. Development of different types of toners and inks will also provide more challenges. For years, Mr. Sang has believed the field will be moving closer to the digital sciences. He hopes the courts will clearly see what a significant and progressive field forensic document examination is as practiced by our members at the AAFS.

Toxicology

Barry K. Logan, PhD — AAFS Past President (2013-14): Forensic toxicology is undergoing a change of focus and direction as we convene in Orlando in 2015. The focus for the last two decades has been on pushing the limits of laboratory technology to tweak the sensitivity of Gas Chromatography/Mass Spectrometry (GC/MS) methods, the workhorse instrument in the laboratory, to the point where we have techniques with adequate sensitivity to answer most questions about whether a person ingested or was exposed to forensically significant amounts of commonly encountered toxic or intoxicating substances. Going forward, the focus is on the next generation of Liquid Chromatography/Mass Spectrometry (LC/MS) instrumentation with less demanding sample preparation requirements to achieve faster throughput and to help identify some of the more esoteric compounds and their metabolites not amenable to GC/MS analysis. Identifying the optimum techniques from Time-of-Flight (TOF) to MSⁿ and beyond in their various combinations and finding economical ways to sort and evaluate the data will be a focus in the immediate future. The second main area of change is in standards development. The Scientific Working Group for Toxicology (SWGTOX), a collaborative consensus-building approach to standards development, has achieved an incredible amount in the five years it has been in existence, and is challenging forensic toxicology labs and practitioners to refine and validate their methods and to ask more of the labs in improving quality and in estimating error and uncertainty in quantitative measurement. As SWGTOX transitions to a subcommittee of the Forensic Science Standards Board (FSSB), it has produced a solid legacy on which to build a strong future. Combined, these two major initiatives will force a harder focus on what the results mean and what they tell us about the likelihood of an adverse effect or life-threatening condition. Drug interactions, pharmacogenomic differences in drug metabolism, the influence of tolerance in interpretation, and other factors will all have to be addressed in a more systematic way in interpretive toxicology. The Toxicology Section of the AAFS is working to equip today's and tomorrow's forensic toxicologists to answer these questions.

The AAFS Interdisciplinary Symposium for 2015 will be one of the highlights of the Academy meetings in Orlando. Attendees will hear from preeminent visionaries in their respective fields. The collection of speakers, the great majority of whom are Past Presidents of the American Academy of Forensic Sciences, represent a diverse and interdisciplinary view of the direction forensics will take in the years to come. These experts are not only familiar with the past in their fields, but are also looking forward to the future.

Future of Forensic Science, Past Presidents of AAFS, Contemporary Research



S2 YFSF 20th Anniversary: The Past, the Present, and Our Future

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After attending this presentation, attendees will have a better understanding of future trends in forensic science. In addition, attendees will learn about casework and research that has been done by their peers at Bring Your Own Poster (BYOP) and Bring Your Own Slides (BYOS) Sessions. Networking skills, how to succeed in the current job market, and career preparation will also be discussed.

This presentation will impact the forensic science community by demonstrating future trends in forensic science through casework, education, and mentorship opportunities as well as by providing tools for successful contributions to the forensic science field.

Each year at the American Academy of Forensic Sciences (AAFS) Annual Scientific Meeting, the Young Forensic Scientists Forum (YFSF) provides a program for a group of students and forensic scientists with less than five years of professional experience. The session allows participants to interact with their peers as well as with the professional speakers and to build professional relationships that foster growth and mentorship opportunities. Special session topics provide attendees with a broad overview of the many opportunities in the field of forensic science. In addition to the special session, YFSF offers two opportunities for young forensic scientists to present their own work or research: the YFSF Bring Your Own Posters (BYOP) Session and the YFSF Bring Your Own Slides (BYOS) Session. The Forensic Sciences Foundation (FSF) Emerging Forensic Scientist Award winner is also invited to present her award-winning paper during this special session.

For the AAFS 67th Annual Scientific Meeting in Orlando, FL, the YFSF Special Session will present *YFSF 20th Anniversary: The Past, the Present, and Our Future*. The special session includes speakers from many of the AAFS sections who will discuss personal experiences and improvements within the field of forensic science. Through the presentation, attendees will learn how various forensic fields are changing and what opportunities are available to undergraduate students, graduate students, and young professionals. Speakers will discuss educational and professional requirements in their respective fields as well as skills needed throughout the forensic science community.

Following the Tuesday session, the YFSF BYOP Session will be presented in the evening, giving young professionals the opportunity to showcase current cases and research in a poster format.

The annual YFSF BYOS Session takes place the evening of Wednesday, February 18, and will include presentations from students and new forensic scientists. The program will conclude Thursday, February 19, 2015, with the annual YFSF Breakfast Session which includes a resume review panel.



Special Session - 2015

As in past years, the Breakfast Session will maintain focus on developing professional skills for the next generation of forensic professionals. Representatives from educational institutions, professional organizations, and various careers will present on career skills including networking and career preparation. The topics discussed will assist emerging scientists as they determine what careers they would like to have. After the presentations, attendees will have the opportunity to receive résumé assistance and feedback from AAFS members already established in their careers.

The Special Session provides students, young professionals, and AAFS members with a way to foster career-long relationships. The main goal of the YFSF is to encourage mentorship between young and veteran forensic scientists. Participants are encouraged to apply for membership in the AAFS and are given guidance on the many opportunities available to aid in career enrichment.

YFSF, Education, Research



Breakfast Seminar - 2015

BS1 Wildland Fires of Electrical Origin — Deaths and Litigation

Helmut G. Brosz, PEng, BASc, Brosz Forensic Services, 64 Bullock Drive, Markham, ON L3P 3P2, CANADA*

After attending this presentation, attendees will better understand some of the causes of wildland fires resulting in deaths, injury, and litigation, resulting in a more effective understanding of forensic methods and issues.

This presentation will impact the forensic science community by providing information to the legal, insurance, electrical utility, and forensic engineering industry as well as authorities having jurisdiction that become involved in wildfire losses, claims, litigation, and investigation. At the outset, the cause of a wildfire is often not known. Investigation after the “smoke” has cleared requires specialized knowledge and methodology in order to determine the origin, cause, and reason for the initiation of a wildfire. Spoliation issues will also be discussed.

California, Australia, and Florida share a high number of wildfires caused by or involving power lines, lightning, animals, carelessness, vehicles, arson, etc., and other unusual causes. Wildfires have caused thousands of deaths (directly and indirectly), property destruction of dwellings and infrastructure, and business interruption. The millions of dollars of loss invariably result in civil litigation if a chance of monetary recovery is likely. Criminal litigation is also likely if the proper circumstances exist.

Issues such as sag and tension of power lines before and after a fire and line-to-line and line-to-ground voltages need to be considered. Clearances from vegetation (trees) and clearances to ground at various temperatures often require not only measurements but catenary calculations and elongation characteristics of copper, aluminum, and Aluminum-Conductor Steel-Reinforced (ACSR) wires. Sometimes energized power lines sag into vegetation due to the heat from a fire below and then make contact with vegetation. The track left by the arcing event may sometimes be interpreted as causal as opposed to resulting.

The importance of accurate surveys of sections of overhead distribution lines along with the ground below are often necessary to determine if the overhead line was built according to applicable codes and standards.

Animals and birds sometimes cause short circuits on lines and at hardware on poles. Animals may fall to the ground while on fire and initiate a wildfire at the base of the pole, giving the appearance of the fire having been caused by utility apparatus. The origin will be at a pole, the cause will be the bird/animal, but the reason will be known only to this critter. Responsibility is sometimes focused on the electric utility and not on the animal. Laboratory simulation tests in a high-voltage lab as well as field tests with portable equipment can be of assistance at these times. Lightning discharges on electrical lines, poles, and hardware can also start wildfires.

The Topanga Canyon wildfire of 1993 near Malibu, Los Angeles County, CA, burned 19,000 acres at a high speed in a span of a few hours; burned 739 structures, homes, and cars; killed three people; and, provides an example of a criminal arson investigation gone awry.

The Cavendale fire of 1996 burnt about 3,000 acres of mostly vineyards in Sonoma and Napa Valley, CA, as well as a few structures. Did a tree grow into a line or did a line sag into a tree? Did a Steller’s Jay bird commit animal electrical suicide? Did California wines develop a smoky taste that year? More than \$30,000,000 was at stake in the ensuing litigation.

Wildfires are here to stay and death, destruction, litigation, and the rebirth of forests and fields continues.

Wildfire, Electrical, Litigation



Breakfast Seminar - 2015

BS2 Mental Health Support to Law Enforcement: Practical Issues

*R. Gregg Dwyer, MD, EdD**, Medical University of South Carolina, Community & Public Safety Psychiatry Division, 29-C Leinbach Drive, Charleston, SC 29407; *Eric Skidmore**, South Carolina Law Enforcement Assistance Program, 2501 Heyward Street, Columbia, SC 29205; and *Andy Gruler, MSW**, 12 Crosswinds Way, Greer, SC 29650

The goal of this presentation is to provide a description of mental health services for law enforcement personnel with guidance on the establishment and maintenance of such services.

This presentation will impact the forensic science community by providing guidance on developing and implementing a mental health program for personnel exposed to exceptionally stressful work environments and content.

Although police work has always had the risk of negatively impacting the mental health of line law enforcement forensic personnel through their exposure to violent crime scenes, officer-involved shootings, line-of-duty deaths, and other critical events, services have been limited. There are multiple combinations and settings in which mental health services can be provided to the law enforcement community. They vary from answering informal questions to formal consulting contracts for service with the mental health professional embedded in an agency for real-time access by law enforcement personnel. Services include pre-employment screening, fitness-for-duty evaluations, training on stress prevention and management, post-critical incident services, responding real-time to crime scenes, Special Weapons and Tactics (SWAT) operations, hostage and barricaded person negotiations, threatened suicide-in-progress, officer-involved shootings, and other traumas. This seminar provides an overview of the types of services available, the necessary and suggested credentials to be an effective consultant, a how-to guide for finding and recruiting such a consultant, and potential conflicts with dual-agency relationships, all presented using actual examples from the field.

Stress Management, Mental Health, Critical Incidents



Breakfast Seminar - 2015

BS3 Bank Robbery on Chicago's South Side — 75 Years After John Dillinger

Brian S. Clark, MFS, 15321 S 94th Avenue, Orland Park, IL 60642*

The goal of this presentation is to introduce attendees to the current difficulties and challenges of investigating violent crime from both an investigator's and a forensic science perspective.

This presentation will impact the forensic science community by creating a better understanding of the crime of bank robbery and the evidentiary issues associated with the crime.

The crime of bank robbery has been the subject of many Hollywood movies, books, and news stories in the United States since the mid-1800s. These events have sometimes been portrayed as glamorous and at other times as the violent and desperate actions of someone with nothing to lose. The closure rate on bank robbery cases varies over time based on numerous variables such as the skill and experience of the robber, the statement of the victims and witnesses, and the location of responding local police officers. However, the quality and amount of evidence available to investigators continues to climb.

As various examination methods of biological evidence get more sensitive, training is provided to bank employees on the importance of preparing the bank counters and other surfaces for the day. It is incumbent on the bank employees to ensure surfaces used by customers and potential bank robbers are cleaned each and every day to help ensure that the biological material from previous customers and employees is removed prior to a robbery.

This presentation consists of a review of several bank robberies on the south side of Chicago to include unarmed note jobs to high-profile takeover-style robberies with multiple subjects with an emphasis on the forensic evidence used in the investigation or lack thereof. Included is the unique experience of an investigating agent on bank robbery investigations who also undertook the responsibility on several occasions to process the evidence involved with the crime scene, the subsequent arrests, and any related search warrants.

Three cases will be presented that illustrate the difficulties and challenges of investigating the crime of bank robbery. The first case is a copy of the movie, *The Town*, in which the robbers, a man and a woman, dressed as nuns, robbed the bank at gunpoint. The second case is a robbery in which the robber conducted reconnaissance from inside the bank as well as surveillance of the bank prior to the robbery. The subject attempted to use the Crime Scene Investigation (CSI) effect leading up to trial and during sentencing to argue that absence of evidence is, in fact, evidence of absence proving that he was kidnapped and placed under duress which resulted in him robbing the bank. A range of video, forensic, digital, and circumstantial evidence was used to convict the subject. The third case involved two individuals who traveled to the Chicago area to rob a bank and planned to flee back to Oklahoma. After preparing for the robbery by obtaining disguises, a rental vehicle, weapons, and a scanner, they robbed a bank, only to be followed by a local tow truck driver who called the police, directing responding officers to the getaway vehicle. The chase was recorded by police dashboard cameras while one of the subjects called 911 to demand they be allowed to escape, ultimately threatening and firing a bullet into another vehicle before crashing into a police car.

Bank Robbery, Chicago, FBI



BS4 Crime Scene Reconstruction of Nine United States Air Force Members Killed in Kabul, Afghanistan

Chad W. Hutchins, MFS, 652 Mossy Oak Trail, Waverly, GA 31565; and Alison R. Babcock, MFS, 1030 S Aspen Street, Buckley AFB, CO 80011*

After attending this presentation, attendees will understand some principles of crime scene reconstruction, such as how to gain explicit knowledge from a series of events or event segments that surround the commission of a crime, the application of deductive and inductive reasoning, the integration and interpretation of physical evidence, strict adherence to the scientific method, and the interrelationship of all the components into a final product.

This presentation will impact the forensic science community by demonstrating how crime scene reconstruction can enhance an investigation with critical interdisciplinary reliance. Crime scene processing techniques, criminalistics, digital sciences, pathology, serology/toxicology, firearms analysis, and other disciplines of forensic science all contribute vital information in this example of a practical application of crime scene reconstruction.

On April 27, 2011, eight United States Air Force (USAF) active duty members and one civilian were murdered by a trusted Afghan pilot being mentored by the USAF members in a surprising blitz-style attack. The incident took place in the two-story Afghan Air Corps Headquarters building on the Afghan Air Force's side of the Kabul International Airport, Kabul, Afghanistan. Just before a routine weekly meeting, seven USAF active duty members and one civilian were gunned down in the Afghan Air Command and Control Center (ACCC); the meeting was to take place just minutes later in an adjoining conference room. The eighth USAF active duty member was murdered just outside the building after he and another active duty USAF member exited the conference room and engaged the gunman throughout two separate hallways of the headquarters building. The gunman was wounded at some point during the gun fight, which left clues to his actions before he walked to the second floor of the building where he sustained two fatal gunshot wounds to the chest.

Four different multi-national response and law enforcement teams processed the scene before the Air Force Office of Special Investigations (AFOSI) was allowed unrestricted access five days later. By this time, furniture had been moved from its original position and cleaned, all visible projectiles and cartridge casings had been collected in a manner which made it impossible to determine exactly where they had come from, blood on the floors and walls had been cleaned and painted over, glass containing bullet holes had been replaced, and the decedent's clothes had been incinerated.

Many questions were immediately asked. Was there more than one gunman? If not, how could one person murder this many military members, most of whom were armed, without being stopped? Crime scene reconstruction was the only way to take this extremely complex case with fragmented physical evidence from a severely contaminated crime scene and produce answers.

Statements from several Afghan witnesses present in the ACCC were used to aid in the reconstruction; however, available physical evidence and autopsy findings were crucial. The rest of the reconstruction, spanning a building of approximately 14,000 square feet, relied heavily upon interpreting physical evidence, deductive and inductive reasoning, and applying the scientific method to come to conclusions.

Many times a crime scene reconstruction is accomplished to answer a specific question or sequence specific events involved in a crime. In this case, the reconstruction was performed to simply identify the major event segments and place them in as much of a sequence as possible in an effort to answer the question, "What happened?" This enabled the conclusion of only one gunman and provided clues as to how one person could carry out a murder spree against military personnel who were carrying weapons.

Reconstruction, Scientific Method, Afghanistan



BS5 The Roso Case: An Unpublished Trial Regarding Hermaphroditism Verified Through the Expertise and Written Advice of Leading Physicians in 19th-Century Florence

Annarita Franza, PhD, Via delle Oche 15, Florence, Italy, ITALY; and Vincenzo Lusa, JD*, Via Ferdinando, Palasciano #72, Rome 00151, ITALY*

After attending this presentation, attendees will understand the techniques and methods used at what, in 19th-century Europe, was considered a famous trial: the case of Maria Rosa Fantini (1764-1839), which centered on a series of reports regarding the very delicate issue at the center of a case of “doubtful sex” and who was later shown to be a hermaphrodite. This trial remained buried in the Archiepiscopal Archives of Florence and has only recently been brought to light.

This presentation will impact the forensic science community by illustrating the importance of introducing, at trial, the technical expertise and opinions of such famous Italian doctors of the time as Vincenzo Chiarugi (1759-1820), the father of Italian psychiatry. In addition, attendees will better understand how the methods of the Positivist School, which had shaped such forensic scientists as the criminal anthropologist Cesare Lombroso (1835-1909), were able to demonstrate a person’s true sexual identity with psychological developments that emerged in court, thus revolutionizing the perception each individual has of themselves. During the presentation, the previously unpublished court records and reports of this particular case will be presented along with the findings of the proceedings.

The research conducted at the Fiesole (Italy) Diocesan Historical Archives has permitted a reconstruction of Fantini’s life. On July 15, 1805, an initial application was filed at the Court of the Episcopal Curia of Fiesole by the husband, Dionisio, to dissolve his marriage to Maria Rosa, whose poor genital conformation invalidated the marriage. Subsequently, on February 13, 1818, Dionisio, convinced of his marriage’s illegality, brought action for an annulment before the Episcopal Court of Florence where, at the behest of the lawyer Simeone Döthel, a second report, entrusted this time to Vincenzo Chiarugi, was filed. The examination showed a surprisingly large enterocele in the right groin area and, at the uppermost part of the pubic arch, a glans imperforate, resembling a foreskin circumcision, and a very well-built frenulum that forced the glans toward the bottom. On the basis of anatomical data, Chiarugi concluded that Maria Rosa was a male. The clinical history found in the Historical Archives of the University of Florence, shows that Maria Rosa was admitted to the women’s ward, where the anatomist Giuseppe Chiarugi, son of Vincenzo Chiarugi, identified her as being a male. She was then transported to the men’s infirmary (bed number 598) and given the masculinized name of Roso. On her deathbed, she declared her regret that, “In the midst of the confusion of the opposite sex, I am and was a woman and I am surprised at how these gentlemen want me to die here among men.” Maria Rosa died at 11:00 a.m. on the morning of April 16, 1839, in the men’s ward of the Santa Maria Nuova Hospital. The autopsy, performed by Stanislao Petri, confirmed the previous forensic report. As evidenced by copies of the death certificate, found at the Florence State Archives and the Archiepiscopal Historical Archives of Florence, the deceased was recorded under the name of Roso Fantini, an unmarried male.

The case of Maria Rosa “Roso” Fantini provides valuable material for scientific research and an unpublished look at Italian positivism regarding the nascent “sexual question.” The extraordinary nature of this now-forgotten case was mentioned by Caesar Taruffi (1821-1902), Professor of Pathology at the University of Bologna and a leading expert on hermaphroditism in the period in the work *Ermafroditismo ed agenosoma* (1902). In keeping with the dictates of scientific writing at the time, Vincenzo Chiarugi’s forensic report was published in the form of letter (*Sopra una supposta specie di ermafroditismo*, 1819) highlighting indirect evidence of Maria Rosa’s sexual behavior. Having been raised and having lived as a female, she believed that she had been born and lived as a woman. Trials in that period demonstrated that, in instances of “ambiguity,” it was possible to divert “nature” from its course, as in the case of Maria Rosa, psychologically changing a male into a female. On the subject of hermaphroditism, Cesare Lombroso, from the School of Criminal Anthropology, also strongly supported the influential role of education in the formation of sexual identities.

Hermaphroditism, Trials, Case Report



Breakfast Seminar - 2015

BS6 Recent Changes in Pharmaceutical Industry Operations: Boon or Bane?

Abraham T. Philip, MD, Regional MEO, 325 Norfolk Street, Newark, NJ 07103*

WITHDRAWN



BS7 The Businessman, the Wife, the Aunt, and the Children: Multiple Murders by Drowning

Chris Milroy, MD, LLB, Ottawa Hospital, 501 Smyth Road, Box 117, 4th Fl CCW, Ottawa, ON K1H 8L6, CANADA*

After attending this presentation, attendees will understand “honor killings” and the role they play in certain societies, the pathology of drowning, and the importance of a complete death investigation system.

This presentation will impact the forensic science community by providing an understanding of the importance of a full autopsy in drowning cases and the role that cultural values play in supplying a motive for some homicides.

On June 30, 2009, near the City of Kingston, Ontario, a car was found in the Rideau Canal, a 126-mile (202km) canal that runs between Ottawa and Kingston, Ontario. When a diver located the car, there were four bodies present. The bodies were recovered and a Nissan® Sentra® car removed from the water. On the same day, a Canadian businessman of Afghani origin reported that three of his daughters, aged 19, 17, and 13 years of age were missing along with their 50-year-old aunt. The father reported that they had been on holiday at Niagara Falls, were returning to Montreal, and stopped at a motel for the night. The 19-year-old daughter, who had apparently been learning to drive, was reported to have taken the car keys to the Nissan® Sentra® stating she needed something, and had then driven off with her aunt and sisters.

When the bodies were recovered, the police initially assumed the deaths were an accident. Postmortem examinations were conducted and the deaths were recorded as drownings. Toxicological analysis of the four victims was negative.

However, soon after the deaths, Kingston police became concerned that the deaths were not accidental. The oldest son of the businessman had reported that he had been involved in an accident with his car in Montreal on the morning the bodies had been found and had reported the crash to the Montreal police, though no other car was involved. However, glass found at the scene in Kingston indicated that the glass was from the Lexus® (which had a defect in the headlight) the son was driving in Montreal.

A very comprehensive criminal investigation which involved multiple jurisdictions was conducted. The aunt turned out to be the first wife of the businessman, who was in a polygamous marriage with his second wife, the mother of all seven children in the family. On July 22, 2009, the businessman, his wife, and their oldest son were arrested and subsequently charged with first-degree murder of all four victims.

Inquires by the police indicated that the four victims in the car had been intentionally killed. The evidence did not support the four victims dying in the car, as they appeared to have been killed elsewhere. Covert surveillance had indicated that the father, mother, and son had been involved in killing their children/siblings. The son subsequently admitted that he had been at the scene when the Nissan® entered the water, but said it was an accident.

The motive presented by the prosecution was that these deaths were “honor” killings based upon the three teenage girls having become too “westernized.” The offences have been called “honoricides” by the press.

The businessman, his wife, and son were tried for first degree homicide. The trial lasted over two months and involved witnesses giving evidence in four languages including Dari, the Persian dialect spoken by the defendants. Both parents gave evidence in their defense, but not the son. The jury returned guilty verdicts against all three defendants.

This presentation will discuss how the complex investigation developed, the pathology of the victims, and the concept of “honor killings.”

Homicide, Drowning, Honor Killing



Breakfast Seminar - 2015

BS8 Thomas Krauss Bitemark Breakfast: From *Frye* to *Daubert* — A Change in Legal Standard

*Jeffrey L. Ashton, JD**, Office of the State Attorney, 415 N Orange Avenue, Orlando, FL 32792; and *Adam J. Freeman, DDS*, 22 Imperial Avenue, Westport, CT 06880

The goal of this presentation is to discuss the challenges facing prosecutors in presenting scientific evidence, generally and specifically those faced when presenting new or novel scientific advances. In particular, discussion will encompass the implication of the change in legal standards from *Frye* to *Daubert* as they apply using the State of Florida as an example.

This presentation will impact the forensic science community by providing a more complete understanding of the prosecution of Casey Anthony.

Born on October 3, 1957, in St. Petersburg, Florida, Jeff Ashton studied law at the University of Florida School of Law and became an Orlando prosecutor. In 1987, he was the first lawyer to earn a conviction by introducing DNA evidence into a case. Decades later, he became the head prosecutor of the sensational 2011 Casey Anthony trial. Ashton has written a book about his experiences, *Imperfect Justice: Prosecuting Casey Anthony*, and was sworn in as a state attorney in 2013.

Jeff Ashton worked in a number of divisions that were part of the state attorney's office before winning his first murder conviction in a 1983 trial. While serving as assistant state attorney in 1987, Ashton took on a groundbreaking role: he began working as prosecutor on the trial of a Florida serial rapist, and during the trial, he introduced DNA-based evidence. The case resulted in the first conviction using DNA testing, with such evidence being used regularly thereafter, in cases throughout the country. Three years later, Ashton established his office's homicide division.

For a time in 2002, Ashton was appointed as head of the juvenile division of the state attorney's office, but missed the courtroom and decided to return to prosecuting. Over his decades-long career, he has taken hundreds of cases to trial.

In June 2011, Ashton worked as prosecutor in the highly sensational Casey Anthony trial. Anthony, a young woman from Florida, was accused of murdering her 2-year-old daughter, Caylee Anthony, whose remains were found near the Anthony home. The case received a huge amount of media attention focusing on the Anthony family's back-story, with the prosecution taking the stance that Anthony was guilty of murder, and the defense countering that Caylee's death was an accident that was subsequently covered up.

On July 5, 2011, the jury found Casey Anthony not guilty of first-degree murder, aggravated manslaughter, or aggravated child abuse charges. Anthony was found guilty of charges related to providing the police with false information, and was placed on parole after receiving a fine and credit for time served. Ashton was stunned by the decision.

As he'd intended, Ashton retired from working as prosecutor after the trial and worked part-time at a law firm. In November 2011, Ashton released the best-selling book, *Imperfect Justice: Prosecuting Casey Anthony*, published by William Morrow. In his book, the prosecutor deconstructs the case, including his thoughts on Anthony's Defense Attorney, Jose Baez, and the use of the death penalty.

In 2012, Ashton ran for the state attorney seat in the Floridian counties of Orange and Osceola, against his former supervisor, Lawson Lamar. Ashton won the election and, in early January 2013, was sworn in as a state attorney. That same month, the Lifetime Network aired, *Prosecuting Casey Anthony*, a TV movie based on Ashton's book, starring Rob Lowe as the prosecutor.

Frye, Daubert, Jeffery Ashton



Evening Session - 2015

ES1 Forensic Sciences — Keep It Simple, Stupid!

*J.C. Upshaw Downs, MD**, GBI ME, 925 A Mohawk Drive, Savannah, GA 31419; *Carla Miller Noziglia, MS**, ICITAP, 305 Ascot Drive, Aiken, SC 29803-7833; *Anjali A. Ranadive, JD**, SciLawForensics, Ltd, 1834 Overlook Ridge Road, Brookings, SD 57006; and *Jennifer L.P. Downs, BA**, 11511 Abercorn Street, Ext 182, Savannah, GA 31419

After attending this presentation, attendees will learn a simple paradigm to balance four key elements of practice in order to enhance opportunities for a long-term, successful career.

This presentation will impact the forensic science community by providing a memorable framework to balance the critical areas of education, analysis, reporting/testimony, and ethics.

The career of the forensic science practitioner is arduous. Reflection and simplification may prove beneficial in coping with the myriad stresses inherent in the profession. The requisite training is intellectually challenging, often continuing into graduate and postgraduate studies. Continuing education is essential to stay current with the latest scientific developments and techniques, in addition to exploring potential new research in order to develop improved procedures and protocols. Application of the knowledge base in investigating a particular event is a daily exercise for the forensic caseworker, regardless of discipline.

The awesome responsibility imposed by applying precise analytical methodology while maintaining scientific objectivity which must be balanced with the reality of the significance of the results can prove burdensome. Eventually, the results of the forensic analysis will yield a scientific report which is often presented in court. Sharing the vast wealth of background information with a lay jury by means of unbiased reports and courtroom testimony is an oft underappreciated skill. The convergence of presenting detailed science with conveyance of the essential data while maintaining neutrality requires both sophistication and simplification in communication. Finally, given the gravity of the consequences of the adversarial system and the inherent nature of the casework, the forensic scientist experiences profound ethical challenges during the course of a career. Those successful in the long term are careful to pay heed to the needs of the individual human spirit. Diversions and moderation can help balance the ethics of personal and professional life in order to maximize the individual's potential for overall success.

Thus, the four cornerstones of knowledge, investigation, sharing, and spirit are the bedrock upon which a successful career in the forensic sciences can be structured. Utilizing an entertaining multimedia format, the panel will discuss each of these four elements in order to demonstrate how each might be integrated into a successful whole. These lessons will no doubt prove beneficial to attendees at all points on the career path, from novice to retiree. Ultimately, the foundation will remind all that a successful mantra for forensics is easy to remember — keep it simple, stupid!

Ethics, Testimony, Analysis



Luncheon Seminar - 2015

L1 Fetal Kidnapping by Cesarean Section

*Michael Welner, MD**, 224 W 30th Street, Ste 806, New York, NY 10001; and *Ann Wolbert Burgess, DNSc**, Boston College, 228 Highlands Avenue, West Newton, MA 02465

After attending this presentation, attendees will be aware of the common conflicts and personality qualities of those who commit kidnapping by cesarean section. Attendees will also be informed about the modus operandi of this most peculiar crime. The data will be integrated into recommendations for forensic assessment and public safety.

This presentation will impact the forensic science community by presenting findings from 15 completed and five attempted cases of fetal kidnapping by cesarean section and by providing insights about perpetrators who commit this unthinkable crime so that prevention can play a role in any future research.

The National Center for Missing and Exploited Children (NCMEC), concerned with the abduction of children (birth through six months) by non-family members, has been studying such cases since 1983, focusing on the abductors and violence, and has been providing guidelines for healthcare professionals on prevention of and response to infant abductions. While educational programs for healthcare agencies and hospitals proved successful in deterring abductions from institutions, the number of abductions from nonagency settings and the numbers of infant abductions from non-hospital settings increased. A study of 247 infant abductions over three decades revealed birth mother injury as an important change between the years of 1983-1992 and 1993-2006. The occurrence of fetal abduction by cutting open the mother's abdomen, though rare, has increased. This raises questions as to the motivation for the act, the dynamics in the planning, the lethal intent to secure a baby, the legal outcomes, and the lessons to be learned for prevention.

Fetal abduction involves behaviors that are not well understood or researched. In the first study of its kind, media-reported, non-family cesarean section cases were reviewed. This presentation reports the findings on the 15 completed and five attempted, non-family member cases reported to the NCMEC. Data were obtained from court documents as well as NCMEC records. A number of demographic variables were investigated: perpetrator gender (all female), state the crime was committed in (13 states), year the crimes took place (with the number doubling since 2000), race (varied, with race of the victim mother being consistent with the offender's), and age (most abductors targeted a younger mother, with the offender's age ranging from 19 to 40 years of age). Furthermore, all perpetrators (except one) were in a relationship, more than half already had children, and nine offenders were (or believed they were) infertile.

Several themes were identified across the 15 cases. All abductors lied that they were pregnant, although the motivation for the lie varied. Abductors sought out medical information on childbirth and cesarean sections to prepare for the crime. A critical component of pregnancy simulation is targeting either a "known" mother and gaining her trust or frequenting places pregnant women attended to select a "random" victim mother. For all perpetrators, the murder plan had long been in their mind, and they usually prepared cesarean "kits" in advance; however, the location of the crime varied. In all but one case, the victim mother died, and only ten of the 15 babies survived. This research investigated the psychological defenses employed in each case, and the topics of lying, pregnancy simulation, and capacity to commit murder for personal gain of a newborn while legally sane were critical to this study. Perhaps most important was the underlying issue of fertility as central to the identity of the perpetrator.

This presentation reviews the findings of this study and its implications for public safety and for forensic assessment. Examples illustrating the expression of these peculiar personalities, how they relate to others in their lives antecedent to and following the crime, and lessons from how victims are ensnared — and escape — are also presented.

Female, Fetal Kidnapping, Cesarean



Luncheon Seminar - 2015

L2 The Dating Game Killer: Rodney Alcalá's 40-Year Dance With Death

*Melissa Mourges, JD**, New York County District Attorney's Office, One Hogan Place, New York, NY 10013; *Martha Bashford, JD**, New York County District Attorney's Office, One Hogan Place, New York, NY 10013; *Mark E. Safarik, MS**, Forensic Behavioral Svs Int'l, 10908 Courthouse Road, Ste 102255, Fredericksburg, VA 22408; and *Jonathan Hayes, MD**, OCME, 520 1st Avenue, New York, NY 10016

After attending this presentation, attendees will learn how investigators linked the murders of two women in New York City in the 1970s with a string of California homicides committed by "Dating Game Killer" Rodney Alcalá, and how he came to justice after 40 years.

This presentation will impact the forensic science community by illustrating investigative, forensic, and prosecutorial techniques that turn cold cases into court cases.

What would you think if you picked Bachelor #1 on the Dating Game and he turned out to be a serial killer? That's what happened in 1978 to a contestant who chose Rodney Alcalá as her date. Luckily, that woman's "Spidey-sense" went off after they met backstage and she refused to go out with him.

Alcalá had killed four women by the time he was a Dating Game contestant in 1978; at least three more victims followed. A decade earlier, Alcalá had abducted an 8-year-old California schoolgirl and drove her to his house. A witness called police, who broke down the front door as Alcalá jumped out a back window. Tali Shapiro was naked and near death; Alcalá had choked her by putting a barbell across her throat.

He escaped to New York City and enrolled in New York University's film school under the name John Berger. Then, in 1971, 23-year-old stewardess Cornelia Crilley was found with bitemarks on her breast, raped, strangled, and posed inside her Upper East Side bedroom, but the case remained unsolved. Later that summer, while "Berger" worked at a music camp in New Hampshire, campers recognized his picture on a Federal Bureau of Investigation (FBI) wanted poster. He was extradited for the Shapiro assault and served four years in jail.

He returned to New York, where aspiring actress Ellen Hover made a date with him, writing "John Berger" on her calendar. She disappeared from her Manhattan apartment in July, 1977. Her badly decomposed body was found buried in a Westchester park near the Hudson River almost a year later.

After Hover's disappearance, Alcalá returned to California. Within a span of 20 months, he killed five women: a teenage health aide, a 27-year-old nurse, a 32-year-old secretary, a 21-year-old data processor, and a 12-year-old on her way to ballet class. Each was raped, strangled, and posed; some were beaten, some had bitemarks.

One victim survived; Alcalá had picked up a 15-year-old hitchhiker, drove into the mountains, where he sodomized, raped, and choked her. He let her live when she promised to be his girlfriend.

DNA eventually linked the California attacks. Alcalá was convicted and sentenced to death in 2010. Detectives uncovered hundreds of photos of young women and boys in his storage locker in Seattle and searched for new victims. Meanwhile, the Crilley and Hover cases remained unsolved. Links to Alcalá were made; his fingerprint was on an envelope found beneath Crilley's body and bitemarks on her breast were consistent with Alcalá. In addition, the "Berger" notation on Hover's calendar was linked to his alias.

The Manhattan District Attorney's Cold Case Unit and the New York City Police Department (NYPD) worked to pull together a legally sufficient case to charge Alcalá in those deaths. Retired FBI Senior Profiler Mark Safarik, who worked the original California cases, and the Office of Chief Medical Examiner (OCME) Forensic Pathologist Jonathan Hayes, scoured autopsy reports and crime scene photos for similarities. Investigators found friends and neighbors of the victims, Alcalá's old girlfriends, eyewitnesses, vehicle registrations, toll receipts, decades-old serology reports, and medical and military records. Alcalá's surviving victim relived the horrible details of her ordeal, and the victims' families cried again as they relived the last time they saw their sisters and learned about their deaths. Alcalá was indicted for killing Crilley and Hover. He pleaded guilty to their murders in 2013 and received the maximum sentence. Headlines at the time read, "Judge Weeps as Serial Killer Sentenced." Alcalá was returned to San Quentin where he still lives on Death Row.

Dating Game Killer, Sexual Predator, Cold Case Serial Homicide



W1 Mass Fatality Incidents: An Integrated Approach Workshop

*Christian Crowder, PhD**, Harris County Institute of Forensic Sciences, 1885 Old Spanish Trail, Houston, TX 77054; *Todd M. Howell, MFS**, 92 Cinnamon Way, Magnolia, DE 19962; *Ladd Tremaine**, CMR 402 Box 2323, APO, AE 09180; *Edward A. Reedy, PhD, MD**, Armed Forces Medical Examiner System, 115 Purple Heart Drive, Dover AFB, DE 19902; *Sean A. Swiatkowski, DO**, Armed Forces Medical Examiner System, 115 Purple Heart Drive, Dover AFB, DE 19902; and *Edward Mazuchowski II, MD, PhD**, 116 Purple Heart Drive, Dover AFB, DE 19902

After attending this presentation, attendees will understand how the Armed Forces Medical Examiner System integrates various areas of forensic science in mass fatality incidents. In addition, attendees will have an understanding of how emerging technologies are leveraged in each of the areas.

This presentation will impact the forensic science community by demonstrating an effective and proven approach to integrating various fields of forensic science and emerging technologies in mass fatality incidents.

A mass fatality incident occurs when the local resources that are normally used in response to a fatality event are overwhelmed or have the potential to be overwhelmed. Although the number of fatalities or potential fatalities has been traditionally used as the delineator for a mass fatality incident, other factors such as the circumstances of the event and condition of the local infrastructure must be considered. For example, an explosion where only three individuals are deceased but there is extensive fragmentation and comingling of the remains may warrant the implementation of a mass fatality plan. This situation is exponentially complicated if the explosion is due to a terrorist activity such as a “dirty bomb.” In order to effectively manage these incidents and conduct appropriate medicolegal death investigations it is necessary to have an integrated approach between various forensic science disciplines.

Since its inception, the Armed Forces Medical Examiner System has employed an integrated approach to medicolegal death investigations, in particular to mass fatality incidents. At the location of the incident there is coordination with the local law enforcement agency, investigating agency, such as the Federal Bureau of Investigation, United States Army Criminal Investigation Command, Naval Criminal Investigative Service, or United States Air Force Office of Special Investigations, and mortuary affairs. At the scene, it is necessary to document evidence, decontaminate the remains as necessary, systematically document and recover the remains and personal effects, provide transport and/or temporary storage of the remains, and generate and maintain a chain of custody of all evidence, remains, and personal effects that will be used until final disposition. At the intake area of the autopsy operations site, all of the remains are triaged, photographed by a forensic photographer, and given a unique identifier. The remains are scientifically identified through fingerprint, dental, and DNA comparison. These comparisons require the integration of fingerprint specialists, forensic odontologists, and DNA specialists. Radiographs are performed on all of the remains by radiology technicians and forensic radiologists interpret the images. Examination of the remains is conducted by board-certified forensic pathologists, toxicology samples are taken, and all evidence is collected and released to the investigative agency. Board-certified forensic anthropologists assist in the triage of the remains and are available for expert consultation. The forensic pathologist has the overall responsibility to complete the death certificate and certify the identification of the remains, cause of death, and manner of death. The remains and personal effects are released to mortuary affairs with the appropriate documentation and communication is made with the families.

This presentation will provide an overview of how various areas of forensic science are integrated in mass fatality incidents. These areas include law enforcement, mortuary affairs, medicolegal death investigation, forensic photography, fingerprint specialists, forensic odontologists, DNA specialists, forensic radiology, forensic anthropology, forensic pathology, and forensic toxicology. In addition, the emerging technologies of each field will be highlighted.

Mass Fatality, Victim Identification, AFMES



W2 The Electrophysiology of Impulsive Aggression and Criminal Responsibility

*Alan R. Felthous, MD**, Saint Louis University School of Med, Forensic Psychiatry Division, 1438 S Grand, St. Louis, MO 63104-1027; *Stephen J. Ferrazzano II, JD**, Office of Public Defender, 601 Jewett Street, Ste A, Marshall, MN 56258; *Ghazala Hayat, MD**, Saint Louis University, 1438 S Grand Boulevard, Saint Louis, MO 63104; *Kelly N. Gable, PharmD**, Southern Illinois University at Edwardsville, School of Pharmacy, Edwardsville, IL 62025; and *Matthew S. Stanford, PhD**, Baylor University, 1 Bear Place, #97334, Waco, TX 76798

After attending this presentation, attendees will better understand of the role of an electrical abnormality in impulsive aggression and will appreciate the concomitant normalization of abnormal event-related potentials and impulsive aggressive behaviors.

This presentation will impact the forensic science community by enabling attendees to explain the strengths and weaknesses of impulsive aggression, with its electrophysiological manifestation, in mental defenses against criminal charges.

In the context of criminal responsibility it is difficult to distinguish an irresistible impulse from an impulse not resisted. Yet criminal responsibility presumes some capacity to control one's conduct and impulsive aggression is aggression without the normal level of control. The neurosciences have in recent decades enhanced our understanding of impulsive aggression. Electroencephalograph (EEG) and event related potentials are manifestations of consciousness and intention respectively, of an "electric will" as it were. A measurable biological sign of impulsive aggression, normalize by specific efficacious Anti-Impulsive Aggressive Agents (AIAAs), is the event related potential, P3 in particular. This provides evidence, beyond a subject's self-serving claim, "I could not control myself!" of a mental/emotional/behavioral abnormality with relevance to the so-called volitional component of criminal responsibility.

Event Related Potentials (ERP) are summated responses at the post-synaptic level of pyramidal neurons in the different parts of the brain to sensory, motor, or cognitive stimuli. ERPs can be exogenous or endogenous. The exogenous ERPs are of shorter latency, lower amplitudes, and high frequency. These are evoked by the environment and can be obtained under sedation. While endogenous ERPs of longer latency, higher amplitudes and lower frequency, these are not affected by changes in physical parameters of stimuli. These depend on prior experiences and expectations and are affected by psychological states. Endogenous ERPs are obtained only in awake states. Also related to voluntary action, Bereitschaft potentials, movement related potentials, are recorded over a contralateral sensory cortex in response to movements.

The most common latencies recorded are P3, N1, SW, and CNV. The ERPs can be helpful in dementia, Parkinson's disease, vascular dementia, multiple sclerosis, encephalopathies, and other neuropsychiatric disorders. The P3 abnormalities have been shown in many of these disorders including Down and Turner's syndrome.

With standardizations, ERPs can be helpful in many neuropsychiatric disorders, there are more easily performed as compared to functional MRI scans.

The panel will review neurophysiological biomarker assessments, specific to pharmacotherapy treatment options used for the treatment of impulsive aggression. Biomarker assessments are closely linked to brain physiology and maybe a reasonable representation of pharmacotherapy changes. The electroencephalograph (EEG) abnormalities seen specifically with antipsychotics, anticonvulsants, and antidepressants will be reviewed. The clinical implications of EEG changes in relation to medication efficacy and resistance will also be discussed for schizophrenia, bipolar disorder, and Attention Deficit Hyperactivity Disorder (ADHD).

Aggressive behavior is a major concern in both mental health and criminal just settings. Although pharmacotherapy is often used in the treatment of the violent individual, no medication is presently approved by the United States Food and Drug Administration specifically for such use. It has been suggested that the neurobiological deficits specific to impulsive aggressive behavior may serve as indicators of an ineffective behavioral control system. This presentation will give an overview of studies that have used ERPs as an outcome measure in pharmacotherapy for impulsive aggression.

With this scientific and clinical background on the electrophysiology of impulsive aggression, the format of the panel will change over to a brief discussion of the American Law Institute (ALI) insanity defense and the extreme emotional disturbance defense, followed by a mock direct and cross examination of an expert witness at trial. The extreme emotional disturbance discussion will center on the jury instruction for the State of New York where it is an affirmative defense that can, if successful, reduce a murder charge to a first degree manslaughter conviction. Using a created case to best illustrate the points of this presentation, the mental health professional will provide testimony in support of impulsive aggression, also diagnosed as intermittent explosive disorder, in a defendant charged with



Workshop - 2015

first degree murder. The double mental defense will consist of both the volitional prong of the ALI insanity standard and a version of the extreme emotional disturbance defense respectively. After attendees provide the verdict, the discussion will focus on the strengths and deficiencies of impulsive aggression, where sufficiently severe and abnormal, as a mental defense without the presence of another mental disorder.

Event-Related Potentials, Impulsive Aggression, Criminal Responsibility



W3 Classification of Typewritten Documents

Karen J. Nobles, BA, Forensic Document Examinations, PO Box 411, Pensacola, FL 32591; and Peter V. Tytell, BA*, Forensic Research, LLC, 15 Maiden Lane, Ste 308, New York, NY 10038-4017*

After attending this presentation, participants should have an understanding of the typewriter typestyle classification systems and understand their uses and limitations.

This presentation will impact the forensic science community by providing an update of typewriter typestyle classification systems.

Typewriters, typewriting, and typewritten documents have shown an increase in popularity in recent years. In 2010 in Philadelphia at Bridgewater's Pub in 30th Street Station, the first "Type-In" was organized and held by Mike McGettigan. The popularity of the Type-In has grown, and in recent years they have been held in various locations across the United States, Canada, Europe, Asia, and Australia. A Type-In is an arranged meeting of manual typewriting enthusiasts. A typical Type-In may include the following activities: a typing speed competition; distribution of stationery, envelopes and stamps, followed by a typed letter-writing session; and, the swapping and purchasing of typewriters.

One suspect told investigators, "I saw on TV how the cops can seize your computer and get all of the information off of it, so I bought a typewriter, which I didn't think could be identified or tied to me."

This workshop will enhance and refresh the forensic document examiner's skills in the examination of typewritten documents. The morning session will introduce the attendees to the history of type style classification schemes, including those developed by Ordway Hilton, Dr. David Crown, and by Josef and Bernard Haas. The basic principles for classifying typewriter type styles will be demonstrated and explained.

The afternoon session will cover classification techniques using the WinType classification program, the Haas Atlas of Pica and Non-Pica Typewriter Print, and the Interpol System for Identification of Typewriter Makes Card Index. The WinType program is an update of the original "type" program developed by Dr. Philip D. Bouffard in the late 1980s. Dr. Bouffard's TYPE program was based on the manual typewriter classification systems of the time, and required a DOS-based operating system. It used a searchable database to limit the number of known type specimens that had to be compared with a questioned typewritten text. Unfortunately, the current Windows environment does not allow the original TYPE program to operate correctly.

Numerous practical exercises using the WinType program, as well as other classification systems, will provide a unique hands-on experience. A copy of the WinType database and other valuable reference files will be provided to each attendee.

Attendees will need to bring a laptop computer running Microsoft® Access, or other comparable database program, and a hand magnifier. A small flashlight, or other portable light source, that can be used for additional lighting may also be helpful.

After attending this workshop, participants should have a greater understanding of the history of typewriter type styles classification systems, the principles of classifying type, the typewriter reference material used, and a firm understanding of their limitations.

Typewriter, Typestyle, Classification



W4 Obtaining Successful DNA Profiles From Challenging Samples

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After attending this presentation, attendees will understand how to overcome challenges associated with trace and/or degraded forensic DNA samples by using novel DNA extraction, quantification, profiling, and analysis technologies to provide probative results from even the most compromised DNA samples.

This presentation will impact the forensic science community by serving as a forum for disseminating the latest information regarding the analysis of challenging forensic DNA samples. The panel discussion will include information on new DNA testing systems and methodologies for cases where traditional methods do not produce results.

The past 25 years have seen great strides in forensic DNA analysis. Improved evidence collection procedures and technology advancements using the Polymerase Chain Reaction (PCR) process have enabled scientists to provide scientific evidence in criminal cases that were previously unimaginable. Next generation platforms, test systems and methodologies now provide scientists with the tools to evaluate biological samples that consist of degraded, trace, touch and/or contact DNA evidence. Typically forensic DNA samples have multiple challenges including: limited quality, limited quantity, the presence of PCR inhibitors and complex result interpretation. These factors contribute to the difficulty of eliciting meaningful profiles from compromised samples. At present, there is a real need for more robust, highly sensitive, reproducible methods for the extraction, quantification, PCR amplification, typing and interpretation of DNA results when attempting to profile these difficult samples.

Next generation platforms and test systems now provide scientists with tools to evaluate samples consisting of degraded and/or trace DNA with greater efficiency and higher success rates. This panel of subject matter experts will present their research and findings regarding challenging forensic samples and how to overcome issues of degraded and/or low copy DNA as well as new technologies designed to provide probative results from even the most compromised forensic samples. Topics covered in this workshop include improvements in extraction efficiency, optimizing laboratory processing procedures, trace DNA recovery methods, improved methods for DNA quantification, setting thresholds for capillary electrophoresis, novel DNA markers systems and result interpretation issues with challenging samples such as mixtures, and statistic probabilities and allele drop-out. This panel of experts will also discuss case studies, including missing persons and mass disaster identifications.

Topics presented include:

- Optimized processing methods developed and utilized for the identification highly challenged human remains.
- How to set analytical thresholds in the face of new instrumentation and improved STR kits.
- Development and application of trace DNA methods to household dust and touch evidence.
- Novel marker system for analysis of highly compromised DNA samples.
- Analysis of highly degraded missing persons samples.
- Improvements in DNA extraction efficiency from challenging samples such as hair and bone.
- Forensic statistics at the NYC OCME - dealing with complex DNA mixtures, probabilities and dropout.
- World trade center disaster samples 14 years later.

DNA, Degraded, Challenging Samples



W5 Ancestry Estimation in the 21st Century

*Kate Spradley, PhD**, Texas State University, Dept of Anthropology, 601 University Drive, San Marcos, TX 78666; *Joseph T. Hefner, PhD**, Michigan State University, Dept of Anthropology, 355 Baker Hall, East Lansing, MI 48824; *Stephen D. Ousley, PhD**, Dept of Applied Forensic Sciences, Dept of Anthropology, 501 E 38th Street, Erie, PA 16546; *Richard Jantz, PhD**, University of Tennessee, Dept of Anthropology, Knoxville, TN 37996-0720; *Bruce E. Anderson, PhD**, Pima County OME, Forensic Science Center, 2825 E District Street, Tucson, AZ 85714; and *Kathryn H. Haden-Pinneri, MD**, Harris Co Inst Forensic Sciences, 1885 Old Spanish Trail, Houston, TX 77054

After attending this presentation, attendees will: (1) understand methodological and theoretical aspects of ancestry estimation in light of the modern understanding of human biological variation and classification statistics; (2) comprehend the complex population groups within the United States and the best methods and terminology to use when providing an ancestry estimate; (3) recognize the best methods to use for non-metric and metric ancestry estimation; and, (4) foster an understanding of the role played by the cultural profile in medical examiners' offices and the role of the forensic pathologists in ancestry estimation.

This presentation will impact the forensic science community by providing up-to-date methodologies and modern theoretical considerations in ancestry estimation using traditional and novel approaches. Additionally, attendees will be provided with new approaches and solutions to new concerns when confronted with the current population demographics of the United States.

This presentation will focus on multiple aspects of ancestry estimation in the modern era and direct attention to some of the more important considerations necessary when making these estimates. Following a general introduction and a thorough overview of human biological variation and secular change, the focus of attention will be refined to illuminate aspects of ancestry estimations from different viewpoints and multiple forms of analysis. Ancestry estimates by the medical examiner rely on aspects of human biological variation related to, but different from, those used by forensic anthropologists. Understanding how each practitioner can assist each other and appreciating the separate questions answered is necessary and timely, given the changing demographics in the United States. When attempting to identify the remains of an individual from a complex population, we must understand the history of that population and how that history affects population structure. In other words, we need to "know" the population in order to estimate ancestry. Methodological approaches to the estimation of ancestry will also be addressed. These will include comprehensive discussions on metric and non-metric methods of analysis and a thorough exchange of ideas on the traditional and novel statistical methods and theories being used today. While metric and non-metric methods have traditionally dominated forensic anthropological analysis of ancestry, the cultural profile in conjunction with the biological profile, stands as a novel although very important tool for ancestry estimates, particularly in Undocumented Border Crossing (UBC) cases from the southwest where the cultural profile was developed. The cultural profile has been instrumental in the identification of UBCs from Central and South America.

Establishing the ancestry of human remains has been referred to as one of the more difficult aspects of biological profile construction; however, this need not be the case. Our understanding of human variation has changed dramatically since the early days of biological anthropology; in fact, the last quarter-century has witnessed intensification in the methods used to estimate ancestry and in the theoretical considerations of secular change and population specificity of method application. Non-metric traits no longer rely on typological trait lists or extreme trait values supposedly linked to a "race." Metric analysis has likewise experienced a concerted change as our understanding of classification statistics increases and as we acknowledge the need for more reference populations from around the world to represent the true nature of human biological diversity.

Biological profiles generated in a medicolegal setting play an important role in identification, therefore, estimating ancestry is a fundamental component of the biological profile; however, understanding why we can estimate ancestry and the complex factors surrounding ancestry estimation are given little attention in the forensic anthropology literature. Understanding the population history and structure in addition to secular changes in the American population, with particular attention to complex population groups (e.g. Hispanic, American Black), can provide a greater understanding of process of ancestry estimation and can lead to more accurate results. Further, gone are the days of trait lists and typology. The new morphoscopic approach relies on a comprehensive understanding of the traits and their character states, along with proper classification statistics for ancestry estimation. Metric analyses also require more than just rote memorization of landmarks and dated classification statistics. New metric methods, such as machine learning techniques, capture the salient features of geographic ancestries, but may also be used for further refinement at the local population level. These methods require knowledge in the data at hand and in the statistical methods used for classification.

Ancestry, Human Variation, Biological Profile

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W6 Practical Homicide Investigation®: Offender-Manipulated Homicide Scenes Relating to Equivocal Death and Staged Crime Scenes

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After attending this presentation, attendees will have a better understanding of the unique dynamics of offender-manipulated homicide scenes and equivocal death investigations as well as the application of professional homicide investigation and medicolegal analysis of these events.

This presentation will impact the forensic science community by providing and familiarizing forensic scientists and investigators with the art and science involved in the professional examination of homicides and sudden, violent death scenes which have been manipulated by the offender to mislead and thwart the investigation.

Offender manipulated homicide scenes pose a unique problem for the first responders and investigators because these events are equivocal death investigations and as such are open to interpretation. Crime scene and autopsy observations may suggest more than one meaning. The facts may be purposefully vague or misleading as in the case of the “Staged Crime Scene” or where the death is suspicious or questionable based upon what is presented to the authorities.

Such deaths may present as homicides, suicides, accidents, or natural causes and may be altered by the offender to misdirect the investigation through staging of the scene and/or posing or mutilation of the body. They are open to interpretation pending further information of the facts, the victimology and the circumstances of the event.

This workshop will focus on the investigative applications and best practice model of Practical Homicide Investigation® and the medicolegal evaluation of specific equivocal cases as well as the application of forensic pathology to the investigative process to accurately determine the cause and manner of death through a multidisciplinary medicolegal investigation as they relate to the investigation of offender manipulated homicide scenes.

Upon completion of this workshop the participants will have a better understanding of the unique characteristics and dynamics of offender manipulated homicide scenes and equivocal death investigations as well as the application of professional homicide investigation and medicolegal analysis to these events. The participants should better understand the importance of crime scene integrity, the management of the homicide investigations, the processing of the homicide crime scene, as well as the application of the medicolegal investigation specifically as it relates to cause and manner of death and the evaluation of the lethality of injuries and wounds.

Equivocal Death, Victimology, Forensic Science



W7 Challenges in Fire Debris Analysis

Eric Stauffer, MS, Police cantonale Fribourg, Place Notre-Dame 2, Fribourg, FR CH-1700, SWITZERLAND; Reta Newman, BS*, Pinellas County Forensic Lab, 10900 Ulmerton Road, Largo, FL 33778; Julia A. Dolan, MS*, Bureau of ATF, Forensic Science Laboratory, 6000 Ammendale Road, Ammendale, MD 20705; and Douglas E. Byron, BS*, Forensic & Scientific Testing, 275 N Perry Street, Lawrenceville, GA 30046*

After attending this presentation, attendees will better understand the interfering products that can be found in fire debris analysis and how the products make identification of ignitable liquids difficult. Additionally, attendees will become familiar with: (1) different ignitable liquid classes and their chemical characteristics; (2) the effects of the extraction techniques on the recovered ignitable liquid residues; (3) the thought processes used to identify interfering products and ignitable liquids from fire debris samples; (4) the composition of various non-traditional ignitable liquids; and, (5) why different analytical procedures are needed. After this presentation, attendees will be able to return to their forensic laboratories and handle the interpretation of the most difficult fire debris samples.

This presentation will impact the forensic science community by helping attendees better understand and interpret data related to the analysis of fire debris samples, which will lead to a reduction in the number of false negative conclusions and will eliminate false positive conclusions. The direct impact of this presentation on the forensic science community will be a more accurate and reliable science being practiced in crime laboratories.

The analysis of debris and evidence in a fire investigation commonly includes the identification and classification of various types of ignitable liquids. Because the vast majority of ignitable liquids are complex mixtures of hundreds of chemicals, and because many, if not most, of the chemicals present in ignitable liquids are created by pyrolysis or incomplete combustion of common items (carpet, carpet pad, upholstery materials, plastics, etc), proper recognition, identification and classification can be challenging. Couple that with the fact that many substrates inherently contain ignitable liquid residues due to their manufacturing process, an analyst must be able to elucidate the significance of laboratory findings in an appropriate context. The attendees of this workshop will learn how to properly interpret complex chromatograms obtained from the analysis of extracts from fire debris samples. Attendees will first learn the fundamental theory of what fire debris samples are, how they are created, and what stages samples go through before they reach the laboratory. This will provide the necessary foundations for understanding the chemistry of interfering products such as pyrolysis products. The physical and chemical processes leading to the presence of interfering products will be covered.

And, while petroleum based ignitable liquids are the most common, other ignitable products, including bio-fuels and vegetable oils are also of interest in some investigative scenarios. This course will include discussions on the different types of ignitable liquids and their chemical characteristics. Examples of both traditional (mainly petroleum based) and non-traditional ignitable liquids (biofuels, vegetable oils, etc.) that may be encountered will be covered. Presentations will include strategies for recognizing the possible presence of ignitable liquid residues (including physical characteristics of the samples, investigative information, and preliminary chemical findings); the processes associated with optimizing extractions based upon both the sample type and the target analytes; and best instrumental methods and method parameters necessary to identify or classify each type of ignitable liquid. Case discussions with sample data will be included to illustrate the interpretation process.

The type of extraction used and parameters associated with the extraction process used for fire debris analysis can have a significant impact on the data obtained. The influence of the various extraction procedures will be presented with discussion regarding the optimization of sample specific parameters for the most representative extraction. The presentation will include the most common extraction techniques using passive headspace sampling with various adsorbents (activated charcoal, 2,6-diphenyl-p-phenylene oxide, SPME) as well as solvent extraction and simple headspace sampling. Focus will be given to the impact of extraction parameters, primarily surface area of the adsorbent, selectivity of the adsorbent, desorption parameters, and adsorption duration and temperature on the efficacy of the extraction process. Simulated case samples in which extraction technique significantly impacted data generation will be included in the discussion.

The workshop will include practical examples of real case or simulated case samples. Attendees will be encouraged to apply the information provided in the presentations to recognize, identify, classify and/or troubleshoot complex samples using proper interpretative methodology. This element of the workshop will consist of small-group discussions of the data under the close supervision of, and with lively input from, the presenters.



Workshop - 2015

At the conclusion of this workshop, the attendees shall have a better understanding of processes associated with interpreting complex fire debris data starting with assessing the physical characteristics of the samples and the investigative information and culminating with the proper interpretation of the analytical data generated in the laboratory process.

Fire Debris Analysis, Ignitable Liquid Residues, Interpretation of Chromatogram



W8 From Fire Dynamics to Legal Dynamics: Shifted Science and the Criminal Justice System's Response

John J. Lentini, BA, Scientific Fire Analysis, LLC, 88005 Overseas Highway, #10-134, Islamorada, FL 33036; Mark E. Goodson, PE*, 1500 Spencer Road, Denton, TX 76205-5105; James M. Doyle, LLM*, Bassil, Klovee & Budreau, 20 Park Plaza, Ste 1005, Boston, MA 02116; Terry-Dawn Hewitt, LLM*, McKenna Hewitt, 9057 E Mississippi Avenue, #11-206, Denver, CO 80247; and Steven W. Carman, MS*, Carman & Associates Fire Investigation, Inc, PO Box 273, Grass Valley, CA 95945*

After attending this presentation, attendees will grasp the changes that have inundated the fire investigation profession and, to a larger extent, the criminal justice system in which wrongful convictions are sadly a part of the process. Attendees will develop an appreciation of a systematic approach to the prevention of wrongful convictions, much like the National Transportation Safety Board (NTSB) focuses on the causes of train wrecks and plane crashes, not with the idea of blaming someone, but of preventing the next disaster.

This presentation will impact the forensic science community by raising awareness of new science, new standards, and new methods of review on the criminal justice system's handling of fire litigation.

This presentation will explore the changes that have swept through the fire investigation profession in the last few years, both in terms of our understanding of how fires behave and how the courts have come to view fire-related expert testimony.

Recent work by Steven Carman and others has dramatically changed the way fire investigators must view the role of ventilation in fully involved fires. Determining the point of origin of the fire, a fire investigator's core competency, is far more difficult than previously believed. As a result, many fire origin determinations have been called into question. If the fire investigator has not correctly determined the origin, chances are his cause determination is also wrong. The frequent scenario is one where, having determined the wrong origin, the investigator finds no competent source of accidental ignition at the "origin," and using "negative corpus" methodology, declares the fire to have been set.

This presentation will describe the work carried out to attempt to measure the accuracy of origin determinations, and to evaluate the utility of fire patterns in determining the correct origin.

As this new knowledge percolates through the fire investigation community, the legal community has learned new ways of challenging the reliability of fire investigation opinions. Terry-Dawn Hewitt will describe the "perfect storm" now enveloping fire investigators because of Daubert challenges, the National Academy of Sciences Report (NAS), changes in NFPA 921 and 1033, and recent court decisions.

Several cases will be reported where individuals served long prison sentences as a result of being convicted for setting fires that were actually accidents, and will provide some of the legal decisions that move beyond "ineffective assistance of counsel" and "new evidence" theories, and actually embrace the shifted science and fire investigation.

Examination of errors in the criminal justice system will be discussed, including faulty arson determinations, using an approach similar to that used in the medical and transportation industries. "Sentinel event" analysis looks carefully at the train wreck that is a wrongful conviction, but not with the sole intent of finding someone to blame. When people act inappropriately, it is often not simply a result of a "bad apple." Often the cause is a system that encourages bad behavior, or fails to catch it.

Finally, the Scientific Review Board set up by the Texas State Fire Marshal in the wake of the Forensic Science Commission's review and recommendations resulting from the investigation of the Willis and Willingham cases will be described. The new Texas Fire Marshal, Chris Connealy, set up the Board to retroactively examine the arson convictions of individuals serving time in Texas prisons. Several cases reviewed by the committee have been found to not stand up to the standards in place today.

Shifted Science, Ventilation, Conviction Integrity



W9 Forensic Anthropology and Cold Case Investigations: Breaking the Ice

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The goal of this presentation is to provide training specific to long-term unsolved cases in the areas of forensic anthropology and archaeology, imaging, human identification, and geochemical profiling. This workshop is relevant for anyone in homicide investigations, forensic science, or forensic anthropology working in the United States or internationally.

This presentation will impact the forensic science community by providing an in-depth exploration of a range of new tools and an integrated approach to investigating previously unsolved or long-term cases. The integration of scientific experts into homicide investigations provides an array of evidence and investigative leads that help increase case solvability.

Since 1960, the homicide rate in the United States of America has remained relatively constant; however, significant changes have been made in the professionalization and development of resources available to homicide investigators in the areas of national data and reference banks, innovative forensic tools, forensic imaging, chemical and elemental isotope analysis, and crime scene processing. Moreover, there is a renewed commitment to addressing the problem of long term unsolved or cold cases and the use of new tools and technologies improving the solvability rate.

Research on cold cases, why some cases remains unsolved, and the best approaches utilized to increase their success rate is largely lacking in the scientific literature; however, one suite of tools that is rapidly becoming part of homicide investigations comes from forensic anthropology which offers investigators a range of services, research, training and professional development in human identification, facial imaging, living person age estimation, clandestine grave search and recovery, grave excavation, trauma analysis, and expert testimony.

Homicide cases in which the victims are unknown are the hardest to solve and make up a significant portion of the unidentified persons pool in the United States. Increasingly, anthropologists are also used by law enforcement to assist at the crime scene with human identification, imaging, and media outreach. The purpose of this presentation is to provide training specific to longterm unsolved cases and is relevant for anyone in homicide, forensic science, or forensic anthropology. Experts from anthropology, geochemistry, and homicide investigations will discuss current trends for methods and case analysis in several key areas: (1) cold case analysis: opening pandora's box; (2) witness interviews and issues of episodic memory; (3) crime scene and field methods: from excavation to remote sensing; (4) forensic anthropology methods of ancestry and imaging; (5) geochemical methods to track mobility, birth origins, and diet; (6) public outreach: using media as a tool to bring in leads; (7) case management: when the years pass by; and, (8) trends and opportunities in education and training in cold cases.

Over the past four years, a service oriented research initiative, the Tampa Bay Cold Case Project, has brought the University of South Florida Forensic Anthropology Laboratory together with local medical examiner departments and law enforcement agencies throughout the region. Through this initiative, anthropologists work together with homicide detectives, criminalists, forensic laboratories, and other scientists to systematically go through unsolved cases, dating from 1940 - present and have created a network for collaborators, called the International Consortium for Forensics, Anthropology, and Human Rights (ICFAHR) (www.icfahrf.org). This partnership has been formalized between University of South Florida, the Hillsborough County Sheriff's Office, and the National Center for Missing and Exploited Children to provide training and technical assistance to current and past cases and serves a model for regional networking and service training.

The methods and techniques for this initiative, the ways in which similar models could be established in other regions, and successful case study examples are discussed. From locating buried remains in clandestine graves to identification and prosecution strategies; cold case investigations are discussed from a multitude of perspectives.

Homicide, Cold Case, Forensic Anthropology

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W10 NamUs: The Results of Forensic Collaboration

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After attending this presentation, attendees will: (1) learn about the history of the National Missing and Unidentified Persons System (NamUs) and the various NamUs user types as well as the manner in which NamUs receives and validates its case data; (2) understand how the NamUs databases interact to produce potential matches; (3) realize how case exclusions are made; and, (4) grasp the importance of the “public user” to the success of NamUs.

This presentation will impact the forensic science community by increasing the attendees’ ability to enter cases into the NamUs system and participate in the investigation or tracing of missing and unidentified persons cases nationally. The skills and knowledge obtained in this presentation will assist attendees in communicating with other forensic investigators on cases of missing and unidentified persons regardless of jurisdictional boundaries.

The National Missing and Unidentified Persons System (NamUs), is an electronic clearinghouse for cases involving missing and unidentified persons. NamUs is a free, online, technological resource that can be searched by medical examiners, coroners, forensic scientists, law enforcement officials, and the general public seeking to locate missing persons, or identify those individuals who remain unidentified in morgues across the United States. This workshop will present information highlighting the system’s development, management and the many application strategies used by forensic investigators who regularly use the system as an investigative tool. All workshop attendees will have the option of becoming NamUs “registered users” after the AAFS workshop.

NamUs consists of three linked databases: one populated with information about missing persons (NamUs-MP), one populated with information obtained from the discovery and examination of the remains of unknown persons (NamUs-UP), and the third populated with those individuals who remain unclaimed even after identification is confirmed. Through interactive queries between these three databases, profiles of missing persons can be compared with profiles of unidentified decedents, and vice versa. Unclaimed case information is regularly posted by popular search engines and used by individuals conducting genealogy research who find additional next-of-kin and communicate with medical examiner and coroner offices regarding this information.

Data about missing persons can be entered into the NamUs system by registered public users (including relatives of a missing person), registered law enforcement officers, registered members of clearinghouses, and NamUs staff members. The profiles of unidentified persons are entered into the NamUs system by registered representatives of coroner’s and medical examiner’s offices, some registered forensic scientists, and NamUs staff members. Users of the NamUs-MP and NamUs-UP systems are allowed differential access to information and data entry, such as through viewing and/or editing privileges that are assigned by NamUs administrators. Unclaimed cases may be entered into the system by medical examiner/coroner offices or automatically transferred from the unidentified persons system to the unclaimed system.

Members of the general public can view selected information within all NamUs databases without registration; however, their access to any confidential information about a missing person is restricted, as is their ability to view certain details about unidentified persons. Even after registration, access to some information is completely denied to public users, such as details about a case intended solely for the eyes of law enforcement personnel or medicolegal investigators. Despite some limitations, NamUs is the first government-sponsored website of its kind to allow the general public to view the profiles of missing persons, the unidentified and the unclaimed dead.

Missing, Unidentified, Unclaimed



W11 RADid: Using Radiologic Technologies to Identify Unknown Decedents

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After attending this presentation, attendees will understand: (1) the role of radiography and advanced Radiologic Imaging (RADid) in the process of decedent identification; (2) techniques for performing high-quality imaging and creating 3D images and strategies to be able to use these images to make successful identifications; (3) anthropologic approaches to RADid; (4) the need for interdisciplinary collaboration; and, (5) the evolving nature of the evidence basis and standards for RADid.

This presentation will impact the forensic science community by enhancing the ability of practitioners to perform RADid, solidifying awareness of the scientific underpinnings of the methodology, and fostering the interdisciplinary collaboration necessary to ensure the most appropriate methods are considered in any given case. The overall goal is to increase the use of these valuable techniques and, in so doing, increase the number of identifications that are successfully made in the medicolegal death-investigative community.

Forensic pathologists and other forensic practitioners are well aware of the legal and ethical imperatives to identify the dead. Identification using RADid is a well-recognized and perhaps underused method to accomplish this task. Postmortem radiographs, the historical workhorse for RADid, require special attention to technique and positioning to enable successful comparison with Antemortem (AM) studies. The incorporation of advanced imaging modalities in medicolegal death investigation, such as Postmortem Computed Tomography (PMCT), offers new possibilities for RADid. Image data sets generated by PMCT can be rendered and oriented to match almost any AM radiologic study. When comparing to AM radiographs, images replicating the appearance of radiographs can be generated using thick slab multi-planar reformatting in the correct orientation (dubbed “pseudo-radiographs”). Similar images that mimic the orientation and field of view of dental radiographs and orthopantograms can be created, which may facilitate dental identification. Familiarity with these techniques can enable forensic practitioners without Computed Tomography (CT) scanners to create pseudoradiographs from antemortem CT data, for comparison with postmortem radiographs. These techniques are straightforward to master and will be demonstrated during the workshop.

Forensic anthropologists are increasingly using PMCT in addition to standard radiograph comparison for ID investigations. A number of traditional anthropologic measurements can be made from planar or 3D CT reconstructions. These measurements can be used to characterize the biologic profile, potentially narrowing the search for AM records, excluding potential identities, or selecting cases for more detailed anthropologic analysis. Additionally, a new Computer-assisted RADid method using sophisticated shape matching software (CADI) is under development.

A wide array of anatomic and pathologic features are used for RADid comparison including: normal and variant anatomy, presence of new or old disease and foreign bodies. The range of capabilities for decedent identification, and the advantages and disadvantages of RADid will be demonstrated through specific case examples. Techniques applied in this process bridge the technical expertise and knowledge bases of several disciplines. Therefore, collaboration between many types of forensic experts is optimal. Advanced imaging is ubiquitous in medical facilities throughout the United States, but is rare in this country’s medical examiner or coroner’s offices. Specific strategies detailed in this workshop will address the perceived lack of access to advanced imaging in the forensic community.

Identification, Forensic Radiology, Postmortem Computed Tomography



W12 Implementing and Evaluating 3D Technology in a 2D World

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After attending this presentation, attendees will have an understanding of the principles behind each of the technologies presented and the strengths and weaknesses that have been observed. The theory of use, method development, maintenance, and applications within the field of firearms/tool marks will be discussed. The presentation will focus on the studies that have been conducted, evaluation of the data generated, and the potential impact these technologies will have in the discipline of firearms and tool marks.

This presentation will impact the forensic science community by expanding the knowledge-base regarding the use of newer technologies in the forensic firearms/tool marks setting.

The current methodologies used in the firearms/tool marks discipline can be traced back to the early work of J. Howard Mathews, Calvin H. Goddard, and James Hatcher from the 1920's. In these early days of research, many of their methods showed a close parallel to existing technologies that relied upon measurements and illumination techniques. The same question continues to motivate the practitioner to determine whether or not a bullet or cartridge case could be "identified" to a particular firearm. Even before the arrival of the comparison microscope, a match could be determined through the use of a filar micrometer, which was simply a special device placed at the top of a compound microscope that contained a scale and a cross hair, which moved along a scale. Another method used was the method of interchange. This method depended upon an illumination technique which involved a long camera set-up with a short lens. It was also during this time that the stereophotomicrograph was introduced. This system enabled illumination from all directions and was simply a stereo camera on a binocular microscope which allowed for a 3D view of an object and it was realized then how troublesome vibrations could be to capturing these types of images. Now, almost ninety-years later, novel instruments for measuring and illumination can be used to highlight areas on bullets and cartridge cases to a level beyond the 2D world. This workshop will present the work that has been conducted using the Sensofar instrument capable of confocal microscopy, interferometry, and focus variation techniques and the intercomparison of that data, highlighting the strengths and weaknesses of the system. Results of the validation work generated using the TopMatch-GS3D Imaging and Analysis System will be presented highlighting the strengths and weaknesses of the system. Validation work that has been conducted on the Alicona Infinite focus system, the integration of this system for the collection of general rifling characteristics measurements on bullets, and the implications that have been seen for updating the Federal Bureau of Investigation's (FBI) General Rifling Characteristics Database (GRC) will be presented.

This presentation will present the evaluation/validation work that has been conducted on the Sensofar® Instrument, TopMatch-GS™ 3D Imaging and Analysis System, and the Alicona® Infinite Focus® microscope. As a result of these validation experiences, an overview of the necessary validation processes that a laboratory should consider when planning to incorporate new technologies in today's forensic laboratories will be provided as well as how to set up a training program for personnel conducting analyses with these systems. An introduction on how to report the results generated from these methodologies will be presented as well as how to approach court challenges and effectively articulate these technologies and interpretation of results in a court of law.

Topography, Ballistics, Individual Characteristics



W13 The Examination of Skillfully Simulated Signatures

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After attending this presentation, attendees will be able to examine and give opinions on signatures that may be genuine or the product of skillful simulations. Attendees will learn the requirements for a skillful simulator and the characteristics they should look for in the examination of a signature that may have been skillfully simulated.

This presentation will impact the forensic science community by making attendees more aware of the features that may be present in a skillful simulation and, therefore, increasing the examiner's reliability and accuracy in determining the authenticity of signatures.

In the examination of a signature on a document to determine its authenticity, trained forensic document examiners are exposed to document case problems that include such questioned signatures on wills, contracts, log books, checks, financial forms, and other documents. The characteristics and features of simulation may include poor line quality, tremor, lack of variation in pen pressure, hesitations, unusual pen lifts, patching, and retouching of the signature; however, in simulations that have been skillfully executed, these flags may be absent and the examiner may have some difficulty in spotting the flaws in the questioned signature. Such signature authentication problems that may involve a skillful simulation can pose a difficult task for an examiner to render a correct conclusion as to its authenticity.

This workshop will focus on the examination process of questioned and known signature samples and their evaluation, leading to the opinion as to the authenticity of the questioned signature that may be genuine or skillfully simulated. Signature problems of this kind are challenging and require the forensic document examiner to properly assess fine and subtle writing characteristics that are indicative of simulation or are the product of natural handwriting variation. Technical application and proper interpretation of authenticating questioned signature samples to determine whether a signature is genuine or a skillful simulation will also be addressed and further discussed. Attendees will discuss the types of opinions that may be expressed in such examinations.

Upon completion of this workshop, the attendees will have a better understanding of authenticating questioned signatures that may be genuine or simulated. The attendees will also learn about the requirements for a person to be a successful simulator and best practices when examining signatures for authentication. The participants should be able to better understand the strengths of opinions that can be rendered in these types of examinations and the pitfalls that lay in wait for the unwary examiner.

Forensic Science, Document Examination, Signature Examination



W14 On the Leading Edge of Forensic Science

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The goal of this presentation is to describe how new developments might impact forensic scientists in their work. Practical examples will be presented.

This presentation will impact the forensic science community by providing an overview of some of the new developments in forensic science and by opening a forum for the discussion of issues that arise regarding such developments.

A wide variety of developments that will soon impact forensic science have been identified within the Think Tank Committee of the Forensic Sciences Foundation, Inc. These range from developments in facial comparison systems, labs on a chip, cloud forensics, drone forensics, cybercrime (including hacking medical devices such as insulin pumps), bitcoins, and augmented reality to 3D printing and even printing DNA for storage of information or making a clone of Van Gogh's ear based on DNA.

The workshop will discuss the advantages and limitations of facial comparison systems possibilities. While there are many publications on automated systems describing their efficacy, questions remain on how well they perform in reality, especially in uncontrolled conditions.

Another focus of the workshop will be on new developments in drone forensics. Proper evidence collection protocols will be discussed.

Within big data and cybercrime forensics several new methods have been developed for analyzing huge amounts of data within the first 24-hours after a crime has occurred. The processing and storage of large amounts of data as well as indexing it in a secure and transparent way to address, while also privacy related issues are the cornerstones of these systems. The workshop will also address cybercrime related issues with body nets (where body sensors are connected to the internet) as well as extracting evidence from other devices such as insulin pumps.

In the last decades, hyperspectral imaging techniques evolved from remote sensing and airborne applications, and are now finding their way into forensic investigations. Hyperspectral imaging enables the non-destructive visualization of chemical differences within the original context, and has proven its value in various forensic applications, e.g. ink comparisons and fingerprint detection. Recent advances in hyperspectral imaging technology enable the development of portable and fast image acquisition systems, motivating a shift from forensic laboratories to the crime scene. In the future, all crime scene investigations could start with a complete spectral scan to record the optical properties in the visible wavelength range, the near infrared and the thermal infrared. This provides investigators with information invisible to the human eye. In this presentation, forensic hyperspectral imaging applications are reviewed and possible future applications are discussed.

A number of forensic applications of virtual and augmented reality have been proposed and tested, such as virtual scene tours, serious gaming, scenario testing and communication. An overview is given of applications that have been tested so far. For every application, a short explanation will be given on the technology and its usability. Finally, preliminary results of a usability study being performed by the Netherlands Police, the University of Delft, and the Netherlands Forensic Institute, will be presented.

Issues with 3D printers and new possibilities for consumer printers will be discussed. The possibilities for making counterfeits and firearms are many. Forensic investigation of 3D printers differs from 2D printers and finding a link between the printer and the material can be more complicated. The next generation of 3D printers is with programmable matter, which has the ability to change the physical properties (density, shape, etc.) in a programmable way, based on user input or autonomous sensing.

Wearable Sensors, Future Developements, Impact



W15 Clinical Toxicology of the Poisoned Patient

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After attending this presentation, attendees will be able to describe the process used to evaluate, triage, and treat patients in the emergency room. Attendees will be able to identify situations where consultation with a clinical or medical toxicologist can benefit an investigation and find new resources available through medical toxicology professional organizations to support their investigations.

This presentation will impact the forensic science community by creating better understanding between forensic science professionals and their counterparts who treat living patients prior to their deaths.

Medical toxicology is the branch of medicine that is concerned with the diagnosis and treatment of the poisoned patient. Forensic toxicology, on the other hand, deals with the identification of toxic substances in fluids and tissues and the subsequent interpretation of the findings. While on the surface, the two fields seem mutually exclusive, there is often overlap, especially in the interpretive aspects of findings. Questions as to who should be doing what in related matters can arise and create an aura of confusion and, at times, unfortunate dissension despite clear educational and practice differences.

Individuals involved in the diagnosis of the poisoned patient include both physicians and pharmacists who have received appropriate training. Often, initial assessment of the poisoned patient is accomplished through poison control centers. These centers are manned by professionals who use their knowledge, education and experience, as well as accumulated data, to triage a given situation. Decisions can lead to protocols ranging from no treatment to emergent care. Alternatively, the poisoned patient may appear in the emergency room of a hospital where treatment is sought. Specially trained medical toxicologists are often consulted to diagnosis the patient and plan a course of action. While the majority of hospital laboratories are equipped to analyze for a small set of potential poisoning agents, forensic toxicology laboratories are often geared to analyze for a more diverse set of compounds that can assist in the diagnosis of the poisoned patient.

The outcomes of poisoning can range from complete recovery to death. Forensic toxicologists are often involved in the analysis of fluids and tissues from individuals who have died as a result of poisoning. The practice of forensic toxicology includes both analytical and interpretive components, with the latter representing an assessment of the role of an agent(s) in the death. Often, such conclusions have medicolegal impact. Additionally, forensic toxicologists are involved in the analysis of body fluids for purposes of human performance issues. Interpretation of the findings often impacts legal proceedings.

It is not uncommon for medical and forensic toxicologists to find themselves as opposing experts with both claiming ranking privilege. The arguments from both sides are often convincing in support of each profession. It is clear, however, that educational and experiential differences exist between both parties. Who then, is in a better position to assist in matters regarding the poisoned or impaired individual?

This workshop will provide an overview of the similarities and differences between the medical and forensic toxicologist through their approaches to the poisoned or impaired patient. The roles of each profession in respect to medicolegal issues will be highlighted followed by a discussion on how the two fields together bring synergies to investigations involving the poisoned or impaired patient that either alone, by definition, cannot attain.

Poisoning, Overdose, Medical Toxicology



W16 Your Attention, Please! — A Public Speaking Skills Workshop

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The goal of this presentation is to present an oral communications-skills workshop for forensic science students and professionals that will: (1) present more than 55 oral communications learning tools that attendees will be able to implement immediately; (2) facilitate thought and ideas for an effective, entertaining, and strategically planned oral presentation; and, (3) demonstrate how to be customer-focused on your audience.

This presentation will impact the forensic science community by educating attendees to the fact that just being an expert in your field does not provide you with the additional skills necessary to present material in an effective manner. Strong and clear oral communication is paramount in the forensic sciences field to present legal and scientific information in a manner that can be heard, understood, and remembered by listeners. This presentation provides attendees with more than 55 learning points that can immediately be implemented to make a difference in how information is presented in the classroom, courtroom, or working environment.

“Most people would generally agree that a great deal — probably most — of the presentations we have to sit through in the business world are awful. They are all too often passionless, boring, and dense with unreadable PowerPoint slides.”¹

Whether you speak to one or 1,000; are comfortable or terrified with public speaking; whether you have been speaking for many years or just a beginner; “Your Attention Please! — A Public Speaking Skills Workshop” will improve your presentation skills through strategic planning, preparation, and performance. Techniques for speaking in all settings with confidence, choosing the right audio-visual technologies, and dealing with questions from an audience, for example are explained clearly to help the participant develop their presentation skills. Fifty-five proven effective presentation tools will be presented, demonstrated, and provided to each participant who attends this workshop. Knowing your subject does NOT guarantee a successful presentation. Aristotle, who many recognize as the Father of Public Speaking and Forensic Debate said, “It is not enough to know what to say, one must know how to say it.” This short four hour workshop focuses on technique and the recognition that, “a speech is composed of three factors — the speaker, the subject and the listener — and it is to the last of these that its purpose is related.”²

Dr. Joseph Sommerville’s, *The Seven Deadly Sins of PowerPoint Presentations*, will be discussed and positive steps will be presented to take advantage of the benefits of PowerPoint presentation software, rather than be a victim or suspect to “death by PowerPoint.”³ Visuals (slides) should augment the spoken word, not be in place of it. Slide development and the use of “Mindmapping,” will be taught to create clear and dynamic visuals that help the speaker be “heard, understood, and remembered.”⁴

So reduce the fear, embarrassment, and agony of public speaking; or increase your repertoire of oral communications skills in the classroom, courtroom, or conference room. This training experience guarantees that you will gain a wealth of knowledge that will “make a difference.”

References:

1. Nick Morgan, Ph.D., one of America’s top communication theorists and coaches, blog article, “Why is good public speaking important to the business world?”
2. Aristotle, *The Art of Rhetoric*, translated by H.C. Lawson-Tancred, Penguin Books, Chapter 1.3, 1358b, 1991.
3. Dr. Joseph Sommerville, “The Presentation Expert”, Principal of Peak Communication Performance, the Principal of Peak Communication Performance, a Houston-based firm working worldwide to help professionals develop skills in strategic communication.
4. Mindmapping is —a revolutionary alternative to outlining and slide development that gives you access to your brain’s unlimited potential for creativity. This technique helps you generate ideas for presentations faster and with greater flexibility while encouraging more spontaneity and originality in delivery.

Public Speaking, Oral Communication, Speaking Skills



W17 Quality Assurance in Human Identification

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After attending this presentation, attendees will be able to understand the basic quality assurance principles and measures applicable to human identification. Attendees will learn the unique challenges faced by professionals involved in human identification when striving to have their facilities, procedures, and casework meet the standards demanded by the criminal justice and medicolegal systems. Attendees should be able to utilize the material presented to formulate a quality assurance program for their organizations.

This presentation will impact the forensic science community by demonstrating how quality assurance in forensic laboratories and forensic programs have led to objective and measurable standards of performance that ultimately strengthen and elevate the forensic profession as a whole.

Quality Assurance Programs (QAP) in forensic laboratories and activities have been a growing trend over the past decade. The publication of the National Academy of Sciences Report — *Strengthening Forensic Science in the United States: A Path Forward*, and its recommendations have made quality assurance programs and accreditation an increasing priority for forensic human identification laboratories. Since 1999 the Joint POW/MIA Accounting Command (JPAC), Central Identification Laboratory (CIL) has implemented a stringent QAP to ensure the scientific integrity of its casework. The CIL's QAP ultimately led to its accreditation by the American Society of Crime Laboratory Directors Laboratory Accreditation Board (ASCLD-LAB) in 2003 — the first forensic skeletal identification laboratory so credentialed. In 2008 and again in 2013, the CIL was re-accredited under the ASCLD-LAB International Program using ISO 17025 Criteria.

The goals and objectives of this workshop are to introduce the attendee to the CIL's QAP and to convey the lessons learned resulting from its implementation and growth. The workshop begins with an overview of the CIL, its mission, and QAP. A video of the CIL's mission and operations is presented. In the latter, the concept of the scientific integrity of the CIL is discussed followed by a summary of the "Surety" model of quality assurance.

Attendees will become familiar with each measure that comprises the surety model of quality assurance. The importance of integrating and synchronizing all of the surety measures discussed during the workshop is continually stressed.

Issues that are currently at the forefront of forensic quality management are presented. These include: professional qualifications and development; ethics; research and development; validation of technical procedures; uncertainty of measure; and, data management; and, information release.

Infrastructure and support considerations necessary for a successful QAP are also presented. Surety measures addressed include, but are not limited to: desired qualities of a laboratory manual and other vital documentation and their control; adequacy and safety of laboratory facilities; purchasing and contracting for laboratory services and supplies; policies and procedures conducive to a positive work environment; customer service programs and complaint procedures; evidence management and security; and, training and professional development.

Gathering and interpreting evidence discusses quality assurance measures directly related to field operations and trace evidence casework. These surety measures include: peer review process; preparation of analytical notes, test reports, and other case documentation; photography and other imaging; maintenance, calibration, and performance checking of equipment; taphonomic effects and evidence conservation considerations; and, writing and editing Standard Operating Procedures (SOPs).

Quality assurance procedures and programs are ineffective unless they are monitored, enforced, and subjected to corrective action when non-compliances are discovered. Monitoring and corrective actions outline how these are accomplished in the CIL. Discussed are a myriad of surety measures including: proficiency testing; review of court testimony; internal and external audits; annual reports to, and management reviews with, top management; and, corrective action policies and procedures regarding personnel, technical procedures, facilities, etc.



Workshop - 2015

In closing, the workshop discusses problems that hindered, and the processes that led to, the accreditations of the CIL. Surety assistance programs offered by the CIL are discussed in the event an attendee's organization desires assistance with their QAP or accreditation efforts. Additionally, the contributions, to date, of the Scientific Working Group in Forensic Anthropology (SWGANTH) to the human identification profession are briefly discussed.

Quality Assurance, Human Identification, Forensic Anthropology



W18 Sadism: Distinguishing Between Criminal Behavior and Offender Analysis

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After attending this presentation, attendees will have a conceptual and applied understanding of the complexities of sadism (sexual and non-sexual). The framework presented will begin by introducing participants to the history of sadism and reviewing its development as a psychological construct before employing a crime assessment model that utilizes the crime continuum to explicate behavior, not the psychological paradigm currently in use.

This presentation will impact the forensic science community by clarifying the complexities of sadism to facilitate understanding. The attendees will learn the profound impact that sadism has in our daily forensic work in the disciplines of psychology, psychiatry, criminalistics, law, or criminal investigations, as well as in a myriad of hidden inferences in other related disciplines.

Since the invention of the term sadism in the late 19th century and the implementation of the different systems of psychiatric classification, conceptualization of the term and its associated behaviors has been made via a long and circuitous path. This term has been altered and changed to serve various functions and to encapsulate or exclude varying behaviors. The last modification to the term sadism was found in the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-V), but still it remains unclear whether sadism is a psychiatric disease (or a so-called “disorder”), a form of sexual deviance, or a criminal behavior. The clarification of this ambiguity would improve forensic-psychiatric classification, diagnosis, and therapy, as well as clarify the difference between the medical approach and the typological and behavioral analyses necessary for crime assessment and crime scene investigations.

After attending this session, attendees should have a conceptual and applied understanding of the complexities of sadism (sexual and non-sexual). The framework presented will begin by introducing participants to the history of sadism and reviewing its development as a psychological construct before employing a crime assessment model that utilizes the crime continuum to explicate behavior, not the psychological paradigm currently in use.

The attendees will learn the evolution of sadism from the original concept development into an abridged form which became applicable only to psychology/psychiatry, where the framework changed to only address the diagnostic and treatment aspects of the behavior and crime. Thus, this abridged viewpoint developed a diagnostic criteria exclusive to the interests of the respective disciplines while ignoring other relevant aspects and disciplines that might contribute to a holistic understanding of sadism. That is, inasmuch as the medical focus was only on diagnosis and treatment, the term became restrictive and devoid of any earlier recognized crime behaviors and patterns. Currently, most clinicians and reference books are caught in a thicket of misapplied understandings that impede correctly and comprehensively evaluating the criminal and/or the crime.

Many clinicians and profilers continue to use the psychological continuum of paraphilias to explain crime, which is founded in the criminological continuum of deviancy. This commingling has led to a quagmire of misunderstanding and bad science.

Predicated upon the original conceptualization of sadism, attendees will learn the intent is the psychological and physical destruction of the victim from ante-mortem, perimortem and post-mortem violence, moving beyond the narrow confines placed upon behavioral analysis in current psychological and psychiatric conceptualizations. Primarily, the focus of the extreme violence is based upon the 3 “D’s,” which are dependence, dread, and degradation. Further, although all sadism does not end in death, the psychopathological direction is toward the ultimate demise and destruction of the victim. As expected, this pathway has a learning curve of behaviors that build upon one another until the extremes are met.

Although many believe that sadism is solely sexual in nature and manifestation, this is inaccurate and a discussion of non-sexual sadism will be advanced and presented in case reports. By removing the limitations earlier imposed by psychiatry and psychology and exploring the behaviors from within the crime continuum, attendees will discover forms of sadism that have been ignored or misunderstood in the past.

This presentation will benefit the forensic science community by clarifying the complexities of sadism to facilitate understanding. The attendees will learn the profound impact that sadism has in our daily forensic work, be it in the disciplines of psychology, psychiatry, criminalistics, law, criminal investigations, as well as in a myriad of hidden inferences in other related disciplines.



Workshop - 2015

The above workshop will be devoted to conceptual and applied cases. Accordingly, there will be some extreme examples that may be overwhelming for some persons.

Sadism, Crime Assessment, Behavior



W19 Automating Image Production for Forensic Document Examiners

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After attending this presentation, attendees will be able to automate simple tasks and will understand how to combine these simple tasks into complex actions. As an example, attendees will have the ability to take physical evidence consisting of 20 request writing samples on separate forms and create a complete chart of all 20 signatures in approximately five minutes, remove colored backgrounds at the click of a button, or batch-process large quantities of images with a simple drag-and-drop action. The main focus of this presentation will be to demonstrate how the automation of common tasks associated with the production of writing comparison charts, image enhancement, and document-examination demonstrative aids can save time. This is accomplished primarily through the use of Adobe® Photoshop® features, actions, and droplets.

This presentation will impact the forensic society community by explaining that with the increasing documentation requirements for laboratory accreditation, court testimony, and the increasing complexity of cases, the time required for completion of many forensic document examination cases has increased significantly. To help alleviate the additional time requirements, this workshop will include various tips and tricks in Adobe® Photoshop®, Adobe® Acrobat®, and Adobe® Bridge® to reduce the amount of time the examiner spends preparing and organizing documentary evidence prior to the actual examination.

This workshop is designed specifically for forensic document examiners. Software in common use among forensic document examiners will be demonstrated and shown how it can be used to increase productivity. This is accomplished without any knowledge of computer programming or the requirement to write scripts, but through the process streamlining image production and the automation of common tasks in Adobe® Photoshop®.

Creating writing charts has historically involved the physical cutting and pasting of signatures or words used for comparison onto a backing. This has been a very time and labor intensive process that progressed from using photography, to photo copiers, and finally digital imaging. Unfortunately many document examiners still construct writing charts in a similar manner to the way they were constructed a hundred years ago, by digitally cutting and pasting the individual writings onto a blank back ground. While searching for a way to streamline examination processes, automating repetitive tasks for these digital charts greatly reduced the amount of time spent on cases overall. In many instances it was found that the creation and use of appropriate actions in Adobe® Photoshop® can save a significant amount of time. The time spent constructing printed charts from physical writing exemplars can be reduced from an hour or more to less than five minutes at times.

The first part of the workshop will outline types of computer hardware and peripherals that can be used to accelerate the task of image production. This will include recommendations on how to configure scanners and computer workstations to achieve maximum productivity with minimal user input. The instruction will not be an endorsement of specific items but more of a description of the tools necessary to reduce time spent on imaging documentary evidence. When large numbers of images are created, managing these images then becomes a time consuming task. Quick and easy methods for the naming, searching, and categorizing of these images after creation will also be demonstrated.

The main focus of this workshop will be to demonstrate how the automation of common tasks associated with the production of writing comparison charts, image enhancement and document examination demonstrative aides, can save the attendee time. This is accomplished primarily through the use of Adobe® Photoshop® features, Actions, and Droplets. Upon completion of this workshop, the attendee will have a basic understanding of the features within Adobe® Photoshop® that can be used to execute repetitive tasks at the press of a button.

With the knowledge gained at this workshop the attendee will be able to automate simple tasks and will know how to combine these simple tasks into complex actions. As an example, the attendee will have the ability to take physical evidence consisting of 20 request writing samples on separate forms and create a complete chart of all 20 signatures in about five minutes, remove colored backgrounds at the click of a button, or batch process large amounts of images with a simple drag and drop action. This workshop is paced to maximize the amount of time spent working with hands on examples to ensure these techniques can be used when the attendee gets back to the laboratory. Graphic step-by-step instructions will be provided for future reference.

Documents, Photoshop®, Imaging



W20 Cognitive Bias Issues in the Forensic Analysis of Pattern and Impression Evidence and in Medicolegal Evaluations

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After attending this presentation, the attendees will be acquainted with the different types of bias that can influence the outcome of forensic investigations. Attendees will learn about classic psychological research studies and real-life case histories demonstrating the effects of bias upon interpretations of pattern and impression evidence and upon medicolegal evaluations and assessments, especially in Shaken Baby Syndrome/Abusive Head Trauma (SBS/AHT) cases. Attendees will also discover how bias can improperly sway the perceptual and cognitive judgments of forensic examiners and produce faulty conclusions, even in the absence of malicious intent.

This presentation will impact the forensic science community by clearly demonstrating how various types of bias can adversely impact evaluations of evidence and decision-making in all forensic disciplines. Understanding the sources of bias and learning how to limit or minimize their influence is essential for improving the reliability and accuracy of decisions made by forensic experts and avoiding miscarriages of criminal and civil justice. All forensic scientists and laboratory directors must be keenly aware of the potential for bias and the types of internal procedures and protocols that can and should be implemented to minimize the impact of bias in forensic investigations and casework.

An extensive body of experimental research conducted by cognitive and social psychologists, as well as empirical data obtained from recent research and forensic casework studies, clearly demonstrates that various sources of bias can and often do adversely impact a forensic examiner's visual perception and decision making. Perceptual and cognitive judgments made by forensic examiners performing comparative analyses of fingerprints, hair, tool marks, bite marks, handwriting, and even DNA typing are susceptible to biasing influences that can improperly taint and sway the examiner's decision making process, as are other types of forensic evaluations, such as medical assessments made by physicians or health care providers as to whether a physical injury or death was accidental or willful. Understanding these various sources of bias and learning how to limit or minimize their influence is essential for improving the reliability and accuracy of decisions made by forensic and medical experts. The other participants in the administration of criminal and civil justice — lawyers and judges — likewise need to fully appreciate and comprehend the negative impact that biasing influences may have on the outcome of a forensic or medical expert's analysis of evidence. In our adversarial judicial system, trial lawyers need this knowledge in order to effectively expose potential biasing influences when cross-examining an opposing forensic or medical expert, and judges need it to be more mindful of the critical role that bias plays in producing miscarriages of criminal and civil justice. All stakeholders in the administration of criminal and civil justice must be keenly aware of the potential for bias and the types of internal procedures and protocols that can and should be implemented to minimize the impact of bias in forensic investigations and casework.

In this workshop, a multidisciplinary faculty of distinguished psychologists, lawyers, forensic scientists, and others will provide attendees with a clear picture and concrete examples of how and why bias affects the outcome of forensic investigations. Attendees will learn about the various experimental research studies that reveal the susceptibility of investigations to the prospect of psychological error due to cognitive and motivational factors, including confirmatory biases, dynamic influences, group membership, role conflict, escalation of commitment, and adversarial allegiance. The unique persuasive power of confessions — even false confessions that are recanted and contradicted by other evidence — will be discussed in the context of basic and forensic psychology research which indicates that confessions influence lay witnesses and forensic science examiners across a range of domains, thereby increasing the risk of wrongful convictions.



Workshop - 2015

Attendees will learn about practices that should be avoided and followed in order to minimize potential biasing influences. Examples from actual forensic casework in both criminal and civil cases will be used to illustrate the impact of bias on the outcome of forensic examinations and the manner in which such opinions are expressed in court. Interesting data obtained from recent research studying the problem of contextual bias as encountered in national security investigations conducted by laboratory scientists working in the area of chemical, nuclear/radiological and biological Weapons of Mass Destruction (WMD) forensics will also be discussed, along with steps that these scientists have taken to address this problem.

How lawyers might seek to exclude proffered expert opinion infected by the precursors of bias and to impeach expert testimony that may have been tainted by bias will also be discussed. Gatekeeper decisions about admissibility, the use of cautionary jury instructions when serious issues of cognitive bias have been raised during the trial, and whether to allow expert testimony from a qualified psychologist about the effect of cognitive bias on judgments made by forensic experts will be addressed.

Due to the fierce controversy that has developed within the medical and legal communities surrounding the diagnosis of Shaken Baby Syndrome (SBS), a.k.a Abusive Head Trauma (AHT), attendees will also learn about the various cognitive biases that can affect clinical observations and evaluations upon which decisions are based as to whether infant brain injuries should be attributed to abuse or accident. Distinguished faculty members will discuss: the triad of symptoms historically relied upon to diagnose SBS/AHT; the cognitive bias issues presented in the absence or presence of additional signs of possible abuse; the impact that confessions and other domain-irrelevant information may have upon the outcome of medical evaluations and diagnoses of SBS/AH; and, the need to consider alternative hypotheses or explanations, such as diseases or conditions that can mimic the symptoms typically associated with SBS/AHT.

Finally, the presentations will be followed by a Q&A session in which panel members will be able to provide additional information to inquiring attendees.

Cognitive Bias, Flawed Forensics, Shaken Baby Syndrome



W21 Death in a Bathtub: The Trial of Drew Peterson

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After attending this presentation, attendees will understand the courtroom procedures for admission of evidence and expert witness testimony. In addition, attendees will learn the factors involved in injury identification and analysis with an emphasis on the investigation of drowning.

This presentation will impact the forensic science community by presenting the multidisciplinary reconstruction of one of the most riveting cases in recent American trial history. This presentation will detail the factors and evidence that influenced the decision-making process and will assist future prosecutors, judges, and death investigators in courtroom procedures.

In 2004, the body Kathleen Savio, the third wife of policeman Drew Peterson was found dead in the bathroom of her suburban Chicago home. Her body was found lying in an empty bathtub with a small laceration to the left back of the scalp. Froth oozed from her nostrils. There were some bruises to her left side. Toxicology analysis was negative for intoxicating drugs and alcohol. The initial investigation concluded that the death was the result of drowning and the coroner certified the death accidental. In 2007, Peterson's fourth wife, Stacey Peterson disappeared — her body never recovered. In light of Stacey Peterson's disappearance, authorities reopened the investigation into Savio's death.

Savio's body was disinterred in 2007 and reexamined in two separate autopsies performed by a group of forensic pathologists. The pathologist identified areas of hemorrhage over the left hip region, not appreciated at the initial examination. In light of the additional evidence, the experts concluded that Savio's death was a homicide.

In criminal cases, there is a constitutional dimension to hearsay. The Sixth Amendment gives criminal defendants the right to confront witnesses; since a hearsay statement is made out of court, there is no opportunity for the defendant's criminal defense attorney to cross examine the witness, and thus no confrontation. This means hearsay statements are harder to get into evidence even via the traditional hearsay exceptions when they are used against a criminal defendant.

Prosecutors collecting evidence identified the fact that Stacey Peterson had confided with family and friends implicating her husband, Drew Peterson as her murderer. Unable to question the dead witness, prosecutors petitioned the Illinois legislature to create a new exemption to the hearsay rule, which became known as "Drew's Law." The law allowed for the admission of evidence in cases where the witness was not available to testify due to the actions of the defendant. Meanwhile, defense experts unsuccessfully attempted to exclude testimony related to Stacy Peterson's disappearance in a 2010 evidentiary trial.

The trial of Kathleen Savio's death began in August of 2012. For over six weeks of grueling testimony, the media provided the day-to-day revelations of the case. Five forensic pathologists testified in the case that called into question central to the cause and manner of death. All the pathologists agreed that Savio died of drowning. The pathology testimony rested on questions of the pathological findings of concussion, postmortem artifacts, orientation of injuries, and causes of accidental drowning.

In light of new legislation, the prosecution was allowed to present incriminating verbal testimony against Drew Peterson. Peterson was eventually convicted, sentenced to 38 years in prison for his role in death of Kathleen Savio. The jurors said that the most convincing testimony was hearsay statements allowed into evidence under a new law, known as "Drew's Law," named after Peterson. Prosecutors successfully fought to have statements made by Stacy Peterson and Savio to acquaintances admitted into evidence. In February 2013, the defense was denied a new trial. The trial left numerous questions unanswered and created a precedent of allowing indefensible hearsay testimony

This workshop will be presented by the pathologists, prosecutors, and expert witnesses actually involved in the case. It will provide a multidisciplinary approach to courtroom presentation of evidence, expert testimony, the role of the medical witness and criminalistics evaluation, and the physiology of drowning. Participation of attendees is encouraged and will bring to life the tension of the courtroom in this precedent-setting prosecution.

Drew Peterson, Drew's Law, Bathtub Drowning



W22 Looking Toward a Greater Awareness of Youth Street Gangs

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After attending this presentation, attendees will be able to: (1) explain the organization and historical evolution of youth street gangs (examining African American, Latino, Asian, and Caucasian youth street gang activities from the crimes they commit to their tattoos, graffiti, and hand signs); (2) distinguish the behavioral differences and similarities between gangs (evaluating why one decides to join a gang and the motivational factors that play a role in gang membership); (3) compare and contrast activities of various gangs; and, (4) explore the issue of female gang members and sexual assault.

This presentation will impact the forensic science community by increasing understanding of the crimes and circumstances that involve youth street gangs.

Youth gang violence has continued its upward trend nationwide. It was once thought that gangs only convened in selected areas, which left churches, schools, and hospitals as “neutral” territory. Unfortunately, this is a fallacy. Gang violence has poured into the schools, community centers, and hospitals. Not only in California is gang violence problematic, as often the media portrays, but also throughout the United States gang violence has risen over 35% in the last year. Youth gang violence continues to rise dramatically with more and more of our youth deciding to join gangs each and every day. Sadly, every single state has gangs and the problem is getting much worse in areas that would never have thought about gangs a year ago. These “new generation” of gang members are younger, much more violent, and staying in the gang longer. Gangs are not just an urban problem, but a suburban and rural problem too. There are over 25,500 gangs in the United States with a total gang membership of 760,000 (94% are male and 6% are female). The ethnic composition nationwide include: 47% Latino, 31% African-American, 13% Caucasian, 7% Asian, and 2% “mixed,” according to the Office of Juvenile Justice and Delinquency Prevention of the Department of Justice.

A youth gang, often referred to as a “criminal street gang” by law enforcement must be ongoing, meaning that the gang associates on a continuous or regular basis. The youth gang could be formal or informal. Moreover, the youth gang must consist of at least three members and have a name, hand sign or symbol which is identifiable. The final element that defines a youth gang is that one of the primary objectives of the gang must be criminal activity. Not to get confused with various social groups such as fraternities, sororities, or social clubs, what differentiates a youth gang from other groups is its criminal activity.

In all of the 25,500 youth gangs with over 760,000 gang members across the nation, there are various types of gang members ranging from hardcore to wannabe members. The most plentiful type of gang member is the active/regular gang member making up between 40-50% of the gang. Regular gang members self-admit that they are in a gang when asked. They also have gang related tattoos, involved in gang related crimes, and have a past history of gang activity. Associate/affiliate gang members make up 20-30% of the gang. Associate and regular gang members also use hand signs to communicate to each other and to other rival gangs. Associate and regular gang members also write gang graffiti, wear gang related clothing (colors), associate with known gang members, and are included in gang photos. What sets associates apart from regular members is that they are able to freely come and go in and out of the gang as they see fit making them great informants for law enforcement. The hardcore gang members known, as an Original Gangster (OG), make up 10-20% of the gang and is the primary leadership arm of the gang group. Furthermore, hardcore gang members are involved in violent gang activity from assaults, shootings, robberies, to murder. Wannabes are the last group of gang member types. Wannabes make up less than 10% of the gang however are extremely dangerous. Not to say that the other gang member types are not dangerous because they all have the potential to be extremely dangerous. The dangerousness of the wannabes lies in their motivation to be part of the gang.

As a result of the ongoing proliferation of youth street gangs in our communities, it is imperative that medicolegal death investigators, forensic nurses, law enforcement, district attorneys, coroners, medical examiners, social workers, educators, and others involved with the direct care become educated on how to identify gang members, their activities, and their motivations so that they could provide solutions to families and the youth themselves to help eradicate the problem of gang violence, while keeping safe.

Youth Gangs, Youth Violence, Violence Prevention



W23 Hands-On Evaluation of the Thanatomicrobiome and Epinecrotic Communities

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After attending this presentation, attendees will be able to: (1) describe the process by which crime scene investigators approach human remains for the collection of microbial and entomological evidence; (2) collect genomic DNA evidence from human cadaver-associated soil using readily available DNA extraction kits to isolate and assess the quality of genomic DNA to be used in downstream molecular applications; (3) describe and critically evaluate the benefits and challenges of the thanatomicrobiome and epinecrotic communities for use in forensic research and potential use during investigations for estimating the Postmortem Interval (PMI); (4) discuss the bioinformatics of specific sequencing platforms and the use of multivariate statistical procedures and modeling that are increasingly being used in metagenomic analyses or postmortem microbial communities; and, (5) understand and appreciate the benefits of outdoor decomposition facilities for more realistic studies of postmortem microbial communities.

Additionally, attendees will participate in a hands-on experience learning techniques to extract DNA from: soil samples, eggs, larvae, pupae, and adult insects.

This presentation will impact the forensic science community by providing an introduction to the principles behind the potential use of postmortem microbial communities in death scene investigations and the methods to use in extracting microbial DNA from a variety of sources. Emphasis will be placed on introducing new forensic scientists to practical aspects of DNA extraction techniques and the use of thanatomicrobiome and epinecrotic communities in the estimation of the PMI.

Accurate estimation of Postmortem Interval (PMI) is a major challenge for forensic examiners. Advances in DNA sequencing technology have created new possibilities and approaches to PMI estimation. Use of these techniques has generated new areas of research for scientists. This presentation is intended to provide attendees with an introduction to this emerging approach to PMI estimation.

Upon completion of the morning session of this workshop, the participants should be able to: (1) describe the process by which crime scene investigators approach human remains for the collection of microbial and entomological evidence; (2) collect genomic DNA evidence from soil using readily available DNA extraction kits to isolate and assess the quality of genomic DNA to be used in downstream molecular applications; (3) describe and critically evaluate the benefits and challenges of the thanatomicrobiome and epinecrotic communities for use in forensic research and potential use during investigations for estimating the PMI; (4) discuss the bioinformatics of specific sequencing platforms and the use of multivariate statistical procedures and modeling that are increasingly being used in metagenomic analyses or postmortem microbial communities; and, (5) understand and appreciate the benefits of outdoor decomposition facilities for more realistic studies of postmortem microbial communities.

Thanatomicrobiome, Epinecrotic Communities, DNA Extraction



W24 Scientific Neutrality in Expert Witness Testimony

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After attending this presentation, attendees will better understand: (1) how to achieve neutrality in the preparation for and presentation of both direct and cross-examination in court; (2) how to testify within the limits of his/her expertise; (3) the role and responsibilities of the expert witness in and out of the courtroom and how the expert testimony relates to the roles and responsibilities of the judge and the prosecuting and defense attorneys; and, (4) the importance of being a neutral expert witness.

This presentation will impact the forensic science community by elucidating the importance and value to our justice system of an expert witness who maintains neutrality during expert witness testimony in depositions and in the courtroom. This presentation will provide all current and future expert witnesses in all areas of forensic sciences an opportunity to learn from an experienced expert witness and other knowledgeable members of the courtroom, including a judge, a defense attorney, and a prosecuting attorney.

Each participant in the court room and in the justice system has a specific advocacy role to ensure that justice is properly and appropriately administered. The very nature of the trial advocacy system often places the forensic scientist in what could be perceived as an advocacy role for the prosecution or the defense; however, judges, and prosecuting and defense attorneys should have the same objectives regarding scientific expert witness testimony in that the witness must be truthful, forthcoming, and as neutral as possible.

The respective roles of the judge, the attorneys and the forensic science expert witness in the courtroom and how the roles impact the testimony of the expert witness will be discussed from the perspective of an experienced prosecuting attorney, defense attorney, judge, and expert witness. Attendees will be provided valuable tools for effective presentation of scientific evidence while maintaining scientific neutrality during court testimony, including during direct and cross-examination, as well as throughout the testing process and reporting in order to be true to the science and to the results obtained. A portion of the session will be devoted to a panel discussion where relevant issues and questions from the panel and the audience will be addressed by each of the speakers who will give practical advice and demonstrate applications of the information provided. Examples of questions to be covered are: "What should I do if I am asked by an attorney to give opinions that are contrary to my conclusions and opinions?" and "How do I handle questions regarding mistakes, errors, and other issues in the laboratory?" Attendees are encouraged to bring tough real-life questions for the speakers to address and witness how much agreement there is between all participants in the courtroom when the goal is factual, truthful and neutral testimony. It is important for all forensic science expert witnesses to recognize and understand for all future testimonies that their only advocacy role is for good science and the truth through competent and unbiased testing and testimony.

Attendees may be inclined to believe that they play the role of an advocate since they are called by either the prosecution or defense; however, it is the forensic scientist's role to ensure that expert testimony is factual, truthful, and unbiased. Valuable tools for effective presentation of scientific evidence while maintaining neutrality will be provided from the perspective of an experienced prosecuting attorney, defense attorney, judge, and expert witness. The respective roles of the judge, the attorneys, and the forensic science expert witness in the courtroom and how the roles impact the testimony of the expert witness will be discussed. Relevant issues and questions will be discussed with practical advice and applications of the information provided.

Neutrality, Expert Witness, TestimonyA Title Sample



Anthropology Section - 2015

A1 Temporal Patterns of Mexican Migrant Ancestry: Implications for Identification

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After attending this presentation, attendees will learn how genetic ancestry estimates can be used to reveal the dynamics of demographic trends in United States-Mexico border casework, thus contributing to the continuous refinement of identification logistics.

This presentation will impact the forensic science community by validating that the science of producing evidence alone is not enough to maintain identification rates in complex identification contexts like the border dead. The ongoing efforts of culturally conscious caseworkers and outreach groups, who explain the identification process, collect family reference samples, and source antemortem data is essential for the continued success of identifications at medical examiners' offices. This interdisciplinary approach to identifications could be used in other investigative contexts as well.

As understanding the geo-temporal patterns of who is dying on the border and who is being positively identified speaks to casework logistics, attendees will see how studying the ongoing evolution in Undocumented Border Crossers (UBC) demographics provides insight for modifying current identification protocols.

The United States-Mexico border remains a key focal point for developing new approaches to the identification of migrant remains, as the rates of cross-border fatalities continue to rise and source populations continue to diversify. As demonstrated by the Pima County Office of the Medical Examiner (PCOME), the identification of border remains is an interdisciplinary effort, requiring skillful integration of multiple lines of biological and social information. The present study focuses on how genetic ancestry estimates from standard forensic DNA markers in UBC cases provide valuable insight into border crossing deaths and identification patterns over time, and how this information has practical implications for future identification rates in forensic anthropology as the population make-up of undocumented migrants continues to change.

Genetic ancestry estimates were generated using Combined DNA Index System Short Tandem Repeat (CODIS STR) loci for both identified (n=97) and unidentified UBC cases (n=262) recorded by the PCOME from 1972 to 2013. These estimates were generated in STRUCTURE software, under a k=2 model, using European and Native Mexican reference samples. To identify temporal trends in the genetic ancestry of the UBC cases, ancestry proportions for the identified and unidentified UBC samples were tested separately for a significant correlation with case year. A positive correlation ($r=0.27$, $p<0.0001$) between case year and Native American ancestry for unidentified UBC cases was found. Compared to Mestizos, indigenous populations on average exhibit greater amounts of Native American ancestry; thus, these temporal trends are consistent with current reports of an increased rate of migrants from southern Mexico, where the largest concentration of indigenous populations reside. In contrast, a negative correlation with Native American ancestry and a positive correlation with European ancestry was found for the positively or presumptively identified UBC cases, with values approaching statistical significance ($r=-0.16$, $p=0.105$). Skeletal data offer external support, as neurocranial Interlandmark Linear Distances (ILD) variables for identified cases also show a negative correlation with Native American ancestry (n=83, $r=-0.20$, $p=0.0473$).

These contrasting trends suggest an identification bias, implying that the success of identifications will decrease over time as the prevalence of UBC cases with increased Native American ancestry grows. PCOME-reported identification rates have decreased from 75% in 2003 to 45% in 2013, and although identification success is attributable to many factors, this trend in decreasing rates supports the genetic ancestry-based findings reported here. Given these results, it is critical to isolate the factor(s) driving this identification bias. It is unlikely that identification bias is produced at the case-evidence level, because all cases investigated at PCOME undergo similar protocols for skeletal and DNA analyses. One possible explanation may be the lack of family reference DNA samples from more rural, southern, and indigenous communities. Families of missing or deceased migrants from these backgrounds may be less accessible to Non-Governmental Organizations (NGOs). If these families are not reached, or are less inclined to provide DNA samples, identification potential drops significantly.



Anthropology Section - 2015

This study analysis demonstrates how forensic investigations should place greater emphasis on and receive increased support for the community outreach work lead by national and international NGOs. These results validate that the science of producing evidence alone is not enough to maintain identification rates, as the demographics of UBCs change over time. The ongoing efforts of culturally conscious caseworkers and outreach groups, who explain the identification process, collect family reference samples, and source antemortem data, is essential for the continued success of identifications at medical examiner offices. Since the 1990s, PCOME has led the development of flexible and innovative approaches to identification that meet the complex challenges of UBC casework. As UBC demographics are in a state of flux, in order to continue to improve identification rates, forensic approaches must be adaptive and balanced with a focus on both the forensic science and the social science of identification.

Mexican Migrant, CODIS STR, Ancestry



A2 “I Learned About My Death From the Newspapers” — Misidentification Following Cutbacks After a Spending Review

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After attending this presentation, attendees will understand that it is essential, even in a period of financial crisis and cutbacks after a spending review, to perform all the investigations on an unknown corpse.

This presentation will impact the forensic science community by demonstrating the fundamental need to perform proper identification procedures. Failure to perform such procedures, deemed unnecessary and costly, could result in declaring the death of a living subject and the burial of a corpse under a false name.

In December 2013, firemen were called to a fire in an abandoned farmhouse in a rural area near Binetto, a small town in the province of Bari (Apulia, Italy). While dousing the flames, the firemen found an extensively burned corpse. The body was lying near a fireplace, in the “boxer’s” position. The skull was skeletonized and extensively charred. The limbs were amputated and charred from the heat. The thoracic and abdominal cavities were exposed and the internal organs were blackened and shrunken. The sex was identified, but transformational phenomena due to the heat and flames did not allow the age and height to be determined. At the end of the preliminary survey, it was not possible to determine the cause or the time of death, nor to make a clear identification of the corpse.

From the statements issued by the owner of the farmhouse as well as by the inhabitants of the small town, it was learned that “R.R.,” a homeless immigrant with a valid residence permit, frequently took shelter in the farmhouse. Therefore, the police and the state prosecutor decided to identify the cadaver as that of R.R., and it was considered unnecessary, in view of stringent cutbacks, to proceed with an autopsy or further investigations to ascertain the identity and the cause of death of the burned corpse and the case was closed. Two days after the identification of the corpse, R.R. arrived at the police station stating that he had learned, to his surprise, the news of his own death from the newspapers. He reported that the house was used as a shelter by various immigrants.

In this case, a misidentification of a body had occurred due to economic problems and the general dispositions of the spending review. At this point, the question was: who was the charred body?

The police compiled identity photographs of missing immigrants. Due to the flame-damaged, charred soft tissues of the face, it was not possible to visually identify the corpse, nor was it possible to carry out DNA analysis because of the lack of close relatives for comparison. Antemortem medical files were not available. The only feasible identification procedure was a parameterized skull-photo superimposition of the face using a 3D Computed Tomography (CT) scan. This technique involves superimposing a computed, parameterized 3D facial image over photo images considered a possible match with the remains. During the autopsy, the charred skull was carefully removed and subjected to adequate preservation treatment for the subsequent identification investigations.

The 3D reconstruction of the skull was carried out by employing an open 3D Cone Beam CT (CBCT) acquisition system, normally used for facial mass studies. The 3D image of the skull, according to precise dimensional anthropometric constraints, was mobilized by software and superimposed on photographs of the faces of missing immigrants. At the end of the survey, there was evidence of a perfect parameterized overlap of the skull with the photo of another immigrant, H.A. Notably, the victim was physically quite unlike R.R., who had previously erroneously been identified as the corpse under examination by the state attorney and the police.

Charred Body, Identification, 3D CT Scan



A3 The Relevance of a Multidisciplinary Approach in the Identification of Skeletal Remains: A Case Report

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The goal of this presentation is to introduce attendees to a multidisciplinary approach to the identification of human remains findings. Specifically, the presentation will focus on the enrichment of any aspect of information on any given case regarding time-since-death occurrence, records related to the involved subject, and, whenever available, information useful for the identification of the subject.

This presentation will impact the forensic science community by highlighting the relevance of a multidisciplinary approach to the management of cases of skeletal remains retrieval.

This study will address two major areas: (1) who the person was; and, (2) how long the remains were lying in the place where they were found. The rationale and background for this approach is based on the following assumptions: (1) postmortem interval estimations are generally based on the degree of soft tissue decomposition, identifiable stages of tissue alteration and loss that occurs in a predictable, sequential, and semi-continuous pattern at a rate that is dependent on both accumulated temperature over time (measured in Accumulated Degree Days (ADD)) and insect access; and, (2) the age of the subject could be estimated by looking at the stages of union for the epiphyses in the different bones and comparing this to the standard growth tables.

Case Presentation: An advanced decomposed leg was found in a field bordering the Arno River in Tuscany, Italy, after a flood had recently occurred. The findings consisted on a foreleg wearing a tennis shoe and included a tibia, a fibula with no soft tissue, and a femur with partial muscle flesh attached. Within seven meters of the leg, a soft tissue mass weighing about ten grams was also found. Every portion of the soft tissue was already saponified and the remains were overgrown with vegetation and mud.

Believing that diverse information gathered from different forensic approaches should assist in shedding light on any given case, it was thought that the victim was likely a young male between 18 and 25 years old. The case could not rely on DNA test matching or comparisons with any other samples collected for identification as none of these samples matched the description of a comparable male subject missing in that area within a reasonable period of time. Remarkably, botanical investigation highlighted the presence of different types of seeds related to a specimen distinctive to areas of fresh water which actually matched the place where the remains were retrieved, providing evidence suggesting they were found in that place before late winter. Also, a complete radiological investigation, including Computed Tomography (CT) and X-rays failed to show any evidence of degenerative articular disease or previous fractures. The X-ray of the femur epiphysis revealed a complete stage of union for the epiphyses, so it was possible to establish the above-mentioned age interval. The anthropological investigation enhanced the identification process. In fact, following a one-month stay in a fresh-water environment, the bones were clean and free of soft issue, which readily allowed the anthropological study. Additionally, measurement of the size of the skeletal remains suggested the age range of the subject and his lifestyle contributed to gender identification. The remains were estimated to be of a male subject with an average Body Mass Index (BMI), around 174cm tall, possibly involved in some physical activity such as squatting. Finally, the DNA analysis confirmed the gender of the subject as male although no confirmatory analysis based on samples collected from other suspect victims could be gathered so a firm conclusion on the actual identity of the subject remains unknown.

In conclusion, despite the inability to perform a DNA matching test, this study demonstrates the relevance of a multidisciplinary approach which significantly helped in gathering a variety of information for preliminary identification of the subject.

Remains, Multidisciplinary Approach, Forensic Anthropology



A4 Estimation of Age From the Adult Pelvis: A Comparison Across Different Pelvic Sites and Statistical Methods

Molly Miranker, BA*, 1078 Fulton Street, #5G, Brooklyn, NY 11218

After attending this presentation, attendees will understand how reliable the estimated age is from three separate pelvic sites compared to one another and why the statistical methods employed in these aging techniques influences the estimates' reliability.

This presentation will impact the forensic science community by demonstrating which of the pelvic aging methods is most reliable and what future research is necessary to improve age estimation for constructing a biological profile.

Age estimation from skeletal remains is a crucial component for generating a biological profile, the use of which assists in the identification of an individual. Bony changes at three pelvic sites (the pubic symphysis, auricular surface, and acetabulum) have been shown to correlate with aging. There have been multiple studies testing the reliability of the pubic symphysis and the auricular surface and comparing those two sites, but few assess the acetabulum and none compare the reliability across all three sites.

Four aging methods were compared in this study: (1) the Suchey-Brooks pubic symphysis method; (2) the Osborne et al. auricular surface method; (3) the Rissech et al. method for the acetabulum; and, (4) the Calce method for the acetabulum.¹⁻⁴ The Suchey-Brooks method was selected because it is one of the most frequently used in biological profiling. The Osborne et al. method was used because it provides more discrete age ranges than other auricular methods. Two acetabular methods (Rissech and Calce) were applied because age estimation techniques have recently been developed for this region and all require additional testing.

A total of 212 known-age individuals housed at the Bass Donated Skeletal Collection were examined. The sample was comprised of individuals of European ancestry who died in the United States during the mid- to late-20th century. The study sample ranged from 26 to 95 years of age, with a mean age-at-death of 62 years. Although the sample was selected to be representative across the decades of life, the age distribution was not normal (Shapiro-Wilk W test $p=0.013$, Skew=-0.203).

The reliability of the four methods was assessed by comparing the accuracy (percentage of point age estimates that fall into the predicted age range of a method), inaccuracy (average absolute difference between known and estimated ages), and bias (amount age is over- or underestimated) of the age estimates produced by each method. These data demonstrate which method(s) were most reliable, in what contexts, and therefore, most useful for describing skeletal remains.

In terms of accuracy, the acetabular aging methods represented the most and least accurate of the techniques (96% Rissech and 59% Calce). The Osborne et al. method had 86% accuracy and Suchey-Brooks 64% accuracy. The Rissech et al. method had the smallest margin of inaccuracy (± 9 years), followed by Calce (± 13 years), Osborne et al. (± 16 years), and then Suchey-Brooks (± 19 years). Bias tests revealed the tendency for all the methods to underage individuals. The Rissech et al. method only slightly underestimated age (-0.70 years), followed by Calce (-5 years), Osborne et al. (-14 years), and finally Suchey-Brooks (-19 years).

The reliability of the individual methods varied as a consequence of the statistical techniques they employed. Regression-based aging methods are prone to producing estimates biased in the direction of the known age of the reference sample because the predicted ages are set by the mortality distribution of the reference population, which may not be comparable to other populations. Bayesian estimation avoids that tendency because it calculates both the mortality distribution of the reference sample and the test sample's age estimates. Rissech et al., the only method to employ Bayesian estimation, had the most reliable results because it was the most accurate method applied, with the smallest margin of inaccuracy and bias. The results suggest that Bayesian prediction may improve age estimation significantly and following further assessment should be applied to other age indicators.



Anthropology Section - 2015

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Forensic Anthropology, Age Estimation, Adult Pelvis



A5 Plaque and Projections: Assessing the Utility of Morphological Variants of the Sternal Fourth Rib for the Estimation of Sex and Age-at-Death

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After attending this presentation, attendees will better understand the statistical associations between biological sex, age-at-death, and the presence of bony plaque and/or central projections within the sternal pit of the fourth rib.

This presentation will impact the forensic science community by providing data suggesting that osseous anomalies of the sternal end of the fourth rib may profitably be employed in the estimation of sex and age-at-death from isolated ribs deriving from unidentified decedents, thereby allowing construction of a biological profile where more commonly used skeletal elements (e.g., cranium and os pubis) are unavailable.

This research was conducted using the Hartnett-Fulginiti collection housed at the Forensic Science Center in Maricopa County, AZ. This collection is comprised of more than 600 specimens of pubic symphyses and associated sternal ends of the fourth ribs from decedents of known sex, age-at-death, and ancestry. A total of 557 individuals (375 males and 182 females) ranging in age from 18 to 99 years were evaluated for the presence of bony plaque and central projections in the sternal pit of the fourth rib. Rib ends were independently assessed by two experts and consensus opinions on the presence or absence of plaque and projections were recorded. In addition, the presence of bony plaque was subdivided into three distinct morphological categories (Grades 1, 2, and 3) and, where sample sizes permitted, each category was independently assessed for associations with sex and age-at-death.

Results suggest that neither the presence of central projections nor that of plaque are independent of biological sex (projections: $\chi^2=37.5257$, $df=1$, $p\text{-value}=9.022e-10$; plaque: $\chi^2=80.9211$, $df=1$, $p\text{-value}<2.2e-16$). Based on the sample proportions, the probability that an individual exhibiting central projections is female is 0.775 with an associated 95% Confidence Interval (CI) of 0.623 to 0.879. Likewise, the probability that an individual exhibiting bony plaque is female is 0.717 with an associated 95% CI of 0.621 to 0.797. A more complex association is apparent when the occurrence of plaque is subdivided morphologically. All individuals in this sample exhibiting Grade 1 plaque are female ($n=7$), but sample size is too small to permit statistical validation. There is a 60.4% chance that an individual exhibiting Grade 2 plaque is female, but the associated 95% CI (0.463 to 0.730) suggests that this form of plaque is equally likely to be found in both males and females. However, there does appear to be age discrepancies between males and females exhibiting this plaque morphology such that Grade 2 individuals who are older are more likely to be female (95% CI: 0.562 to 0.962). The probability that an individual exhibiting Grade 3 plaque is female is 0.791 with a 95% CI of 0.646 to 0.888.

Similarly, the age distributions of individuals exhibiting central projections or bony plaque are statistically different. The mean age of individuals with projections (74.68, $n=40$) is higher than that of unaffected individuals (52.14, $n=463$) (Mann-Whitney: $U=3428.5$, $p\text{-value}=3.78e-11$). While the mean age of individuals exhibiting any form of bony plaque (54.41, $n=404$) is not statistically different from that of unaffected individuals (51.81, $n=98$) (Mann-Whitney: $U=21998.5$, $p\text{-value}=0.08735$), investigation of the association of specific plaque morphologies with age-at-death suggests more useful patterning. Results of a Kruskal-Wallis test indicate that the age distributions for each morphological grade of plaque and for unaffected individuals are not the same ($H=34.944$, $v=3$, $P=2.2e-16$). Post-hoc tests using Dunn's non-parametric comparisons indicate that the mean age of individuals with Grade 2 plaque (35.02, $n=47$) is significantly lower than that of unaffected individuals (54.41, $n=404$) and that the mean ages of individuals exhibiting Grade 1 (36.86, $n=7$) and Grade 2 morphologies is significantly lower than that of Grade 3 individuals (72.84, $n=43$).

These results suggest that, although far from ubiquitous, the presence of central projections and the formation of bony plaque within the pit of the sternal ends of fourth ribs may be useful in estimating both age and sex in unidentified remains for which more commonly used skeletal elements are missing or damaged. Further research is both required and encouraged to see if the associations identified here are present in other known-age skeletal collections and to assess the accuracy of using these morphological features of sternal rib ends for the estimation of sex and age-at-death.

Sternal Fourth Rib, Age-at-Death, Sex Estimation



Anthropology Section - 2015

A6 Sex Determination Using the Distal Articular Surface of the Fibula

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The goal of this presentation is to demonstrate the use of the distal fibula to sex human skeletal remains.

This presentation will impact the forensic science community by offering a new method for determining the sex of human skeletal remains.

In forensic and anthropological literature, the fibula has not been extensively explored for its potential to determine sex. A study conducted by Sacragi and Ikeda used a population-specific discriminant function to correctly classify Japanese males at 90.1% and Japanese females at 91.4%.¹ This study investigates the distal fibula for its potential to sex Caucasian American skeletal remains. A population-specific discriminant function was developed using a Caucasian sample from the William M. Bass Donated Skeletal Collection. This formula correctly classified 85.2% of females and 89% of males using the left distal fibulae. Intra-observer analysis indicated a percentage error of 1.3%. While age had a slight effect on the measurements, it did not produce a significant effect to inhibit sex determination.

This study included both right and left fibula to determine which side yielded greater accuracy. A total of five measurements were taken of the distal articular surface of the fibula; three perpendicular aspects that form the malleolar surface (PA, PB, PC), total length of the lateral malleolus (LLM), and width of the malleolar fossa (LMF). These measurements combined to produce the discriminant functions that follow. The function for the left fibula is $DF = -14.953 + 0.242(LMF) + 0.253(PA) + 0.169(PC) + -0.055(PB) + 0.214(LLM)$. Scores above 1.0765 are determined male, below -0.8435 are determined female, and scores in between are indeterminate. The function for the right fibula is $DF = -15.128 + 0.253(LMF) + 0.331(PA) + 0.141(PC) + -0.081(PB) + 0.200(LLM)$. Scores above 1.0805 are determined male, below -0.8395 are determined female, and scores in between are indeterminate.

While significant age-related changes were not visible on the distal articular surface of the fibula, regression analysis did indicate that age had some influence on the fibular measurements. The analysis of a younger age bracket and an older age bracket demonstrated that the left fibulae had a smaller percentage of measurements affected by age. Examination of the literature demonstrated that few age-related skeletal changes occur on the articular surface on the distal fibula. This is due to thin articular cartilage and uniformity of cartilage matrix in the ankle, both of which contribute to a rarity of osteoarthritis in the area.

It is believed through this study that the method developed here should be used if the distal fibula is found, with preference given to the left fibulae. The discriminant formula should be used if the remains are suspected to be those of a Caucasian individual. While age does have some effect on the measurements, this technique is not age-dependent and can be used on suspected younger and older individuals. Additionally, low intra-observer error indicates the ease at which this technique can be performed.

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Sex Determination, Anthropology, Discriminant Function



A7 The Frontal Bone as a Proxy for Sex Estimation in Humans: A Geometric Morphometric Analysis

Lucy A.E. Hochstein, MA*, OCME Western District, 6600 Northside High School Road, Roanoke, VA 24019

After attending this presentation, attendees will gain an understanding of how to use a digitizer in sex estimation of the frontal bone using geometric morphometric analyses.

This presentation will impact the forensic science community by providing a means by which to estimate sex from fragmentary and/or commingled remains using existing technology to complement established methods.

This study combines visual assessment of the human skull with statistical methods of shape analysis, geometric morphometrics, to reach a better understanding of how cranial variation of the frontal bone is influenced by sex. Additionally, a sex-estimation technique was developed and assessed based on a single curve collected from the midline of a human frontal bone using a Microscribe® digitizer.

A sample of 204 crania from the Terry Collection at the Smithsonian Institution's National Museum of Natural History was studied. Male and female individuals of Black and White ancestry were selected to encompass sexually dimorphic variation between these ancestries. A visual assessment of cranial sex was performed using the glabellar scoring technique prior to the digital capture.¹ Following the example of previous cranial and brain outline studies, landmarks and semi-landmarks were collected from the frontal bone to form an outline of the frontal profile.²⁻⁴ Coordinate data for individual crania were imported into MorphoJ et al., a 3D, geometric morphometric visualization and analysis software package.⁵ The raw frontal bone outlines, composed of three landmarks and a curve resulting from varying numbers of points spaced every five millimeters, were re-sampled to have equal numbers of landmarks and semi-landmarks. To focus on shape variation, differences in size, position, and skull orientation were removed through a Generalized Least Squares Procrustes Analysis (GPA) superimposition algorithm. Curve shape was standardized by shrinking or stretching the individual curves, based on a calculated average curve length from nasion to bregma. Before statistical analyses were performed, digitized outlines of 12 individuals were deleted that, after visualization, did not appear to represent any natural frontal bone shape. Two geometric morphometric statistical packages, geomorph and MorphoJ, were used to conduct statistical analyses to understand variation in the collected frontal bone outlines and suggest the potential of this digitizer method for use in sex estimation.^{6,7} For all tests, significance was determined if $p < 0.01$.

Plotting of Principal Component (PC) scores on PC1 for all specimens shows a distinct pattern when the sex of individual skulls is labeled. Males are more scattered and females cluster in the negative portion of the graph. Similar to the Principle Component Analysis (PCA) plot, Canonical Variates Analysis (CVA) plots show males are more widely distributed and females cluster. Both the initial Discriminant Function Analysis (DFA) and the leave-one-out cross-validation show that the classification method presented in this study is better able to accurately classify females than males. In the cross-validation, females were correctly classified 88.3% of the time compared to males at 70.4%. Results of sex assessment based on the visual scoring technique corresponded fairly well to known sex. Out of the 192 curves, 69 (73.4%) of the 94 females were scored correctly as 1 or 2 and 13 (13.8%) were intermediate. Of the 98 males, 62 (63.4%) were scored correctly as 4 or 5 and 23 (23.4%) were intermediate.

Conducting this study laid the foundation for a future software package that may involve only a simple stroke of a digitizer pen on a frontal bone and the likelihood of that individual being male or female will be given immediately. Future research will expand the dataset to broader ancestry and age groups and include more modern (i.e., contemporary) individuals before testing begins on forensically significant collections. Additionally, this geometric morphometric technique has the potential to explore cranial sex differences in subadults, as has been demonstrated in innominates.⁸ Refinement of this method and inclusion of a broader sample may improve the classification rates of males and females and make this a useful technique to complement visual assessments of sex from the skull, especially when encountering fragmented and commingled remains.



Anthropology Section - 2015

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Sex Estimation, Geometric Morphometrics, Frontal Bone



A8 A Qualitative Assessment of Bilateral Pubic Symphyseal Face Asymmetry Using the Suchey-Brooks Method for Adult Age-at-Death Estimation

Amy C. Beresheim, MA, 3152 Douglas Drive, Yorktown Heights, NY 10598*

After attending this presentation, attendees will gain an understanding of how bilateral asymmetries influence age-at-death estimations using the Suchey-Brooks Method.

This presentation will impact the forensic science community by serving as a follow-up to Overbury et al., further exploring how intra-observer error contributes to differing age-at-death estimates between right and left pubic symphyseal faces using the Suchey-Brooks Method.^{1,2}

Accurate and precise age-at-death estimation is critical to the construction of a biological profile in both forensic and archaeological contexts. Most methods for adult age estimation have been developed using North American skeletal collections from the early to mid-20th century and are based on progressive developmental and degenerative changes in bone tissues. Recent research indicates that these morphological skeletal changes may progress asymmetrically, complicating age-at-death assessments when there is a discrepancy between right and left pair elements or when age indicators are unilaterally represented.¹

The pubic symphysis is often considered the best indicator of skeletal age.^{3,4} Given its widespread use, the Suchey-Brooks method for adult age-at-death estimation was selected to qualitatively examine asymmetry of the os pubis.² For direct comparison with previous research, the method of recordation and the definitions of asymmetry followed Overbury et al.¹ The study sample was comprised of 142 White males from the J.C.B. Grant Collection, curated at the University of Toronto. This includes all of the male skeletons in the collection with well-preserved right and left pubic symphyseal faces. The documented age range of the sample is 23-90 years, with a mean age of 60.9 (\pm 15.1 SD) years. This study tests the accuracy of the Suchey-Brooks Method on a known age sample and evaluates whether pubic symphyseal face antimeres produce differing age estimates. This study explores whether bilateral asymmetry is a source of error in the Suchey-Brooks phase system.

Contrary to Overbury et al., asymmetries did not appear to affect adult age-at-death estimations using the Suchey-Brooks Method.¹ Individuals were placed into the “correct” Suchey-Brooks phase 64% of the time, irrespective of side. (Phase scorings were considered “correct” when specimens were assigned to the phase with the mean age closest to the verified age). Phase scores, as well as inaccuracy and bias scores, for the right and left pubic symphyseal faces are not statistically different. Asymmetry and intra-observer error was found to be correlated, suggesting that user experience may influence the prevalence of disparate age estimates between right and left pair elements. While this study does not preclude the existence of bilateral asymmetries at the pubic symphysis, quantitative work on pelvic asymmetry suggests that bilateral differences are likely to be inconsequential in an asymptomatic population and rather manifest as measurement artifacts.⁵ This would indicate that the differences between the left and right sides are more likely to be due to observer error (or random chance) rather than an actual effect of pelvic asymmetry itself.

In order to provide the most reliable age-at-death estimations, antimeres should be analyzed together whenever possible. If this is not possible, the use of either the right or left pubic symphyseal face seems justified.

Future research directions include expanding the study sample to incorporate additional younger adults, females, and multiple ethnicities. It would also be interesting to couple qualitative with metric assessments of asymmetry, as well as to examine the correlations between pelvic asymmetry and asymmetries of the lower limb.



Anthropology Section - 2015

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Pubic Symphysis, Pelvic Asymmetry, Skeletal Aging Methods



A9 Visual Analysis of Maxillary Sinus Variability for Identification of Unknown Decedents

Kelsey Collins, BA, 7388 County Road 41, Willows, CA 95988*

After attending this presentation, attendees will gain an understanding of a method of decedent identification using comparison of the maxillary sinus region of antemortem and postmortem panoramic dental radiographs.

This presentation will impact the forensic science community by providing forensic professionals with an additional method of making a positive identification of an unknown individual.

Positive identification of unknown decedents is of great importance in any investigation. This process is often made difficult by a lack of antemortem records for comparison. A variety of positive identification methods using radiographic comparison of various anatomical structures have been extensively studied to combat this limitation, but many of these methods focus on comparisons of radiographs that are not common in antemortem records and can only be used in isolated cases. Standard dental radiographs are commonly used to make positive identifications of unknown remains because of their availability in antemortem records, but these identifications focus on the dentition of the individual. In recent years, panoramic dental radiographs, which allow for the examination of both dentition and a large portion of the maxillary and mandibular regions, have been commonly included in the standard dental exam. Along with providing a more global view of the dentition, panoramic radiography also provides a clear view of the maxillary sinus region. Several pilot studies suggest that panoramic dental radiographs could be used to compare a number of anatomical structures within the facial region of the cranium if dental identification was not successful; however, the use of these structures in the positive identification process has not been fully researched.

The purpose of this study was to examine the maxillary region of a known sample of pairs of panoramic radiographs from seven individuals, looking specifically at the maxillary sinus region visible on the radiographs for both congruency and corresponding unique traits between the pairs of radiographs. This sinus region was chosen due to the previous success of studies on positive identification using radiographs of the frontal sinuses and maxillary sinuses. To examine this, the dentition was cropped out of each radiograph, and an online survey was created that showed radiographs one at a time and allowed for the selection of a match for the radiograph from a group of four possible radiographs. Forty-nine participants completed both the radiograph matching survey and a brief questionnaire noting qualifications and level of experience.

The results indicated that the maxillary region of panoramic radiographs can be matched with an average of 80% accuracy, indicating moderate success in positive identification. Accuracy for matching the radiographs was not significantly higher for the participants with more experience. In examination of the sinus area, four anatomical structures were most associated with being diagnostic of a positive match: (1) the laterobasal border of the nasal cavity; (2) the inferior nasal conchae; (3) the borders of the eye orbits; and, (4) the maxillary sinuses. These results indicate that the maxillary region of panoramic dental radiographs has the potential to be used for positive identification purposes in the field of forensic anthropology and should be pursued further due to high inclusion of panoramic radiographs in the antemortem record. Future studies will further evaluate this region with both geometric shape analyses and visual matching assessments.

Maxillary Sinuses, Positive Identification, Radiograph Comparison



Anthropology Section - 2015

A10 Success Rates of Sex Estimation by Forensic Anthropologists Using Real-Life Forensic Casework Data

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The goal of this presentation is to provide attendees with an understanding of the success rates of sex estimation of skeletal remains by forensic anthropologists through comparison with DNA sex-typing results in real-life casework data.

This presentation will impact the forensic science community by providing insight into the success rate of sex estimation from skeletal remains in various contexts, including the completeness of the remains available for analysis, the education and experience level of the anthropologist, and specific methods used for sex estimation.

A common task in forensic anthropology involves the estimation of the biological sex of a decedent based upon examination of their skeletal remains. Various methods for making this estimation, both morphological and metric, have been developed by exploiting the sexual dimorphism between males and females. These methods are often based upon analysis of skeletal collections of known sex and most include a theoretical success rate derived from the ability of the method to discriminate between males and females found in that skeletal collection. Often, methods are also tested on geographically and temporally diverse skeletal collections to determine how well the methods apply to groups other than the ones on which they were developed; however, the success rates of sex estimation methods in actual forensic casework have rarely been studied.

The research described in this presentation used sex determinations based upon the DNA results from the amelogenin locus from 360 actual forensic cases to develop “real-life” success rates for sex estimations conducted by forensic anthropologists based upon skeletal material. Cases included varying amounts of skeletal material available for analysis, varying levels of education and certification of the anthropologist, and information regarding specific techniques used for sex estimation. The overall rate of correct sex estimation from these cases is 94.7%, with increasing success rates as more skeletal material is available for analysis. For example, success rates varied from 60.0% when only the mandible was available to 87.5% when the cranium was available to 97.8% when a complete or nearly complete skeleton was available. Success rates also increased as the education level and certification status of the examiner increased. The difference in success rates between biologically male versus female decedents (as determined by DNA) was investigated and was not statistically significant. A large portion of incorrect assessments resulted from cases where only one skeletal element was available for analysis, suggesting caution should be employed, including more use of the category “undetermined sex,” when estimating sex for these types of cases.

Sex Estimation, Biological Profile, Human Osteology



A11 Quantifying Bias in Applying the Asian Equations of Trotter and Gleser to Korean Samples

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After attending this presentation, attendees will appreciate the magnitude and cause of potential bias in applying Trotter's and Gleser's Asian equations to Asian samples, particularly Korean samples, and therefore be aware of the necessity of testing for a magnitude of bias prior to applying those equations.¹

This presentation will impact the forensic science community by conducting a validity test for Trotter's and Gleser's Asian stature estimation equations, which are most popularly used in Asia, and by quantifying the amount of potential errors associated with their application to Asian samples. In addition, this research provides a theoretical basis for the potential bias.

Although the Asian equations of Trotter and Gleser have been widely used to estimate stature from skeletal remains of individuals with Asian ancestry, validation tests for those equations have rarely been performed.¹ In this research, accuracy of Trotter and Gleser equations was tested to quantify the degree of bias when they are applied to one of the Asian populations, Korean skeletal remains of the Joseon period (late 14th century to 1910) and the 20th century.

In stature estimation, the anatomical methods are generally known to produce more accurate estimates compared to the mathematical methods (i.e., regression formulas). Thus, statures of a total of 113 Korean skeletons (50 females and 63 males) were estimated using the revised Fully method by Raxter et al., which were assumed to be true statures.² Then, statures estimated by the Trotter and Gleser Asian equations were compared to these anatomically reconstructed statures. Although Trotter and Gleser provided only male equations, the equations were applied to female skeletons also for a comparison purpose.

Results revealed that male statures estimated by Trotter and Gleser were overestimated in all bone dimensions used: on average, 2.3cm, 1.3cm, 2.2cm, 3.0cm, 1.8cm, and 2.5cm for the equations using the femur, tibia, sum of femur and tibia lengths, humerus, radius, and sum of humeral and radial length, respectively. Percent prediction errors (i.e., (true stature - estimated stature) x 100/estimated stature) for each bone dimension were -1.4%, -0.8%, -1.4%, -1.8%, -1.1%, and -1.5%, respectively. When Trotter and Gleser equations were applied to Korean female skeletons, the magnitude of overestimation became larger: 6.5cm, 6.7cm, 6.3cm, 7.8cm, 5.2cm, and 5.6cm for the equations using the femur, tibia, sum of femur and tibia lengths, humerus, radius, and sum of humeral and radial lengths, respectively. Percent prediction errors for each bone dimensions were -4.2%, -4.3%, -4.1%, -5.0%, -3.7%, and -3.4%, respectively.

This bias (i.e., overestimation) appears to be attributed to a combinatory effect of both the extrapolated application of the Trotter and Gleser equations and the allometric nature of the relationship between stature and long bones. The average statures of the Korean samples were significantly lower than that of the reference sample in Trotter and Gleser (i.e., 168.73cm) by 6.51cm (3.6%) and 20.01cm (11.9%) for males and females, respectively. Thus, many of the Korean male samples and most of the Korean female samples fell in the left side of extreme cases or extrapolation cases in terms of the reference sample of Trotter and Gleser. Generally, the Ordinary Least Squares (OLS) regression, which was used in the process of equation development of Trotter and Gleser, is known to produce biased estimates for extreme cases (Konigsberg et al.), which is the case of the Korean skeletal samples.³ In addition, due to the allometric nature between stature and long bones, the slopes of the Trotter and Gleser equations were smaller than the actual relationship between stature and bone lengths of Korean samples. As a result, the degree of bias became larger as stature got smaller in Korean samples.

In applying the Trotter and Gleser equations to Asian populations, it appears necessary to compare the bone lengths of target samples to those of the reference sample of Trotter and Gleser prior to applying the equations and to be aware of the potential magnitude of bias associated with the equations.



Anthropology Section - 2015

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Stature Estimation, Trotter and Gleser, Korean



A12 A Pilot Study Investigating 3D Variation in the Frontal Sinuses

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After attending this presentation, attendees will have an understanding of the issues plaguing current 2D methods to evaluate frontal sinus uniqueness and the potential of 3D approaches to visualize morphological variation.

This presentation will impact the forensic science community by providing a greater understanding of morphological variation which will enhance quantitative methods aimed at capturing individual uniqueness in these structures.

Identification of unknown individuals is an important aspect in forensic cases. The frontal sinuses are among several areas in the skeleton that have been proposed to aid in identification. These structures have long been considered unique to each individual because of the high degree of observed morphological variation. The most basic approach to compare the morphology of the frontal sinuses for personal identification purposes is either side-by-side comparison or superimposition of radiographic images taken of the frontal sinuses from similar angles and with similar magnification. This simple approach has given way to several methods that attempt to quantify observed morphological variation in the frontal sinuses.

A recent test of three methods on an independent sample revealed that these methods were not able to produce unique matches in all cases.¹⁻³ One explanation could be the predominant reliance on 2D data. To date, 3D variation in the frontal sinuses has only been explored in a single study which utilizes a coding system and reports two individuals in the sample produced the same ten-digit code.⁴

This study utilized an anonymized postmortem Computed Tomography (CT) sample of 130 individuals (males n=70, females n=57, unknown n=3) from the University of Copenhagen. The age range of the sample based on medical records is 19 to 88 years (mean males 49.9 years, mean females 54.9 years). The 3D rendering of the frontal sinuses was carried out using Materialise Mimics® for all individuals twice, permitting comparisons to be made between the same individual (different renderings). Ten trials were conducted using random groups of individuals. In nine trials, the second rendering of the primary individual was present and in one it was not. Stereolithography (STL) files were imported into Rapidform XOV3™ 64 and aligned with a sampling ratio of 100%. A whole deviation function was performed with a tolerance of zero. The tolerance value represents the amount of deviation from the target. By setting the tolerance to zero, any difference in size or shape between two frontal sinuses being matched was identified.

It was found that the mean overall out-of-tolerance percentage for same-skull comparisons was lower (16.24%, std dev 0.31) than that of the different skull comparisons (83.72%, std dev 1.29). Whenever the matching target render of the frontal sinus was present in the sample, it showed the lowest out-of-tolerance statistics and there was no overlap in the values between the same skull comparisons and the different skull comparisons. This is consistent with the assumption that two separate renderings of the same individual's frontal sinuses will be more similar to each other than renderings of frontal sinuses from two different individuals.

This preliminary study illustrates the potential for 3D quantification of variation and uniqueness seen in the anatomy of the frontal sinuses. Previously developed methods aimed at quantifying morphological variation have not been entirely successful because they attempt to reduce the variation seen in the frontal sinuses to a fixed number of variables or regions. Preliminary analysis shows that the variation is a function of both size and shape in all three dimensions and is not confined to specific regions of frontal sinus anatomy, viewable on 2D images. These findings highlight an important limitation to consider when developing and assessing methods and establish an explanation for why 2D methods are not fully capable of capturing the individual uniqueness present in these structures. Further methodological development needs to be carried out to improve the alignment procedure, including testing the use of specific landmarks and increased standardization in rendering. These 3D approaches to evaluating the uniqueness of frontal sinus morphology show promising results and represent future directions for research on personal identification.



Anthropology Section - 2015

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Frontal Sinuses, 3D Comparison, Personal Identification



Anthropology Section - 2015

A13 Use of Measurements Derived From Computed Tomography (CT) Head Scans of Modern Americans for Comparison to the Forensic Data Bank of FORDISC® 3.0

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After attending this presentation, attendees will understand how a diverse, modern American population of Computed Tomography (CT) head scans compares to the Forensic Data Bank (FDB). Attendees will also learn about some of the issues encountered when attempting to collect traditional craniometrics from CT scans of living individuals, which tend to be at a lower scan resolution than those collected from cadavers or dry skulls.

This presentation will impact the forensic science community by demonstrating the applicability of CT data in multivariate analyses such as those in FORDISC®. The results suggest that clinical CT scans may be a valuable source of additional population data to enhance anthropological analyses; however, further research is needed to explore how best to replicate traditional craniometrics in clinical CT scans where certain landmarks are typically difficult to locate.

This study presents the first comprehensive population comparison of CT scans of living individuals (n=291) to the FDB of FORDISC® 3.0. Landmarks were placed on rendered 3D models of the skulls of both sexes and three self-identified ancestry groups (African, Asian, and European) using Mimics® software in an attempt to replicate traditional cranial measurements. Triplicate placement of landmarks on a subset of skulls representing all the CT protocols in the sample found landmark errors of <2.1mm Standard Deviation (SD) (excluding euryon) and an average Interlandmark Linear Distances (ILD) error <1.7%. Euryon was found to be the least precise landmark, primarily in the z-axis (SD=4.1mm), but the imprecision of the landmark did not affect the precision of Maximum Cranial Breadth (XCB) (average error of 0.17%).

For comparison to the FDB, individuals were classified in FORDISC® twice, against all FDB populations and then against those of the same sex in accordance with other population-level studies using FORDISC®. In the all-group comparison, average classification accuracies ranged from 53% for sex and ancestry, to 65% for ancestry, and 81% for sex. The highest accuracy rates occurred among white females for ancestry (91%) and sex and ancestry (77%) and Asian males for sex (100%). When only same-sex groups were compared, classification to the correct ancestry was around 65%. In general, the Asian individuals classified with the least accuracy, but also represent a more diverse set of nationalities than those represented in the FDB. Expansion of the Asian groups in the FDB may enhance the classification of Asian Americans such as those in this study. Correct classifications of CT measurements occurred at above-chance frequencies that were comparable to FORDISC's® own cross-validation rates of the FDB using the same variables. In a comparison of group means for the 17 ILDs included in the FORDISC® analysis, CT scan values tended to be larger than FDB values. Orbital Breadth (OBB) had the largest difference across all groups, suggesting that the way this ILD was collected in this study was problematic due to the inability to locate dacryon. This study reveals that clinical CT scans may be a valuable source of anthropological data provided that the appropriate landmarks can be located. The above-chance classification rates and the low landmark placement/ILD errors suggest the multivariate relationships among FDB individuals can be used to estimate the sex and/or ancestry of modern American individuals using CT scans, opening the possibility for expanded use of CT data in anthropological analyses.

Computed Tomography, FORDISC®, Craniometrics



Anthropology Section - 2015

A14 The Effects of Household Bleach on Bone in the Processing of Forensic Remains

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After attending this presentation, attendees will understand the effects of household bleach on the gross appearance of bone and trauma marks and its effect on the stiffness of bone.

This presentation will impact the forensic science community by describing the effects of a commonly used defleshing method on bone as well as by presenting a method by which to compare the biomechanical properties of bone prior to and following the defleshing process.

Individuals processing human remains from forensic cases are confronted with the unique challenge of choosing a defleshing method that is non-destructive to bone in order to preserve identifying features and fine marks of trauma, while remaining within the time constraints of a legal setting. Sodium hypochlorite (NaOCl), an oxidizing agent, in the form of household bleach such as Clorox[®], is used in some laboratories to speed the process of defleshing and as an additional disinfectant. Household bleach is popular among many researchers due to its easy availability and low cost. Some have claimed that while other chemicals are harmful to bone tissue, household bleach is safe enough for human forensic cases, even those exhibiting signs of peri-mortem trauma.¹ Nawrocki intimates, due to health safety issues, that biohazardous remains must be simmered in water with bleach before entering the main osteology laboratory to be handled for analysis.² Despite these claims, others assert that bleach is too harsh to be used on bone.³

The goal of this study is to describe and compare two commonly practiced methods of defleshing human remains — plain water boiling and boiling with Clorox[®] bleach — in order to assess the effects of bleach on bone tissue. Three concentrations of Clorox[®] (low, medium, and high) were compared to the method of boiling in plain water. White-tailed deer (*Odocoileus virginianus*) distal hind-limb segments exhibiting knife-cut marks and saw marks (n=20) were used to test the effects of each defleshing method on the bone tissue and the appearance of sharp-force trauma on the bone, in addition to using them to test the effectiveness of soft tissue removal. The effectiveness of the method was assessed by comparing the quality of the end result and the time-to-completion for each method. The effects of the methods on the bone were assessed by noting the gross appearance of the bone and trauma marks prior to and following each processing method. Additionally, bone core samples were taken before and after processing to be used in a biomechanical test of the structural integrity of the bone tissue. An Instron[®] 5564 with a 2kN capacity was used to conduct unconfined compression tests to test the stiffness of the bone tissue of these bone core samples.

Each of the four defleshing groups took 6-8 hours to complete (M=6.55 hours, SD=0.605), with no statistically significant difference among the methods in time-to-completion. Moreover, comparison of the strain at 1790N, the first peak on the stress/strain graph, and the tangent modulus of the pre- and post-processing bone core samples revealed no statistically significant difference among the groups in the stiffness of the bone. However, cortical bone exfoliation was noted in two of the five high-concentration bleach samples.

In conclusion, the addition of bleach to heated tap water does not affect the time-to-completion or the stiffness of the bone, but, in high concentrations, can damage bone tissue macroscopically. Bleach is also known to lower the quality of DNA retrieval.⁴ Therefore, unless it is found that bleach can sterilize biohazards that boiling alone cannot, it should be avoided in forensic cases as it has the potential to damage bones if not used properly.



Anthropology Section - 2015

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Defleshing, Bleach, Bone



A15 The Influence of Alkaline or Acid Liquids on Cut Marks and on the Structure of Bone: An Experimental Study on Porcine Ribs

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After attending this presentation, attendees will be aware of the macroscopic and microscopic effects of acid and basic solutions when used with the purpose of destroying a corpse and thus hindering discovery and identification.

This presentation will impact the forensic science community by highlighting the effects of acid and basic solutions on bone and the importance in correctly assessing their effectiveness in the destruction of tissues and in the modification of signs of trauma.

Among the different methods that are seldom used with the goal of destroying a corpse and thus preventing discovery or at least identification, the use of highly acidic or basic solutions is something forensic pathologists sometimes have to deal with, especially in criminal scenarios. Moreover, determining whether a bone (or even a single fragment) was in contact with an acidic/basic solution could be a crucial question posed to the anthropologist. Of all taphonomical modifications during decomposition processes, little is known about the action of high or low pH to human tissues and bones. The main question is, are these solutions able to make a cadaver completely “disappear” and, when human tissues come in contact with these substances, what kind of changes do they undergo? How are they recognizable? Only a few studies have focused on this issue and have referred only to macroscopic surveys.

In this study, a total of 60 samples of porcine bone (*Sus scrofa*) were completely skeletonized manually, without any chemical or other artificial treatment. Furthermore, on each sample, a cut mark was produced with a scalpel in order to evaluate the modifications that these signs can undergo in such conditions. Specimens were then divided in groups of ten each and put in six different liquid solutions with different pH (1, 3, 5, 9, 12, 14) prepared by adding sulfuric and acetic acids and sodium hydroxide to water. A neutral control solution (pH 7) was also prepared.

Specimens were analyzed every five days over a period of 70 days. The appearance of the outer cortical layer of the bone and the aspect of the cut marks were investigated first macroscopically and then microscopically with a Wild Heerbrug® M650 stereomicroscope and Scanning Electron Microscopy (SEM). Finally, thin undecalcified sections were prepared and analyzed with a transmitted light microscope in order to evaluate the changes of the osteonic structures and the appearance of characteristic patterns.

Regarding the macroscopic observation, minimal lytic modifications became evident in all the samples, but only those exposed to a pH 14 for a long time showed evident alterations of the cortical bone, such as large erosions and cracking. Cut marks showed alterations in 50% of the cases, especially when exposed to basic solutions, detectable as enlargements, distortions, or detachment of bone flakes.

The most interesting results arose from light microscopy of thin sections: if further significant elements were not gained with stereomicroscopy, scanning electron microscopy and light microscopy provided the most interesting results. The first enabled the observation of significant alterations on the surface of the cortical bone, with evident deposits of organic and inorganic matter as the pH became more acidic and as contact time increased. This layer of matter gives an important contribution to the macroscopic alterations of cut marks on bone which may, as in the case of pH 1 and 14, no longer be even recognizable.

Moreover, the study showed for the first time that, even without detectable macroscopic alterations, the osteon structure visible in light microscopy is severely deteriorated by acids and bases, frequently with peculiar patterns, like radial or multidirectional cracking. The information gained from the present study can be of great help in the detection of an exposure of human tissues to high or low environmental pH and in understanding the effects that these solutions can exert on human bones. Extreme pH can significantly alter the structure of human bone and make signs of traumas undetectable, but the contact between solutions and bone can be detected if thoroughly analyzed, especially through light microscopy.

Acids, Cadaver Destroying, Cut Marks



A16 Thermal Analyses of Property Changes and Weight Loss in Incinerated Bone and Their Implications for Forensic Anthropology

Sarah Ellingham, MSc, Teesside University, School of Science and Engineering, Middlesbrough TS1 3BA, UNITED KINGDOM*

After attending this presentation, attendees will understand how thermal analysis techniques such as Thermogravimetric Analysis (TGA) and Differential Scanning Calorimetry (DSC) can be utilized to glean information about the properties and phase transitions undergone by bone when exposed to heating.

This presentation will impact the forensic science community by investigating how exposure to varying temperatures and differing heating regimes affects the rate of compositional changes of bone and by discussing the implications for further forensic analyses such as dating, isotope analyses, and genetic profiling.

Many studies have demonstrated that bone undergoes three stages of degradation and weight loss during heat exposure, which are attributed to a loss of water, the combustion of organic components, and finally, the loss of carbonate and crystal fusion. Not so well understood is to what extent these processes are influenced by the heating regime or the duration of exposure. This study utilized time- and temperature-resolved TGA and DSC to investigate the temperature regions in which the main structural changes occur and how these are influenced. TGA measures the weight changes in a sample as a function of a temperature profile and DSC allows for the monitoring of the heat flow in and out of a sample, which allows for the detection of phase transitions in the material.

Sheep (*Ovis aries*) rib bones were cut into cross sections of approximately 20mg and analyzed using an Evisa® STA 1500 Simultaneous Thermal Analyzer. Samples were heated from room temperature to 1,100°C, for which three different heating rates were employed, 6°C/min, 12°C/min, and 24°C/min. Additionally, two sets of samples were heated at a rate of 12°C up to a maximum of 300°C and 400°C and held at these temperatures for 45 minutes. All runs were carried out in triplicate.

Although weight loss as well as the heat flow curve of the three different heating regimes exhibit comparable patterns, an approximately 25°C delay in the temperature onset of phase changes with every doubling of heating rate was observed. In order to find the temperature regions of increased weight changes the first derivative of the TGA curve ($\Delta_{\text{mass}}/\Delta_{\text{temp}}$) was calculated. Distinct peaks were noted at about 125 +/- 25°C, 375 +/- 25°C, 500 +/- 25°C, and 775 +/- 25°C at the heating rates of 6, 12, and 24°C/min, respectively. These observed weight changes correspond with the matrix phase alterations shown by the DSC. The initial heating phase corresponds to an evaporation of water, an endothermic process which peaks between 100°C and 150°C and continues up to a temperature of approximately 350°C, at which point the combustion of organic components commences, which introduces the exothermic process of the hydroxyapatite crystals becoming larger and more ordered. A brief endothermic phase attends the combustion of carbonate at about 450°C, which is followed by a strong exothermic phase as the bone's collagen continues to combust, spiking at approximately 500°C, and recrystallization of minerals proceeds. The process remains exothermic up to 750-800°C at which point the mineral begins to sinter and crystals melt in an endothermic event.

Not only did the increase in heating rate delay the temperature onset of the bone's phase transitions, but it also demonstrated an influence on the weight loss. At 1,100°C, the sample heated at 6°C min exhibited the gravest average weight loss with 59.6%, the one at 12°C lost 57.7%, and the sample heated at 24°C lost 51.1%. The observation that exposure time has an influence on the loss of mass is confirmed by the samples which were held at fixed temperatures for 45 minutes. Samples heated to 300°C, when initially reaching the temperature, lost 16.54% of their weight, which after 45 minutes increased to 33.32%. Samples held at 400°C started out with 35.2% mass loss which increased to 49.4%. The corresponding DSC curves indicate that although a steady loss of mass occurs, which is most likely attributed to the combustion of collagen, the bone does not undergo any further property changes if the temperature remains at the same level.

Being able to determine the point at which organic components in bone are lost is of crucial importance for forensic analyses, as successful collagen extraction is the basis to conduct isotope analyses, dating, or genetic profiling.

Burnt Bone, TGA, DSC



A17 **Effects of Soil Environment on Bone Mass: A Human Prospective Taphonomic Study**

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After attending this presentation, attendees will understand how decomposition can be affected by different soil environments, specifically human bone remains.

This presentation will impact the forensic science community by providing information on factors that influence bone alterations that are generated by burial. These alterations can be measured by the loss of bone mass to help pathologists in more accurately predicting the time-since-death on human remains in forensic settings.

A multitude of factors can affect each stage of the decomposition process by either accelerating the process or slowing it down. Variables in soil environments include moisture, temperature, pH, and scavenger activity.

An experiment was designed on four human ribs to document how moisture and temperature affect the bone weight related to the postmortem interval. Three of the ribs were buried and the fourth rib was placed in a controlled environment. Three human cadavers were used, and the R1, R2, R3, and R4 ribs were removed from each cadaver. All of the bones were manually defleshed, immediately weighed, and placed in their environments on the same day. The R1 and R4 ribs were buried outside in clay at a neutral pH, the R3 rib was buried in clay at a neutral pH under a hood at 20°C, and the R2 rib was placed under a hood at 20°C without clay as a control. Temperature and humidity were recorded daily. Ribs were weighed daily using a precision balance (Kern® ALT310-4, d=0.1mg). Daily weight loss of the three subjects (i.e., the percentage of remaining weight) were studied over 90 days and averaged depending on the burial environments.

Burial environments were compared by studying the distributions of bone weight loss using a Kruskal-Wallis test. Results showed there were significant differences between environments ($p < 0.0001$) for inside vs. outside burials and burial vs. control: bone weight loss was significantly faster inside (i.e., low moisture content and constant temperature) and without clay soil (i.e., without retaining moisture in the environment). Combined comparison tests were performed on a day-by-day, per environment basis using a Chi-squared test and Marascuilo's post-hoc procedure. The results showed there was a significant difference in bone weight loss ($p < 0.0001$) between the indoor and outdoor environments from day one to day five of the postmortem interval. This difference was less significant from day six ($p < 0.05$) and not significant from day ten. Bone weight loss continued and finally stabilized to approximately 50% of the initial mass at three months, regardless of the experimental conditions.

Bone tissue can be considered a composite material of an organic phase and mineral phase. An inorganic hydroxyapatite mineral is embedded in an organic matrix composed of type I collagen. Wet bone is composed of approximately 15% water, 20% organic, and 65% mineral portions by weight. The loss of bone mass can be explained primarily by the decrease in bone moisture content. The literature explained that the average ash percentage of bones increases dramatically but generally levels off. Moisture content decreases rapidly for nearly two months, after which the drying process continues at a slower rate. These observations are consistent with this study.

However, the loss of bone mass can also be explained by chemical and physical alterations of the tissue. The diagenesis of bone tissue is a multivariable process that affects mineral and organic phases. The mineral phase can undergo mineral precipitation, ion exchange, recrystallization, dissolution, and leaching. The organic phase is primarily associated with collagen loss due to chemical breakdown or microbial attack followed by leaching. The development of these phenomena should be better studied to understand the process of bone weight loss.

Postmortem Interval, Burial, Bone Moisture



A18 The Identification of Undocumented Border Crossers Along the United States-Mexico Border: A Case for Bone Histology

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After attending this presentation, attendees will understand the difficulties of identification along the United States-Mexico border and the benefit of using bone histology for age-at-death estimation on Undocumented Border Crossers (UBCs).

This presentation will impact the forensic science community by serving as a guide to improve the identification of UBCs in the growing humanitarian crisis afflicting the United States-Mexico border.

UBCs found along the United States-Mexico border present new challenges in identification as population-specific methods are lacking for this group. Since 2013, the Forensic Anthropology Center at Texas State (FACTS) has been working to identify UBCs exhumed from Brooks County, TX, led by Drs. Lori Baker and Krista Latham. As part of this effort, FACTS has accepted 73 UBCs from the Brooks County, TX, exhumations and from the Webb County, TX, Medical Examiner's Office. These UBCs are typically in early to late stages of decomposition and are held at the FACTS outdoor decomposition facility until they can be macerated and analyzed. While some of the UBCs have soft tissue, the majority of remains require skeletal analysis to generate the biological profile. The current ancestry estimates of the Texas UBCs, due to lack of appropriate reference data from Latin America, predominantly fall into the Hispanic category. While the Hispanic classification is useful in the identification of an unknown individual as a UBC, it does not assist in differentiating among self-identified racial/ethnic categories as in the United States, which can narrow down a list of potential matches from a missing persons' list. Therefore, a critical aspect of UBC identification is an accurate age-at-death estimate, to narrow the list of potential matches for identification.

The goal of this study is twofold: (1) to examine the protocol used for identification of UBCs at FACTS to determine if the current methods provide accurate estimates; and, (2) to ascertain if the addition of bone histology can assist in creating more accurate profiles. A case study is also presented to illustrate the need for more accurate age-at-death estimation methods for UBCs.

The remains of N=19 (10 males and 9 females) UBCs were assessed using morphological and histological methods. The morphological methods included dental development, pubic symphyseal and auricular surface morphology, and sternal ribs. Bone histomorphometry of the midshaft of the left 6th rib was analyzed using osteon population density methods.^{1,2} Agreement between the gross morphology and bone histology methods were assessed by recording whether the histomorphometric age ranges overlapped with the final gross morphological age ranges.

Results show that the histomorphometric estimates for all individuals overlapped with the gross morphology ranges (19/19=100%) suggesting that the microstructural age-related changes correspond with the visible morphological changes. Since many of the UBCs are unidentified, this test of agreement is necessary to support the use of bone histology for aging UBCs. When applying the two approaches to a UBC-positive Identification (ID), the morphological methods provided an age range of 20-35 years, but failed to capture the actual age of 38 years, likely due to the lack of fusion of all skeletal elements. However, histomorphometry provided an age range of 27-57 years, with a point estimate of 39.5 years. If the morphological estimate was the sole search criteria within a missing person's database, this individual may have remained unidentified. Combining the morphology with the histology estimate allows for a complete understanding of both the micro and macro skeletal age and provides a range that encompasses the known age of the UBC.

The results of this study indicate two important factors for UBC identification: (1) applying only traditional aging methods to UBC identification can fail to accurately predict age, likely due to the low socioeconomic status of these individuals and corresponding delayed development; and, (2) incorporating bone histomorphometry of the 6th rib more accurately reflects the age-at-death of the UBCs. By changing the protocol for UBC identification, the accuracy of the age-at-death estimates can be improved, the cost in time and money for identification of UBCs can be reduced, and the number of identifications could be greatly increased.



Anthropology Section - 2015

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Undocumented Border Crossers, Bone Histology, Identification



A19 The Influence of Taphonomic Changes and Microstructure Varieties on Histomorphological Analysis in Species Determination

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After attending this presentation, attendees will gain a better understanding of variations in microstructure of osseous remains and will be able to identify the characteristics of taphonomic changes on bone. This will aid in better accuracy at distinguishing inconclusive osseous remains (including human) from non-human osseous remains, enable a better understanding of taphonomy, and may significantly reduce the number of misinterpretations.

This presentation will impact the forensic science community by providing descriptions and examples of different taphonomic conditions as well as variations of bone microstructure between and within species. Example pictures will enable visual comparison and identification of structures and changes and will, therefore, enhance the accuracy of identifying non-human osseous remains.

It is generally a challenge to interpret degraded and fragmented osseous remains found during recoveries. Both biotic and abiotic processes can alter skeletonized remains after death and thus affect any identification analysis. The role of taphonomy and variation is often underestimated and, for this reason, can lead to misdiagnosis of bony fragments.

Histomorphological techniques used at the Joint POW/MIA Accounting Command-Central Identification Laboratory (JPAC-CIL) are applied to distinguish inconclusive osseous remains from non-human osseous remains. Chemical, biological, or physical activity can affect the condition of remains (e.g., environmental conditions, such as soil composition or microbes, as well as type-of-loss incident). Bone structure can differ between species, within individuals of one species, and even within a single bone, depending on numerous factors (e.g., growth speed, aging, diseases, biomechanical load, etc.). Bone tissue is a dynamic structure and subject to remodeling; for example, bone growth and bone resorption due to osteoblastic and osteoclastic activity on the micro-structural level. The histomorphology of bone is similar between genera within *Mammalia*. Fast growing mammals can show plexiform primary bone structure, a bone type that is not found in (slow growing) humans. Thus, a sample can be identified as non-human if plexiform bone structures are present.

Histomorphology at the JPAC-CIL uses standard histomorphological methods to distinguish inconclusive osseous remains (which can include human) from non-human osseous remains. But histomorphology can additionally aid in the identification of taphonomic processes and provides information regarding changes in the bone microstructure. The methodology for standard histological analysis at the CIL utilizes three consecutive processes: (1) embedding; (2) sectioning; and, (3) analysis of a sample. Basic epoxy embedding is used to stabilize the sample for thin sectioning. Then, using a precision saw, transverse thin sections of approximately 0.08mm thick are made, which are mounted and analyzed using a light microscope.

Difficulties in ascertaining with certainty whether a fragment is human or non-human arise when the original bone anatomy exhibits extensive alterations due to taphonomy, degradation, or changes in the microstructure because of bone remodeling. Microbial alteration can destroy the original microstructure to a point where it is no longer recognizable, whereas other processes like heating or diagenesis can reduce birefringence, cause cracking, and introduce foreign material into the bone matrix, all of which can complicate analysis.¹ Remodeling will replace primary bone with Haversian bone, in which case only the presence of organization (banding) of Haversian systems can indicate whether bone is non-human in origin.² Example photographs as well as possible solutions to these issues will be presented.

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1. Jans, M.M.E., Kars, H., Nielsen-Marsh, C.M., Smith, C.I., Nord, A.G., Arthur, P., Earl, N. (2002) In situ preservation of archaeological bone. A histological study within a multidisciplinary approach. *Archaeometry* 44 (3): 343-352
2. Mulhern, D.M., Ubelaker D.H. (2001) Differences in osteon banding between human and nonhuman bone. *Journal of Forensic Sciences*; 46 (2):220-2.

Histomorphology, Taphonomy, Species Differentiation



Anthropology Section - 2015

A20 **Stable and Heavy Isotope Analysis: The Successful Use of Chemical Research in the Tampa Bay Cold Case Initiative**

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After attending this presentation, attendees will understand the principles underlying heavy isotope analysis. Isotope analysis, utilized in forensic investigations, assists in the estimation of geographic origin and migration patterns of unidentified decedents. Isotope analysis has been utilized in bioarchaeology for decades. More recently, heavy isotope analyses involving strontium isotope and lead isotope ratios have been utilized in the estimation of geographic origins for migrant populations and unidentified decedents.

This presentation will impact the forensic science community by highlighting the importance of biogeochemical methods to enhance forensic anthropological investigations. This current research study will yield local strontium and lead isotope values that can assist with current unsolved cases for Florida and the southeast region of the United States.

Forensic anthropologists systematically apply a comprehensive set of methods for unsolved cases (i.e., the estimation of age, sex, ancestry, trauma, pathology, and unique identifying characteristics) to aid in the identification of decedents. Application of methods such as chemical and elemental analysis offers long-term unsolved cases new lines of evidence. These methods are proving to be valuable by yielding georeferencing information for the identification of victims.

Human teeth and bones are an archive of long-term strontium and lead exposure. Strontium and lead isotopes were analyzed from the teeth of unidentified individuals. Permanent teeth begin enamel mineralization during early childhood, as early as three to six months during infancy with central incisors and up to 12-15 years of age with the first permanent molars. Strontium is absorbed into the individual's biology via the food chain with the ultimate source being the local bedrock, soil, and water. Long-distance importation of food and water may affect the individual's strontium isotope ratios and may not be entirely controlled by the local environment. Similarly, the lead isotopic compositions of individuals can be linked back to local environmental sources of lead from the soil or from anthropogenic sources such as leaded gasoline.

In contrast to strontium, it is believed that lead is more directly absorbed through soil and/or dust ingestion or inhalation and, therefore, is not likely to be affected by importation of foods from other regions. A comparison of the isotope ratios of the enamel and bone can yield a pattern of migration from when the individual moved from one geographic region to another region throughout their lifetime. The enamel formation during early childhood gives a biochemical profile of the individual's early years, with bone remodeling over a course of 7-10 years continually as a person ages. The bone offers a biochemical profile of the individual's last years of life.

For this research, stable and heavy isotope analysis of $\delta^{13}\text{C}$ (carbon), $\delta^{15}\text{N}$ (nitrogen), $\delta^{18}\text{O}$ (oxygen), $^{87}\text{Sr}/^{86}\text{Sr}$ (strontium), and the lead isotopes ($^{206}\text{Pb}/^{204}\text{Pb}$, $^{207}\text{Pb}/^{204}\text{Pb}$, and $^{208}\text{Pb}/^{204}\text{Pb}$) were completed on current and cold cases, which includes a sample of 30 individuals (15 males, 10 females, and 5 unknown) since 2010. Stable isotopes of carbon and nitrogen were utilized to investigate diet and nutrition, while oxygen, strontium, and lead isotopes were used to evaluate geographic origin and environment. Isotopic analysis aided in identifying the individual's diet over their lifetime and the geography of their living environments. Heavy isotope results were instrumental in the estimation of the decedent's birth location versus where they were recovered in Florida.

In the cases of successful identification encompassed within the Tampa Bay Cold Case Initiative, the isotope results have successfully revealed geographic origins and migration patterns for the decedent. A trend in the isotope results also reveals numerous cases in the Tampa Bay region are foreigners or out-of-state individuals. From the isotope results for the unsolved cases, cold case investigators were able to redirect their investigations and reach out to other agencies with the new information concerning the case.

The Tampa Bay Cold Case Initiative is a noteworthy example of collaborative work and research by forensic anthropologists, scientists, and medicolegal agencies. The goal of this project is to reinvestigate and highlight "cold cases," which has led isotope analysis to be applied to recent skeletal cases due to the transient nature and population of the Tampa Bay region. Collaborative and multidisciplinary research is a key function for casework involving unidentified human remains.

Isotope Analysis, Cold Cases, Biogeoferencing



A21 A Preliminary Study for Estimating Postmortem Interval of Fabric Degradation in Central Florida

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After attending this presentation, attendees will understand how forensic anthropologists can use degraded clothing in determining the extended postmortem interval of cases involving skeletal remains.

This presentation will impact the forensic science community by establishing standardized methodologies for the research and analysis of degraded clothing which have the potential of being used as a new method when estimating the extended postmortem interval.

Forensic anthropologists rely on a variety of evidence to estimate the Postmortem Interval (PMI) of a decedent, which includes gross decomposition rates, the life stages of insects, and the degradation of associated material evidence. The degradation of material evidence, in particular, is an underutilized area in estimating PMI. Degraded clothing is a common type of material evidence recovered in association with skeletonized remains that is also modified by taphonomic processes. While previous research has been undertaken regarding how degraded fabric can be used as a PMI indicator, there is no standard methodology for the research design or to quantify the extent of fabric degradation. The purpose of this project was to analyze the degradation of four different fabrics in central Florida and to develop a comprehensive scoring system and descriptive methodology to be used as a standard for scoring fabric degradation. In addition to providing standards for future research, the methods used in this project are applicable to the analysis of forensic cases.

Four types of fabric swatches were tested (100% cotton, 60% polyester/40% cotton, 100% rayon, and 100% cotton denim) at three different burial depths (ground surface, ~30cm below ground surface, and ~60cm below ground surface). Swatches were placed in six groups for each of the three different burial depths. Each group included a total of eight swatches, two of each fabric type (cotton, cotton/poly, rayon, and denim) that were arranged in two different positions, flat (horizontal) and crumpled. While there is no standard for fabric swatch size, this study utilized a 15cm x15cm-size swatch that is suggested as a standard research size. The swatches were washed and dried one time based on recommendations by Mitchell et al., who concluded that clothing found at a crime scene is unlikely to be unlaundered and brand new.¹ In addition, a meat source was incorporated from a butcher to more accurately reflect the degradation process of fabrics found in conjunction with a decomposing body. Combinations of microscopic and macroscopic methods were used to analyze the degraded swatches. These included a stereomicroscope to analyze warp and weft, the Munsell color system to document color changes, and a transparency overlay developed for this project to evaluate the percentage of material loss.

Groups of fabric swatches were retrieved at one-month intervals for the duration of six months. After retrieval, cotton exhibited the highest level of degradation, as one-third of all cotton fabric swatches demonstrated more than 50% total degradation. Furthermore, cotton fabric swatches degraded more at both ~30cm and ~60cm below ground surface than on the ground surface; however, all other fabric types demonstrated slightly more degradation on the ground surface than the other two areas. For all fabric types, swatches that were positioned flat tended to degrade more than those that were positioned crumpled. While soil moisture fluctuated the most on the ground surface, the soil at both ~30cm and ~60cm below ground surface depths retained increased moisture throughout the study period. Overall, cotton was the only fabric type to degrade significantly enough to demonstrate substantial degradation during the research period, while all other fabric types exhibited minimal degradation over six months of monitoring.

In forensic cases lacking soft tissue, degraded clothing is a common example of material evidence recovered at a crime scene with skeletal remains. Overall, this study has developed replicable standards that can be used for scoring and evaluating fabric degradation to be used as an extended PMI indicator. Understanding fabric degradation is critical to establishing a long-term PMI when confronted with skeletal remains and an area of research to consider in the future as forensic anthropologists continue to expand their interdisciplinary tool kit and stimulate new areas of research.

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1. Mitchell JL, Carr DJ, Niven BE, Harrison K, Girven E. 2012. Physical and mechanical degradation of shirting fabrics in burial conditions. *Forensic Science International* 222:94-101.

Taphonomy, Postmortem Interval, Fabric Degradation

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Anthropology Section - 2015

A22 Decomposition in Concrete: Los Angeles Style

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After attending this presentation, attendees will understand how concrete impacts the decomposition process and the importance of a multidisciplinary team for investigation of concrete “burials.”

This presentation will impact the forensic science community by serving as an instructional guide to assisting in the determination of Postmortem Interval (PMI) for bodies recovered from concrete.

Disposal of human remains in concrete is a rare occurrence in Los Angeles County; however, interpretation of remains from such contexts may lead to significant information in homicide investigations. One of the important aspects of investigation is the determination of PMI. As with any estimation of PMI, many intrinsic and extrinsic factors must be taken into consideration. Despite these factors, an analysis of three cases recovered by the Los Angeles County Department of Medical Examiner/Coroner illustrates commonalities between bodies recovered from concrete.

Four commonalities were documented: (1) bodies, even when dismembered, are generally complete; (2) the concrete is generally poorly-mixed; (3) the bodies are secreted in a secondary fashion as well; and, (4) concrete may act as a preservative of both remains and identifying features.

Case 1 was an adult recovered from a sandy soil in the yard of a residence. The remains were buried in a shallow grave adjacent to the dwelling, clothed but without additional wrapping. A thin layer of poorly-mixed concrete was poured over the remains, but did not cover the entire decedent. Soil was placed over the concrete. The decedent was discovered approximately 13 years postmortem and was completely skeletonized, with excellent preservation of bone. The concrete preserved a mold of the face and one hand; from these preserved molds casts were made and fingerprints were obtained for positive identification of the decedent.

Case 2 was a child approximately four years old, wrapped in a plastic garbage bag, and encased in concrete in a cardboard box. The decedent was moderately decomposed, having been kept in a bathtub for four days, stored for a time in an ice chest over ice, then encased in concrete. The decedent was recovered from a car trunk approximately six months postmortem. The decedent was dismembered and showed skin and hair slippage, body fat liquefaction, and softening of the tissues, but was remarkably well preserved.

Case 3 was an infant recovered in a plastic bin wrapped in a plastic sheet and buried in a shaded area on public land. Scavengers had attempted to uncover the remains, but were prevented from doing so by heavy plastic sheeting. The decedent was clothed and in a disposable diaper, wrapped in a fleece blanket, placed on a folded second fleece blanket in the container, covered with a towel, and then covered with approximately seven inches of poorly mixed concrete. The container was sealed before the concrete fully set, leaving a moist, anaerobic environment for decomposition. The remains were recovered approximately one month after they were placed in the container and buried. The infant showed advanced decomposition changes in the area of the abdomen, with hard adipocere formation across the remainder of the body.

Based on these case studies, a preliminary hypothesis that concrete encasement slows the decomposition process was formed. Los Angeles County contains a minimum of five microclimate zones, including coastal, valley, mountain, high desert, and low desert. Temperature variations between the microclimates can be as much as 30°F and humidity differentials are dramatic between the coastal and desert areas. This leads to differences in the rates of decomposition between these microclimate zones. In general, decomposition studies conducted at California State University, Los Angeles in thesis research and personal experience demonstrate that, with the exception of the high desert and mountain zones during the winter months or in cases where mummification occurs, infants and children decompose rapidly, reaching skeletonization in as little as one week in some cases and rarely exceeding three months.

As these three cases illustrate, PMI for bodies in concrete is difficult to decipher because of the many extrinsic and intrinsic factors inherent in the decomposition process. It is hypothesized that the anaerobic environment and lack of insect access slow the decomposition process in concrete-encased bodies regardless of the state of decomposition when they are placed in the concrete. Additional research is currently underway to test the phenomenon of decomposition delay in concrete.

Decomposition, Concrete, Adipocere



A23 A Test of Wright and Vasquez on an American Population

Carrie B. LeGarde, MA*, 974 Auloa Road, Kailua, HI 96734

After attending this presentation, attendees will understand the applicability of the Wright and Vasquez equations for estimating maximum length of a long bone from fragmentary remains on an American sample.¹

This presentation will impact the forensic science community by providing another possible means of estimating maximum length of the tibia and fibula from fragmentary remains for the purpose of stature estimation on an American sample.

Obtaining a stature estimate is difficult when dealing with fragmentary remains since stature estimation models are most successful when maximum length measurements are utilized. Multiple studies have produced equations that estimate the maximum length of a long bone based on measurements of fragments or bone segments, which can then be used in stature estimation models. Currently, there are no methods for estimating maximum length of the fibula from fragments for an American population. Wright and Vasquez produced estimation equations for the fibula based on a Guatemalan sample, but suggested that before applying their equations to a different population, the equations should be validated on complete long bones from the population in question.¹ In response, this study utilizes measurements from the tibiae and fibulae of 19 American Korean War soldiers to test the applicability of Wright's and Vasquez's equations on an American population.

The regression equations using fibular segments produced by Wright and Vasquez were used to calculate the estimated maximum length of the fibula and tibia.¹ The estimated maximum lengths were then compared to the actual maximum length of the elements. When Wright and Vasquez tested their equations on a known Guatemalan population, the equations for estimating tibial length from segments of the fibula actually performed better than the equations utilizing segments of the tibia. For this reason, regression equations for segments of the tibia were also used to investigate if, indeed, the fragmented fibula is more reliable than the fragmented tibia for estimating maximum length of the tibia on an American sample as well.

This study shows that the Wright and Vasquez equations perform well on an American population. Following the procedure Wright and Vasquez used for testing their equations, the mean difference between estimated and actual maximum lengths was calculated, as well as Mean Absolute Deviation (MAD) and Mean Squared Error (MSE).¹ The probability for a paired t-test between estimated and measured lengths for each equation was also calculated. Wright and Vasquez considered only $p < 0.001$ to indicate significant differences between the estimated and actual lengths.¹

No tibial or fibular length estimates using the regression equations differed significantly from the actual maximum lengths as evidenced by no p values less than 0.001. The mean difference was 2mm or less for the majority of the equations. The equations with the greatest mean difference were the equations estimating tibial length from fibula segments, ranging from 2mm to 5.36mm. Overall, the MAD was relatively small for all equations and it was less than the standard error of the estimating equations in all cases. The tibial length was underestimated in all equations, reflected by a negative mean difference. The maximum length of the fibula was overestimated for two of the three equations. Estimating the maximum length of the tibia from incomplete fibulae did not perform as well as using tibial segments, but they still performed adequately.

The results of this analysis are promising for the applicability of this method on non-Guatemalan remains. Although the sample size is small, this study suggests that Wright's and Vasquez's equations for estimating maximum length of the tibia and fibula from fragmentary remains can be used on an American population.

Reference:

1. Wright LE, Vasquez MA. 2003. Estimating the length of incomplete long bones: Forensic standards from Guatemala. *AJPA* 120:233-251.

Fragmentary Remains, Stature, Fibula



A24 **Bilateral Asymmetry in Age-Related Trait Expression of the Pubic Symphysis and Auricular Surface of the Ilium**

Kyle A. McCormick, MA*, 805 Deery Street, Knoxville, TN 37917; and Michael W. Kenyhercz, MS, University of Alaska Fairbanks, 403 Salcha Street, 310 Eielson Bldg, Fairbanks, AK 99775

The goal of this presentation is to inform attendees about the consistency in age-related trait expression between the left and right portions of two popular skeletal regions for age-at-death estimation — the pubic symphysis and the auricular surface of the ilium.

This presentation will impact the forensic science community by providing a more nuanced understanding of age-related changes to the aforementioned joint surfaces and how this possibly affects current age-at-death estimation methods.

Currently, there are two major types of methodology for age assessment from the pubic symphysis and auricular surface — phase- and component-based methods.¹⁻⁴ Lovejoy et al. and Brooks and Suchey presented phase-based methods for age estimation from the auricular surface and pubic symphysis, respectively.^{1,2} These methods are based on the gestalt of surface morphology, where traits are lumped together into phases. When present, both the left and right sides of these surfaces should be assessed. However, phase-based methods do not provide a means for incorporating information from both sides into a cohesive age estimate. This limitation is most obvious when left and right sides show differing morphologies. Component methods, on the other hand, score traits separately and each contributes independently to an age estimate. Buckberry and Chamberlain and Milner and Boldsen present component methods for the auricular surface and the auricular surface and pubic symphysis, respectively.^{3,4} Like phase-based methods, Buckberry and Chamberlain suggest that both left and right sides should be scored, but do not provide a framework for incorporating information from both sides into an age estimate. Only Milner and Boldsen provide a means for incorporating left and right scores into a single age estimate.⁴ The current study assesses asymmetry in trait expression of the left and right sides of the auricular surface and pubic symphysis to identify possible areas of uncertainty in age estimation.

The primary goal of the present study was to assess the agreement in left and right expression of three age-related traits of the pubic symphysis and five of the iliac auricular surface. The traits of the pubic bone are relief, texture, and margin morphology of the symphyseal surface. The traits of the auricular surface are transverse organization, microporosity, macroporosity, apical change, and retroauricular activity. These traits were scored on different locations of each surface (i.e., superior/inferior demiface) using a condensed scoring system based on Milner and Boldsen.⁴ The study sample consists of 97 American White males and females aged between 20 and 66 years from the William M. Bass Donated Skeletal Collection. Left and right agreement was assessed using the Intraclass Correlation Coefficient (ICC). A two-way random model was used, with agreement type 95% tolerance interval. The ICC was used over other agreement statistics because it also accounts for the variability in scores between observations or trait expressions of antimeres.

Overall, the pubic bone shows higher agreement than the auricular surface. Pubic symphysis ICC scores are moderate to strong (symphyseal relief = 0.65, texture = 0.46, and margin = 0.70). Auricular surface ICC scores are poor to moderate (for transverse organization = 0.33, microporosity 0.49, macroporosity = 0.51, and apical change = 0.32).

These results suggest that age-related traits progress differentially between left and right portions. This result is especially true for the auricular surface. Component methods that incorporate left and right trait scores into a single age estimate, such as Transition Analysis, are preferable over phase-based or component methods that do not incorporate left and right scores into a cohesive age estimate.

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2. Brooks S, Suchey JM. 1990 Skeletal age determination based on the os pubis: a comparison of the Acsadi-Nemeskeri and Suchey-Brooks methods. *Human Evolution* 5(3):227-238.
3. Buckberry JL, Chamberlain AT. 2002. Age estimation from the auricular surface of the ilium: a revised method. *American Journal of Physical Anthropology* 119:231-239.
4. Milner GR, Boldsen JL. 2012. Transition Analysis: A Validation Study with Known-Age Modern American Skeletons. *American Journal of Physical Anthropology* 148:98-110.

Age Estimation, Pubic Symphysis, Auricular Surface

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A25 A Validation Study of Sex Estimation From the Scapula and Clavicle in a Modern United States Population

Danna N. Bran, BA, 201 S Bradfield Avenue, Compton, CA 90221*

After attending this presentation, attendees will understand the applicability of a sex estimation technique developed on an archaeological collection in New Zealand in a modern context.

This presentation will impact the forensic science community by providing an applicable sexing technique with high accuracy for use in cases where the os coxae and cranium are not available or are poorly preserved.

In forensic anthropology, sex is one of the first attributes reviewed by forensic anthropologists.¹⁻⁶ Finding complete remains of an individual increases the chances of correct identification; however, frequently only incomplete remains are found. For this reason, new sex estimation methods and the validation of existing methods using various bones in the body are necessary.⁷⁻¹¹

The clavicle and scapula are comparable to the os coxae, sacrum, and coccyx, which form the pelvic girdle, the base that supports the appendicular skeleton of the lower half of the body. Because of a relatively high degree of sexual dimorphism in the human os coxae, more attention has been given to those elements in the development of new accurate methods for sex estimation. The pectoral girdle shows less obvious sexual dimorphism, but promising traits on the clavicle and scapula have been documented, with reportedly high degrees of accuracy.¹²

In this study, a technique developed by A.M.C. Murphy using an archaeological sample from New Zealand was tested on the William M. Bass Donated Skeletal Collection at the University of Tennessee.¹³ Sliding calipers were used to measure the diameters of the sternal and acromial end of the clavicle and height and breadth of the glenoid fossa of the scapula. Data were obtained from 125 females and 121 males during the current study. Only adult individuals with fused epiphyses were used and left elements were given preference to prevent potential inconsistencies in right-handed asymmetry. Furthermore, only individuals of White ancestry were measured due to their larger sample size within the collection. Discriminant function analysis was then applied through the use of the statistical program Statistical Package for the Social Sciences (SPSS) by IBM® to test Murphy's method.

Statistical analysis shows the discriminant function as statistically significant with a Wilk's lambda of 0.394 and significance level of .000, which indicates that the function can effectively discriminate between males and females with an accuracy rate better than chance. The coefficient with the greatest discriminating ability is glenoid breadth, and the least discriminating coefficient is sternal diameter. An 89.9% accuracy rate in estimating sex was reached with a sectioning point of 0.0205.

A second statistical analysis tested the accuracy of this function as it applies to younger age-at-death versus older age-at-death individuals. The researcher chose the differentiating point of 50 years of age. Discriminant function analysis was applied first to those less than 50 years old and then to those older than 50 years of age. The accuracy rate in correctly estimating sex increased for individuals less than 50 years old to 92.9% and for individuals older than 50 years of age to 90.7%.

Although the method applied to this modern United States population sample did not reach Murphy's 97.7% accuracy rate, this study will present an anthropological method that can be applied to current forensic cases involving unidentified individuals where other methods of identification are unattainable that portrays a statistically significant accuracy rate of 89.9% in correctly estimating sex.



Anthropology Section - 2015

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Forensic Anthropology, Sex Estimation, Pectoral Girdle



Anthropology Section - 2015

A26 Classification of a Sample of Chinese Males From Cuba Using FORDISC® 3.1

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The goals of this presentation are to give attendees the opportunity to explore the variation present in the Chinese-Cuban sample and to understand how it compares to known modern and historic reference samples.

This presentation will impact the forensic science community by providing classification accuracies for a traditionally ill-represented, unique ancestral population using modern statistical techniques.

FORDISC® is a widely used tool for the estimation of ancestry based on a number of cranial measurements that differ between population groups. Cuba has a diverse genetic background that includes Native American, African, Spanish, and Chinese influences. Native American groups, decimated and enslaved by the Spanish, eventually dwindled to numbers that led to the importation of slaves from West Africa. After the banning of slavery in the late 1800s, Chinese were brought to the island as indentured servants and later middle-class Chinese businesspersons came to the island in the 1920s. These waves of migrants from three continents helped create the diverse Cuban population that is present today. Remains from a recent Chinese cemetery exhumed and now incorporated into the Aristides Mestre Laboratory of the Montané Anthropology Museum, University of Havana, Cuba, include 49 Chinese males, according to records. It is the goal of this project to investigate how this group classifies using FORDISC®.

Fourteen cranial measurements, as defined by standards, were collected for this study from the 49 Chinese-Cuban males. Individuals were then analyzed in FORDISC® 3.1 using the Forensic Data Bank (FDB) and Howells' groups in accordance with the procedures outlined in Ousley and Jantz.^{1,2} The posterior probabilities and typicality probabilities were then utilized in eliminating groups that did not meet acceptable cutoffs (posterior probabilities >0.1 and F-typicality >0.05). Classification accuracies were evaluated using leave-one-out cross-validation. Further analyses included comparing the Cuban male sample as a whole to forensic and Howells' groups using the Mahalanobis distance.

Using the FDB groups, individuals classified as Chinese 32.7% of the time and as an Asian group 63.3% of the time. A further breakdown of classifications of groups is: Vietnamese Male = 16.3%, Japanese Male = 14.2%, Guatemalan Male = 14.2%, Black Male = 8.2%, American Indian Male = 6.1%, and Hispanic Male = 2.0%. One individual classified as a Japanese female and two individuals could not be classified due to low typicality scores. The correct sex classification rate for the sample was 98%. When analyzed as a group using FORDISC®, the linear discriminant function classified 32.7% (16/49) of the sample into its own group when Basion-Prosthion Length (BPL) was removed due to the large number of the samples missing this measurement. Results demonstrate that the Wienker sample is closest to Asian groups, particularly the Chinese males, with a Mahalanobis distance of 2.5. Other close groups include the Vietnamese males with a Mahalanobis distance of 3.3, and the Japanese males with a Mahalanobis distance of 3.6. The Cuban-Chinese males were most similar to Howells' Chinese groups (Anyang and Hainan), followed by other East Asian groups.

In summary, classification results corroborated the recorded Chinese origins of the sample. Individuals from this sample classified as East Asian 63% of the time. Even when attributed to a single ancestry, the Chinese-Cubans classify as Chinese males 33% of the time, the highest classification rate of the groups compared. These classification percentages are better than random chance and thus indicate that a large component of the morphological variation reflects the group's geographic source. As a group, classifications of the sample using FDB and Howells' data reflect Southeast Asian affinities, particularly indicative of Chinese heritage. The comparative samples come from different areas in China and reflect different ethnic groups. The FORDISC® Chinese sample includes individuals from Hong Kong and the Howells' groups include the Anyang from northern China and the Hainan from southern China.

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Ancestry, Cubans, FORDISC®



Anthropology Section - 2015

A27 Multi-Method Resolution of Small-Scale Commingling

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The goal of this presentation is to highlight the use of a multi-method approach for resolving small-scale cases of commingled remains. After attending this presentation, attendees will understand the benefits of using portable X-ray fluorescence spectrometry in conjunction with traditional methods to resolve complex commingling cases.

This presentation will impact the forensic science community by highlighting the application of elemental analysis to resolve commingling in addition to the array of traditional methods in forensic anthropology.

Methods commonly used to sort commingled remains tend to rely on the presence of joint surface morphology, diagnostic anatomical features, osteometric sorting, or, more recently, the use of DNA analysis. Taphonomic conditions, such as carnivore scavenging, may inhibit the use of these techniques by fragmenting the remains, resulting in loss of diagnostic features and articular joint surfaces. In such instances, elemental analysis holds enormous potential. Previous research has established that portable X-Ray Fluorescence (pXRF) can be a useful tool for sorting small-scale commingling cases.¹⁻² This presentation focuses on a case study involving the successful use of traditional osteological methods, DNA analysis, and pXRF for resolving a case of commingling.

In 2012, an informant told law enforcement that he and three other Mexican nationals were involved in an illegal marijuana growing operation in northern California. The informant further indicated that one individual shot and killed two of the others while they were in the marijuana field. The informant and suspect then buried both decedents in two shallow graves at the location before fleeing to southern California. Law enforcement located the gravesite and, although buried, both individuals were heavily scavenged by large carnivores, most likely black bears. This resulted in significant commingling of the remains. Each set of human remains was assigned a unique barcode and geographic location at the scene and was then submitted to the California State University, Chico Human Identification Laboratory for analysis.

Several traditional sorting methods were employed, including: (1) reconstruction of fragmented remains through physical matching; (2) visual pair-matching of bilateral elements; (3) articulation to evaluate joint congruence; (4) osteometric sorting; (5) evaluation of taphonomic patterns; and, (6) DNA analysis. After applying these methods, a left radius, ulna, scapula, clavicle, a left and right humerus, a mandible, and several right-hand elements still could not be assigned to an individual. In several instances, carnivore scavenging damage or lack of diagnostic joint morphology inhibited the use of these techniques and pXRF was used to segregate the remains.

Following methods recently outlined in Perrone et al., 95% confidence intervals were established for seven chemical elements detected (silicon (Si), phosphorus (P), potassium (K), calcium (Ca), manganese (Mn), iron (Fe), and cobalt (Co)) based on skeletal remains previously assigned to Individual I and Individual II.² Elemental concentration values were consistent with the informant's statement that the remains represented two individuals. Results of the pXRF analyses also indicated that nearly all unassigned skeletal elements belonged to Individual I, whereas Individual II was only represented by two mandibular fragments.

In this case study, the use of pXRF combined with several traditional sorting methods permitted the resolution of commingling. In addition, the correct assignment of skeletal elements to each individual facilitated the assessment of the biological profiles, trauma analysis, and evaluation of taphonomic patterns. This multi-method approach resulted in the accurate sorting of skeletal elements and the subsequent repatriation of the remains to their respective families.

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Forensic Anthropology, Portable X-Ray Fluorescence, Commingling



A28 The Optimized Summed Scored Attributes Method for the Classification of American Blacks and Whites: A Validation Study

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After attending this presentation, attendees will understand the reliability of the Optimized Summed Score Attributes (OSSA) method created by Hefner and Ousley, which is currently employed in biological profile ancestry estimation for unknown individuals in active forensic cases.¹

This presentation will impact the forensic science community by proposing a new sectioning point that maximizes classification accuracy, thus demonstrating the utility of OSSA for modern forensic casework.

Ancestry estimation is essential for assessing the potential identity of unknown persons and is one of the most difficult parameters of the biological profile (age, sex, stature, ancestry) to estimate. Non-metric traits have been pervasive in their use within the field of forensic anthropology and are continually used in active cases and in research.² Recent work examining the “gestalt” approach to ancestry estimation shows that decisions by researchers concerning the population affinity of an individual may be established within seconds upon first viewing the crania.³ Thus, initial impressions, based upon past experiences, bias the researcher either consciously or subconsciously. The aforementioned impressions are generally the product of typological approach-based training and education, which have been ubiquitously taught in the foundation of forensic anthropology and are still included in many introductory forensic anthropology texts. As a means to standardize this typological approach, Hefner consolidated the various typological character lists used in forensic anthropology to 11 traits, either binary or expanded into ordinal character states, to more adequately capture the range of normal human variation observed and to avoid the inherently biased typological approach.⁴

Six of the original Hefner non-metric traits were incorporated by Hefner and Ousley into the OSSA method for classification of ancestry.^{1,4} The scores of each of the traits are then converted into a binary score and summed. Individuals with scores ≤ 3 are classified as Black (B) and ≥ 4 as White (W). The OSSA approach includes statistical measures of classification and a prediction plot for group membership. For the current research, these six traits were collected for 208 American Black ($n=101$) and White ($n=107$) crania from the Hamann-Todd Human (HTH) Osteological Collection, 28 positively identified Black ($n=5$) and White ($n=23$) crania from Mercyhurst University’s Department of Applied Forensic Sciences (DAFS) forensic cases, and 38 positively identified Black ($n=10$) and White ($n=28$) crania from the Office of the Chief Medical Examiner (OCME) in New York City. Ancestry was first determined using the cut-off scores (≤ 3 for Black and ≥ 4 for White) supplied by Hefner and Ousley.¹ The original sectioning point was chosen on the basis of maximizing correct classifications. Next, the cut-off score was heuristically adjusted to maximize classification accuracy for the current sample.

The HTH collection achieved a total correct classification of 68.3% (B=50.5%, W=85.0%) using the suggested OSSA sectioning point. Shifting the sectioning-point heuristically to ≤ 4 for Black, improved total correct classification of the HTH sample to 77.9% (B=80.2%, W=69.2%). The Mercyhurst sample had a total correct classification of 71.4% (B=20.0%; W=82.6%) using the suggested sectioning score and 89.3% (B=60.0%, W=95.7%) using the adjusted sectioning point of 4. The OCME sample showed a total correct classification of 89.5% (B=70%, W=96.4%) using the suggested sectioning score and 94.7% correctly classified (B=90.0%, W=96.4%) using the adjusted sectioning score of 4.

In the present study, classification accuracy improved in all three samples using the adjusted sectioning point of ≤ 4 for Black. American Blacks had higher classification accuracy than Whites in the HTH sample (using the adjusted cut-off), yet had lower correct classifications in the DAFS and OCME samples. Secular change may potentially explain these differences given the time disparity between the HTH sample and the two modern ones. It is suggested when examining modern, forensic cases to increase the cut-off score to 4 for best classification accuracy. Furthermore, practitioners should have adequate experience in scoring the traits as defined by Hefner and Ousley, as well as be sufficiently familiar with the normal range of human variation to confidently score each of the traits.¹



Anthropology Section - 2015

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Biological Profile, Ancestry, OSSA



Anthropology Section - 2015

A29 **Vertebral Maturation in Age Estimation: Further Testing the Revised Method of Scoring the Timing and Progress of Epiphyseal Ring Union**

A. Midori Albert, PhD, University of NC Wilmington, Dept of Anthropology, 601 S College Road, Wilmington, NC 28403-5907; and Kate D. Sherwood, 10401 Litzinger Road, St. Louis, MO 63131*

After attending this presentation, attendees will gain a more in-depth understanding of the pattern, sequence, and timing of vertebral ring union for the cervical, thoracic, and lumbar vertebrae. The revised method of scoring epiphyseal ring union and the relationship with age-at-death was further explored on a known skeletal sample. Findings from this study may be used to corroborate information from other skeletal age indicators to more accurately estimate age-at-death in cases of unknown identity.

This presentation will impact the forensic science community by explaining how vertebral maturation correlates with age-at-death when using the revised method of scoring the progress of epiphyseal union of the superior and inferior vertebral centra (or “ring” epiphyses). Results of this study may potentially aid in the determination of skeletal age-at-death for pre-teenagers up through young adults which, when considered in conjunction with other skeletal age indicators, may improve the accuracy of age estimation in human identification.

The progress of union of the superior and inferior ring epiphyses of the vertebral centra was documented for the pre-sacral vertebral column, where applicable (i.e., C2 inferior, C3-C7, T1-T12, L1-L5) from a sample of 54 females and 38 males, ages 10 to 30 years at death, from the Lisbon Collection, National Museum of Natural History (Bocage Museum), Lisbon, Portugal.

Epiphyseal union was documented using the revised five-stage method: (1) Stage 0: no fusion, bare centrum; (2) Stage 1: partially fused ring, sections fused and unfused, gaps present; (3) Stage 2: complete fusion, clear demarcation between ring and centrum, no gaps but possibly a groove, no remodeling; (4) Stage 3: complete fusion, ring edges are melded with centrum, a slight groove may be seen in some areas but centrum is fully remodeled in other areas; and, (5) Stage 4: complete fusion, ring edges are melded with centrum, bone is remodeled with no grooves. This modified five-stage scoring method focuses on the distinction between differing appearances of complete union, mainly where complete union may present with or without a groove, indicating that remodeling has not or has occurred.

Spearman’s rank correlation coefficients indicated a relatively high positive (and significant, $p < 0.05$) relationship between vertebral ring union mean values and age at death, $r_s = 0.90$. High positive and significant ($p < 0.05$) correlations were also found when each vertebra type was analyzed distinctly (i.e., cervical, thoracic, and lumbar), for sexes combined, and for the female and male samples separately, ranging from $r_s = 0.84-0.92$.

The sample was then divided into five age groups, sexes combined, to gauge the progression of union: (1) Group 1: 10 to 13 years of age; (2) Group 2: 14 to 17 years of age; (3) Group 3: 19 to 21 years of age; (4) Group 4: 22 to 25 years of age; and, (5) Group 5: 26 to 30 years of age. Analysis of Variance (ANOVA) and most paired samples t-test results showed no significant differences in vertebral ring union mean values between age groups, with sexes combined and when sexes were tested separately, which could be an effect of small sample sizes. Noteworthy were the raw data observations that provided insights into the subtle patterns and progression of vertebral ring union. Specifically, union began as early as 11 years of age in both females and males for each vertebra type — this is earlier than previously shown; prior samples did not include many individuals as young as 10 years of age. Union was complete in all vertebrae (all vertebrae Stage 3 or 4) at 18 years of age for females and 21 years of age for males. Stage 3 persisted up through 30 years of age in both sexes. Stage 4, complete union with remodeling, was first evident in females at 18 years of age for all vertebra types and for males at 20, 21, and 17 years of age for cervical, thoracic, and lumbar vertebrae, respectively.

Findings from this study may be used to either support information from other skeletal age indicators or provide a general guideline for estimating age-at-death if only vertebrae are recovered in forensic cases.

Vertebral Maturation, Epiphyseal Union, Age Estimation



A30 Reliability of Craniometric Measurements Using a Variety of Imaging Technologies

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After attending this presentation, attendees will understand that measurements taken from representations of crania are, for the most part, just as reliable as those taken directly on the crania themselves. Attendees will also learn which measurements should be used with caution for each tested data format and how to mitigate these issues when collecting data.

This presentation will impact the forensic science community by supporting the validity of expanding anthropological osteometric databanks to include information derived from indirect sources such as medical scans or laser scans. The ability to reliably collect skeletal information from these indirect sources will greatly enhance the potential for collecting osteometric data on currently underrepresented demographic groups.

With the continuing advancement of biomedical imaging and 3D modeling technologies, anthropologists have begun making use of indirect osteometric data derived from scans of skeletal elements. In response to this trend, a study was undertaken to test the reliability across several different imaging technologies of 26 standard craniometric measurements frequently used in forensic casework.¹ Measurements from five crania of known individuals were collected in duplicate by two anthropologists from Computed Tomography (CT) scans and 3D laser scans of the original crania. The laser scans were also used to create prototype models of the original crania. These prototypes were subsequently laser-scanned and measurements were collected from the prototypes and the laser scans of the prototypes.

Craniometric data were initially evaluated for inter-observer and intra-observer error, with one measurement (Bimaxillary (inferior malar) breadth (ZMB)) showing significant inter-observer error. The nature of this error was identified and a second round of measurement collection was undertaken, this time eliminating any inter-observer error. Measurement sets from each technology were then compared with one another using the previously collected osteometric measurements taken on the crania themselves as the ground truth. Statistical analyses conducted on the craniometric data included repeated measures Analysis of Variance (ANOVA), as well as total technical error of measurement and percent total technical error of measurement.

Results show that the majority of measurements demonstrated no significant differences across data formats. However, a few select measurements were found to be problematic for particular technologies. For example, measurements taken from CT scans in a supero-inferior direction (e.g., Basion-Bregma Height (BBH), Orbital Height (OBH)) were prone to greater deviation from direct measurements of the cranium than other technologies, especially for CT scans taken at greater slice increment and thickness. Also, several measurements defined by Type 1 landmarks, particularly those occurring at complex or unclear suture junctures (e.g., Biasterionic (maximum occipital) Breadth (ASB), ZMB), were found to have high variance across all technologies while measurements based on Type 3 landmarks proved to be highly reproducible. This is contrary to measurements taken directly on crania, where measures defined by Type 1 landmarks are typically the most reliable. This observation is most pragmatically explained by the reduction or complete loss of suture definition in the scan data.

If alternative data sources are to be increasingly utilized for the collection of osteometrics, it is vitally important that efforts are made to ensure scanning parameters are sufficient to capture the level of detail required for accurate measurement collection. If measurements are to be collected from pre-existing scans, then a detailed understanding of how individual measurements may be impacted by the particular data format being utilized is necessary to avoid the introduction of unseen error and the possibility of drawing erroneous conclusions.

Reference:

1. Richard AH, Parks CL, Monson KL. Accuracy of Standard Craniometric Measurements Using Multiple Data Formats. *Forensic Sci. Int.* 2014, <http://dx.doi.org/10.1016/j.forsciint.2014.06.015>.

Craniometrics, Measurement Error, Medical Imaging



A31 Living Body Mass Estimation From Skeletal Elements in Forensic Contexts

Tyler E. Dunn*, 622 W Clark Street, Unit 3, Campaign, IL 61820

After attending this presentation, attendees will have an understanding of the possible place for body mass estimation in the anthropological biological profile.

This presentation will impact the forensic science community by showing the need to develop new inclusions into the biological profile and to statistically validate novel methods.

Body mass estimation in forensic anthropological contexts has the ability to augment the biological profile by providing information about an additional individualizing characteristic. Methods for estimation of body mass from skeletal elements are common in paleoanthropological and bioarchaeological populations; however, these methods have not been thoroughly tested in modern individuals of known body mass. Despite research on this topic in other bioanthropological contexts, methodological and statistical support for the inclusion of body mass in the traditional biological profile is lacking in the forensic literature. The limited populations on which these estimation equations are based may further limit the forensic applicability of these methods.

Across biological anthropology, body mass estimates are usually calculated from femoral head diameter and bi-iliac breadth. In the case of the femur, a mechanical approach is applied and relies on the idea that a bone will respond and remodel to the forces that the bone undergoes; in this case, the weight force of the individual. The estimation of body mass from bi-iliac breadth relies on the morphometric relationship between volume, density, and weight. The body is conceptualized as a cylinder. In order to estimate the volume of a cylinder, the diameter is needed; in this case, the analogous diameter of the human body is the bi-iliac breadth of the pelvis.

This study compares two commonly used methods for body mass estimation in a documented sample drawn from the William M. Bass Donated Skeletal Collection (n=388) consisting of 154 females and 233 males. The mean age-at-death of the sample was 62. All individuals in the sample are of known forensic body mass which ranged from 36.3kg to 190.5kg. Forensic body mass relies on self-reporting, reporting by next-of-kin, or is ascertained from medical and/or governmental paperwork. Although forensic body mass has inherent biases, forensic body mass is a similar measure to the body mass used in medicolegal death investigations by law enforcement.

Results indicate that the methods of body mass estimation from osteometric distances compare well throughout the range of body masses in the sample. Despite this correlation, osteometric dimensions were poor predictors of forensic body mass in individuals whose body masses were considered underweight or obese ($p>0.05$). The middle ranges of body mass had no statistical difference between the skeletal indicators and the forensic body mass ($p<0.05$). Both of the estimates of body mass from osteometric distances limit the variation in the estimates. When compared to the forensic body mass, both of the osteometric methods artificially skew the estimates toward a central mean while the variation in the forensic body mass is greater and therefore the distribution is wider. The standard deviation for the body mass estimations from the femoral head diameter and bi-iliac breadth are 8.18kg and 7.76kg, respectively, while the forensic body mass has a larger standard deviation of 25.44kg.

Although there is some reliability in the estimation of body mass from skeletal metrics, the use of these methods in modern forensic contexts is cautioned as the range of modern body mass variation could be much greater than in the archaeological populations on which the estimation equations are based. Although the theoretical framework for the estimation of body mass from skeletal elements is accepted in some bioanthropological contexts, the wide variation of body mass that is unique to modern populations confounds forensic applications. Better techniques to estimate body mass from the outliers of the body mass spectrum are needed in order allow its inclusion in forensic contexts.

Body Mass, Biological Profile, Validation



A32 Skeletal Indicators of Shark Feeding on Human Remains: A Case Study From the Eastern Coast of Florida

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After attending this presentation, attendees will gain an understanding of skeletal trauma/damage patterns that likely indicate shark predation and/or scavenging through the examination of a case study originating from the C.A. Pound Human Identification Laboratory (CAPHIL) at the University of Florida (UF).

This presentation will impact the forensic science community by contributing to current forensic anthropological research regarding trauma and taphonomic analyses from skeletal elements deposited in a maritime environment — in particular, remains that were subjected to shark predation and/or scavenging. Furthermore, the benefit and utility of collaborating with experts in shark biology and cases of shark attacks will be discussed.

Shark predation and scavenging are becoming increasingly prevalent, especially along the United States' coastlines. Extended postmortem intervals and taphonomic damage from factors such as sand sediment and wave action in the marine depositional environment may obscure potential indicators of trauma and/or postmortem damage from shark feeding activities. A case study from the CAPHIL is presented in which three disparate human skeletal elements (left and right ossa coxae and a right proximal femur) washed ashore within several miles of each other on Florida's eastern coastline. Forensic anthropologists at the CAPHIL putatively reassociated these elements based on pair matching, articulation, and similar biological profile indicators (i.e., age and sex), in addition to the elements' similar taphonomic signatures indicative of deposition in a maritime environment (e.g., sandy adhesions, small barnacles, and erosion characteristic of wave action).

All three elements evidenced a distinctive pattern of trauma or damage, including sharp force defects and torsional loading. Anteroposterior loading on both ossa coxae resulted in bone failure around the acetabula, while a spiral fracture completely transected the right femur, of which only the proximal portion was recovered. During a collaborative analysis process with personnel from the Florida Program for Shark Research at UF's Florida Museum of Natural History (FLMNH), CAPHIL and FLMNH analysts determined that the pattern of damage to the bones likely resulted from shark predation or scavenging. Analysts applied the suite of traits indicative of shark-inflicted trauma described by Allaire et al., and determined that several of these traits were present on the remains — among them a set of parallel, incised bone gouges resulting from the spiraling action of a shark's teeth along the femoral shaft.¹ No teeth were retained in the sharp force defects, precluding a definitive diagnosis of shark species; however, based on the geographical location of the remains' discovery, the size and patterning of the shark-inflicted sharp-force damage, and the high torsional forces required to produce the damage, shark researchers from the FLMNH estimated that this distinctive pattern of trauma/damage was likely created by a bull or a tiger shark. It should be noted that analysts were unable to determine whether this shark activity constituted predation or scavenging, as all trauma/damage to the bone occurred while the bone was in a fresh or otherwise hydrated state.

This case study applies previous research on shark predation/scavenging to a case from the Florida coast with a distinctive taphonomic signature. It highlights the importance of communication and collaboration — not only within a single forensic anthropology laboratory but, perhaps even more importantly, with researchers from outside fields whose expertise may provide valuable insight into analyses of skeletal trauma and taphonomy.

Reference:

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Shark Predation/Scavenging, Skeletal Trauma, Taphonomy



Anthropology Section - 2015

A33 A Protocol for the Collection and Culture of Microbes From the External Surfaces of Human Bone

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After attending this presentation, attendees will understand the techniques used to collect microbial samples from the external surfaces of human bone and how to culture and isolate these microorganisms to identify potential links between microbes and their bioerosion of bone. Histological studies of human bone show microstructural changes that have occurred postmortem. While the exact mechanisms behind these structural changes are unknown, they are likely to be microbially mediated changes.

This presentation will impact the forensic science community by contributing to the increased awareness of the microbially mediated changes that continue to occur in skeletal remains during the extended postmortem interval. By standardizing microbial collections from human bone, a higher potential number of living microbial species can be collected and studied for their role in bone taphonomy and bioerosion.

A common misconception is that the process of decomposition is complete once human remains become skeletonized; however, bone continues to be altered through diagenesis and micro-bioerosion. By having the ability to successfully collect and culture microbes from the external surfaces of human bones, a better understanding of the biological decomposition of bone can be achieved. Additionally, this protocol will allow for the identification of microbes associated with the extended postmortem interval, which may aid in determining time-since-death in skeletonized remains.

This protocol outlines the methods and materials used during the collection of samples from six different sets of Korean War skeletal remains disinterred from the National Memorial Cemetery of the Pacific (NMCP) in Honolulu, HI. These remains have been buried at NMCP since the late 1950s and were being disinterred for identification purposes.

Once the remains were removed from their caskets, one long bone from each set of remains was selected for sampling. The bones were dry-cleaned to remove any debris that may have been present on the bones. Sterile water and cotton swabs were used to swab each long bone and multiple samples were taken from each bone. The swabs were then streaked onto a standard nutrient agar plate and placed in an incubator at 22°C to allow microbial growth. After five to seven days of growth, bacterial isolations were conducted in order to more accurately identify each strain. The isolations were repeated for several more generations until one strain of bacteria was present per plate. Plates were selected for species identification by visually selecting different colonies for each set of remains. Colonies were identified via Matrix Assisted Laser Desorption/Ionization Time-of-Flight (MALDI/TOF). From the sampled remains, 12 different species of bacteria and fungi were identified. Species included *Proteus mirabilis*, *Providencia rettgeri*, and *Bacillus thuringiensis*, which have all been observed in decomposition studies. These results show that with this relatively simple protocol, it is possible to culture and identify microorganisms present on bone, allowing investigation of the changing microbial population involved in human decomposition during the extended postmortem interval.

Histology, Bacteria, Postmortem Microbiology



A34 A Comparative Analysis of Macroscopic, Microscopic, and Chemical Alterations in Modern and Ancient Bones: A Preliminary Study

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After attending this presentation, attendees will understand that the taphonomical evaluation of skeletal remains cannot be limited to the macroscopic aspect but must take into account microscopic and chemical alterations since these may react differently.

This presentation will impact the forensic science community by showing how information related to site of deposition, time-since-death, etc., should be searched not only macroscopically but also microscopically and chemically since different levels of tissue structure may tell different stories.

Bone conservation may be different according to the level at which one examines it: macroscopic, microscopic, chemical, or biochemical. Degradation can be used in relation to Postmortem Interval (PMI), environment of deposition of human remains, or applicability of biological tests. For this reason, it is fundamental to verify how, when, and in which components bone degrades. For example, is a well-preserved bone surface a predictor of the survival of the histological component? Or does a negative luminol test necessarily mean that the collagen in the bone is completely gone? Very few comparative studies exist in the literature. This study, therefore, has a dual goal: (1) investigating the preservation of the organic and inorganic bone components; and, (2) testing the accuracy and precision of the methods already employed.

The right tibia or femur of 40 human skeletons was collected from four known populations of the following historical periods: (1) ten Roman (3rd-5th century A.D.); (2) ten 16th century A.D.; (3) ten 17th century A.D.; and (4) ten contemporary (1990-1992 A.D.). Macroscopic, microscopic, and luminol testing were performed on all.

For the macroscopic analysis, this study evaluated the general appearance of the remains and their state of preservation, in accordance with Behrensmeyer's classification, through the observation of specific parameters and morphological characteristics.

Histological analysis was performed both on undecalcified and decalcified sections. The histological analysis conducted on the thin undecalcified sections was performed by scoring preservation according to the Oxford Histological Index (OHI). In parallel, the decalcified stained sections were scored as either well or badly preserved according to the percentage of collagen (>60%=well preserved).

To evaluate the survival of the heme molecule in bone, this study performed a luminol test, a quick, inexpensive method developed to detect blood traces.¹ As expected, results showed a divide in conservation between contemporary and archaeological bone. However, interesting results were seen when comparing different levels of preservation. The macroscopic evaluation showed that 62.5% of both contemporary and archaeological samples were well preserved (stage 0-1). Undecalcified histological microscopy in general showed a good osteonic conservation in contemporary (80%) bone, with high OHI (4-5), whereas archaeological bone showed a high OHI in only 20% of the samples. Therefore, a large difference was noticed in macroscopic and microscopic degradation, particularly among older bone.

When evaluating survival of the collagen component of bone via Hematoxylin-Eosin (H&E) microscopy of the decalcified bone, a slight amelioration in conservation was noticed, which may indicate that if the structure of the calcified matrix is degraded, the respective connective tissue component may be better preserved.

The luminol test was negative in 70% of the ancient samples and in 20% of the contemporary samples, as could be expected; however, when comparing the luminol vs. histological response among older samples, in 27% of cases where luminol was negative, microscopic preservation was very good, and in 25% of cases where histology was negative, luminol was positive.

In conclusion, results show that macroscopic, microscopic, and chemical preservation may not depend upon each other. This means that, according to the type of environment and to other unknown variables, the evaluation of taphonomical degradation must be performed at different levels.



Anthropology Section - 2015

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Forensic Taphonomy, Microscopy, Chemistry



Anthropology Section - 2015

A35 A Comparative Study of Human Decomposition Research Facilities in the United States: The Role of “Body Farms” in Forensic Applications

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After attending this presentation, attendees will understand the importance of human decomposition research facilities as well as the perceptions associated with them, within the forensic community as a whole.

This presentation will impact the forensic science community by providing, for the first time, an all-encompassing look at how human decomposition facilities are started, what they are used for, how their utilization may have changed since their inception, and what their role is in the future of forensic anthropology. This overview will in turn open up dialogue so the benefits of these facilities may be fully realized by the forensic science community, scholars, and the public as a whole.

The first human decomposition facility, the University of Tennessee’s Anthropological Research Facility, or the “Body Farm,” as it is more commonly known, was established in 1980. Not until 2006 did another of its kind open and in the past six years, the number of such facilities has tripled. Human decomposition facilities, and their amenities, are being used for research purposes more frequently each year, although there is little in the literature that describes the facilities themselves.

Interviews with facility representatives were used to gather data in order to better understand how these facilities are initiated, the difficulties and successes that come with such a facility, and their uses beyond decomposition research. Also, surveys were distributed to forensic professionals (including physical anthropologists) in the American Academy of Forensic Sciences and to university students to understand perceptions on the utilization and usefulness of human decomposition facilities and what place they have in the future of forensic sciences.

Results show that the majority of those involved in the forensic sciences, and especially forensic anthropology, find that human decomposition facilities provide vital research opportunities. Based on both interview and survey responses, more human decomposition facilities should be established in unique climate regions, in order to better understand decomposition rates. Also, individuals affiliated with facilities that are already established intend to continue collaboration with one another, to extend research opportunities to other departments and universities, and to expand their own research goals. Finally, the perceptions of non-forensic professionals (as represented by university students) regarding both human decomposition facilities and the role of forensic anthropology appear to be influenced by the popular media.

In order to realize the full potential of these facilities, their representatives and others who utilize them must continue to provide factual information and publishable material to counter misconceptions that are so readily provided by media culture. The human decomposition facility provides a unique opportunity for research, training purposes, and hands-on experience for all who use them. Their continuation is vital to better understanding taphonomic changes, thereby assisting in a medicolegal context.

Human Decomposition, Body Farms, Forensic Anthropology



A36 A Look Into the Past, Present, and Future of Decomposition Research and the Estimation of the Postmortem Interval

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The goal of this presentation is discuss the nature and trends of decomposition research in relation to the estimation of the Postmortem Interval (PMI) and to make recommendations for future research directions.

This presentation will impact the forensic science community by demonstrating that a great deal of decomposition research is unsystematic, descriptive, and/or idiosyncratic to a particular region. Statistically testable methods based on human subjects using depositional environments likely to be encountered in human remains cases are not particularly common.

The progression and rate of decomposition plays a key role in establishing a PMI for a set of human remains. The goal of this project is to examine previously published human and non-human decomposition research in relation to the estimate of the PMI in order to evaluate the scientific progress of past methods and projects and to make recommendations for future research directions.

In order to evaluate previous decomposition research, this study examined the American Academy of Forensic Sciences (AAFS) *Proceedings* from 2002 to 2014, as well as all issues of the *Journal of Forensic Sciences (JFS)* from 1972 to 2014. All research that examined decomposition was categorized and those projects which addressed issues of decomposition rates, progression, or other topics related to the estimation of PMI involving the usage of tissue or remains were included in the study sample (excluding those that were purely entomological in nature).

A total of 76 AAFS presentations and 77 papers published in the *Journal of Forensic Sciences* were examined for this project as they were determined to contribute to the literature on tissue decomposition and the estimation of the PMI. Overall, 32% and 45% of decomposition research involved human cadavers or tissue from the *JFS* and the AAFS *Proceedings*, respectively. Most studies (over 60%) either described decomposition in unique circumstances (e.g., corpses hanging or encased in concrete) or were descriptive decomposition studies for a region/area. Less than 20% of studies actually presented or tested a statistical method for estimating the PMI for a test of remains (not just describing time to reach a decomposition stage). Concerning non-human decomposition research, over 75% of the projects used pigs as proxies for human cadavers.

The discipline of forensic anthropology is currently in an era of method validation and refinement, much of which has been inspired by the *Daubert* criteria. This professionalization of the field has only been furthered by the introduction of best practice guidelines from the Scientific Working Group for Forensic Anthropology (SWGANTH); however, from this study, very few systematic approaches to decomposition research were found, and almost no statistical methods for estimating the postmortem interval of forensic cases (which should be the goal). Further, while soft tissue has been demonstrated to decompose similarly across a variety of species, no studies have directly correlated porcine specimens as reasonable proxies for human cadavers.¹ Looking toward the future, it is suggested that decomposition research focus on more defined, applicable approaches to the estimation of the PMI. There is a need to standardize variable collection and measurement for consistency between research projects (e.g., using accumulated degree days, consistent measures of total body score). Additionally, little research has been conducted on the decomposition of osseous materials for extended PMIs.²

Finally, this study contends that while non-human models were necessary proxies for foundational decomposition research, it is time to move on to systematic, directly applicable research using human cadavers to inform measurements of uncertainty for human forensic cases (which should be best practice). There are currently five human decomposition facilities and plans for at least two more in the very near future. With increasing access to human decomposition facilities and the questionable correlation of non-human to human decomposition models, the necessity of non-human decomposition research is called into question. Beyond entomological work, careful consideration of equating non-human and human decomposition studies is suggested and researchers without access to human subjects are encouraged to carefully evaluate how differences between human and non-human decomposition could affect results.³



Anthropology Section - 2015

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Decomposition, Postmortem Interval, Time-Since-Death



A37 Inter-Observer Reliability of the Total Body Score System for Quantifying Human Decomposition

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The goals of this presentation are to: (1) demonstrate inter-observer reliability of the Total Body Score (TBS) system for quantifying decomposition; (2) illustrate where discordance between observers occurs; and, (3) make suggestions to improve the system.¹

This presentation will impact the forensic science community by demonstrating that the TBS system has low levels of inter-observer error and is a reliable method of quantifying decomposition.

Megyesi and colleagues' TBS system for quantifying decomposition is often cited and is currently used, along with other methods, to record decomposition at several human decomposition research facilities. However, the consistency of observations between two or more observers has never been tested. The use of multiple observers throughout the decomposition process is common at decomposition research facilities. Testing methods to determine rates and potential sources of error are now required in forensic sciences in this post-*Daubert* and National Research Council (NRC) report climate.^{2,3} This presentation addresses the observed deficiency.

Sixteen participants scored 59 observation packets using the Megyesi et al. system. The participants included both sexes ranging in education (undergraduate to PhD) and experience (0-6 months to 10+ years). All had some experience working at a human decomposition research facility and using the TBS system. The packets used 13 human cadavers in different stages of decomposition (Postmortem Interval (PMI) 2-186 days) from three human decomposition research facilities. The distribution of the PMI matched the outdoor sample of the original study as closely as possible. Each packet included photographs (averaging nine per packet) that minimally showed the overall body and close-up shots of the head/neck, trunk, and limbs. Where available, notes by the original on-site observer were supplied. Observers were provided the scoring tables from Megyesi et al.'s publication and instructed to follow only those descriptions, disregarding any modifications in use by individual facilities, and to return categorical scores for each bodily area (head/neck, trunk, and limbs), as well as overall TBS scores. When decomposition fit into more than one category, observers recorded both categories and averaged the contribution to TBS, as instructed by the original publication. Data analysis used a two-way random model Intraclass Correlation Coefficient (ICC) in Statistical Package for the Social Sciences (SPSS) (v. 17.0). The ICC is similar but deemed superior to a weighted Cohen's kappa.^{4,5}

The TBS method shows "almost perfect" agreement between observers.⁶ The overall single measure for Absolute Correlation Coefficient (ACC) is 0.859 for TBS and the Consistency Correlation Coefficient (CCC) is 0.878 for TBS. Assessment of the individual component categories shows variation in the correlation coefficients for each category, with head/neck being the highest (ACC=0.857; CCC=0.875), followed by the limbs (ACC=0.803; CCC=0.817), then trunk (ACC=0.690; CCC=0.720 — only "substantial agreement"). Education impacted the reliability, with individuals holding Master of Arts/Master of Science (MA/MS) degrees (ACC=0.989; CCC=0.923; n=8) or Doctor of Philosophy (PhD) degrees (ACC=0.896; CCC=0.940; n=2) having higher correlation coefficients than those with a Bachelor of Arts (BA) degree or less (ACC=0.801; CCC=0.841; n=6), although all education levels still fall in nearly perfect agreement. No difference in absolute or consistency correlations were observed when the participant sample was divided by experience, with the separation point being more or less than two years of experience (n=8, 8). The individual component scores followed similar patterns as the TBS score when examined under the filter of both education and experience.

Overall, trunk scores were the least concordant. Comments made by observers suggest this may result from difficulty identifying post-bloat release, greater variety of observed color change than described in the TBS system, and differences in decomposition of the upper (ribs) versus lower (abdomen) trunk. Common errors observed during data collation included simple issues, including use of non-existent categories for a particular body portion, probably the result of using the wrong table to make observations; recording point values for the category value (for example, C7 instead of C2, where 7 is the number of TBS points for category C2); and arithmetic errors in calculating TBS. All are easy to remedy (although the second problem could be difficult to parse for earlier stages of decomposition, where the categorical score and TBS point value are similar).

Thus, the TBS system is reliable, with near-perfect agreement between observers. Minor variation exists between observers based on education levels. The trunk category has the lowest level of agreement between observers and may provide an opportunity for improvement.



Anthropology Section - 2015

This study was conducted with the approval of the Southern Illinois University Human Subjects Review Committee, approval 14151.

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Inter-Observer Error, TBS, Forensic Anthropology



A38 An Innovative Analysis of the Postmortem Interval and Its Role in Juvenile Decomposition

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After attending this presentation, attendees will understand the importance of taphonomic processes in regard to juvenile and infant postmortem interval estimation.

This presentation will impact the forensic science community by presenting a novel statistical technique that can identify key decompositional changes in days that can be used as predictors for time-since-death in juvenile and infant remains.

Eight *Sus scrofa* (three juvenile and five fetal) remains were obtained fresh from the North Carolina State University (NCSU) swine farm in the summer, fall, and winter months of 2013. The initial day of deposition was determined by the traditional calendar for the start of each season. The three juvenile remains that analyzed were all placed on the surface. Each season two fetal pigs were deposited, one wrapped in a cotton blanket and one placed in a plastic garbage bag. The winter season does not contain bagged fetal data due to scavenging. All remains were enclosed in cages to prevent scavenging. Decompositional observations were quantified using Megyesi et al. to obtain a Total Body Score (TBS) and Anderson and VanLaerhoven decompositional stages were used for comparison.^{1,2} Fly activity was recorded as adults present, eggs present, larvae present, many larvae present, and none. Accumulated Degree Days (ADD) were calculated from daily maximum temperature with data obtained from the State Climate Office of North Carolina Lake Wheeler Road Field Lab weather station located one-half mile from the open-air site.

Statistical analyses were performed using a time series analysis that accounts for time between observations that can identify significant changes in quantified observations. The time series analysis utilizes an Autoregressive Integrated Moving Average (ARIMA) that incorporates a longitudinal mixed-effects model. The surface juvenile remains showed a significant seasonal pattern in days for decomposition with the summer juvenile reaching a TBS of 26 in eight days ($p\text{-value}=0.0001$), the fall juvenile reaching a TBS of 28 in 11 days ($p\text{-value}=0.0006$), and the winter juvenile reaching a TBS of 27 in 79 days ($p\text{-value}=0.0090$). These TBS values correspond with more than half the remains being skeletonized. The variables analyzed showed significant associations between TBS and ADD for summer, fall, and winter ($p\text{-values}=0.0023$, 0.0030 , and 0.0022 , respectively). Fly activity was only significant for the summer and fall months ($p\text{-values}=0.0046$, 0.0345 , respectively); however, there were no significant associations between the TBS and Anderson and VanLaerhoven decompositional stages.

The blanket fetal remains showed significant seasonal changes that mirror those seen in the juvenile remains. The summer fetal remains reached a TBS of 27 in seven days ($p\text{-value}=0.0001$), in the fall they reached a TBS of 29 in ten days ($p\text{-value}=0.0004$), and in the winter they reached a TBS of 27 in 79 days ($p\text{-value}=0.0001$). The variables analyzed showed significant associations between TBS and ADD for summer, fall, and winter ($p\text{-values}=0.0023$, 0.0300 , and 0.0024 , respectively). Fly activity and Anderson and VanLaerhoven stages were not found to be significant for any of the blanket fetal remains. The bagged fetal remains for summer and fall showed a similar decomposition patterns not related to seasonal deposition with the summer bagged fetal remains reaching a TBS of 26 in nine days ($p\text{-value}=0.0004$), and in the fall reaching a TBS of 27 in six days ($p\text{-value}=0.0001$). While the Anderson and VanLaerhoven stages did not have a significant association with TBS, fly activity did show a significant association with TBS for the fall bagged fetal remains ($p\text{-value}=0.0231$). This may account for the advanced rate of decomposition relative to the summer period.

The results of this study further support the importance of seasonal and burial deposition on the rate of decomposition. The implications that fly activity are of less significance than temperature should be explored and may relate to access to remains. This study also illustrates that more comprehensive stages of decomposition need to be investigated. Multi-environmental approaches in combination with time series analysis in experimental studies may provide predictive power for estimating the postmortem interval in medicolegal contexts.

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Anthropology Section - 2015

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Postmortem Interval, Juvenile, Time Series Analysis



Anthropology Section - 2015

A39 Human Identification From Burnt Remains

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After attending this presentation, attendees will acquire a deeper understanding of the cremation process as well as of some alterations of human bone and teeth commonly associated with fire exposure.

This presentation will impact the forensic science community by describing the skeletal traits observed after a commercial cremation that can be used to estimate the biological profile as well as to achieve an individual identification.

Cremation of biological materials is a destructive and minimizing process. Burnt human remains identification is challenging at best. The most common methods for identification of burnt remains are odontology and DNA. However, these methods require the availability of antemortem records to be compared to postmortem data. In cases where no antemortem records are available, the first step in the identification process is to determine a biological profile of the victim through the assessment of sex, ancestry, age, and stature. Next, antemortem trauma or other pathology markers, particularly in radiographs, could serve as individualizing characteristics. Finally, antemortem and postmortem data are compared to assess the likelihood of the match.

Due to fracturing, deformation, shrinkage, and color changes, the amount of osteological data that can be extracted and accurately assessed from burnt remains is often limited. A greater understanding of fire-related bone alteration can serve to extend this information and is critical for its interpretation. Commercial cremation is one of the more destructive treatments of human remains. This process involves burning the body until all organic materials are destroyed by heat, followed by pulverization of the burnt remains before returning the ashes to the family. Literature on human cremations published before the previous decade mostly focuses on archaeological remains. More recent publications pay more attention to forensic contexts, focusing on fire related macro- and microstructural changes to bone. Examination of human bodies in commercial cremations can provide an optimal assessment of the skeletal markers that are more resilient to fire destruction and thus more useful for determining the biological profile of burnt victims.

The material for study includes the analysis of 30 bodies submitted to commercial cremations from Memora Funeral Home (Salt, Girona, Spain). Standardized pre-cremation observations of the body were recorded before placing the body into the crematorium. After cremation, and before pulverization, standardized post-cremation observations of the remains were completed. The crematorium conditions (temperature and time of exposure) were essentially the same for all individuals included in the study. Pre-cremation data included sex, ancestry, age-at-death, body constitution, cause of death, dental data, and *postmortem* alteration during autopsy or embalming, as well as cremation parameters such as the presence of clothes or shroud, and body position in the crematorium. Post-cremation data collection included the surviving skeletal and dental elements that would allow assessment of sex, ancestry, age, pathology, and individual identification of the body and dental prosthesis recovered and their significance for identification, and bone and teeth alterations due to fire exposure.

The observed color spectrum went from the predominant white color expected from complete calcination to orange/brown tones observed in the spongy bone of vertebral bodies, the inner layer of cranial diploe, and the ribs. Gray colors could be observed in long bones. The colors observed in teeth were white and gray. Skeletal preservation was higher in male individuals, which could be attributed to the increased robusticity and body size of the male skeleton. Maxilla, mastoid processes, orbital ridges, vertebrae, coxae, and long bone epiphyses proved to be the most resilient areas, while ribs, cranial vault, and long bone diaphyses displayed the highest degree of deformation. Long bones generally showed the presence of curved transverse fractures and longitudinal fractures. Patina fractures were conspicuous on the surface of vertebral bodies. Cranial fractures often coincided with the suture lines, while mandibles often presented condyle fractures. This study showed that it was not possible to estimate stature in any case, due to heat alteration of long bones; however, the burned elements that contributed to sex assessment were sciatic notch, femoral and humeral heads, orbital ridge, mastoids,inion, and nuchal crest. Age assessment was more often possible from long bone epiphyses, teeth, and degenerative traits such as vertebral osteophytes or osteoarthritis in joints. Body and teeth prostheses and consolidated fractures provided individualizing characteristics.

Burnt Human Remains, Commercial Cremation, Skeletal Identification



Anthropology Section - 2015

A40 Does Aluminum Transfer to Bone When Used as a Packaging Medium? A Test Using X-Ray Fluorescence Spectrometry

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After attending this presentation, attendees will be familiar with the use of aluminum foil as a packaging medium for fragile skeletal material and understand whether this form of packaging affects the elemental properties of bone.

This presentation will impact the forensic science community by determining whether the use of foil to package skeletal material results in the transfer of aluminum to bone and by questioning whether foil should continue to be recommended as a packaging medium when subsequent elemental analyses may be performed on bones.

Skeletal evidence recovered in the field is typically placed into a packaging medium to preserve and transport the material to the morgue, laboratory, evidence storage, or other destination. Preferred packing methods involve considerations for preventing loss, cross-contamination, or deleterious change. Materials such as paper products (including paper bags, envelopes, and cardboard boxes) are often recommended for packaging skeletal material to offer protection and also to prevent mold growth, tissue breakdown, or other changes associated with confining the material with moisture.

Skeletal material that is burned or highly weathered is often considerably more fragile and at high risk for fragmentation during packaging and transportation. One method that has been suggested to stabilize and protect fragile skeletal material is to carefully wrap it in aluminum foil. This approach offers protection against further fragmentation and also maintains the relative anatomical positioning of bones and teeth at the time of recovery in the event that fragmentation does occur in transit. However, traces of aluminum are known to leach from foil and other aluminum packaging materials into food products and it is currently unknown whether transfer/leaching of aluminum or other elements to the bone may also occur. Chemical and elemental analyses are becoming more common in forensic anthropological examinations and transfer of aluminum or other elements to bones from packaging materials, if it occurs, could result in skewed elemental test results. This study used X-Ray Fluorescence (XRF) spectrometry to test whether the use of aluminum foil to package bones results in the transfer of aluminum from the foil to the bone.

Twenty-six deer bones were used in this study. Each bone was burned on one end using a hand-held butane torch, leaving the other end unaltered. Prior to placing the bones into aluminum packaging, each end of each bone was sampled twice using a hand-held XRF device to determine the baseline elemental profiles. A standard 120-second collection time was used and the data were saved as total photon counts in spread sheets. One specimen (used as a control) was packaged in brown paper with no foil and maintained at room temperature. The remaining 25 specimens, in sample groups of five, were then packaged in five different ways. Five specimens were packaged in new, flat foil and stored at room temperature. Since crumpling foil disrupts bonds and may increase the likelihood of aluminum transfer, five specimens were placed into foil that had been crumpled prior to use, and then stored at room temperature. Since elevated temperatures may also increase the likelihood of aluminum transfer, five specimens in flat foil and five specimens in crumpled foil were stored in the trunk of a car where temperatures reached up to 110°F. Finally, one group of bones was placed in foil, then exposed to excessive heat using a torch. Specimens were maintained in their packaging and stored for a period of six weeks.

After six weeks, each end of each bone was again tested using XRF. Paired t-tests of each of the five sample groups before and after six weeks in the aluminum packaging were used to determine whether the mean aluminum levels of the bone surface changed as a result of prolonged contact with aluminum foil. None of the differences in mean aluminum levels for any of the sample groups were significant ($p > 0.05$ for all groups). These results indicate that, even when physically compromised aluminum foil is used, and even when foil-packaged specimens are exposed to very high temperatures, significant aluminum transfer to the bone does not occur. Aluminum foil can therefore continue to be recommended and used as a packaging medium for bones without the risk of affecting subsequent elemental analyses.

Forensic Anthropology, Evidence Packaging, Elemental Analysis



Anthropology Section - 2015

A41 Fluorescence in Heat-Altered Bone Under Ultraviolet Light

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After attending this presentation, attendees will be knowledgeable about the use of fluorescence under ultraviolet light in the examination of burned and heat-altered remains. Attendees will also learn what fluorescence can reveal about the bone properties and the parameters of the heat event.

This presentation will impact the forensic scientific community by exploring the use of fluorescence under ultraviolet light as a method for analysis of burned and cremated remains. The description of these types of cases has been largely qualitative; this research attempts to provide a low-cost, easily interpreted method of analysis of this type of anthropological casework.

Alternative light sources and fluorescence are methods that have a long tradition within the forensic sciences. Few studies have addressed fluorescence in burned and cremated human remains. Warren et al. mention it as part of the examination of commercially cremated remains and Harbeck et al. examine how fluorescence changes with differing temperatures of the heat event.^{1,2} In addition to understanding fluorescence changes with heat exposure, it is important to know what is underlying the fluorescence patterns to ensure reliability and applicability of the technique. Fluorescence results from an interaction of the excitation of light and the material under examination. The specific combination of excitation wavelength and fluorescence emission peak is indicative of the material under examination. Thus, fluorescence in burned bone can not only reflect the temperature of heat exposure, it also indicates what changes are taking place to bone properties.

The diaphysis of five pig femora were cleaned and segmented, with each exposed to a differing time and temperature combination. One segment was held at ambient temperature for a control; five segments were each subjected to temperatures between 100°C and 900°C in 200° increments for 60 minutes. To examine the impact of duration, a segment was heated at 500°C or 900°C for one of the following durations: 15, 30, or 45 minutes. Fluorescence data was collected using a spectrofluorimeter, as well as bone chemistry and composition analyses, to document changes to the skeletal tissue. Ultraviolet light between 300nm and 395nm at 5nm increments was used for excitation, with emission collected across the visible spectrum (400nm and 700nm).

Under excitation light similar to most Ultraviolet (UV) light sources (365nm), fluorescence changed based on temperature of exposure but not duration. The control samples and those heated to 100°C showed a bright blue fluorescence (~450nm peak). No fluorescence was seen in samples heated to 300°C and 500°C, with red fluorescence (>640nm) noted in samples heated to temperatures of 700°C and 900°C.

To investigate what excitation and emission combination resulted in fluorescence of the samples, an Emission Excitation Matrix (EEM) was collected for each bone segment. Parallel factor analysis of the EEMs for samples at all temperatures indicated two components best describe the dataset. The first component of the model (excitation=330nm, emission peak=696nm) is a red fluorescence. More research is needed to determine the underlying mechanism for this fluorescence. The second component (excitation=365nm, emission peak=458nm) characterizes blue fluorescence corresponding to the collagen and organic components in samples of low temperature exposure.^{3,4}

This research indicates that fluorescence under ultraviolet light can be used to assess not only the temperature of the heat event, but also indicate the bone properties. In order for fluorescence to be a reliable method, it is necessary to determine what changes in bone properties underlie the fluorescence after heat exposure. Based on this research, it is expected that bone exposed to low temperatures would exhibit blue fluorescence linked to collagen content; with complete or near-complete loss of fluorescence in the middle range temperatures, and a red fluorescence is expected at the higher temperatures.



Anthropology Section - 2015

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Fluorescence, Burned Bone, Ultraviolet Light



Anthropology Section - 2015

A42 **Effects of Hydrated Lime and Quicklime on the Decay of Human Remains Using Pig Cadavers as Human Body Analogues: Field and Laboratory Experiments**

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After attending this presentation, attendees will understand the short- and long-term effects of hydrated lime and quicklime on the decomposition of human remains. An increased number of police inquiries involving human remains buried with lime has demonstrated the need for more research into the effect of different types of lime on cadaver decomposition and its micro-environment.

This presentation will impact the forensic science community by showing results that have implications for the investigation of time-since-death of limed remains and potentially for the interpretation of clandestine burials, mass graves, and management of mass disasters by humanitarian organizations and Disaster Victim Identification (DVI) teams. Knowledge of the effects of lime on decomposition processes is of interest to forensic pathologists, archaeologists, humanitarian organizations, and those concerned with disposal of animal carcasses or human remains in mass disasters.

Contradictions and misconceptions regarding the effect of lime on the decay of human remains have demonstrated the need for more research into the effect of different types of lime on cadaver decomposition.

In this study, a series of field and laboratory microcosm experiments are presented studying the effects of lime. Six pig carcasses (*Sus scrofa*), used as human body analogues, were buried without lime, with hydrated lime (Ca(OH)_2), and with quicklime (CaO) in shallow graves in sandy-loam soil in Belgium and recovered after 6, 17, and 42 months of burial. Analysis of the soil, lime, and carcasses included entomology, pH, moisture content, microbial activity, histology, and lime carbonation. The results of this study demonstrate that despite conflicting evidence in the literature, the extent of decomposition is slowed down by burial with both hydrated lime and quicklime. The more advanced the decay process, the more similar the degree of liquefaction between the limed and unlimed remains. The end result for each mode of burial will ultimately result in skeletonization. A further three pig carcasses (*Sus scrofa*) were observed and monitored for 78 days without lime, with hydrated lime, and with quicklime in a taphonomy laboratory. The results showed that in the early stages of decay, the unlimed and hydrated lime cadavers follow a similar pattern of changes. In contrast, the application of quicklime instigated an initial acceleration of decay. Microbial investigation demonstrated that the presence of lime does not eliminate all aerobic bacteria. The experiment also suggested that lime functions as a sink, buffering the carbon dioxide evolution. In the absence of other studies on lime in graves, this research produces valuable and novel information of interest to the forensic society.

Lime, Taphonomy, Differential Decomposition



A43 Assessing How Repetitive Carrion Placement Affects Vulture Scavenging Behavior

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After attending this presentation, attendees will be aware of how sites with repetitive carrion placement, such as an outdoor forensic facility, affect animal scavenging rates and behavior. Attendees will also become aware of the applicability of temporal scavenging data collected at the Texas State Forensic Anthropology Research Facility (FARF) to forensic contexts that lack the continuous carrion presence found at this FARF.

This presentation will impact the forensic science community by addressing the possibility of learned behavior and accelerated arrival times in avian scavengers at the FARF as a result of repetitive decomposition studies occurring at this site.

In recent years, forensic anthropology research facilities have gained much attention because they offer a means of conducting decomposition research that seeks to benefit law enforcement. These outdoor decomposition facilities provide invaluable skeletal data with associated demographics, but no researchers have investigated the impact these facilities may be having on vertebrate scavenging behavior. This study focuses on vulture scavenging behavior and addresses the possible variability in vulture scavenging rates at the FARF as a consequence of repetitive decomposition studies occurring at this site.

To address the possibility of learned behavior in vultures scavenging at the FARF, a series of 14 decomposition trials were conducted at three site types over a two-year period to test the hypothesis that the type of scavenging site affects the amount of time between carrion exposure and the initiation of a vulture scavenging event. Each trial involved placing a single juvenile pig at each of the three site types, which included the following: (1) Texas State FARF — repetitive carrion placement in a single location; (2) Rotate Sites — repetitive carrion placement at different locations; and, (3) Stationary Site — repetitive carrion placement in a single location. The FARF and Stationary Sites were over 1km apart and the Rotate Sites were distributed across an area of 6,000km² spanning from Austin to San Antonio. The three pigs used in each trial were placed in uncaged locations on a single day. All sites were equipped with a motion-activated infrared wildlife camera and a weather station programmed to record climatic variables using one-minute sampling intervals. The cameras and the weather stations were in operation 24 hours a day throughout the duration of the study.

Temporal data were calculated for minutes between researcher departure from the site until the time of vulture arrival at the carrion. Departure time was chosen based on the assumption that vultures would not arrive while a human was at the site. In addition, Accumulated Degree Minutes (ADM) were calculated for the time between pig placement and vulture arrival to account for the assumption that vulture arrival at carrion is based on their detection of temperature-dependent volatiles being omitted during different stages of decomposition. To account for some of the pigs being placed in the evening after vultures had returned to their roosts, a second ADM value was calculated for temperatures only recorded during the day. Solar radiation values of 0.6 W/m² (i.e., nighttime) were used to distinguish daytime and nighttime temperature values.

One-way Analysis of Variances (ANOVAs) testing for differences between the type of scavenging site and time of vulture arrival were performed on the three temporal values described above and reveal an absence of statistical differences between site type and vulture arrival times. Results also indicate that scavenging rates obtained through the FARF research are applicable to scavenging rates occurring outside of FARF within the 6,000km² geographical range used in this study so long as the carrion size, type, and stage of decomposition are the same between the FARF and the location in question. Furthermore, the similarity between scavenging rates at the FARF and other sites were based on carrion placement occurring two weeks apart, which suggests this may be a best practices temporal benchmark for future scavenging studies. Lastly, this study reveals that although variation exists in vulture scavenging rates, the repetition of carrion using two-week intervals at a particular site does not accelerate or cause differences in vultures' arrival time at recently exposed carrion.

Vulture Scavenging, Carrion, Taphonomy



Anthropology Section - 2015

A44 **Burning Models: Human vs. Pig in Taphonomic Fatal Fire Modeling**

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The goal of this presentation is to understand the fundamental differences between how the human body burns and how a pig burns which are two different processes and have two distinct outcomes. Results from 12 bodies (n=6 pigs and 6 humans) that were burned under similarly replicated conditions with direct and radiant heat produced early-to-advanced heat-related changes to the respective human or pig body. Pig tissues differ from human tissues in their thickness, configuration, and organization.

This presentation will impact the forensic science community by answering the question, “Is it reasonable to model burn patterns on tissues that are not of human origin, such as the pig, in fatal fire modeling?” This research will demonstrate that no, the pig is not an acceptable model to use in fatal fire modeling for a number of reasons that include their comparative soft tissues of skin, muscle, and fat, and the differences of the musculoskeletal systems.

Forensic taphonomic modeling has been peppered with the use of porcine models, since they are said to be, “the most similar to the size, shape, and anatomy of the human torso.” In fact, the pig model has become the standard species used to replicate a wide variety of decompositional studies, along with other similar-sized animals such as deer or sheep. Anatomy of the thoracic cavity and abdomen of pigs may be similar to humans, but when it comes to areas of the head and the limbs; the parallels cease to exist. Likewise, the soft tissues themselves respond differently in the porcine model than what occurs in the human tissues of the skin, the underlying subcutaneous fat, muscles, tendons, and, ultimately, the bones. To fairly test the differences between the two species, a total of six young (100+lb) pigs and six adult unembalmed human bodies were burned together within the same fire environments on three separate occasions to discern similarities and differences of the burn patterns and to answer the question, “Is it a good idea to use pigs/quadrupeds for fire modeling in taphonomic research when drawing human comparisons in fatal fire modeling of forensic casework?”

Pig skin is much thicker than human skin. Human skin is thin and elastic and is the first boundary to heat exposure; it therefore exhibits the earliest heat-related changes of the body. Human skin splits several minutes into the fire, while the thicker pig skin takes longer to split (>ten minutes). The biggest difference between the two is the fact that humans have a healthy layer of subcutaneous fat underneath the skin, whereas young pigs are solid muscle under their skin and lack this important layer of soft tissue. This difference is the one of the major flaws with using pigs to model humans in fatal fire modeling. The human body’s thin elastic skin and abundant underlying subcutaneous fat plays a huge role in the burn patterns that are unique to the human body as well as the burning process as time passes during the fire. Another major difference in this study was that the pig bone was young and had immature developing bone with the epiphyses and young joint structures. When the smaller limbs of pigs flexed, the entire joint surface became exposed along with the epiphyses and diaphyses of the pig knuckles. Flexion of the limbs was a major difference between pigs and humans as short quadrupedal anatomy cannot replicate the lengthier human form, particularly of the upper body. Likewise, the musculoskeletal anatomy of the human and pig head does not warrant comparison due to differential buttressing of the pig’s face and skull that differs from that of the human’s unique craniofacial complex. These are several reasons to discourage the use of pigs as human models when it comes to fatal fire modeling where literal comparisons may be based off of the burn patterns.

Fatal Fire Modeling, Burn Patterns, Pig or Human



Anthropology Section - 2015

A45 A Multidisciplinary Validation Study of Non-Human Animal Models for Forensic Decomposition Research: A Time Series Approach

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After attending this presentation, attendees will better evaluate the validity of pig and rabbit proxies for human decomposition studies, particularly for time-since-death estimation. Attendees will also understand the applicability of time series analysis to decomposition studies.

This presentation will impact the forensic science community by demonstrating whether non-human animal data could be admissible in court when applied to human remains in forensic contexts.

Animal proxies are often utilized in decomposition studies in cases where human remains are unavailable. The most frequent animal subjects found in the literature are pigs, although rabbits, guinea pigs, dogs, and other models have been studied; however, the scientific merit of non-human models for forensic applications, including estimation of the postmortem interval, has never been formally tested.

The goal of this project is to directly compare decomposition data among three species — pigs, humans, and rabbits — in three separate trials that differ by microenvironment and season. Variables which have been documented include insect activity (fly oviposition, the developmental stage of immature insects, fly larval migration, emergence of adult arthropods, and succession), morphological changes of the body, scavenging, and climatic conditions. The results of the first trial are presented here and focus on the comparison of morphological changes over time.

On March 13, 2014, five pigs, five humans, and five rabbits were placed in a previously unused, wooded area of the Anthropological Research Facility at the University of Tennessee, Knoxville. After initial placement, the subjects were photographed twice daily and external signs of decomposition were recorded for 75 days. Total body scores as well as scavenging and insect data were determined for each subject at each observation period.¹ Temperature readings were collected from three data loggers placed approximately 1.5 meters above ground throughout the research area; body core and adjacent soil temperatures were collected as well. Data continued to be collected twice daily until 2,000 Accumulated Degree Hours (ADH) had been reached; data collection then continued once daily, then decreased to every other day until the end of the sampling period on May 27, 2014.

Insect activity and external signs of decomposition during the first trial suggest that the pattern of decay is not identical across species. The rabbits exhibited few external signs of decomposition, then appeared to decompose very rapidly with the onset of larval activity. The pigs and humans were more similar to each other in their rates of decomposition. Both species showed external signs of early decomposition, including color changes and skin slippage. Patterns of insect activity were also more similar between pigs and humans, with multiple distinct maggot masses observed on each subject, as opposed to a single mass observed on each of the rabbits. Humans exhibited greater variability with respect to both visual decomposition changes and insect activity than either pigs or rabbits. In addition, only the human subjects had any evidence of scavenging; this further distinguishes them from the pig and rabbit subjects.

Time series statistics were used to evaluate these observations with an objective, quantitative approach. In the analyses, the average daily temperature data were compared to the average daily total body scores for each species. Dynamic linear regression was chosen to build a model of how total body score changed over time, with the corresponding change in temperature incorporated as an impacting factor. Mean square error was used as a search criterion as this statistic informs whether or not the model is a good fit to the data. Preliminary results of this analysis of each species showed that the temperature data correlated more closely with the total body scores of the humans and pigs than with the rabbits (mean square error 3.68, 3.89, and 6.83, respectively). As with the visual observations, this suggests that the pattern of decomposition differs between the species and, as such, the three groups are not likely to be interchangeable in decomposition research.

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Decomposition, Animal Models, Time Series Analysis

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Anthropology Section - 2015

A46 Identification of Osteological Remains From the Ironclad U.S.S. *Monitor*

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The goal of this presentation is to expose attendees to the possible misidentification of human remains as marine mammal remains.

This presentation will impact the forensic science community by cautioning against misidentification of human remains as turtle remains.

In 2001, National Oceanic and Atmospheric Administration (NOAA) archaeologists and United States Navy salvage divers recovered the main steam engine from the Civil War ironclad U.S.S. *Monitor* which sank off the coast of North Carolina in 1862. The engine was then transferred to The Mariners' Museum (TMM) in Newport News, VA, for documentation, conservation, and exhibition. Additional artifacts like the ship's 120-ton revolving gun turret and personal items were recovered in 2002. The archaeological collection now totals nearly 1,500 artifacts. While surveying this collection, museum conservators discovered an unprovenienced bone. The size and morphology of the bone was quite similar to a human phalanx, having what appeared to be a dual-faceted proximal articulation with tapering diaphysis from the preserved articular end to a widening distal articular end that was broken at the neck of the distal diaphysis. Sixteen sailors perished during the sinking of the U.S.S. *Monitor* and there was some concern that this bone was remains from a lost sailor; however, TMM conservators also discovered the incomplete remains of a sea turtle concreted to the ship's main engine. Portions of the head, carapace, and appendages were documented and removed for conservation. As a result, TMM conservators questioned whether the particular unprovenienced bone was human or non-human in origin.

The possible phalanx, as well as the recovered turtle bones, was brought to the Smithsonian Institution for examination. Human hand and foot elements from the anatomical series were compared to the bone. The morphological structure was more similar to a medial hand phalanx than to a foot phalanx, but there were no clear indications of lateral ridges for the flexor tendon insertion and the cross section of the diaphysis was more oval in shape than the more typical "D" shape of the human proximal and medial phalanges.

For sea turtles, there are six species possibilities along the Atlantic Ocean coast. From examination of the turtle bones from the U.S.S. *Monitor*, the lower jaw and humeri were most diagnostic for the loggerhead turtle (*Caretta caretta*). Using the mandible from the turtle remains for size comparison, digit bones from similar-sized specimens in the Amphibian and Reptile Osteological collections were reviewed and it was established that the bone is extremely similar to the medial phalanx from the front flipper of a loggerhead turtle. Thus, the bone in question is considered to be from a loggerhead turtle and likely the same turtle found in the engine room. DNA analysis would be necessary to confirm association.

Although a situation such as this investigation would be exceedingly rare in "normal" forensic settings, the results of this comparative examination show that there are elements of turtle species that are highly similar in morphology to human elements. In coastal regions where marine turtle species skeletons may be found, it should be noted that these remains may be misinterpreted as human.

Bone Identification, Skeletal Morphology, Human vs. Non-Human



Anthropology Section - 2015

A47 **The Afghan Forensic Science Organization: A Model for Local Capacity Building for Mass Grave Documentation and Preservation in Afghanistan**

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After attending this presentation, attendees will be aware of the goals and objectives of the Afghan Forensic Science Organization (AFSO) and their work in Afghanistan, a country in which more than 30 years of armed conflict has produced countless victims who are listed as missing. Since the United States intervention in October 2001, which liberated the country from Taliban rule, there has not been an effective transitional justice effort tied to the country's developing judicial system.

This presentation will impact the forensic science community by highlighting the importance of long-term dedication to building local capacity in forensic investigations and the role an independent non-governmental organization can play in such settings. The difficulties encountered in this project and the steps taken in an attempt to overcome them will also be presented.

Since 1997, Physicians for Human Rights (PHR) has dedicated itself to creating, supporting, and enhancing a transitional justice strategy in Afghanistan, a society that has suffered decades of continual armed conflict as well as innumerable tragedies inextricably intertwined with conflicts of such duration and intensity. PHR conducted its first forensic work in Afghanistan in 1997 under the auspices of the United Nations Office of the High Commissioner for Human Rights, documenting a mass grave found on the outskirts of Sheberghan in the northern part of the country. Since then, PHR has forensically documented numerous mass grave sites in Afghanistan.

In October 2009, with the support of the United States Department of State's Bureau of Democracy, Human Rights, and Labor, PHR initiated a three-and-a-half-year project, "Securing Afghanistan's Past." The AFSO, a new independent organization, was founded as an outcome of the training carried out under this grant. This organization was formed in 2011 by members of the Afghan National Police (ANP), the Afghan National Police Academy (ANPA), the Legal Medicine Department (LMD), the Afghan Independent Human Rights Commission (AIHRC), and various civil society organizations as a result of a 2010 six-week training course by PHR covering evidence and crime scene documentation and forensic anthropology.

Throughout Afghanistan's decades of conflict, Afghan scientists and scholars have been isolated from modern education and the academic world. International donors and the government of Afghanistan should, therefore, identify and prioritize funding for the increased development of Afghanistan's higher education system, particularly for those who must necessarily play a role in its forensic future, such as judges, prosecutors, attorneys, scientists, and medical professionals.

The AFSO provides a non-governmental, independent organization which can assist in the development of a national forensic capacity. Despite the immense scale of the disappeared-persons issue in Afghanistan, there has been little to no official acknowledgment of disappearances that have occurred throughout the conflict, nor any real effort to find the missing and disappeared or to hold alleged perpetrators accountable. The right of families to know the truth surrounding the fate of a missing family member is an essential part of any developing judicial system. AFSO and its work represent the first small steps on the path to reconciliation and transitional justice.

Mass Graves, Afghanistan, Physicians for Human Rights



A48 Skeletal Markers of Gender Reassignment

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After attending this presentation, attendees will recognize potential skeletal indicators of male-to-female gender transition and will be aware of some traits that should be regarded with caution when transgenderism is suspected.

This presentation will impact the forensic science community by providing a case study for sex assessment in male-to-female transgender individuals.

In 1988, a decomposing human body was found in a wooded area of central Florida. The remains were found in association with several articles of female clothing, including a denim skirt, blouse, and pantyhose. Two silicone breast implants were also found in close association with the remains. A limited autopsy was performed in which it was noted that the pelvis was of “female type.” The body was decomposed to the extent that external genitalia were not present and the absence of a uterus was attributed to a probable hysterectomy. The case was referred to an anthropologist, who noted the presence of preauricular sulci and dorsal pitting which was interpreted as evidence of childbirth. The anthropologist’s report listed the decedent as female. During a review of cold cases for submission of DNA samples, the case was re-evaluated. The skeletal morphology strongly suggested that the decedent was a male. The female clothing, breast implants, cosmetic rhinoplasty, and pits of parturition were interpreted as evidence for gender reassignment surgery and normal sequelae associated with estrogen supplementation and/or androgen suppression treatment. A new anthropology report listing the sex of the decedent as male was submitted with bone samples to the University of North Texas Center for Human Identification, who were able to sequence mitochondrial DNA for the decedent. The DNA sample confirmed that the decedent was born a male.

The medical literature addresses possible skeletal effects related to the medical and surgical treatment of gender dysphoria, primarily related to bone density; however, little is known about the expression of those effects in dry bone. This case presents preliminary information about skeletal changes and contextual evidence that may alert an anthropologist that the decedent is male-to-female transgendered, aiding greatly in the investigation of the death. This case also demonstrates how new scientific findings can change interpretations over time. In this case, after the initial anthropological analysis, research was published related to the production of the hormone relaxin in males; this research was helpful in conducting the second examination of the remains. This case exemplifies the need for the field to evolve in accordance with changing demographic trends, as well as with new research that may not be entirely confined to biological anthropology.

Transgender, Parturition Pits, Sex Assessment



Anthropology Section - 2015

A49 Ritualistic Use of Human Skeletal Remains: Is It Forensically Significant?

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After attending this presentation, attendees will have an increased understanding regarding the recognition of human skeletal remains and associated artifacts used within the rituals of Palo Mayombe, as well as of the determination of the forensic significance of these skeletal remains. This presentation will focus on at least six Palo Mayombe case studies in which human remains were recovered within the central Florida area in order to discuss criteria for recognizing these types of ritual remains.

This presentation will impact the forensic science community by discussing the criteria used to recognize Palo Mayombe scenes with human skeletal remains. In addition, an overview of the common methods of procurement of human skeletal remains used by the practitioners for these rituals will be discussed.

Palo Mayombe, an Afro-Caribbean syncretic religion originating from the Bantu regions of the African Congo, is of particular interest to the forensic community as one of the main components of the religion is the use of human skeletal remains in ritual. Practitioners use human skeletal remains in order to communicate with the deceased and to harness the power of the dead to influence undertakings in the lives of the practitioners.^{1,2} The power of the deceased may be used for either benevolent or malevolent purposes, depending upon the will of the practitioner.^{1,3} Human skeletal remains are most commonly found contained within and in the immediate vicinity of the *nganga*, an iron cauldron containing specific objects chosen according to the spirit with whom they are associated, such as animal carcasses, graveyard soil, herbs, mercury, assorted metal objects, colored beads, feathers, blood, sticks (*palos*), and stones (*otanes*).^{1,3} The most common human skeletal elements found within the *nganga* include the skull, tibia, femur, ribs, and phalanges.³ Of particular significance is the presence of a tibia wrapped in black cloth, which is used as a scepter, or an animal horn, both of which are used by the priest (*palero*) to summon the spirits.¹ This aspect of ritual is especially important, as the forensic anthropologist will often be involved in determining forensic significance of the human skeletal remains discovered within this context.

The sacred space in which the *nganga* resides is often key in the identification of Palo ritual. Examples of these spaces will be detailed in the case studies presented. The space will contain elements of nature such as greenery, animal heads and skins, wooden branches, or stones, and will also be painted in the religious language of Palo, the *firma*, which are religious symbols, drawn within the space to signify intent to make contact with the spiritual realm.⁴ Oftentimes, there will also be pieces of metal, figurines, crosses, and other artifacts symbolic of the nature spirit (*Mpungo*) with whom the *palero* associates.^{1,3}

This presentation will focus on at least six Palo Mayombe cases that were discovered in central Florida. Crania, skulls, and post-cranial skeletal material were discovered within *ngangas*, yielding information useful to the forensic community in properly identifying archaeological and anatomical teaching specimens when discovered in this unique context. The cases from central Florida comprise different settings in which human skeletal remains were discovered and include a shed in the practitioner's backyard, a warehouse, and the disposal of a *nganga*, presumably upon the death of a *palero*, on the bank of a river. The most common sources of procurement for skeletal material used for Palo Mayombe include grave robbing, botanicas, and the legal purchase of anatomical specimens from anatomical supply companies through the internet. Associated artifacts and the presence of religious symbols will also aid in recognition of religious ritual. In two of the cases, forensic significance of the human skeletal remains was confirmed with documentation of the purchases that was provided by the practitioners (receipts and tags).

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Forensic, Palo Mayombe, Ritual



A50 Regression Analysis for Estimation of Stature From Foot Lengths in a North Indian Population

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After attending this presentation, attendees will understand the usefulness and methodology of stature estimation, especially from various length measurements of the foot, which will be helpful in conducting further research in this area and in studying forensic cases usually encountered in airplane crashes, intentional mutilation and dismemberment, explosions, or other mass disasters.

This presentation will impact the forensic science community by presenting standards for stature estimation from various length measurements of the feet when feet or parts thereof are presented for forensic examination.

Establishing identity is one of the primary goals of any medicolegal investigation, especially when human remains are brought in for examination. These remains may be in the form of dismembered and mutilated body parts or skeletal remains. The identification process involves the estimation of a biological profile of the decedent which includes estimation of age, sex, stature, and race or ethnicity. A biological profile derived in this manner is a circumstantial identification that narrows the comparative pool of potentially matching profiles and thus helps in medicolegal investigations. Estimation of stature is an essential parameter of identification that helps in establishing personal identity of the deceased. This complements other details on the age, ethnicity, and sex in the identification of skeletal remains and body parts. In this context, the present study was conducted in order to make regression models for estimation of stature from five specific foot lengths in a north Indian population. This study is based on a random sample of 700 adults (500 males, 200 females) ranging in age from 18 to 30 years old. In addition to stature, five separate foot length measurements were taken from each subject (length of the foot from each toe; i.e., T1, T2, T3, T4, and T5, respectively) according to standard procedures and landmarks. The data were statistically analyzed using Statistical Package for Social Sciences (SPSS), version 11.0, computer software. Sex differences in stature and foot measurements were analyzed using Student's t-test. Karl Pearson's correlation coefficients were calculated between stature and various length measurements of the foot. The stature was estimated from various length measurements of the foot using both linear and multiple regression analyses.

Mean stature of the sample was 170.3cm and 157.9cm in males and females, respectively. Length measurement at the first toe (T1) was found to be the longest in both the sexes on the right and left sides. Statistically significant sex differences were observed in the length measurements on the foot between males and females in right and left feet ($p < 0.001$). Statistically significant correlation coefficients ($p \leq 0.001$) were observed for correlation between stature and various foot length measurements in males, females, and the pooled sample. Thus, the stature was found to be positively and strongly correlated to various foot length measurements in both the sexes. In males, the correlation value (r) ranged between 0.628 and 0.641, while the range in females was between 0.539 and 0.657; however, the correlation coefficient in the pooled sample ranged between 0.746 and 0.776. Thus, the pooled sample showed relatively higher values of correlation coefficients than in males and females separately. The linear and multiple regression models were derived for estimation of stature from foot length measurements in males, females, and the pooled group. While estimating stature from linear regression models of all the right and left foot length measurements, the Standard Error of Estimate (SEE) in the case of females (3.5cm) was lower than that of males (5.3cm), indicating that female feet give a better estimate of stature than male feet. The pooled sample showed a similar SEE as that observed in males. Multiple regression models showed a marginally better, but similar, trend of accuracy as that shown in the males, females, and the pooled group in the linear regression analysis.

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Personal Identification, Stature Estimation, Foot Length Measurements



Anthropology Section - 2015

A51 Sexual Dimorphism of the Zygomatic Bone in a Southeast Asian Sample

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The goal of this presentation is to inform attendees of the potential use of the zygomatic bone in assessing sex during the construction of a biological profile.

This presentation will impact the forensic science community by providing the foundation for developing a discriminant function equation using three linear measurements from the zygomatic bone to estimate the sex of fragmentary human remains.

The attribution of sex is an important component of the biological profile, but is often complicated by the lack of the most diagnostic elements typically used for sex estimation in fragmentary human remains. Prior research has suggested the zygomatic bone, a bone that is often recovered in the archaeological context, is sexually dimorphic. The Workshop of European Anthropologists noted an increased robusticity of the marginal process in European males, where Woo noted a maximum height and width difference between males and females in an English anatomical collection, and Wilson noted a size and shape difference between modern American White males and females from the William M. Bass Collection.¹⁻³ Specifically, Wilson demonstrated, through the use of geometric morphometrics, that the shape of the zygomatic bone can discriminate between American males and females at an 86% classification rate; however, digitizing a single element is not always practical or available to forensic practitioners, necessitating methods that use traditional caliper-based measurements.³ Further, prior research only evaluated samples from European populations. Thus, this research utilized a Southeast Asian sample to determine the discriminatory power of three linear measurements of the zygomatic bone for sex estimation.

The maximum height and width of the zygomatic bone, defined previously, and the maximum width of the frontal process, defined for this study, were collected from the left and right zygomatic bones of 202 modern Southeast Asian individuals (102 females aged 26-84 years and 100 males aged 22 to 96 years) housed at Khon Kaen University (KKU), Khon Kaen, Thailand, using sliding calipers.² Measurements were obtained from individuals for whom complete, unfractured zygomatic bones were available. Independent t-tests determined that no significant difference ($p\text{-value} > 0.05$) existed between the left and right sides for all three measurements; therefore, all subsequent analyses used pooled samples. All analyses, including a linear discriminant function analysis, were conducted using a statistical computing software.

The mean maximum height of the zygomatic bone is 48.2mm (95% SD=3.4mm) in males and 45.4mm (95% SD=3.3mm) in females, the mean maximum width of the zygomatic bone is 54.7mm (95% SD=3.7mm) in males and 50.8mm (95% SD=3.8mm) in females, and the mean maximum width of the frontal process of the zygomatic bone is 14.7mm (95% SD=2.2mm) in males and 12.6mm (95% SD=1.8mm) in females. A linear discriminant function analysis demonstrated that all three measurements are important in discriminating between Thai males and females, with the maximum width of the frontal process contributing more than the other two measurements. Further, the combination of these three measurements correctly classified 75% of the sample (74.5% of males and 75% of females).

When compared to previous research, the maximum height and width of the Thai zygomatic bone is larger than the modern American zygomatic bone for both males and females. This is not unexpected, as several researchers have noted population differences in the zygomatic bone, with individuals of Asian ancestry having larger malar regions. Future validation testing will need to test the universality of any discriminant function equations from the linear measurements due to this size difference. Additionally, the size differences between males and females in the Thai sample are less than those in the American sample, which supports the belief that Southeast Asians are less sexually dimorphic than Americans. Nevertheless, differences in the linear measurements from both samples are significant and can discriminate between males and females, which lends credence to the utility of the zygomatic bone as a criterion for assessing sex in fragmentary remains.

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1. Workshop of European Anthropologists. Recommendations for age and sex diagnoses of skeletons. *J Hum Evol* 1980;9:517-549.
2. Woo TL. A biometric study of the human malar bone. *Biometrika* 1937;29:113-125.
3. Wilson RJ. Sexing the zygomatic bone. *Proceedings of the American Academy of Forensic Sciences; 57th Annual Scientific Meeting*. New Orleans LA 2005.

Forensic Anthropology, Sex Estimation, Zygomatic Bone

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Anthropology Section - 2015

A52 Age Estimation in Modern Individuals Between Birth and Fourteen Years of Age Using Measurements of the Knee Joint

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After attending this presentation, attendees will understand the growth and development of the knee joint and how it can aid in age estimation, as well as becoming familiar with the radiographic data bank, PATRICIA, and how it can be used in further growth and development research.

This presentation will impact the forensic science community by providing an alternative to other age-estimation methods. Additionally, this presentation will illustrate the importance of large-scale databases and their contribution in estimating the normal growth curve and in detecting secular changes.

Age estimation of the juvenile skeleton has primarily been dominated by dentition, as it is less likely to be subjected to environmental factors; however, in many circumstances, the dentition is not recovered, forcing anthropologists to use other areas of the skeleton for age estimates.¹ The ossification patterns and fusion timing of the knee joint can provide a wealth of information in regard to age, especially for individuals between birth and two years of age. Despite the abundance of information available from this region of the skeleton, especially through radiographic analysis, very little research has been published in regard to metric assessment. The purpose of this study is to provide a method of juvenile age estimation using metric analyses of radiographic images.

Nine measurements were taken on a sample of more than 1,000 radiographic images of modern children between the ages of birth and 14 years. Six ratios have a statistically significant relationship with age and were analyzed using linear regression. For example, tibial metaphyseal breadth and tibial epiphyseal breadth show two distinct linear trends, forming a clear “elbow” prior to data transformation, suggesting a well-defined lower bound just after birth that can be used to estimate post-birth age versus fetal age; however, most of the data required transformation to better model the relationship between chronological age and measurement ratios from the knee. Each of the six ratios were squared and cubed to be analyzed using linear regression. Moreover, all statistically significant linear regression models exhibit adjusted R² values that were 0.90 or greater, suggesting a strong relationship between chronological age and measurement ratios from the knee. All statistically significant linear regression models were also evaluated using 95% prediction intervals; however, due to the nature of the growth curves and the forensic questions being asked, the derived lower bounds appear to be more useful.

The data also suggest that the growth and shape changes of the proximal fibula are significantly less important than those changes seen in the distal femur and proximal tibia; however, appearance of the proximal fibular head epiphysis proved to be useful and was seen in individuals as young as 1.87 years of age.

In metrically evaluating knee development, outliers were detected which included individuals with Osteochondrosis (OCD) and Cornelia de Lange Syndrome, both of which delay skeletal development. These individuals were noted as lying significantly below the normal growth curve, suggesting that this type of data may also be useful in detecting child abuse in living individuals.

Other statistical methods, including Multivariate Adaptive Regression Splines (MARS) and Partial Least Squares provide estimates that better adjust for the multicollinear nature of the measurements. These techniques will more accurately model the post-birth growth spurts, while simultaneously accounting for the deceleration in growth following puberty.

This study illustrates the importance of not only large-scale, modern, reference data banks, but also of metric observations and modern statistical methods for estimating age of the juvenile skeleton.

Reference:

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Growth and Development, Radiographic Data Bank, Knee Joint



A53 Femoral Midshaft Shape: An Indicator of Adult Age-at-Death?

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After attending this presentation, attendees will understand that the cross-sectional shape of the adult human femoral midshaft is highly variable and that discovery of a uniform shape transformation pattern among this variability may allow for production of age-at-death ranges from unidentified skeletal remains.

This presentation will impact the forensic science community by eliminating a method proposed to utilize femoral midshaft cross-sectional shape to produce age-at-death estimates from unidentified adult human skeletal remains.

Literature review indicates anterior-posterior elongated femoral cross-sections are associated with great workload and mobility. This finding has been utilized previously to reconstruct behavioral differences, such as sexual division of labor and subsistence strategy within and between past populations; however, recent transversal biomechanical data reveals the femoral midshaft changes in size and shape throughout adulthood: once the human femur is fully developed, biomechanically adapted, and periosteally adjusted by adjacent musculature a net loss of cortical area begins as the amount of bone deposited on the periosteal surface lessens in comparison to the amount of bone removed from the endosteal surface. Additionally, analysis of shape ratios and the angle formed between the I_{\max} biomechanical axis and the medial-lateral anatomical axis indicates young adults often have anterior-posterior elongated cortices but mature adults increasingly display circular to medial-lateral elongated cortices.

Because members of modern industrialized society are not expected to display evidence of a sexual division of labor or differential subsistence strategies, this study hypothesized that femoral cross-sectional shape variability could partially reflect adult age-at-death, as reduced mobility is often associated with increasing age. This hypothesis was tested by extracting geometric morphometric data from 200 adult femoral midshaft cross-sections originally harvested by M.F. Ericksen from The George Washington University dissecting-room cadavers. The sample was composed of 97 males and 103 females largely of European descent, ranging in age from 30 to 97 years (mean=71 years, standard deviation=12 years). A quantitative evaluation software was utilized to extract the periosteal outlines from oriented bitmap images of the femoral cross-sections, quantify the contour shapes with elliptic Fourier descriptors, and perform principal component analysis to summarize the shape information.

Although the results illustrated observable differences among the sample of femoral cross-sections, shape variance was not found to be significantly correlated with age. Specifically, none of the seven first principal components, accounting for 95% of the variance, correlated with age after a Bonferroni adjustment. The first principal component (representing an anterior-posterior flattening of the section and accounting for 63% of the total variance) was found to be significantly correlated only with the biomechanical properties, such as I_x/I_y and I_{\max}/I_{\min} , that are themselves different indicators of shape. Only PC3 and PC4 (accounting for a total of 7% of the variance) were statistically linked with sex (males display a rounder shape). Overall, these findings reinforce how femoral midshaft shape primarily reflects mechanical environment regardless of age and suggest there is too much variation in mobility among this sample of modern humans for femoral shape to be a useful indicator of age-at-death.

Age-at-Death, Geometric Morphometrics, Elliptical Fourier Analysis



A54 **Body Height Estimation From Femur Measurements in Postmortem Computer Tomography**

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After attending this presentation, attendees will: (1) understand how body height from computed tomography data can be estimated; and, (2) gain knowledge about the accuracy of estimated body height and limitations.

The presentation will impact the forensic science community by providing knowledge and competence which will enable attendees to develop formulas for single bones to reconstruct body height using postmortem Computer Tomography (p-CT) data.

The estimation of Body Height (BH) is an important component of the identification of corpses and skeletal remains. Stature can be estimated with relative accuracy via the measurement of long bones, such as the femora. Compared to time-consuming maceration procedures, p-CT allows fast and simple measurements of bones. This study undertook four objectives concerning the accuracy of BH estimation via p-CT: (1) accuracy between measurements on native bone and p-CT imaged bone (F1 according to Martin 1914); (2) intra-observer p-CT measurement precision; (3) accuracy between formula-based estimation of the BH and conventional body length measurement during autopsy; and, (4) accuracy of different estimation formulas available.¹

In the first step, the accuracy of measurements in the CT compared to those obtained using an osteometric board was evaluated on the basis of eight defleshed femora. Then the femora of 83 female and 144 male corpses of a Swiss population for which p-CTs had been performed, were measured at the Institute of Forensic Medicine in Bern. After two months, 20 individuals were measured again in order to assess the intraobserver error.

The mean age of the men was 53 ± 17 years and that of the women was 61 ± 20 years. Additionally, the body length of the corpses was measured conventionally. The mean body length was 176.6 ± 7.2 cm for men and 163.6 ± 7.8 cm for women.

The images that were obtained using a six-slice CT were reconstructed with a slice thickness of 1.25 mm. Analysis and measurements of CT images were performed on a multipurpose workstation. As a forensic standard procedure, stature was estimated by means of the regression equations by Penning & Riepert developed on a Southern German population and for comparison, also those referenced by Trotter & Gleser "American White."^{2,3} All statistical tests were performed with a statistical software.

No significant differences were found between the CT and osteometric board measurements. The double p-CT measurement of 20 individuals resulted in an absolute intra-observer difference of 0.4 ± 0.3 mm.

For both sexes, the correlation between the body length and the estimated BH using the F1 measurements was highly significant. The correlation coefficient was slightly higher for women. The differences in accuracy of the different formulas were small. While the errors of BH estimation were generally ± 4.5 – 5.0 cm, the consideration of age led to an increase in accuracy of a few millimetres to about 1 cm. BH estimations according to Penning & Riepert and Trotter & Gleser were slightly more accurate when age-at-death was taken into account.^{2,3} That way, stature estimations in the group of individuals older than 60 years were improved by about 2.4 cm and 3.1 cm.^{2,3} The error of estimation is therefore about a third of the common ± 4.7 cm error range.

Femur measurements in p-CT allow very accurate BH estimations. Estimations according to Penning led to good results that (barely) come closer to the true value than the frequently used formulas by Trotter & Gleser "American White."^{2,3} Therefore, the formulas by Penning & Riepert are also validated for this substantial recent Swiss population.



Anthropology Section - 2015

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 3. Trotter M. & Gleser GC. (1952): Estimation of stature from long bones of American Whites and Negroes. *American Journal of Physical Anthropology* 10, 463-514 und 355-356.
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Body Height, Postmortem Computer Tomography, Femur Measurement



A55 Comparative Performances of Sacral Osteometrical and Geometric Morphometrical Studies Based on Multi-Slice Computed Tomography Explorations to Assess Sexual Dimorphism

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After attending this presentation, attendees will become familiar with different statistical methods that can be used to estimate sacral sexual dimorphism from multi-slice computed tomography data. This presentation will present classic and original multivariate analyses based on metric data and a geometric morphometric analysis.

This presentation will impact the forensic science community by showing classification and segmentation trees as an alternative statistical tool to classic multivariate analysis for sex determination in anthropological research, with results similar to geometric morphometric analyses.

Sex estimation from skeletal remains for identification is an important component of many anthropological investigations. Sacrum, due to its contribution to the pelvic girdle, demonstrates sex differences. This sacral sexual dimorphism is classically studied by using multivariate analysis on metric data. Geometric morphometric analysis is another pertinent approach widespread in anthropological research to study sexual dimorphism.

The present study seeks to compare two statistical methods using classic published metric parameters and geometric morphometric analysis for sacral sex estimation based on multi-slice computed tomography.

A total of 13 landmarks were located on sacral multi-slice computed tomographies of 174 individuals (86 males and 88 females). Fourteen variables (seven distances and seven indices) were selected from the literature for their effectiveness in sex determination.

First, a univariate analysis using t-tests was performed on all. Then multivariate statistics, including Linear Discriminant Analysis (LDA) and classification trees, were realized from variables that showed a significant sexual difference in the univariate analysis. A geometric morphometric analysis was realized from the 13 landmarks and a Canonical Variate Analysis (CVA) was performed from procrustes landmarks coordinates.

The accuracy rate for sex assessment using LDA was 81.3% from five distances and 79.4% from five indices. Sex predict yielded 85% with classification and segmentation tree using four metric parameters (three distances and one indice). The CVA results based on the 13 procrustes landmarks coordinates showed a correct classification for 87.9% of the individuals.

These results were in accordance with previous studies about sacral sex assessment. Classification and segmentation trees showed better results than linear discriminant analysis and similar results to geometric morphometric analysis, but used only four parameters. Classification and segmentation tree seems to be a pertinent and easily alternative statistical tool to classical multivariate analysis for sex determination in anthropological research.

This study revealed that geometric morphometric analysis did not demonstrate a more objective superiority in sacral sexual determination than anthropometric analyses. Moreover, this study provided a particular application of virtual anthropology by transposing classical metric parameters studied on dry bones to multi-slice computed tomography.

Sexual Dimorphism, Classification Trees, Geometric Morphometrics



A56 Sex Estimation Using Discriminate Function Analysis of Non-Metric Cranial Traits: An Inter-Observer Error Study

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The goal of this presentation is to explore inter-observer error rates of sexually dimorphic non-metric cranial traits using the Walker five-trait scoring system.

This presentation will impact the forensic science community by providing further treatment to inter-observer error rates for non-metric cranial traits. While Walker assessed inter- and intra-observer error rates for sexually dimorphic cranial traits, the sample size was small ($n=10$) and the confounding variables of observer interaction and observer experience were not partitioned out from this assessment.¹ The current study attempts to evaluate inter-observer error derived from the scoring method by controlling for experience level.

Skeletal sex estimation is traditionally based on the subjective morphological assessment of traits of the pelvis and skull. To standardize morphological assessment, Walker developed a five-trait scoring system to evaluate the expression of non-metric cranial sex traits. The traits include the supraorbital margin, the mastoid process, the supraorbital ridge/glebella, the nuchal crest, and mental eminence. Each trait is scored on a scale of 1 to 5, which shows the progression of the traits from gracility (1) to robust (5).

Minimal treatment has been given to inter-observer error rates for visually scored traits of the skull. As such, the objectives of this study are to: (1) evaluate inter-observer error derived solely from the original scoring method as proposed by Walker; and, (2) assess how inter-observer error affects sex estimation.

The current work evaluates a large sample of individuals of known age and sex ($n=78$; $F=37$, $M=41$) using the Walker five-trait scoring system. Data were obtained from the William M. Bass Donated Skeletal Collection housed at the University of Tennessee, Knoxville. Ages range from 24 to 88 years old. Each of the traits was scored in accordance with Walker.¹ The left side was evaluated in the event of paired traits. Trait scores were compared among three anthropologists with similar levels of experience to negate error attributed to varied levels of experience. As such, any variation in scores among observers can be ascribed to inherent limitations in the scoring method as opposed to differences in experience level.

Statistical software was used to perform Fleiss' Kappa, the Intra-Class Coefficient (ICC) and Discriminant Function (DF) analyses.² The ICC was applied to account for close observations between observers and was used with a two-way model that evaluated both consistency and absolute agreement. Trait score differences among observers were assessed and subsequently compared to those reported by Walker.¹

Results indicate that, for most traits, there is consistent scoring agreement among observers. The majority of the traits showed substantial absolute agreement (>0.7) with the exception of the mental eminence, which showed a moderate agreement of 0.54. Further, the observations were substantial to almost perfectly consistent between anthropologists.

Agreement between observers was the same for each DF at approximately 72%. In cases of disagreement, sex bias was idiosyncratic to the observer. A score of 1 for female and -1 for male was assigned to each disagreeing observer's sex estimation. Observer Three had a sex bias score of -10, or a strong male bias. Observer Two had a sex bias score of 13, or a strong female bias. Observer One had a sex bias score of 2, or no appreciable sex bias. Though the DF identified a sex bias in two of the three observers, correct classifications of sex were not impacted by the observer bias as each scorer showed correct classifications higher than 77%.

The current study provides further treatment to inter-observer error rates for non-metric cranial traits. Though results indicate that there is some subjectivity associated with the scoring system, trait scores can be reliably assigned by observers with comparable levels of experience. Thus, when possible, sex estimation from the skull should be confirmed through blind peer review.

In contrast to inter-observer error results reported by Walker, the majority of traits scored revealed substantial absolute agreement among observers.¹ As such, by controlling for observer experience level, the inter-observer error inherent in the scoring system is quite low.



Anthropology Section - 2015

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1. Walker PL. Sexing skulls using discriminant function analysis of visually assessed traits. *Am J Phys Anthropol.* 2008;136(1):39-50.
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Inter-Observer Agreement, Cranial Non-Metrics, Sex Estimation



A57 Comparing Cranial Morphology in Asian and Hispanic Populations: Exploring Why Misclassification Can Occur When Using Discriminant Function Analysis

Beatrix Dudzik, MA, 3909 Briargate Avenue, Knoxville, TN 37919*

After attending this presentation, attendees will have baseline knowledge of the similar and distinct cranial morphologies that are represented among various modern Asian and Hispanic samples.

This presentation will impact the forensic science community by providing quantification of size and shape variables of the human cranium among the aforementioned populations in an effort to elucidate why misclassification of Asian and Hispanic individuals can occur when performing discriminant function analyses between these groups.

Arguably one of the most difficult and daunting tasks that forensic anthropologists face during the construction of the biological profile, is the estimation of ancestry. The interaction between heritability and the influence of environmental factors which result in the common microevolutionary forces outlined in the literature is not a cause-and-effect relationship and thus will continuously provide research fodder for a range of disciplines that seek to examine how human populations adapt and change over time. Practitioners that work within the unique context of North America, which has high percentages of populations from all over the world and is often referred to as a genetic and biological melting pot, must be especially cognizant of these factors when estimating ancestry of unidentified individuals. Specifically, it has been reported by forensic anthropologists who operate in the American Southwest that using reference samples provided by FORDISC® 3.0 software can misclassify Hispanic individuals as representative of modern Japanese and other Asian populations.¹

To examine the morphological overlap that can occur when attempting to estimate ancestry of Hispanic and Asian skeletons, this study employed discriminant function and canonical variate analysis to examine morphological differences and similarities among the populations sampled. Fifteen standard cranial measurements were used that represent all developmental and functional modules of the human cranium. The Asian samples used included male and female individuals from Korea (n=52), China (n=60), Vietnam (n=45), Thailand (n=109), and Japan (n=240). The University of Tennessee Forensic Databank provided the Korean and Vietnamese samples, while the Japanese, Thai, and Chinese samples were measured for this study. The Japanese samples are representative of northern, middle, and southern Japan and were collected from Sapporo University, Tohoku University, University of Tokyo, Kyoto University, Kyushu University, and University of the Ryukyus, respectively. The Thai sample was collected at Khon Khaen University and the Chinese sample was collected at Hong Kong University. Additionally, the Hispanic data (n=450) was provided by the Forensic Databank.

Results indicate that, in general, good separation between the Hispanic and Asian samples described can be achieved with discriminant function and canonical variate analysis. Specifically, while it has been found that misclassifications of Hispanic individuals occur at a higher rate among the Japanese than any other Asian population sampled, it was shown in this study that significant differences can be identified within the cranial vault. In particular, Hispanic samples were shown to be much wider within the posterior portion of the cranium, while the anterior portion of the vault were shown to be much narrower than the Japanese samples; however, considerable overlap was also found among the Japanese and Hispanic samples in many of the other cranial dimensions represented by the measurements used, indicating that misclassification is indeed easy to achieve. In regard to the other samples used, it was found that, in general, the Asian samples clustered together and were distinct from the Hispanic samples, but separation was also identified between the East Asian and Southeast Asian individuals. Morphological distinction was also identified that separates the Japanese samples from the other Asian samples in some dimensions of the cranium.

The patterns observed in this study show that morphological similarities and distinctions can be identified when comparing Hispanic samples to East and Southeast Asian populations. While overlap is observed among the Asian and Hispanic samples, there are also specific regions of the cranium, namely anterior and posterior portions of the vault, where differences are identified, particularly among the Hispanic and Japanese samples. Thus, this indicates that partitioning the cranium and using specific dimensions may increase accurate ancestry estimation. This information may provide anthropologists with a better understanding of cranial variation in Hispanic and Asian populations that may ultimately result in more accurate estimates of the biological profile and in a higher percentage of positive identifications.



Anthropology Section - 2015

Reference:

1. Jantz RL, Ousley SD. *FORDISC® 3.0: personal computer forensic discriminant functions*. Knoxville (TN): The University of Tennessee, 2005
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Cranial Morphology, FORDISC®, Forensic Anthropology



A58 Quantification of Frontal Sinus Morphology From Radiographs for Positive Identification

Priyanka Atit, BA, C.A. Pound Human Identification Laboratory, Cancer & Genetics Research Complex, 1376 Mowry Road, Rm G-17, Gainesville, FL 32610; Carlos J. Zambrano, MS, Cancer/Gen Resarch Complex, 2033 Mowry Road, PO Box 103615, Gainesville, FL 32610; and James D. Pampush, MS, University of Florida, Dept of Anthropology, Turlington Hall, Rm 1112, Gainesville, FL 32611*

After attending this presentation, attendees will possess a general understanding of the key challenges facing forensic anthropologists in determining identity using frontal sinus radiographs. This study highlights an alternative and easily accessible method of metric analysis of the frontal sinus.

This presentation will impact the forensic science community by investigating a quantitative method for establishing positive identification of an unknown individual using frontal sinus form.

Human frontal sinuses are lobate cavities contained within the frontal bone; their morphology is evident on radiographs and they remain consistent over the adult lifespan. Prior research has attempted to use the frontal sinus for forensic identification, for which methodology is expected to adhere to the *Daubert* guidelines. The *Daubert* guidelines govern the admissibility of scientific evidence in the federal court system and require standardized methodology to have acknowledged limitations and a replicable technique. Earlier studies have successfully used the frontal sinus to identify individuals; however, the subjectivity of the methods does not meet the *Daubert* standards. The research presented here is an attempt to develop frontal sinus identification methodologies that meet the *Daubert* guidelines. Moreover, this study follows recent efforts to quantitatively assess the frontal sinus and the goal is to develop a simple analytical method that can be used on commonly accessible software (e.g., ImageJ[®], R, and MS[®] Excel[®]).

For this study, 40 cranial radiographs with frontal sinuses meeting the selection criteria were randomly chosen from the C.A. Pound Human Identification Laboratory case archives. Using ImageJ[®], the scale was independently calibrated for each radiograph and a lower boundary line was drawn to demarcate the sinus. Area, perimeter, width, height, and circularity measurements were obtained by tracing the superior margin of the sinus. Each of the cranial radiographs were then traced four times — twice each by two separate observers to test for both inter-observer and intra-observer error. To test the applicability of this method for antemortem vs. postmortem identification, a four-crania demonstration sample was generated by radiographing each crania twice; once with a bag of substrate under the cranium to produce noise in the “antemortem” radiograph, and then without the substrate to represent a postmortem image. Single blind tracings by were used to examine if an antemortem image could be correctly matched to its postmortem counterpart with the described measurements.

Intra- and inter-observer error were tested using paired *t*-tests and Pearson’s correlations. The paired *t*-tests suggest that frontal sinus measurements are consistent and repeatable by observers who have practiced the tracing method. Pearson’s correlations (r^2 -values > 0.860) indicate good congruency of measures between observers. Measurements from the original 40 tracings were Z-score scaled, and Euclidean distances between three sets of measures were calculated: (1) distances between individuals measured twice by the same observer; (2) distances between individuals measured twice by two different observers; and, (3) distances between random pairings of individuals. *T*-tests on Euclidean distances demonstrate matched individuals to be significantly closer than randomly paired individuals. Furthermore, gamma distributions calculated from the Euclidean distance sets allowed for the generation of log-likelihood ratio values. Matched individuals’ Euclidean distances consistently showed positive log-likelihood ratio values compared to randomly paired individuals, demonstrating quantitative reliability. Using Euclidean distance measures and the log-likelihood ratios, three of the four crania in the demonstration sample were positively identified. Measurement error associated with the height measure for one of the “antemortem” images precluded positive identification for this individual. Euclidean distances from all four random pairings of the demonstration sample indicated no-matches.



Anthropology Section - 2015

Although these preliminary results indicate that quantifying frontal sinus morphology can be accomplished with readily available software, there are some limitations to this method. For tracing simplicity, only individuals with connected sinuses were used. As such, features that could be used for individualization (e.g., separate sinus cavities, partial septa, etc.) are not captured in the presented method. Future studies should focus on expanding the sample size and enhancing the method by quantifying and incorporating more individualizing features. By excluding such diagnostic features, the individualizing power of frontal sinus morphology for identification purposes is constrained. Ultimately, while somewhat limited, the research presented here demonstrates a quantitative technique for using frontal sinus morphology in forensic identifications following the *Daubert* guidelines.

Forensic Anthropology, *Daubert* Guidelines, Log-Likelihood Ratio Values



Anthropology Section - 2015

A59 An Outline Analysis of Ancestry and Sex Differences in Cranial Shape

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The goal of this presentation is to introduce a new approach to the estimation of sex and ancestry from skeletal remains as a part of the formation of a biological profile. Attendees will understand the utility of cranial outlines in the estimation of ancestry and sex from the skull. Further, attendees who are accustomed to visually analyzing non-metric traits will learn that geometric morphometric outline analysis can also capture the overall variation in trait expression and help identify cranial traits that are responsible for the most variation between ancestry and sex groups.

This presentation will impact the forensic science community by introducing a new method for studying human cranial variation that is capable of increasing the objectivity of traditional non-metric techniques used to estimate the biological profile from skeletal remains.

Forensic anthropological techniques that utilize non-metric skeletal traits to estimate sex and ancestry have historically been criticized for their subjectivity and replicability; however, non-metric traits have proven to be valuable tools in identifying remains in forensic investigations. In this study, geometric morphometric analyses of cranial outlines (lateral, posterior, and superior views) were performed to assess population and sex variation in a sample of modern humans. 3D scans of 198 crania were collected from the Hamann-Todd and Terry skeletal collections. 2D images of the left lateral, superior, and posterior outlines were subsequently captured from these scans. These three views were chosen for analysis because they are most likely to capture the variation of cranial traits traditionally used to estimate sex and ancestry, such as glabella projection, frontal bossing, dolichocephaly, and facial prognathism. Elliptical Fourier analysis was utilized to define the cranial outlines and principle component analysis was performed on the elliptical Fourier descriptors to quantify shape variables into uncorrelated numerical values. Two-way Multivariate Analysis of Variance (MANOVA) analyses were performed on the principle components to test for sex and ancestry differences in outline shape. Two-way Wilk's lambda discriminant function analyses were also performed to determine the utility of these cranial outlines in discriminating between sex and population groups.

Results indicate that cranial outlines are able to differentiate between American Blacks and Whites, as well as American males and females, with high accuracy. Discriminant function analysis performed on the lateral view between Blacks and Whites performed extraordinarily well, resulting in a 92.4% cross-validated correct classification. The first principle component (39.8%) appears to reflect changes in vault shape (brachiocephalic vs. dolichocephalic) and degree of maxillary prognathism. These results support the traditional hypothesis that Black individuals tend to have a more elongated cranial vault and more prognathic face, while White individuals tend to have more rounded crania with less prognathism. The lateral view was also the best at differentiating between males and females. Principle component four, which only accounted for 5.53% of the variation within the sample, actually performed the best when differentiating between the sexes. The shape changes occurring in this principle component include glabella projection, with males possessing larger, more pronounced glabellae than females. Overall, results indicate that significant sex and population differences in cranial shape do exist and follow traditional qualitative descriptions. Outline analysis may provide a more objective means of estimating sex and ancestry from these traits, thereby increasing estimation accuracy (as high as 93% between ancestry groups).

Forensic Anthropology, Morphometric Outline Analysis, Non-Metric Traits



A60 Stature Estimation Using the Mandible in a Caucasian Italian Population

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After attending this presentation, attendees will have additional analytical tools for assessing unknown human remains. A new method for estimating stature using the mandible will be introduced, particularly useful where few diagnostic skeletal elements are present.

This presentation will impact the forensic science community by providing a new method to contribute to the biological profile using a skeletal element not typically used for this purpose. When unknown human skeletal remains are recovered, anthropologists estimate the biological profile (age, sex, ancestry, stature, etc.) using all available analytical tools. In this way, it is possible to reduce the list of potential missing persons matches and to help achieve a positive identification.

In forensic anthropology, stature estimation is an important parameter used in personal identification where the most common methods involve analysis of the full skeleton or long bones.^{1,2} If long bones are absent, the only remains available may be ones not normally used to estimate stature. The mandible is potentially valuable in connection with stature estimation, but has been little studied, despite its successful use in estimating sex and, in some cases, age. Since stature is population-specific, a mandible-based method should derive from study of a population biologically similar to the remains being analyzed.³ A stature method based on the mandible in a Caucasian Italian population is still not present in the current literature.

The current study investigated the relationship between the mandible and the stature of individuals from a Caucasian Italian population in order to develop a formula for the estimation of stature from this bone. The sample included 103 living Caucasian individuals from Italy (62 males and 41 females) with a mean age of 41.4 years. The variables of interest include four mandibular parameters (total condylar width (CoCo), total gonial width (GoGo), Condylar-Gonion (CoGo), and Gonion-Gnation (GoGn)) as well as measured stature (cm), age of the individual, and ancestral information (to confirm ancestry).

Using the data collected, Mean Stature (Hcm) was modeled using linear regression models. In the literature, previous models establish the relationship of Hcm with sex and age (e.g., it is known that stature decreases with age).⁴ Therefore, these two variables (sex and age) were included in the linear regression models in addition to the measurements of the mandibular parameters using two different approaches:

Model A - Including a parameter called "Outline": $2*(CoGo+GoGn)$.

Model B - Including all the singular parameters (CoCo, GoGo, CoGo, and GoGn) and reducing their number on the way by means of a backward selection. The final model included only parameters with a P value less than 0.10.

In both models (A and B), sex and age were strongly associated ($p < 0.01$) with stature, confirming Hcm is significantly higher in male and decreases with age. In Model A, this study found that Hcm was positively associated with the "Outline" ($p < 0.001$) resulting in a total model R^2 (coefficient of determination) of 0.657. Model B, after the backward selection, included as mandibular parameters CoGo ($p = 0.091$) and GoGn ($p < 0.001$), resulting in an R^2 of 0.665 for the final model.

In conclusion, based on R^2 value, the mandible appears to be potentially useful for stature estimation. In the current literature, similar R^2 value has been considered reliable when long bones are not available.⁵

The next stage of this study should be to validate these models using new mandibular and long bone measurements for Caucasian Italians. This method could be applied to improve personal identification of incomplete human remains in order to evaluate a range of stature for filtering the list of missing persons in a Caucasian Italian population.



Anthropology Section - 2015

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Stature, Mandible, Identification



Anthropology Section - 2015

A61 Testing Inter-Observer Reliability of the Transition Analysis Aging Method on the William M. Bass Forensic Skeletal Collection

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After attending this presentation, attendees will gain an understanding of the Transition Analysis (TA) component-scoring and age-estimation method and its applicability in a modern, forensic skeletal collection.

This presentation will impact the forensic science community by providing method-validation information for the repeatability and teachability of the TA method by assessing the degree of variation in age estimates produced from differently assigned scores.

Age estimation is an integral part of the biological profile generated by forensic anthropologists presented with a set of unidentified skeletal remains. For nearly a century, scholars have investigated age-related changes to the adult skeleton with varying degrees of success. It is generally understood that accurate and precise age-at-death estimates derive from the evaluation of multiple anatomical elements and skeletal traits (or components) within an anatomical element rather than isolated, single-age indicators. Multiple-trait approaches better capture the sequential aging process that occurs at different rates among individuals and, therefore, provide a more reliable age estimate. The TA age-estimation method of Boldsen and colleagues is one such component scoring system.¹ This method utilizes skeletal observations of the pubic symphysis, auricular surface, and cranial sutures to make inferences about the timing of transitions of specific osteological traits from one stage to the next. The transitional ages derived from skeletal traits are then inverted using Bayes theorem in combination with prior knowledge on the age-at-death distribution of the target in order to calculate maximum likelihood estimates and 95% confidence intervals. Boldsen et al. urged others to validate the TA method and, in the last decade, several studies have responded to this call.²⁻⁵ The current study seeks to contribute to this discourse and is the first inter-observer error study using TA with more than two observers. Specifically, inter-observer error tests among practitioners representing varying levels of TA scoring experience have been carried out in an effort to assess its applicability to achieve accurate age-at-death estimates.

The sample data consist of 58 positively identified cases from the William M. Bass Forensic Skeletal Collection curated at the University of Tennessee, Knoxville. The Bass Forensic Collection is a skeletal collection of 20th- and 21st-century Americans. Five researchers independently applied the TA method as described by Boldsen et al. to the available elements with no prior knowledge of chronological age. These scores were input into the software and maximum likelihood estimates and 95% confidence intervals attained using the United States homicide prior distribution. To evaluate inter-rater reliability and agreement of the raw scores, Krippendorff's alpha values were calculated for each trait using R. Unlike other specialized coefficients such as Cohen's or Fleiss's Kappa, Krippendorff's alpha is a generalization of a number of reliability indices and allows for more than two observers, any measurement level, small sample sizes, and incomplete or missing data.⁶ Alpha values close to one denote increased reliability while values nearing zero signify less reliable measures.

Krippendorff's alpha values indicate that the majority of traits had a moderate to excellent agreement among observers with a 0.6 or better level of agreement. For a single trait, superior surface morphology had the least amount of congruence (0.355) and the ventral symphyseal margin had the most congruence among scores (0.903). A repeated measure Analysis of Variance (ANOVA) demonstrates that, despite differences among the five raters with regard to knowledge of the TA method, there are no statistical differences between the maximum likelihood ages estimated by each observer and the true ages. These results indicate that the TA method can be reliably used by researchers of varying experience levels to estimate the age of an unknown individual.



Anthropology Section - 2015

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Forensic Anthropology, Biological Profile, Age-at-Death



Anthropology Section - 2015

A62 Obliteration of Sharp Force Trauma Artifact by High Particulate Water Wash

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After attending this presentation, attendees will understand: (1) the taphonomic processes occurring in marine and aquatic environments; (2) the abrasive effect that suspended particulate has on bone and sharp force trauma wounds existing on bone; and, (3) the applicability of this information in the forensic science field.

This presentation will impact the forensic science community by providing information about specific morphological changes that occur when injured bones are left submerged in water for extended periods of time as well as estimation of time-since-deposition, trauma site identification, and associating remains with a particular event.

Forensic examination of bone is not always a straightforward enterprise. A complex interplay of factors contribute to the information able to be gleaned from a set of skeletonized remains that is largely influenced by the environment in which the remains were found. Bone weathering has been well studied and documented regarding skeletonized remains in terrestrial environments; however, less work has been published on the process of abrasion to bones that are submerged in particulate-laden dynamic water.

Skeletal material under water is subjected to a different set of stresses than in a terrestrial setting and research to fully document these postmortem changes is therefore required. Increasing instances of maritime disasters, such as the sinking of the Italian cruise ship *Concordia*, the loss of migrant boats in the Mediterranean and Timor Sea, as well as the recent loss of a ferry in South Korea, warrant further investigation of the processes of degradation of human remains found in marine environments as a result of both water flow and abrasion by suspended particulate matter. Studying the physical changes that occur over time to bones that may have been injured in a traumatic event such as a shipwreck can provide useful information to investigators, as information regarding time-of-death is often crucial to an investigation. This study seeks to determine if and how suspended particulate abrades identifiable characteristics of bone.

Pig (*Sus scrofa*) ribs were inflicted with sharp force trauma wounds using a meat cleaver and were then subjected to an environment simulating an underwater decompositional site using a high-particulate water wash in two different apparatuses. One model created bi-directional wave action via a rotational tumbler system in which the water and particulate were passed back and forth over the sample bones. The other model imitated uni-directional wave action using a circular track to wash a continuous wave over the sample bones. Uniform-grain sand and diatomaceous earth — comprised of the microscopic remnants of dead diatoms — were used separately as suspended particulate to mimic the sediment that is present in a marine environment. Samples were allowed to abrade for set intervals of time and examined using micrometer caliper and stereomicroscopy. These measurements were then inputted into a database, where statistical software was used to run Analysis of Variance (ANOVA) tests in order to determine significant differences. Regression analysis was conducted to determine the relationships between the rates of ablation and associated skeletal measurements.

Marine Taphonomy, Skeletal Abrasion, Forensic Anthropology



A63 Estimating the Postmortem Interval: A Validation Study of the Total Body Scoring Method Using Medicolegal Cases From Southeast Texas

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After attending this presentation, attendees will understand the limitations of applying the Total Body Scoring (TBS) method to time-since-death estimates in cases of temperature-regulated indoor decomposition in humid environments.

This presentation will impact the forensic science community in terms of performance by contributing to the existing anthropological literature on the utility and contribution of the TBS to time-since-death estimations.

Time-since-death estimation often falls under the purview of the forensic anthropologist, requiring an extensive analysis of the intrinsic and extrinsic factors that affect decomposition. Methods employed in the estimation of time-since-death must be rigorously tested to ensure they are accurate, reliable, and contribute meaningful information to the death investigation. A common practice used by forensic anthropologists is to estimate the Postmortem Interval (PMI) based on the gross presentation of the decedent. Following this practice, Megyesi and colleagues attempted to correlate various stages of decomposition with Accumulated Degree Days (ADD).¹ They developed a standardized scoring system for the progression of decomposition, termed TBS, and found a strong positive correlation between estimated ADD and TBS. A regression formula that allowed the methods to be applied to medicolegal cases was developed for this study.

The TBS is considered to be one of the most reliable methods for quantitatively estimating time-since-death; however, several recent validation studies in various climate zones have found that the TBS method failed to produce reliable estimates. These studies cite humidity as a factor that may explain the variation seen in individual rates of decomposition. As a subtropical region, southeastern Texas experiences high levels of humidity year round, making this particular variable an important component of the decomposition process. For this reason, independent validation of the method for the specific environment is needed. Based on previous studies, the initial expectation for this study was that the TBS would yield time-since-death estimates incongruent with known PMIs.

For the present study, the TBS was applied retrospectively to medicolegal cases in which the decedent was found in a temperature-regulated indoor environment and had a documented last-seen-alive date. The study included 95 deaths investigated by the Harris County Institute of Forensic Sciences in Houston, TX, from 2013 to 2014. All cases had known PMIs of less than one year and temperature data recorded from indoor thermostat devices. Additionally, ambient temperature was recorded during the scene investigation and, when it deviated from the thermostat temperature, the average of the two temperatures was recorded. A TBS was assigned to each case using scene and autopsy photographs. In each case, the TBS was input into the regression formula developed by Megyesi and colleagues to calculate the total number of ADD and the associated standard error. Known and estimated ADD and associated PMIs were compared quantitatively. Accuracy was measured by dividing the number of cases with known PMIs within the estimated PMI ranges by the total number of cases in the sample. Additionally, eight individuals independently scored six cases to measure potential inter-observer error.

Overall, this study did not support the TBS in cases of temperature-regulated indoor decomposition in southeast Texas. The TBS accurately estimated the PMIs of 89 (94%) decedents, but yielded results with low specificity. There was no significant difference between mean known and estimated ADD ($U=4111.00$, $p=0.289$); however, there was a significant but weak positive relationship between assigned TBS and known ADD ($r=0.4576$, $p<0.001$). Additionally, a fixed-factors analysis of variance indicated that inter-observer error was low ($F=0.397$, $df=7$, $p=0.903$). These results suggest that, in the context of this study, the TBS fails to estimate the PMI with enough resolution to provide meaningful information to medicolegal death investigators; however, it does provide a quantitative means for comparing stages of decomposition between decedents. Further investigation into the utility of the TBS in estimating time-since-death in indoor environments is recommended.

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Postmortem Interval, Total Body Scoring, Human Decomposition



A64 Bioreactors as a Method for Examining Environmental Effects of Changes in Bone Biochemistry Over Time

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After attending this presentation, attendees will be familiar with novel techniques to design, develop, and maintain an artificial environment using interdisciplinary environmental databanks to systematically determine fundamental environmental parameters, specifically temperature and soil properties, as a method to determine these parameters' impact on bone biochemistry over time.

This presentation will impact the forensic science community by illustrating the potential of a reproducible artificial environment, a forensic bioreactor, which can be controlled over time to evaluate environmental parameters that impact chemical methods for determining the Postmortem Interval (PMI).

The universal application of novel methods for determining the postmortem interval, such as citrate concentration, hinge on a well-characterized understanding of the environmental parameters that impact bone biochemistry over time. The objective of this research is to design a forensic bioreactor that can quantitatively account for environmental parameters known to impact decomposition across a global environmental spectrum.

To accomplish this goal, a synergistic collaboration between several core disciplines was pursued. Bioengineering developed a controllable, artificial environmental system, forensic anthropology defined factors impacting bone decomposition, and soil science contributed knowledge and extensive data to determine environmental parameters. The specific objectives were: (1) to identify the environmental parameters that define a burial setting in an outdoor context that are also useful for forensic anthropology; (2) to discretize environmental parameters of an outdoor forensic burial setting using soil science databanks; and, (3) to design a functional forensic bioreactor that can be easily monitored and accurately mimic an outdoor burial setting.

In this study, the following environmental parameters were characterized: soil temperature, texture, soil horizon, soil depth, soil pH, and organic matter content. Soil temperature, soil organic matter content, and soil pH parameters were characterized using the online databank Web Soil Survey (WSS) and regional temperature and soil regime mappings. Four temperature regimes, two soil organic matter contents, and three soil pH values were selected. Soil analysis was conducted to quantitatively measure soil pH, texture, and the exact soil organic matter content. Soil depths were characterized using soil horizon data to simulate surface scatter and shallow grave burial, respectively. Bioreactor chambers were constructed using sterilized, thin-walled polystyrene boxes housed in calibrated temperature chambers. A total of 14 different environmental conditions were created and controlled successfully over a 90-day experiment.

This study describes an interdisciplinary approach to forensic anthropology and develops a forensic bioreactor system based on defined factors known to impact bone decomposition. Specifically, environmental factors important to forensic anthropology and useful for modeling decomposition in an outdoor context were defined and then discretized using soil science data. Finally, these factors were successfully incorporated into an easily monitored forensic bioreactor prototype that mimicked an outdoor setting. In this manner, the bone samples were treated as a tissue undergoing dynamic changes when exposed to a soil medium. This research provides a foundation for bone decomposition models and advances forensic anthropology related to determining PMI for skeletal remains. These results demonstrate successful implementation and control of forensic bioreactors simulating precise environments in a single research location, rather than testing in an outdoor context. Using this methodology, bioreactor systems can be created to replicate many different clandestine burial contexts, which will allow for more rapid understanding of environmental effects on skeletal remains.

Bioreactor, Postmortem Interval, Taphonomy



A65 The Effects of Decomposition Upon the Efficacy of Biometrics for Positive Identification

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After attending this presentation, attendees will understand: (1) the utility of biometrics for obtaining positive identification of unknown individuals; (2) the effects of decomposition on facial, iris, and fingerprint characteristics; (3) the necessary procedures for successfully obtaining these biometrics from human remains; and, (4) the types of predictions that can be made from these data.

This presentation will impact the forensic science community by describing the advantages and limitations of the use of biometric identifiers at early stages of decomposition.

Biometric identifiers are measurable, unique characteristics that are used to classify both living and deceased individuals. This study examines the effects of decomposition upon the ability to capture biometric information from three physiological characters: facial photographs, fingerprints, and iris scans. This study examines the maximum number of days in which usable biometric data can be successfully collected using digital technologies and how the recognition performance decreases over time. For the purposes of this study, *usable data* refers to images that are able to correctly identify the individual through a digital biometric program by matching the captured images with images taken upon the initial receipt of the donated individual. This study was conducted in conjunction with Oak Ridge National Laboratory and the University of Tennessee Anthropological Research Facility between the months of April and June. Digital facial photographs, iris scans, and fingerprints from the donated remains of eight (n=8) individuals were obtained daily until usable data could no longer be captured. The individuals were placed supine and mostly uncovered with the exception of wire mesh placed over the hands to prevent scavenger activity. The left iris of all individuals was hydrated with 0.4mL of sterile saline solution ten minutes prior to iris scanning to determine if this would increase the quality of images compared to the untreated right iris. No other preparations were made to the remains prior to data collection.

With daily high temperatures ranging between 59°F (15°C) and 84°F (28.89°C) during the spring trial (n=4), usable data was obtained for an average of four days. However, the early summer trial (n=4) included high temperatures between 81°F (27.22°C) and 91°F (32.77°C) and the number of days usable data could be captured was reduced to two. Overall, fingerprints proved to be the most reliable biometric data, producing usable data longer than iris scans or facial images (four days for fingerprints, two for facial, and one day for iris images). Insect activity, bloating, and color changes due to decomposition prohibited the capture of usable facial images after an average of two days (regardless of season), while dehydration, clouding, and collapse of the cornea prevented capture of usable iris scans after an average of two days in the spring and only one in the summer. Additionally, hydration of the left iris did not lead to an improvement in the quality of iris images when compared with the right iris.

This study demonstrated that digitally captured biometric data can be used within two to four days postmortem to identify individuals, compared to existing antemortem biometric data. For some modalities such as iris recognition, it has been generally believed, but never studied, that iris biometrics are only viable within the first 24 hours; however, the results of this study show that they remain viable for a longer period of time, depending upon environmental conditions. When scavenger activity is inhibited, fingerprints persist longer than facial and iris identifiers; however, temperature, precipitation, and insect activity were the primary factors affecting the retention of biometric information in decomposing human remains. While this study is an initial step in determining the utility of physiological biometric identifiers during the decomposition process, biometric research has the potential to make important contributions to forensic anthropology and the law enforcement, military, and medicolegal communities.

Biometrics, Human Decomposition, Positive Identification



A66 The Extraction of Handedness and Amount of Experience From Sawing Imprints in Bone

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After attending this presentation, attendees will be informed about the possibility of determining handedness and experience from sawing imprints. Attendees will learn the markers that need to be taken into account explained in a visual manner.

This presentation will impact the forensic science community by explaining how the usual tool mark examination is directed toward classifying the tool that was used. This research shows that characteristics of the perpetrator may also be extracted from the same trace. This will have an impact on the tool mark analysis in bone as it will add an extra dimension to this type of examination.

The research material for this examination was obtained with the help of volunteers. Each of the 38 volunteers provided three different sawing cuts in a sheep bone. All volunteers inflicted one shallow cut, one cut halfway through the bone, and one cut completely severing the bone. Thirty-one of the bones were examined for characteristics described in earlier research pertaining to sawing imprints. For handedness, extra care was given to the side on which these characteristics were more prominent. To determine experience, the general prominence, roughness, or smoothness of these same characteristics were examined. Additionally, anything that stood out or seemed to be a pattern was noted. The remaining seven bones were saved for a blind test.

In determining handedness, the downward direction of the sawing imprint was shown to be most related to handedness. The side of the cut on which flaring was most prominent, as well as the horizontal direction and the harmonics of the cut, were also related to handedness. Less important (but still somewhat indicative for handedness) were the roughness of the imprint walls, the striations in these walls, and the placements of false starts surrounding a main cut.

In order to analyze the bones in the case of experience, the amount of experience was quantified. This was done by asking the volunteers to rank themselves on a scale of one through ten, one being very inexperienced and ten being very experienced. In the case of experience, the roughness (or smoothness) of the floor and walls of the cut as well as their striations were indicative. The straightness of the walls and the vertical direction of the cut were also shown to be of value.

The reason for the changes in the sawing imprints was investigated. For handedness, it is argued that the most comfortable way of holding a saw is by tilting the blade. For left-handed people, this tilt will be the opposite of right-handed people, causing a difference in the placement or prominence of markers described in earlier research. For experience, it is argued that more experienced sawyers will start sawing more easily and will proceed evenly, causing smoother, straighter cuts.

The blind test was performed by examining the seven saved bones and using the stated markers to judge handedness and to estimate the amount of experience of the sawyer in question. In performing the blind test, handedness was correctly classified in five out of seven bones, one was inconclusive, and one was wrongly classified. For experience, the examination resulted in defining a range of experience (mostly of three digits). This range included the self-given score five out of seven times.

This research provides an indication that it is possible to extract information related to handedness and experience from a sawing imprint; however, this is research in a new area and is simply an indication. More research is needed, with larger sample sizes and more variables to justify and verify the conclusions drawn in this research.

Tool Marks, Handedness, Experience



A67 **Standardized Descriptive Method for the Anthropological Evaluation of Fractures of the Adult Neurocranium**

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The goal of this presentation is to provide case-example illustrations of a novel method developed for the standardized description of adult neurocranial fractures.

This presentation will impact the forensic science community by describing a method that is designed for use by anthropologists in the description of adult neurocranial fractures which circumvents the confounding effects of the inappropriate application of clinical fracture descriptions in the forensic context.

The system is a modification of the system recently presented for use with pediatric cases.¹ The presentation will: (1) describe the method; (2) place the method within the context of the existing skull fracture literature; and; (3) describe a validation study based on the application of the method to forensic casework examined at the Harris County Institute of Forensic Sciences (HCIFS).

Interpretation of head trauma is an important component of the forensic autopsy that benefits from thorough detection, standardized description, and appropriate interpretation of skull fractures. The majority of the literature pertaining to skull fracture is clinically based and thus motivated by the need for effective assessment of both fracture characteristics (type, frequency, location, and mechanics) and context (severity of injury, associated soft tissue damage, and patient prognosis). Much of the data pertaining to adult skull fractures is drawn from studies of traumatic brain injury. This clinical perspective is reflected in the significant array of skull fracture classification systems that exist. Most clinical schema identify some variation of the following fracture categories: simple, complex, compound, comminuted/composite, depressed, basilar, stellate, and diastatic. From a strictly descriptive standpoint, these categories are not mutually exclusive but instead represent overlapping levels of detail in fracture description that are useful in the clinical setting but confound the non-clinical description of fractures in the forensic context. For this reason, application of these schema in the forensic anthropological interpretation of skull fractures, as manifest on the bone itself rather than imaging/associated clinical findings, is inappropriate.

It is argued that forensic anthropological interpretation of adult neurocranial fractures requires a standard classification system that reflects fracture morphology alone; thus, a three-step classification system which conveys increasing detail with each additional step is suggested. The first, and most basic step is the fracture *category* of which four variations exist: simple, complex, comminuted, and hyper-fragmented. The second step describes the fracture *pattern*, e.g., linear, curvilinear, and stellate. The third step adds the fracture *descriptors*, e.g., depressed. Thus, the proposed system utilizes the fracture characteristics that covary with, but are not independent of, the basic fracture categories, as modifiers rather than additional fracture categories.

The proposed schema was applied retrospectively to evaluate its applicability and repeatability. The study sample included all HCIFS adults autopsied between January 1, 2011, and August 15, 2013, and who had the terms “fracture” and “cranium” or “skull” in the cause of death. Thirty-nine cases met the sampling criteria and were included in the study. For each case, four doctoral-level forensic anthropologists examined photographs taken during the autopsy and photographs of processed bone, when available. Each analyst described each fracture following the proposed schema. Intra-observer disagreement was evaluated.

The study was intended to: (1) statistically evaluate the effectiveness of the schema in capturing the variation in neurocranial fractures seen at the HCIFS; and, (2) to identify the potential for inter-observer error in the application of the schema. Forty-four fractures were found in the 39 cases. Application of the method demonstrated that the proposed schema adequately documented fracture variation in each of the cases and validated the effectiveness of the schema in describing fractures of the adult cranial vault. There was 100% agreement between the four anthropologists in the assignment of the fracture category. The fracture patterns assigned to each fracture were also highly consistent among the analysts, with only six of 156 discordant entries (3% error rate) between observers.

This study illustrates the value of the proposed schema for the standardization of the anthropological description of adult neurocranial fractures and the effective distinction of fracture description and clinical implication. The system is adequate for the anthropological classification of the majority of skull fractures observed during medicolegal autopsies of adults.



Anthropology Section - 2015

Reference:

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Skull Fracture, Adult, Blunt Trauma



Anthropology Section - 2015

A68 Identifying Undocumented Border Crossers From the Texas-Mexico Border: A Collaborative Effort

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After attending this presentation, attendees will understand how the Forensic Anthropology Center at Texas State (FACTS), in collaboration with other universities, human rights organizations, and government offices, facilitate identifications from a humanitarian crisis along the Texas-Mexico border. Additionally, this presentation will highlight the mentoring of future forensic anthropological practitioners through these humanitarian efforts.

This presentation will impact the forensic science community by highlighting the various collaborative efforts and methodologies used to help identify and repatriate Undocumented Border Crossers (UBCs) from South Texas.

A high number of UBC deaths occur each year in Brooks County, TX (80 in 2011, 129 in 2012, and 87 in 2013); these deaths fall under the jurisdiction of a Justice of the Peace (JP), as there is no medical examiner within the county. Due to the dramatic increase in deaths and lack of resources, the local JP and Brooks County Sheriff's Office were overwhelmed with deaths and began to bury the UBCs, most without proper analyses or collection of DNA samples, leaving little chance for UBC identification and repatriation. During the summers of 2012 and 2013, Drs. Lori Baker and Krista Latham and their students performed voluntary exhumations of UBC burials within Brooks County for the purposes of skeletal analysis and DNA sampling in hopes of facilitating positive identifications. The purpose of this presentation is to describe the work of faculty and students at Texas State to identify and repatriate the remains of these individuals as well as to specify the external collaborations that are required for this humanitarian crisis.

The majority of the Brooks County exhumations contained individuals in early to late stages of decomposition, requiring a storage area or location for further decomposition until the maceration could be accommodated. Because the FACTS has large-scale storage and maceration capabilities due to the Forensic Anthropology Research Facility (FARF) and the Osteological Research and Processing Laboratory (ORPL), UBCs with significant amounts of flesh (47 in 2013 and 20 in 2014) were brought to the FACTS to await maceration and analysis. Once in the FACTS custody, all UBCs are taken to the FARF and placed in a special enclosure within the facility. The UBCs are not used in any decomposition or taphonomic studies at the FARF. During placement of UBCs at the FARF, faculty and staff conduct intake procedures that involve opening the body bags and documenting the condition of remains and personal effects. At this time, personal effects are removed and placed in plastic bags for freezer storage until they can be hand-washed and dried for photography.

All case information is entered into the National Missing and Unidentified Persons System (NamUs) and students search through the possible missing persons matches to narrow down potential identifications. DNA samples are sent to the University of North Texas for profiling and uploading into the Combined DNA Index System (CODIS). Clothing descriptions, along with biological profiles, have facilitated several potential identifications and DNA results are currently pending. Resources for decedent identification within the United States, such as NamUs and CODIS often lack missing persons information or appropriate DNA family reference samples for comparison to UBCs. Therefore, the FACTS faculty and students also work with human rights groups such as the Equipo Argentino de Antropología Forense, the Colibrí Center for Human Rights, and foreign consulates, providing each agency with case information and skeletal analyses.

The Scientific Working Group in Forensic Anthropology (SWGANTH) suggest best practices in education and training in forensic anthropology should include theory, methods, techniques, and forensic casework. This large-scale effort to identify the UBCs in South Texas provides both mentorship and specialized forensic casework training opportunities for graduate and undergraduate students. Students are involved in every aspect of working toward the identification of the UBCs including: intake, maceration, washing clothes, entering personal effects and case information into databases, and collection of DNA samples. Student involvement in case analysis is reserved for graduate students and supervised by faculty. As recommended by SWGANTh, graduate students log their hours spent working on casework to track their specialized training. Student involvement in UBC identification efforts allows students to be involved in the holistic and collaborative nature of forensic anthropology and, in this case, provides exposure to a humanitarian crisis within the United States.

Undocumented Border Crossers, Human Rights, Identification



A69 **Surveying a Titan: An Argument for the Presence of Human Remains on the Wreck of the R.M.S. *Titanic***

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After attending this presentation, attendees will have a better understanding of the events surrounding the sinking of the passenger liner R.M.S. *Titanic* along with possible unexplored locations within the wreck that may harbor human remains.

This presentation will impact the forensic science community by increasing awareness of the necessity of including individuals trained in the identification and recovery of human remains when surveying shipwrecks and the importance of eyewitness accounts when reconstructing historical events.

The first highly publicized maritime disaster to result in a significant loss of human life occurred in the early morning hours of April 15, 1912. After colliding with an iceberg the night prior, the White Star liner, R.M.S. *Titanic*, sank in the icy north Atlantic, taking 1,523 of her passengers and crew with her. For the next 73 years, the wreck of the 882-foot-long ship lay undisturbed nearly 2.5 miles below the ocean surface. In 1985, the sunken ship was discovered and, in the ensuing years, multiple salvage expeditions have descended to the site. To date, no human remains have been recovered from the debris field that encircles *Titanic's* fragmented hull.

In the years since she sank, *Titanic* has ceased to be merely a ship, instead approaching enigmatic status and her wreck remains one of the most visited wrecks in the world. However, no expeditions have journeyed deep enough into the liner's hull to definitively rule out the presence of human remains. Historical records and eyewitness accounts indicate that at least one area of the ship has the potential to reveal the remains of crew members trapped by the rising waters.

This area is the forward mail holds, located on G-deck. R.M.S. *Titanic*, as a Royal Mail Steamer, was in service to the British government to bring mail to and from America. This task was charged to five postal clerks, each of whom were contracted by the White Star Line. Minutes after her fatal collision with the iceberg, *Titanic* was fast taking on water and among the first to notice this were the mail clerks. Their desperate attempts to save the mail were in vain. As the vessel's bow sank deeper into the Atlantic, the mail clerks ran back and forth between the holds on G-deck and the empty state rooms on D-deck, trying to outpace the rising water. All five men perished in this endeavor.

In April 2015, *Titanic* will have lain on the ocean floor for 103 years. The standard argument against the presence of human remains on the wreck is that this length of time is sufficient to have allowed bacteria and deep-sea scavengers to remove all traces of organic matter. This line of thought fails to take into account the presence of organic matter in recessed areas of the hull that have escaped destruction. Remains of the victims of maritime disasters that occurred within a similar timeframe to *Titanic* have also been recorded.

The most well known of these analogous disasters was the 1914 sinking of the R.M.S. *Empress of Ireland*. After a collision with another vessel, the *Empress* sank with the loss of 1,012 lives. Resting in waters easily accessible by experienced divers, the liner has become well known for the skeletal remains of her victims that linger on the wreck.

The other flaw with the current party line about remains on the wreck of *Titanic* is that the principle players making this judgment are not experts in human anatomy. Robert Ballard, a noted oceanographer, and James Cameron, the Hollywood director, are titans in their respective fields and have well-deserved reputations as experts in those fields; however, neither has the training necessary to identify human skeletal remains, particularly fragmented remains.

It is entirely possible that no trace of human remains persist on the wreck of the former White Star Line flagship; however, it is argued that the presence of remains cannot be ruled out. By looking at the historical documentation of the last known locations of individuals during the sinking, notably the postal clerk staff, an argument is made that further exploration inside the wreck is warranted before a statement on the presence or lack of human remains can be made.

***Titanic*, Ocean, Historical**



Anthropology Section - 2015

A70 Utilizing Geographic Information Systems (GIS) to Analyze Geographic and Demographic Patterns Related to Forensic Case Recovery Locations in Florida

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After attending this presentation, attendees will have a better understanding of how GIS can be utilized by forensic anthropologists and the forensic science community at large and how GIS has elucidated patterns of body recovery at the C.A. Pound Human Identification Laboratory (CAPHIL) at the University of Florida.

This presentation will impact the forensic science community by demonstrating how the application of GIS provides a powerful tool for understanding the spatial distribution of case recoveries within specific geographic and cultural areas.

GIS can be used to provide information about the physical and cultural environment that humans inhabit. As such, it is a valuable tool that can be utilized to provide information about overall patterns of body recovery. In the case of this research, this study examines how geographic and demographic factors, such as topography, population density, and crime rates influenced or affected the spatial distribution of the forensic anthropology cases that are analyzed by the forensic analysts at CAPHIL.

In order to look for patterning, 92 cases spanning a five-year period (2007-2012) were selected and geolocated to a map of Florida using the program ArcGIS®. Recovery sites were selected for geolocation based on the following criteria: they contained human remains, they were recovered in the state of Florida, and the data on their recovery location was sufficiently complete for geolocation. Private cases and scene searches that did not yield human remains were excluded. ArcGIS® is equipped with a feature that allows the user to input addresses from a database file and use those addresses to create a point feature class data layer. Thus, each geolocated CAPHIL case appeared as a specific point in an ArcGIS® data layer that could interact with existing data layers collected from the Florida Geographic Data Library (FGDL), which serves as a repository for GIS map data created by state and federal institutions, such as the Florida Fish and Wildlife Conservation Commission and the United States Census Bureau.

The results of overlaying geolocated cases over map layers of physical land cover (swamp, forest, agricultural land, etc.), population density, and crime rate indicate that for the five-year period examined, the majority of cases brought to the University of Florida were recovered from designated urban areas, were found in areas of low population density (less than 300 people per square kilometer), and that the majority of remains were recovered from areas with very low to medium crime rates (a Police Report Index of less than 25,000 reports filed annually; less than 30 murders annually). Seventy-seven percent of geolocated cases were recovered from urban areas. Interestingly, though land cover maps designate these areas as environmentally urban, this does not make them synonymous with densely populated areas due to the fact that 48% of case recoveries were from areas of low population density. Regarding the relationship between crime rate and the selected cases, this study found that 78% of cases were recovered from areas with less than 25,000 police reports filed annually and 75% of cases were found in areas designated medium to low in terms of annual murder rates. Chi-square statistical analysis revealed all results to be significant.

While the results of this study convey important information about the spatial patterning of case recoveries in Florida and indicate how GIS can be employed to aid the forensic anthropology community at large, the lack of proper addresses and/or GPS coordinates from individuals recovered from a few very remote locations, such as the Ocala Nation Forest and open water areas off the Florida Keys, contributes to a certain amount of underenumeration. In many of these cases, issues regarding underenumeration of certain physical environments could be solved by encouraging the individuals conducting the recovery of the remains to take a GPS coordinate of the recovery location when possible. Though underenumeration does occur and not all cases will possess the requisite information for inclusion, GIS remains a powerful tool in regard to understanding potential patterns related to the spatial distribution of case recoveries.

GIS, Case Recoveries, Florida



A71 **The Application of the Sub-Pubic Concavity/Contour for Sexing Subadult Human Innominates**

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After attending this presentation, attendees will understand the timing and onset of sexual dimorphism in the morphology of the pubis which is commonly used for non-metric sex estimation of human remains.

This presentation will impact the forensic science community by testing the applicability, reliability, and validity of a trait commonly used in adult sex estimation for the prediction of sex in unknown subadult individuals encountered in forensic cases. The revised method presented in this research can be used to estimate sex in individuals younger than previously reported in the literature.

Sex estimation is essential for the assessment of a biological profile in forensic cases. While the innominate is considered the best indicator of sex in adults and is widely used for sex estimation in forensic cases, the use of the innominates for subadult sex estimation has been limited. Numerous studies have assessed the utility of other areas of the innominate (ilium/ischium), yet few studies have attempted to address the usefulness of the pubic bone for subadult sex estimation. A preliminary study on subadult pubic bones from the Hamann-Todd Osteological Collection has shown that it is possible to use a modified version of the Klales et al. method to estimate sex in subadult age categories younger than previously believed.¹ The results from this preliminary study were encouraging; however, a larger sample size was needed for the results to be statistically significant. The present research examines the subadult pubis using a revision of the Klales et al. Sub-Pubic Contour (SPC) scoring in a large sample of subadults.¹ The first goal of the study was to determine if this revised technique can be used to differentiate sexes in subadult remains. The second goal was to evaluate the timing or onset of sexual dimorphism in the SPC. The third goal of the study was to test the level of observer agreement for trait scoring.

The sample used in this research was derived from the PATRICIA Radiographic Data Bank. The database contains radiographs from a “geographically and ethnically diverse” sample of modern American subadults (born after 1990) with known demographic information collected from various coroner and medical examiner offices throughout the United States.² A total of 334 individuals of both sexes (149 females, 185 males) were included in this research. Individuals in the sample ranged in age from 1.19 to 20.47 years. The sample was divided into six age cohorts that were slightly modified versions of the age categories presented in Baker and colleagues: (1) Young Child Early (1.0 to 3.5 years); (2) Young Child Late (3.6 to 6.5 years); (3) Older Child Early (6.6 to 9.5 years); (4) Older Child Late (9.6 to 12.5 years); (5) Adolescent Early (12.6 to 15.5 years); and, (6) Adolescent Late (15.6 to 20.5 years).³ Two observers, one experienced and one upper-level anthropology student, scored each radiograph using a revision of the Klales et al. adult SPC scale/figures.¹ For the present research, the original adult SPC scores were reduced from five ordinal scores to three. First, trait frequency distributions for each ordinal score by sex were calculated for each of the age cohorts. Next, sex classification accuracy using SPC ordinal scores was tested using Ordinal Logistic Regression (OLR). Lastly, the Intraclass Correlation Coefficient (ICC) was utilized to test the degree of agreement for trait scoring between the experienced and inexperienced observer.

Score frequencies varied by age cohort. Score frequencies shifted away from Score 2 (straight), which was most common in the younger age cohorts, to a higher frequency of Score 3 (convexity) in males and a higher frequency of Score 1 (concavity) in females with increasing age. Using OLR, sex classification accuracy was highest for the oldest age cohort (Adolescent Late) at 97.2% combined correction classification and then decreased in order of age. Using the ICC, inter-observer error between the two scores rendered a high level of agreement (0.806) and mirrored the results of the original Klales and colleagues study. Results from this research indicate that the SPC method used in adult sex estimation can be modified and applied to subadult remains to accurately estimate sex of unknown subadults following the onset of puberty (around 12 years of age) with a high degree of reliability and validity.



Anthropology Section - 2015

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 2. Ousley SD, Frazee K, Stull K. 2010. Assessing Bone Growth and Development in Modern American Children. Proceedings of the 79th Meeting of the American Association for Physical Anthropology; 2010 Apr 14-17; Albuquerque, NM. Albuquerque: *American Journal of Physical Anthropology* 141(S50):183.
 3. Baker BJ, Dupras TL, Tocheri MW. 2005. *Osteology of Infants and Children*. College Station, TX: Texas A&M University Press.
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Biological Profile, Subadult Sex Estimation, Pelvis



A72 Sex Estimation of the Modern Human Pubic Bone Using a 3D Geometric Morphometric Approach

Valda Black, MA, 1134 NE Markley Drive, Apt 3, Pullman, WA 99163*

After attending this presentation, attendees will have gained a greater understanding of the morphological differences seen between male and female modern pubic bones and how those shape differences can be captured using a 3D geometric morphometric approach.

This presentation will impact the forensic science community by quantifying non-metric shape analyses that are widely used in sexing human pubic bones through a 3D geometric morphometric approach for analysis. Using a 3D geometric morphometric approach allows for the utilization of landmarks and semi-landmarks to accurately capture the slight shape differences that non-metric observations are unable to obtain.

Sex estimation methods for human skeletal remains have been a highly researched and debated topic in biological anthropology. A plethora of studies have been performed using various statistical methods, in part because os coxae variation spans a large spectrum and does not always fit into distinct nonmetric categories. Growing in popularity is the utilization of 3D geometric morphometric techniques; studies have been performed using landmarks and semi-landmarks to sex os coxae with a high degree of accuracy.

Generally, it is believed that male pubic bones tend to be shorter and triangular in shape, while females have a longer and squarer shape. Previous studies acknowledge the need to quantify non-metric methods while sexing os coxae, but do not specifically test the widely used non-metric visual method of sexing males on the basis of short or triangular pubic bones and females on the basis of long or square pubic bones.

In this research, 35 known male and 35 known female specimens from the William M. Bass Donated Skeletal Collection at the University of Tennessee (UT) and 24 unknown specimens from the Forensic Anthropology Unit at the New York City Office of Chief Medical Examiner (NYC OCME) were scanned using a NextEngine® 3D laser scanner. The os coxae were edited in Geomagic® and eight landmarks were placed on the left pubic bone. The landmarks were chosen to highlight the portion of the pubic bone associated with the triangular and rectangular shape differences between males and females. These points were placed on the dorsal aspect of the bone since there are fewer irregularities and span from the superior and inferior portions of the pubic symphysis, along the obturator foramen opposite the symphysis points, and up to the most superior point of the obturator foramen. The points were aligned using a Generalized Procrustes Analysis, and then a Principal Components (PC) analysis was employed to distinguish meaningful shape differences. A Discriminant Function Analysis (DFA) was then run on the PC scores to determine how accurate the captured portion of the pubic bone is in determining sex. The known specimens were placed in their proper sex categories for the DFA analysis, while the unknown specimens were left ungrouped to compare the non-metric analyses of those os coxae found in their case reports versus their assigned group in the DFA analysis.

Using the 12 PC scores that contained significant shape information, the DFA analysis was able to classify 95.7% (94% males, 97% females) of the known UT pubic bones correctly into their skeletal sex groups with a 92.9% (91% males, 94% females) leave-one-out cross-validation accuracy. For the unknown NYC OCME specimens, 83.3% of the specimens matched their case reports. Intra-observer error was tested by placing the landmarks on the specimens a second time and running the same analyses. The results were an original classification accuracy of 97.1% (94% males, 100% females), a cross-validation accuracy of 91.4% (86% males, 97% females), with 87.5% of the unknown specimens matching their case reports. The differences in percentages between these two analyses are slight and the changes were due to specimens that fell within the overlapping regions found on the PC plots.

The results capture the major morphological differences between male and female pubic bones. These differences match the non-metric categories of a triangular shape in males and a square shape in females, which were found on the first PC, but it also captures 11 other significant PC variations that cannot be observed from visual assessments. This experiment has high accuracy results and can be a good categorization method for unknown specimens in future studies.

Geometric Morphometrics, Sex Estimation, Discriminant Function Analysis



Anthropology Section - 2015

A73 Estimating Sex From the Innominate Utilizing New Measurements

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After attending this presentation, attendees will understand how the use of newly devised measurements, including maximum and minimum measurements of the innominate for sex estimation, can provide extremely high accuracy rates and reduce errors associated with differential interpretations of landmark locations in traditional measurements.

This presentation will impact the forensic science community by introducing novel measurements that circumvent errors introduced by differential interpretations of traditional landmark-based measurements, resulting in an improved rate of accuracy of sex estimation in human identification, along with reduction of intra-observer errors.

The innominate is commonly viewed as the best skeletal element used in the estimation of sex of an unknown individual and non-metric methods have dominated. However, metric methods can provide a more objective means of estimation. Previous metric studies cite accuracy rates in at least the 90% range, though many of these methods use measurements based on landmarks that are difficult to find and nearly impossible to replicate, leading to high inter-observer error rates. Most recently, Murail et al. defined a series of measurements through text and images and analyzed them using logistic discriminant analysis, in a computer program known as Diagnose Sexuelle Probabiliste (DSP).¹ Their study showed accuracy rates of at least 95% in diverse samples from around the world, apparently avoiding bias in sex classification due to ancestry. Though this study seems to provide highly accurate results, their DSP program does not provide the actual logistic regressions used, a necessity in the post-*Daubert* era.

In this study, measurements with clear landmarks or those involving a maximum or minimum were used as is or were modified from previously published definitions, some of which were rather unclear. A sample of 100 male and 100 female innominates from Whites and Blacks in the Hamann-Todd Collection were used for this study. The individuals used were of known age, ranging from 19 to 96 years old, with known sex and ancestry. Only innominates from the left side were used for consistency. On each innominate, 11 measurements were taken with a digital sliding caliper connected directly to a computer to record the measurements efficiently and reduce data input errors. The data were analyzed using FORDISC® 3.1 discriminant function analysis, using both stepwise and non-stepwise functions. All reported error rates were cross-validated.

Using stepwise discriminant function analysis, a combination of five variables were shown to provide classification accuracies of 96% in males and 99% in females, for a pooled-sex accuracy of 97.5%. The five measurements selected by the stepwise discriminant function analysis included minimum apex to symphysis, maximum innominate length, maximum ischial length, maximum innominate breadth, and maximum pubic length. These measurements are effectively able to capture dimorphism in the innominate, with the measurement of minimum apex to symphysis, which captures true pelvic morphology; maximum innominate length and maximum innominate breadth, which capture information on the ratio of height to width; and maximal ischial length and maximum pubic length, which capture the ratio of pubis to ischium. Some logistic regression and other classification methods produced similar or higher classification accuracies with little sign of classification bias for sex by ancestry. Forty individuals were measured a second time to calculate the technical error of measurement and coefficient of reliability for each measurement. The technical error of measurement showed differences between rounds of measurements to be less than 2mm, a mean of less than 3.5% for all measurements, signifying low intra-observer error rates. Additionally, for all but two of the measurements, the coefficient of reliability values were greater than or equal to 0.96, indicating an extremely high level of intra-observer consistency for those measurements.

The high levels of intra-observer agreement between rounds of measurements reveal the value of measurements with clear, unambiguous landmarks, or those that involve maxima and minima. This approach proves that current methods can be further improved upon to reduce measurement errors and increase classification accuracy.

Reference:

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Sex Estimation, Forensic Anthropology, Innominate



A74 Sex Estimation Using Metric Measurements of the Sternum

Megan Chapin, BA*, 254 Lovell Place, Erie, PA 16503-2622

After attending this presentation, attendees will understand the benefit of including sternal measurements in traditional postcranial metric analyses. By understanding how the sternum can be utilized for sex estimation, an appreciation of how all bones contribute to constructing a biological profile will be gained.

This presentation will impact the forensic science community by presenting testable metric measurements of the sternum that display a high degree of differentiation between the sexes.

Though the innominate is most commonly used for skeletal sex estimation, post-cranial measurements have also proven to be accurate and useful due to the fragile nature of the pubis. Due to the incomplete or fragmentary nature of forensic cases, the need to use any available bones to produce a more accurate biological assessment of the individual cannot be overstated. Work by Jit et al. using Indian sternums produced a 84.9% classification accuracy for males and 88.6% accuracy in females.¹ Dahiphale's 2002 validation study of Jit et al. further demonstrated the feasibility of this method with classification accuracies of 92% for males and 87% for females.²

This study used metric measurements on sterna from an American group of Blacks and Whites from the Hamann-Todd Collection for use in sex estimation. A total of 152 individuals were used, with 76 males and females, respectively. Six measurements were taken to the nearest millimeter from the manubrium and sternal body using digital sliding calipers. Data was then analyzed using the statistical program R and FORDISC®.^{3,4} All accuracy rates were evaluated using discriminant function leave-one-out cross-validation. Measurements were analyzed individually, together, and using forward stepwise selection to classify an individual based on the best measurements available. All functions were tested for within-group variance-covariance matrix homogeneity, a requirement for linear functions, using the Kullback test. A forward mean stepwise analysis was also run to produce a cross-validated classification for ancestry, as well as for ancestry and sex combined.

The Kullback test *p*-value was greater than 0.01 for all analysis, meaning the hypothesis that the variation in each group is more or less the same was accepted. Each measurement alone classifies individuals by sex at a rate higher than 80% with the exception of breadth of the third sternal body and breadth of the last costal notch. The best function for sex classification was the four variable forward mean stepwise selection function (Breadth of First Costal Notch (-0.383))+Height of the Sternal Body (-0.174)+Maximum Manubrium Height(-0.161)+Breadth of the Manubrium(-0.048)+38 which showed a 91% sex classification accuracy. When the value is greater than zero, the individual is classified as female; if less than zero, the individual is classified as male.

Discriminant function analysis for ancestry using forward mean stepwise chose three measurements with an accuracy rate of 50.9% for females, not better than random; however, the ancestry samples used in this study had different proportions of males and females in each ancestry group. Discriminant function analysis for males needed only two variables in the forward mean stepwise function to produce a classification rate of 64.9%. When the classification of groups by sex and ancestry was run using stepwise, 58.5% of the total sample were classified correctly, suggesting that the sternum shows no metric differences based on ancestry.

This study proves the utility associated with sternum measurements in American groups. By displaying the effective classification by sex, it is the hope that future steps can be made to include the sternum in postcranial measurements.

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Sternum, Sex Estimation, Postcranial Measurement



A75 Skeletal Sex Assessment Utilizing the Fifth Lumbar Vertebra

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The goal of this presentation is to explore the levels of sexual dimorphism found in the fifth lumbar vertebra and to specifically examine the utility of 11 linear measurements taken from an American sample of vertebra for sex assessment.

This presentation will impact the forensic science community by increasing the range of skeletal elements that can be utilized for determining the metrics for sex estimation and by beginning to explore the potential differences in levels of sexual dimorphism between vertebra and populations.

Sex estimation is a vital part of any investigation of unidentified human remains as it narrows the pool of potential victims. While well-accepted methods of sex estimation for the cranium and many postcranial elements exist, levels of sexual dimorphism have not been established in all skeletal elements; however, this is becoming vitally important in cases where remains may be fragmentary or incomplete and many heavily relied-upon methods may fail.

Over the last two decades, research has been pursued in exploring sexual dimorphism in select vertebra from a variety of populations.¹⁻³ These studies have resulted in varying levels of success, with overall sex estimation accuracies of approximately 85%. Of the three previously cited studies, two examined vertebra below the level of the sixth thoracic, both using data collected from Computed Tomography (CT) scans on East Asian populations.^{2,3} Though their results show promise, the applicability of these methods to measurements taken on dry bone from external populations needs validation.

The present study examined 11 linear measurements from 160 fifth lumbar vertebra housed in the Hamann-Todd Collection at the Cleveland Museum of Natural History in Cleveland, OH. Of the 160 individuals, 41 are documented as White males, 39 as White females, 39 as Black males, and 41 as Black females. Individuals in the sample were between the ages of 16 and 93 years old. The measurements include maximum dimensions of the vertebral body, pedicle widths, maximum widths of articular processes, transverse processes, and length of the spinous process. Statistical methods including discriminant function analysis and principal components analysis were run on the collected measurements to examine the level of sexual dimorphism in the sample.

Of the measured vertebral dimensions, anterior posterior dimensions of the vertebral body are the most dimorphic from this sample. Classification accuracies were found to be 78% and 77% for American Blacks and American Whites, respectively. Cross-validated sex estimation accuracies using discriminant functions increased this accuracy by only one to two percentage points when ancestries were pooled. These results are much lower than those reported in studies using Korean and Chinese CT populations. This could be for any number of reasons, all of which require further investigation. These include differences in measurement technique from 3D CT to dry bone, differences in population sexual dimorphism, and differences in vertebral level sexual dimorphism.

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Anthropology, Vertebra, Sex Assessment



A76 Sexual Dimorphism of the Manubrium in a Modern Forensic Sample

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After attending this presentation, attendees will be familiar with metric sex estimation using the manubrium in a modern forensic sample in the United States. Previous studies have used measurements of both the manubrium and the mesosternum to develop sex estimation methods; however, these methods tend to focus on only the manubrium length in combination with the other standard sternal measurements. The objective of this research is to test a sex estimation method using measurements that encompass the overall shape and articular surfaces of the manubrium, rather than just the length, in order to define sectioning points for males and females using the manubrium without the mesosternum.

This presentation will impact the forensic science community by offering further research into sex estimation methods using postcranial skeletal elements. This is important in cases where only postcranial elements are found postmortem due to taphonomic damage or animal scavenging.

Forensic anthropologists estimate sex as part of the biological profile that is created to aid with human identification. For this research, a sample consisting of 303 known individuals (202 males and 101 females) from the Hillsborough County Medical Examiner's Office in Tampa, FL, was used. All individuals in the sample were between the ages of 20 to 86 years old; any cases with arthritic changes to bone, open-heart surgery, or other medical disturbances were excluded. Seven total measurements were collected on processed manubria removed at the time of autopsy. Using sliding calipers, the traditional manubrium length measurement was collected, in addition to six newly defined measurements: superior manubrium breadth, middle manubrium breadth, inferior manubrium breadth, height of right and left costal notch one, and thickness of manubrium body.

A Discriminant Function Analysis (DFA) was run in statistical analysis software to determine which measurement, or combination of measurements, was the most accurate when estimating sex, as well as if any of these results were as accurate as the results found in previously published studies that also incorporated the mesosternal measurements. The highest cross-validation classification rates resulted when using all seven manubrium variables with 90.91% for males and 81.82% for females (86.37% overall). When assessing the individual measurements separately, the inferior manubrium breadth had the highest cross-validation classification rates of 77.14% for males and 81.25% for females, followed by thickness of manubrium body with 76.66% for males and 71.74% for females. The overall cross-validation classification rate was only 68.4% when using only manubrium height (the standard manubrium measurement).

These results are similar to those found by Bongiovanni and Spradley with an overall cross-validation classification rate of 84.12% when using both the manubrium and sternal body measurements.¹ By incorporating additional measurements that were created to capture more of the manubrium shape and morphology of the articular surfaces, the cross-validation classification rates increased to be comparable to those found when using the manubrium and mesosternal measurements. The goal of this study was to explore sexual dimorphism of the manubrium as an applicable technique to use in forensic anthropological casework; the results suggest that the incorporation of the proposed measurements of the manubrium can be used to estimate sex, especially in combination with other sex estimation techniques.

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Manubrium, Sex Estimation, Discriminant Function Analysis



Anthropology Section - 2015

A77 Feature Analysis of the Pubic Bone for Estimation of Age-at-Death

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After attending this presentation, attendees will better understand which features of the pubic bone have the strongest correlation with age-at-death.

This presentation will impact the forensic science community by providing attendees with an in-depth evaluation of features from the most preferred region for age-at-death estimation. This information may assist the forensic anthropologist in producing a more accurate and reliable estimation of age-at-death.

During an individual's lifetime, the pubic symphysis will go through phases of epiphyseal formation, quiescence, and secondary changes related to age; it is these changes that are used in estimating age. The value of the pubic symphysis as an age indicator lies in the changes it continues to undergo when the rest of the skeleton has ceased to grow.

Assessment of a given morphological pattern and its age distribution is a difficult and challenging task when the individual features of the pubic symphysis express different age stages. In 1998, Suchey and Katz hoped that "studies of single traits will help produce narrower age ranges."¹ By observation and analysis of each feature individually, there may be a way to facilitate placement into the correct phase or age range.

The purpose of this research is to evaluate certain individual morphological features on the pubic bone to determine which are most strongly correlated with age-at-death. A known-age sample consisting of 156 female and 168 male pairs of pubic bones from the Forensic Science Center in Phoenix, AZ, was evaluated. Nine separate features of the pubic bone were scored on all specimens. Both left and right sides were examined and the oldest score was used. At the time of scoring, sex was known but age was not. The features scored were: (1) ridges and furrows (RID); (2) dorsal lipping (DOR); (3) bone quality (BONE); (4) rim formation (RIM); (5) the pubic tubercle (PUB); (6) depression of the symphyseal face (SYM); (7) ossific nodules (OSS); (8) ventral ligamentous outgrowths (VENT); and, (9) the ventral hiatus (HIAT). Features were scored according to a specific scoring system developed. Inter-observer and intra-observer error of the scoring system was assessed by four volunteers with different levels of experience from graduate students in physical anthropology to forensic anthropologists with PhDs.

Comparison of the results from the Pearson's correlation, Analysis of Variance (ANOVA), and simple regression analyses indicate that the nine features chosen for analysis are significantly correlated with age-at-death in the combined-sex sample. However, the Pearson's r values 0.762 (BONE), 0.731 (RIM), and 0.677 (VENT) and the F coefficients from the ANOVA 155.83 (BONE), 99.68 (RIM), and 90.13 (VENT) indicate that bone quality, rim formation, and ventral ligamentous outgrowths are more strongly associated with age-at-death than the other features. R -values for these specific features in the female sample were .819 (BONE), .790 (RIM), and .735 (VENT); r -values for the male sample were .701 (BONE), .696 (RIM), and .647 (VENT). The F coefficients for the female sample and the male sample were 110.09 (BONE), 68.17 (RIM), 59.68 (VENT) and 54.54 (BONE), 40.88 (RIM), 40.12 (VENT), respectively. The largest adjusted R^2 values for the total sample are from bone quality, rim formation, and ventral ligamentous outgrowths, respectively explaining 58%, 53%, and 46% of the variation over what would be expected by chance. The adjusted R^2 values of bone quality, rim formation, and ventral ligamentous outgrowths in the female sample are 67%, 62%, and 54% and 49, 48, and 42% in the male sample. These findings suggest that these three features are the best predictors of age-at-death in the total sample, as well as by sex.

Inter-observer error is low for most features, except dorsal lipping and ossific nodules, which were scored inconsistently across observers. Intra-observer test results indicate the majority of features are easy to evaluate, the exceptions being ossific nodules and dorsal lipping in the male sample.

The results of this study highlight bone quality, rim formation, and ventral ligamentous outgrowths as the features on the pubic bone that are most strongly correlated with age. In addition, the evaluation of bone quality of the overall pubic bone places the focus beyond the symphyseal surface and provides a means to assess older individuals previously thought impossible to age. Future studies should emphasize bone quality and analyze other collections in and out of the United States.

Reference:

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Pubic Bone, Age Estimation, Forensic Anthropology

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Anthropology Section - 2015

A78 Assessing the Effects of Pregnancy on Aging From the Pubic Symphysis: Incorporating Living People Into Biological Profile Research by Combining Medical Imaging and Participant Interviews

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After attending this presentation, attendees will gain an increased understanding of the effect of pregnancy on the face of the female os pubis and its impact on aging accuracy as well as a practical introduction to the value and implementation of living patients in biological profile research.

This presentation will impact the forensic science community by introducing a novel means of future data collection combining medical imaging technology and patient observation and interviews.

One of the major restricting factors in biological profile research continues to be the limited availability of data representing current populations. Each year, historical collections become further removed in diet, activity, environment, and general lifestyle from the people of today, prompting an increasing shift to skeletal material that has been generously donated, sanctioned for study after conflict, or collected during autopsy. By incorporating the medical imaging of living volunteers, this study sought to investigate an additional methodology for modern data collection that allowed for both unlimited sample size and the inclusion of life history information provided first-hand by the subjects themselves.

Anecdotal evidence has long associated pregnancy with the difficulty and disproportioned inaccuracy of aging from the face of the female os pubis. However, as previous studies have exclusively utilized physical postmortem specimens (mainly from autopsy), both lifestyle and birth history information has been basic and of debatable accuracy (as, by necessity, it was/is gathered from secondary and tertiary sources such as medical records and/or family and friends). Perhaps as a result, in the absence of a solution, publications moved away from examinations into pregnancy and parturition and into so-called “what” investigations such as methods comparisons, 3D surface mapping, advocating for a 7th phase, and the creation of new equations for narrowing age-at-death to smaller, more specific ranges. In setting living participants as its data set, this study sought to focus more on the “why” of symphyseal change than on the “what.”

By working within Britain’s National Healthcare System (NHS) and Oxford University Hospital’s system (OUH), for this study, access was obtained to any and all patients coming into radiology for scans involving the pubic symphysis (approximately 40-50 per day). Questionnaires detailing demographics and body type, diet, activity level, sports history, and smoking and alcohol consumption were then completed by both sexes, with an additional pregnancy history page given to females. All participants then signed consent for both their scan(s) and questionnaires to be included as part of this project. The scans were 3D volume rendered and a modification to the *Suchey-Brooks Method for Aging the Os Pubis* was applied by the both a person familiar with this study and three independent observers (blindly) to determine whether male, nulliparous females, and parous females could be distinguished from each other with any significant reliability. Additional patterns between life history and symphyseal change were also investigated.

While pregnancy and the pubic symphysis was chosen to illustrate this methodology, it is by no means the only application — utilizing the data of living subjects, coupled with participant observation and patient interviews, lends itself equally to more complex investigations such as the effect of substance abuse on over-aging or the effect of environment (both social and physical) on inter- and intra-population variation. Therefore, in addition to insights into the effects of pregnancy on the reliability of aging accuracy, attendees of this presentation will be walked through the process of setting up partnerships with local hospitals, gain a basic knowledge of the different imaging modalities and their current uses, and receive an introduction to volume rendering as well as some of the software currently available.

Pregnancy, Pubic Symphysis, Medical Imaging



A79 McKern's and Stewart's "Unknowns": A Reappraisal of the Individuals Omitted From the "Skeletal Age Changes in Young American Males" Sample

Alexander F. Christensen, PhD*, JPAC-CIL, 310 Worcester Avenue, Joint Base Pearl Harbor-Hickam, HI 96853

After attending this presentation, attendees will understand the nature of the reference sample for one of the foundational studies in forensic anthropology and how a reassessment of military records can improve this sample.

This presentation will impact the forensic science community by showing how one of the most important reference samples for studies of skeletal aging can be enlarged and improved.

McKern's and Stewart's *Skeletal Age Changes in Young American Males: Analysed from the Standpoint of Age Identification* is a foundational study in American forensic anthropology; however, since its publication, little attention has been paid to the original data for the study.¹ According to the monograph's introduction, data was collected from the skeletal remains of 450 American service members repatriated by Communist forces after the Korean War; however, only 375 of these individuals were ever identified. The Joint POW/MIA Accounting Command-Central Identification Laboratory (JPAC-CIL) is actively engaged in the exhumation and identification of unknown remains from the Korean War, and was therefore interested in obtaining additional data on these 75 service members.

McKern and Stewart did not directly analyze the skeletal materials for their project. Instead, Stewart collected data on 450 skeletal cases in the Central Identification Unit (Kokura, Japan), from September 1954 to January 1955. For each case, he scored a predetermined list of age indicators on two punch cards, wrote additional notes on the front of each card, took extensive photographs (a total of 3,861), and made a series of casts, generally including one pubic symphysis, one medial clavicle, and one lateral manubrium and/or corpus sterni. All materials were prepared in duplicate: two identical sets of punch cards were made, two 8"x10" images were printed from each negative, and two casts were made from each mold.

At the time of skeletal processing, the casualties were unidentified. As identities were determined, Stewart added notes to the cards that included dates of birth and death, home of record, cause of death, "national extraction" (determined from race combined with surname), and how the identification had been made (generally, the strength of the dental comparison with antemortem records, although occasional fingerprint comparisons are noted). Upon Stewart's return to the United States, the Army provided him with updates on casualties subsequently identified.

Stewart then sent one set of casts, photographs, and data cards to McKern so he could analyze them, while retaining the others at the National Museum of Natural History (NMNH). While he sent a complete set of casts, so that McKern could use all of them for seriation, he only provided the punch cards and photographs for the series of identified individuals, retaining both sets for the unidentified individuals. Stewart's set of casts are currently curated by the Department of Physical Anthropology while his data cards, notes, and drafts of his chapters of the monograph are in the National Anthropological Archives, both at the NMNH.

Recently the JPAC-CIL acquired the duplicate data cards collected by Stewart from 73 "unknown" individuals (it is not known what accounts for the discrepancy between the "known" totals of 375 reported in the monograph and 377 filed at the NMNH). Comparison of these cards with original military records revealed that, in fact, 37 had been identified as United States service members; it appears that these identifications were made after Stewart sorted out the cards. An additional nine were identified as Australian or British, and thus excluded from a study of "young American males." Two were determined to be Korean, and the remaining 25 were buried as Unknowns. Since 1999, JPAC has exhumed six of these 25 and identified four. These records thus provide an opportunity both to assess how well McKern's and Stewart's aging methods work on a known sample that was set aside from their analyses and to compare Stewart's casts, photographs, and data collection to the actual skeletal remains of some individuals. When their data cards and casts were used to generate age estimates for the 50 known individuals, the actual ages of 31 were within the estimated ranges. Six were younger (average of 1.55 years below the bottom limit of the estimate), while 13 were older (average of 1.67 years above the top limit of the estimate).

Finally, a longer-term project of collecting military records for the identified individuals will eventually provide additional biological information on the original 377 as well, including skeletal measurements and antemortem weights.



Anthropology Section - 2015

Reference:

1. McKern TW & TD Stewart 1957 "Skeletal Age Changes in Young American Males: Analysed from the Standpoint of Identification." Headqu QM Res and Dev Command, Tech Rep EP-45 Natick, MA
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Age Determination, War Dead, Skeletal Sample



Anthropology Section - 2015

A80 A Reassessment of McKern's and Stewart's Pubic Symphysis Aging Method

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After attending this presentation, attendees will understand how inter-observer error affects age estimates using the McKern and Stewart pubic symphysis method and the impact of such error on forensic casework.

This presentation will impact the forensic science community by drawing attention to limitations of a particular skeletal aging method.

In their 1957 study, *Skeletal Age Changes in Young American Males: Analysed from the Standpoint of Age Identification*, Thomas W. McKern and T. Dale Stewart presented a new “component system” of assessing age from the male pubic symphysis using a reference sample of plaster casts made from the symphyses of 349 American casualties from the Korean War.¹ In 1983, Katz and Suchey published a revised method of symphyseal age determination, a modification of Todd's system that condensed ten phases into six.² Subsequently, this six-phase method has become the standard in American forensic anthropology; however, in the Joint POW/MIA Accounting Command-Central Identification Laboratory (JPAC-CIL), the McKern and Stewart method is used when analyzing the remains of World War II and Korean War casualties, because they are drawn from the same population as McKern's and Stewart's reference sample. This reassessment of McKern's and Stewart's method looks at two questions: (1) how difficult is the method to apply properly; and, (2) how accurate is it when applied to samples drawn from the same population as the reference series?

For the first question, two data sets were assessed. The first is a table of phase scores made available by Lyle Konigsberg, which he and colleagues collected in 1999 from the Korean War symphyseal casts, using both McKern's and Stewart's and Todd's methods.³ Notably, Konigsberg et al. scored the same series of casts that McKern had scored to develop his method. The second is a series of 66 Korean War casualties identified since 2000 for whom full component scores were available.

McKern's discussion of the component system highlights the relationship between the three scores: I≤II≤III. He only recorded 21 combinations within the reference series (vs. 216 possible combinations), which he explained by this chronological relationship. Logically, Konigsberg et al. should have obtained the same combinations; if they did not, this indicates some degree of inter-observer error. However, they observed 21 combinations that violate the general chronological progression, indicating that they applied the method differently. This study rescored 162 of these casts and obtained the same total score on only 45. On 61 casts, this study scored two of the three components the same, and on 40 only scored one the same. On 33 casts, the casts were scored as further advanced than Konigsberg et al. (mean difference of 1.45), while on 84 the casts were scored as less advanced (mean difference of 2.06).

The CIL data set consisted of skeletal individuals who were not in the original reference sample, but were drawn from the same population. A similar pattern was evident: CIL analysts observed 15 of McKern's 21 patterns, as well as 13 not seen by McKern. In both series, scores of 0 appear less frequently than expected, particularly on the second and third components; this is probably due to the absence of reference casts for these values.

For the second question, the symphyseal casts of 50 individuals who were omitted from McKern and Stewart's sample but for whom ages are now available were scored and assigned age ranges to each based on summary component scores. Of the 50, the actual age of 31 individuals fell within the range identified by McKern and Stewart. Thirteen were older than predicted, with a mean difference of 1.67 years between the predicted maximum age and the actual age. Six were younger than predicted, with a mean difference of 1.55 years between the predicted minimum and the actual age.

These results indicate that the McKern and Stewart method is indeed difficult to use accurately, even for experienced physical anthropologists. Furthermore, while the age estimates that it produces are smaller than those of Suchey-Brooks, which is appealing in forensic cases, they may falsely exclude the actual age, particularly if the method is not accurately applied.



Anthropology Section - 2015

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1. McKern TW & TD Stewart 1957 "Skeletal Age Changes in Young American Males: Analysed from the Standpoint of Identification." Headqu QM Res and Dev Command, Tech Rep EP-45 Natick, MA
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Age Determination, Pubic Symphysis, War Dead



A81 Estimating Stature When Ancestry is Unknown: What Statistical Methods Work Best?

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After attending this presentation, attendees will gain an in-depth understanding of key sampling and hypothesis testing issues that arise when estimating new equations to assess stature from skeletal elements. In particular, it will demonstrate the convenience of incorporating outgroup comparisons in the development of stature equations, including the appropriate hypothesis tests to assess whether the regression lines differ significantly from those for other samples or groups. This strategy serves to prevent overfitting to the reference sample, which is not addressed by goodness-of-fit measurements such as correct classification percentages or error rates.

This presentation will impact the forensic science community by demonstrating that, contrary to traditional assumptions, pooled-ancestry stature equations can be more appropriate than population-specific equations obtained from a single sample.¹⁻³ This implies that in many instances stature can be accurately and precisely estimated from the femur and tibia even when the decedent's ancestry is unknown.

The study examines this hypothesis and proposes new methods and criteria to assess whether it is necessary to subdivide samples from the same or different populations to create new, specific stature equations. Equations for five modern adult skeletal samples separated by different levels of genetic distance are compared using Analyses of Covariance (ANCOVA) to test whether regression lines actually differ across groups.^{4,5} In the absence of such differences, a single, common regression line is appropriate to estimate stature for those groups. ANCOVA also provides weighted estimates for the slope and intercept, accounting for the slopes and range of variables in all sample groups. This method provides a criterion to decide if separate equations should be calculated for new "populations" and compares the regressions obtained from several samples to address the issue of sample bias.

Results demonstrate that despite differences in mean height, the relationship between female stature and lower limb bone length is constant across several populations, indicating that specific stature equations are not always required for each ancestral group. More precisely, in the present example, the ANCOVA approach serves to reduce the total number of female equations from twelve to five, since no significant differences were observed between several of the groups. The observed differences across male groups were slightly greater than those of their female counterparts, but the total number of equations for male groups could still be reduced significantly.

By presuming that populations are significantly different, forensic anthropologists sometimes create a false need to calculate new stature equations.⁶⁻⁸ The results of this study indicate that it is more beneficial to first compare different sample populations using ANCOVA to determine whether or not the groups are truly significantly different, thus requiring new, population-specific stature regressions.



Anthropology Section - 2015

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Stature Estimation, Unknown Ancestry, Analysis of Covariance



A82 The Issue of Age Estimation in a Modern Skeletal Population: Are Current Aging Methods Satisfactory for the Elderly?

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After attending this presentation, attendees will gain insight into the reliability of the current skeletal age-estimation methods tested on a modern cemetery population of known sex and age. The presentation will focus primarily on the issue of age estimation of elderly individuals for whom the identification of the correct age range seems more problematic. At the same time, an overview of the applicability of each method, based on the survival of the skeletal sites of application of the method, will be provided.

This presentation will impact the forensic science community by pointing out the advantages and the limitations of current methods of age estimation when approaching skeletal remains of elderly individuals.

The skeletal age estimation of individuals is one of the main tasks in forensic anthropology. From 1920 to the present, several methods have been developed, each with its limits, mean error, and age ranges in which that particular method proved to be more reliable. The main idea behind age assessment in adults is related to the analysis of the physiological degeneration of particular skeletal structures with age. The main issues with these procedures are due to the fact that they have not been tested on different populations and in different taphonomic contexts and that they tend to underestimate the age of older individuals. The methods currently used by anthropologists have in fact been standardized on archaeological and historical collections that may not completely reflect the characteristics of a modern population, especially from a demographic point of view. In addition, the increased life expectancy at birth has pointed out the need for further research on the estimation of age ranges in skeletal remains belonging to elderly individuals.

In the present study, the following methods were taken into account: Suchey-Brooks (symphysis pubis), Lovejoy (auricular surface of the ileum), Iscan (fourth rib's cartilaginous end), Meindl-Lovejoy (ectocranial sutures), Rougé-Maillart (acetabulum combined with auricular surface), and Beauthier (palatine sutures).

The purpose of this study was to test the applicability and the reliability of these methods on a contemporary population of skeletal remains of 165 elderly individuals of known sex and age (ranging between 50 and 98 years), exhumed 20 years after their burial. Although the skeletal remains were generally in good condition, some skeletal sites showed a lower survival due to taphonomic influences and consequently it was not always possible to test all the methods on the entire population. The results show that the methods with the highest percentage of applicability were Lovejoy (89%) and Rougé-Maillart (79%), followed by Suchey-Brooks (56%) and Meindl-Lovejoy (37%). Those with the lowest were Beauthier (36%) and Iscan (24%).

In regard to the age estimation accuracy, Rougé-Maillart (88%) and Lovejoy (82%) showed the best results in terms of the correct identification of the age ranges in which the chronological age of the individuals was included. These percentages are reduced to 70% with Suchey-Brooks (2σ), 64% with Beauthier, 46% with Meindl-Lovejoy, 45% with Iscan, and 20% with Suchey-Brooks (1σ).

Despite this, the only method that proved to be reliable when dealing with over-60-year-old individuals was Rougé-Maillart. The main limit of this method was due to the fact that the age ranges are too small (nine years). Therefore, at this time, the probability of associating an individual to the wrong age class is too high. The first step to improve the method could be the redefinition of the age classes in wider ranges without neglecting the need for further testing on a wider sample.

This research has shown how, for older adults, the study of both acetabulum and auricular surfaces may be more reliable for aging. This is also in accordance with the fact that auricular surface and the acetabulum are the areas that more frequently surviving taphonomic insult.

Forensic Anthropology, Age Estimation, Old Adults



Anthropology Section - 2015

A83 One Hundred Years Since Martin's Lehrbuch: Measurement Confusion and DCP 2.0

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After attending this presentation, attendees will appreciate that there have been many versions of measurement definitions published and neither the primary nor secondary definitions are always ideal, due to poor descriptions, inaccurate or incomplete translations, or definitions for measurements that can at times be impossible to take correctly.

This presentation will impact the forensic science community by serving as a reminder that metric standards are necessary but have been corrupted through various means and a new approach to standards must be taken for consistency.

Measurement standards and definitions are difficult to write clearly, explicitly, and unambiguously, but are necessary in order to use metric methods of forensic analysis. At the beginning of the 20th century, there were a number of different cranial measurement standards of varying quality, largely in French and German. International standards for cranial and postcranial measurements were established in Monaco in 1906 and in Geneva in 1912. In 1914, Rudolf Martin published his *Lehrbuch der Anthropologie* and established measurement standards that have persisted in one form or another to the present. His definitions have remained unchanged in newer editions posthumously published and include comments on subsequent measurement definitions, such as those from Howells.^{1,2} The interpretations of Martin's definitions have dominated most anthropological metric standards since.

However, Martin's definitions have never been translated fully into English; they are at times extremely opaque, such as his definition for the landmark ectoconchion. In some cases, his definitions are somewhat contradictory, referring to the most inferior and most superior points initially, then specifying techniques to obtain a maximum measurement, such as in the maximum length of the femur. In 1973, W.W. Howells modified and clarified craniometric landmarks and measurements from Martin and others. One often-overlooked anthropologist, Aurel von Török was far ahead of his time. In 1890, his *Grundzuege einer Systematischen Kranimetrie (Essentials of a Systematic Craniometry)* defined more than 100 landmarks and listed more than 5,000 measurements on the cranium. He also advocated using a "universal craniometer" that would calculate all standard measurements, all interlandmark distances, and all possible angles, which modern digitizers and computers finally can do.

Most knowledge of Martin's definitions is indirect, from the *Standards* of Buikstra and Ubelaker (BUS) or University of Tennessee's (UT) *Data Collection Procedures (DCP1)*.^{3,4} BUS was supposedly based on translations of Martin in DCP1 and a few secondary sources; however, many of the definitions leave out specific information on techniques, meaning a "maximum" measurement may not be the actual maximum measurement. Some subsequent publications cite the definitions in BUS and find a maximum length of a femur that is smaller than the bicondylar length, which is impossible if the full Martin definition is used. Other information in BUS is simply incorrect, such as the locations of dacryon and ectoconchion: their figure 41 actually illustrates maxillofrontale and frontomolare anterior. These and other errors are propagated in other osteology references works, some of which cite BUS. The DCP1 is largely a translation of Martin with secondary sources cited, but the DCP1 procedure for maximum long bone lengths, taken by moving the bone up and down, left and right, apparently comes from Hrdlicka's *Anthropometry*, which was not cited.⁵ Hrdlicka was likely influenced, directly or indirectly, by studying anthropometric technique in France under Manouvrier in 1896, to whom his book was dedicated, but Manouvrier's definitions are not as explicit as Hrdlicka's. Whatever the source for Hrdlicka's standards, they seem to be consistent with his stated goal of providing "simple, practical, well-tested instructions." Finding maximum measurements in such a fashion should be more consistent among measurers than basing measurements on the most superior and inferior points.

Measurement standards must also evolve as technology and equipment changes. For more than 100 years, the standard instruments included sliding and spreading calipers. With the advent of 3D digitizers and scanners, and geometric morphometric methods, the Howell's measurement definitions which use landmarks defined independently of the measurement taken, are geometrically better than Martin's, whose landmark locations sometimes depend on the measurement being taken.



Anthropology Section - 2015

Examples from Martin to BUS to DCP1 remind the forensic science community that measurement definitions need to be clarified, improved, and, if necessary, changed, especially in light of the *Daubert* decision and the 2009 National Academy of Sciences Report, *Strengthening Forensic Science in the United States: A Path Forward*.⁶ Because definitions can always be improved and augmented, and the nature of publication has changed, versioning is necessary. Electronic standards can easily be versioned, and can include textual descriptions, methodological comments, illustrations, images, and even video instruction. All are necessary to establish consistent and reliable standards. These forms of information and instruction will be integral parts of *Data Collection Procedures for Forensic Skeletal Material 2.0*, which will provide “simple, practical, well-tested instructions” based on empirical data.

References:

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3. Buikstra JE, Ubelaker DH. Standards for data collection from human skeletal remains. Fayetteville, AR: Arkansas Archaeological Survey, 1994.
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Craniometrics, Measurement Standards, Osteometry



Anthropology Section - 2015

A84 Evaluation and Reformation of Osteometric Data in Forensic Anthropology: The Foundations of DCP 2.0

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After attending this presentation, attendees will be aware of “problem” skeletal measurements and informed about error rates associated with measurements that interface with the FORDISC® program. This presentation will also introduce a new version of a popular laboratory manual: *Data Collection Procedures for Forensic Skeletal Material 2.0*.

This presentation will impact the forensic science community by addressing recommendations of the 2009 National Academy of Sciences (NAS) Report, *Strengthening Forensic Science in the United States: A Path Forward*. Specifically, the NAS Report stressed the need for “rigorous systematic research to validate the discipline’s basic premises and techniques.” This foundational research investigates the reliability, accuracy, and validity of forensic methods (Recommendation 3) and seeks to assist in the effort to establish valid and reliable methods and protocols that will serve as proficiency testing, training, and certification (Recommendation 6).

Many methods and techniques in forensic anthropology employ osteometric data as their basis, but little work has been done to investigate the error rates associated with these measurements. Metric data form the basis of the FORDISC® program used to develop a biological profile of unknown remains. Metric data also provide a straightforward means to quantify error; therefore, their use in forensic casework is increasingly common, particularly in light of the judicial atmosphere since *Daubert* (1993). Many components of a forensic anthropology case report are derived from osteometric data (i.e., sex, ancestry, stature). As such, error associated with any method that employs these data is compounded by the error that is inherent in the measurement(s), whether a function of the observer, the instrumentation, or both. Documented error rates will provide a foundation from which to proceed with metric estimations of the biological profile components, as well as method development.

Data collection was done on William M. Bass Donated Collection skeletons at the University of Tennessee. Four observers took 78 standard (34 cranial and 44 postcranial) and 20 less commonly used measurements on a sample of 50 skeletons using sliding calipers, spreading calipers, an osteometric board, and a mandibulometer; one observer repeated the measurements on the 50 skeletons three times with a two-month time lapse between sessions. Instruments were calibrated daily with a calibration rod. Absolute and relative Technical Error of Measurement (TEM), Scaled Error Index (SEI), percent error, and one-way and repeated measures Analysis of Variances (ANOVAs) were used to assess the degree of inter-observer and intra-observer error for each measurement.

Results indicate that maximum lengths and breadths have the lowest inter- and intra-observer values across the board (SEI values <2.0, relative TEM values <4.0, percent error <2%, and non-significant ANOVAs at $\alpha=.05$). The ten most reliable measurements are maximum femur length, bicondylar femur length, maximum humerus length, maximum fibula length, bizygomatic breadth, maximum ulna length, maximum clavicle length, maximum radius length, biauricular breadth, and scapula height. The 12 most unreliable measurements are ischium length, mastoid height, breadth of mandibular body, pubis length, interorbital breadth, vertical diameter of clavicle, proximal epiphyseal breadth of tibia, sagittal diameter of clavicle, transverse diameter of first sacral segment, transverse subtrochanteric diameter of femur, anterior sacral breadth, and A-P subtrochanteric diameter of femur (SEI values >2.0, relative TEM values >4.0, percent error >2%, and significant ANOVAs at $\alpha=.05$). Alternative maximum/minimum midshaft diameters were assessed as options to positionally dependent measurements such as sagittal and vertical clavicle diameters, dorso-volar and transverse ulna diameters, diameters at the nutrient foramen of the tibia, and sagittal and transverse radius diameters. The alternatives for the clavicle and ulna were found to be considerably more reliable than their aforementioned counterparts.

These results support an earlier report on inter-observer error of 22 postcranial measurements (Adams and Byrd) and have implications for forensic anthropology practice and research.¹ At the extreme, this study suggests that some measurements should be abandoned or replaced with more reliable alternatives if they are to be used in case analyses and method development. Others must be clearly explained and accurately translated in the available laboratory manuals. *Data Collection Procedures 2.0* will provide error rates for all measurements that interface with the FORDISC® software and include more reliable options for “problem measurements.” In addition, the new manual will clarify problematic definitions, include updated images, and interface with upcoming versions of the Forensic Data Bank and FORDISC® software.

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Anthropology Section - 2015

Reference:

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Osteometric Data, Observer Error, FORDISC®



A85 Investigations Into Age-Related Changes in the Human Mandible

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The goal of this presentation is to examine age-related osteometric changes in the adult mandible.

This presentation will impact the forensic science community by demonstrating that, despite previous claims, mandibular shape changes are more correlated with tooth loss than advancing age.

Recently, Shaw and colleagues argued that the adult mandible changes significantly with age. Using a sample of Computed Tomography (CT) scans from 60 males and 60 females, Shaw et al. examined six mandibular measurements and found that as both male and female individuals aged, there were significant decreases in ramus height, mandibular body height (equivalent to chin height using the Forensic Data Bank definitions established by Moore-Jansen et al. — the latter term will be used here for consistency), and the length of the mandibular body, whereas the mandibular angle increased significantly.^{1,2} However, Shaw et al. did not attempt to control for antemortem tooth loss, nor did they utilize the full set of standard mandibular measurements.

In order to more fully investigate the purported mandibular age-related changes, this study used a sample of 319 individuals from the W.M. Bass Donated Skeletal Collection (University of Tennessee (UT) Knoxville) and the Robert J. Terry Anatomical Skeletal Collection (Smithsonian National Museum of Natural History (NMNH)). A single observer collected the ten standard mandibular measurements and scored antemortem tooth loss according to the Eichner Index, which classifies tooth loss based on the presence of occlusal pairs. The sample consisted of 105 females with ages ranging from 17 to 99 years and 214 males with ages ranging from 16 to 84 years (total n=319).

A two-way Analysis of Variance (ANOVA) was used to determine if age, the Eichner Index, and the interaction between the two variables had an effect on the mandibular measurements. The current study found no significant difference between age and any of the standardized mandibular measurements ($p > 0.05$, in all instances); however, significant differences were noted between the Eichner Index and three mandibular measurements: chin height ($p < 0.001$), corpus height ($p < 0.001$), and the mandibular angle ($p = 0.031$). Additionally, no interaction was noted between age and the Eichner Index for any mandibular measurement.

The results indicate that no significant osteometric changes occurred in the mandible with increased senility, including ramus height, chin height, mandibular body length, and mandibular angle, as had been previously reported by Shaw and colleagues; however, significant differences between the Eichner Index and chin height, mandibular angle, and body height were found, suggesting that changes in the mandibular dimensions are more highly correlated with antemortem tooth loss than with age. It is widely known that socioeconomic gaps leave a large portion of the population without access to proper dental care; as such, antemortem tooth loss and subsequent mandibular shape changes may begin at an earlier age in these individuals. Alternately, such morphological changes may not occur or may be significantly delayed for those with better access to dental care. Given these findings, it may be necessary to re-evaluate previous notions of mandibular osteometrics and age, as these may be more a consequence of dental health than increased senility.

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2. Moore-Jansen PH, Ousley SD, Jantz RL 1994. Data Collection Procedures for Forensic Skeletal Material. Report of Investigations No. 48. The University of Tennessee. Knoxville, Tennessee.

Mandible, Age Estimation, Osteometrics



A86 Accuracy of Estimating Age From Cervical Vertebrae and Mandibular Molar Maturation

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After attending this presentation, attendees will be able to: (1) recognize the potential of Cervical Vertebral Maturation (CVM) as a method of estimating age (particularly when third molars are mature or absent); (2) appreciate the difficulty in assessing biological age in juveniles in order to estimate chronological age; and, (3) understand the need for additional methods to estimate age in young adults between 16 and 21 years of age from radiographs.

This presentation will impact the forensic science community by emphasizing the importance of the fact that age estimation is required for identification of disaster victims and forensic cases as well as minors without documentation and age-disputed asylum seekers.

Shape changes during the maturation of cervical vertebrae are used to assess the pubertal growth spurt and are a potential method to estimate chronological age. The goal of this pilot study was to assess the accuracy of estimating age by CVM and mandibular second (M2) and third (M3) molars in a group of males. The sample consisted of lateral cephalograms of 60 boys aged 10 to 15 years from the Bolton-Brush online collection. A new method of cervical vertebral growth based on the changing size and shape of C2 to C6 was devised using raw data of 69 boys (aged 9 to 15 years) in Lamparski.¹ CVM ages were calculated by transition analysis. Dental age was calculated using molar ages from Liversidge.² The mean difference and absolute mean difference between CVM age and dental ages and chronological ages was calculated. CVM and molar tooth stage assessment reliability was assessed by duplicate readings from previous studies.

Results show that CVM age could only be calculated for 48 boys and those with a bone age of “10 years” could not be aged because of the minimum age of Lamparski’s sample. The number of individuals with M2 developing was 37 and with M3 developing was 43. Thirty-five boys had results for CVM, M2, and M3.

Results for accuracy of age estimation show that the mean difference using CVM was -1.10 years (SD 0.85, N=48), mean difference using for M2 was -0.86 (SD 0.69, N=38) and M3 was -0.37 (SD 0.98, N=43). Absolute mean using CVM was 1.21 year, M2 was 0.91 and for M3 was 0.83. These findings show that mandibular molars were more accurate in estimating age than this method of CVM. It is concluded here that there is a compelling need to develop an appropriate CVM age estimation method particularly around the age threshold of 18 years. This approach could feasibly extend the age range for young adults during which age can be reliably estimated.

References:

1. Lamparsky DG. Skeletal age assessment utilizing cervical vertebrae. Pittsburgh: University of Pittsburgh; 1972.
2. Permanent tooth formation as a method of estimating age: HM Liversidge Karger Publishers 13, 153-157

Cervical, Dental, Age



A87 Mitochondrial DNA (mtDNA) Mutations as a New Approach for Age-at-Death Estimation

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After attending this presentation, attendees will consider the possibility of using mitochondrial DNA mutations as an alternative to classical anthropological methods for age-at-death estimation.

This presentation will impact the forensic science community by introducing a potential quantitative indicator of age, demonstrating its accuracy of age-at-death estimation in two Spanish populations.

Age-at-death estimation is one of the fundamental parameters in the identification of human remains, particularly in mass disaster scenarios where skeletons are often incomplete, complicating the correct identification of the victims. Teeth are frequently preserved long after all other tissues have disappeared and are often used to estimate characteristics like age-at-death.

There are several approaches to age estimation based on dental development. In forensic anthropology, the Lamendin technique and its variants are non-invasive methods of age-at-death estimation; however, these methods can only be applied to single-rooted teeth and their accuracy is not guaranteed due to differences in population-specific references. In contrast, the new methodologies for age estimation are based on the natural process of aging, which causes alterations of tissues and organs on different biochemical levels. One of these alterations is the increase in the production of free radicals with age, playing a key role in the degenerative processes of senescence. The increase in free radicals, oxidative stress, induces an accumulation of non-repaired lesions in mitochondrial DNA (mtDNA). Some studies have pointed out the relationship between mtDNA mutations and age in different tissues. These studies are potentially interesting in forensic identification because they could help to improve the estimation of age-at-death.

Since teeth are the hardest tissue of the human body and one of the most abundant types of biological remains available in forensic cases, the goal of this study is to evaluate the mutations in mtDNA from dentin and pulp and their relation with the age, and assesses the reliability of this methodology in two Spanish populations.

Thirty healthy erupted third molars from Asturias, NW Spain, and 30 healthy erupted third molars from Cataluña, NE Spain, (aged 20-70 years) were collected from dental clinics. The Smithsonian Institution's ethical committee approved all procedures related to experimentation with human subjects. The teeth were cleaned and the enamel and cementum removed. The dentin was isolated, mechanically ground, and divided in aliquots of 200mg each; pulp also was isolated. The dentin and pulp were submitted for DNA extraction and quantification. As a control of correct human DNA extraction, the Amelogenin gene was amplified. To study the mtDNA mutations, Hypervariable region 2 (HV2) of the mitochondrial D-loop was chosen. This region was analyzed by Real Time Polymerase Chain Reaction (RT-PCR). Each sample was tested in triplicate. The analysis of relative gene expression data was calculated using the $2^{\Delta\Delta CT}$ method.

Quantitative-PCR (qPCR) results were similar in the two populations. There was an age-dependent decrease in the amplification of HV2 region in dentin and some variation in pulp. Using a regression analysis, a negative significant strong linear correlation was found between the mtDNA amplification and the age in dentin, with almost the same value in both populations. In contrast, a correlation was not found between mtDNA amplification and the age in pulp. The reason for this variation is the projection of the odontoblastic processes from pulp to dentin, which houses numerous mitochondria. As a result, the majority of the oxidative stress is generated in the dentin, making it possible to relate with age.

The findings from this research provide a new quantitative tool for estimating age-at-death which, in combination with traditional age markers, could improve identification accuracy in forensic cases. Future research may be able to expand on these results, using different types of teeth, analyzing different populations, and extending the age range.

Age-at-Death, Anthropological Methods, Mitochondrial DNA Mutations



Anthropology Section - 2015

A88 A Test of the Passalacqua Sacral Age-Estimation Method in a Japanese Sample

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After attending this presentation, attendees will understand the applicability of the Passalacqua sacral age-estimation method to individuals of Asian (Japanese) ancestry.

This presentation will impact the forensic science community by providing error rates for a new age-estimation method in a sample different from that of the reference method and suggesting some revisions to the method.

Age estimation is an important component of the biological profile and provides key information in the identification of unknown skeletal remains. It is more difficult to estimate age in adults, but methods that are able to combine developmental and degenerative changes appear to be more successful than those that do not. It is important to test methods developed on certain populations in different groups, as the composition of the reference sample may affect the ability to accurately estimate age. To date, there is a dearth of data in United States research that concerns individuals of Asian ancestry.

Age estimation from the sacrum has generally focused on two areas: fusion of the adjacent sacral bodies and the sacral auricular surfaces. The method developed by Passalacqua scores seven developmental and degenerative traits of the sacrum and is based on individuals of African and European American ancestry from two American skeletal collections (Hamann-Todd and William M. Bass). Fusion of the S1/S2 and S2/S3 bodies, changes of the sacral auricular surface and apices, and porosity of the sacral auricular surface are scored as 1-incomplete/absence or 2-complete/presence. S1 ring fusion is scored as 1-incomplete, 2-fused, or 3-absorbed. The resultant scores are combined to produce a sequential seven-digit code with an associated phase and descriptive statistics. Differences in ancestry and sex were not found in the reference method, but it is currently unknown whether the method can be applied to individuals of Asian ancestry.

Sacra from Chiba and Jikei Universities Medical School documented collections were scored for the seven traits described by Passalacqua as well as three additional traits: overall bone quality, changes to superior articular facets, and S1 superior vertebral border (n=205; minimum age=17 years, maximum age=95 years). Individuals were pre-selected to create a distribution similar to that of the reference sample, but age was unknown at the time of data collection. Inaccuracy, bias, percent correct for 68% and 95% intervals, and Spearman's rho for assigned phase and known age were calculated. A Kruskal-Wallis test was run to check for differences between the sexes. To test the use of a summary score (all trait scores added) versus the seven-digit code/phase system, Spearman's rho was calculated for known age and seven-trait summary score and for age and ten-trait summary score. A random sample of 35 individuals from the Chiba collection was scored again to examine intra-observer error, and Goodman and Kruskal's gamma was calculated (two trials, ten traits). Statistics were run in computing and graphics programs and R version 2.14.2.

For the Japanese sample, the method performed with an inaccuracy of 11.85 years and bias of 0.37 years, indicating a slight tendency to overage. Older phases show increased inaccuracy as compared to younger, with a tendency to underage younger individuals and overage older individuals. For all but one phase, inaccuracy was higher in the Japanese sample than the reference method. When using 68% intervals, 54.2% of the individuals in this sample were classified correctly; the percent increased to 91.7% with 95% intervals. Of the 205 individuals scored, 61 were assigned codes that were not given in the reference article. For these individuals, a higher component score was interpreted as being older and the next "oldest" code/phase was assigned. To test if this affected overall correct classification, these individuals were eliminated from calculations; correct classification remained similar at 52.1% using the 68% interval and 93.8% using the 95% interval. There were no statistically significant differences in phase assignment between males and females (p=0.36). Spearman's correlations between known age and phase, known age and seven-trait summary score, and known age and ten-trait summary score were 0.62, 0.69, and 0.76, respectively. Goodman and Kruskal's gamma values for all but one of the ten traits were 1 or very close to 1, indicating excellent agreement between trials; bone quality was the exception (gamma=0.48).



Anthropology Section - 2015

The results of this study indicate that the Passalacqua sacral age estimation method can be applied to Japanese individuals, though with slightly less accuracy than African and European American individuals. This difference may also be due to test samples performing less accurately than reference samples. The use of the 95% interval is more appropriate, but given the wide age intervals, this method should be used in conjunction with other age-estimation methods. The method is easy to apply and the use of binary scores reduces scoring discrepancies between multiple trials. Revisions to this method should consider the addition of additional degenerative traits and the use of a summary score rather than trait code.

Age Estimation, Sacrum, Error



A89 The Effects of Parturition on Pelvic Age Indicators

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After attending this presentation, attendees will understand the need for consideration of parity when assessing age of the pelvis in a recent forensic sample from the United States, the necessary methods utilized on the pelvis, the process and reasoning behind the collection of data, and subsequent statistical analysis employed in this study.

This presentation will impact the forensic science community by indicating whether parturition affects an accurate assessment of age on the pelvis in a recent forensic sample from the United States and if it needs to be considered as an addition to the methods employed in human identification.

Estimating age from skeletal remains is a critical component of the biological profile of an individual. To date, there are different methods used on select parts of the skeleton to assess the age of the individual, and currently the pelvis is relied upon heavily to obtain accurate and reliable age ranges.¹⁻³ Other researchers have found that age-related changes follow different trends in males and females, with parity presented as one of the possible causes for such differences.^{1,4-6} There is reason to believe that parturition may increase the rate at which the areas of interest of the pelvis degenerate; however, this hypothesis has yet to be formally tested on a recent skeletal collection. The purpose of this study, therefore, is to assess the effects of parturition on the pubic symphysis and auricular surface and determine whether it influences the physiological age of the individual enough to cause an inaccurate estimate of the chronological age.

Data were collected from the William M. Bass Skeletal Collection located at The University of Tennessee, Knoxville. This is a collection of recent forensic skeletons with known age-at-death, ancestry, sex, and medical background. The features of the pubic symphysis were noted and matched with the best-fitting phase in both the Suchey-Brooks and Todd pubic symphysis scoring systems.^{2,7} Next, the features of the auricular surface were noted and matched with the best-fitting phase in the system presented by Lovejoy and colleagues and were individually scored, resulting in a composite score following the method proposed by Buckberry and Chamberlain.^{3,4} Therefore, others attempting to replicate this research will be able to reliably assess these areas. Time was designated at the beginning of the second and third days to employ the test-retest method to calculate the intra-observer error rate and ensure reliability of these assessments.

In this study, a statistical comparison was made between females who have given birth and those who have not to determine whether this process affects the rate of degeneration of the areas of interest of the pelvis. A transition analysis, also known as a cumulative probit analysis, was conducted on the data in order to establish the age-at-transition distributions between the stages of each age-estimation method. The results were then compared between the males and the parous and nulliparous female groups. The purpose for this comparison is to observe whether the age-at-transition distributions differ between sexes and/or the two groups, with the focus being on whether the parous group illustrates a difference in rate of degeneration, or transition to subsequent observable stages, when compared to the nulliparous group.

The study contained 434 individuals (males: 234/females: 200/parous: 157/nulliparous: 43). The data was entered into the statistical software program R version 3.0.2. The transition analysis produced significantly different results between parous and nulliparous females using the pubic symphysis but not when using the auricular surface. The current research suggests that parturition affects the pubic symphysis and not the auricular surface when determining age-at-death. Moreover, the male group and the nulliparous female group transition around the same age, while the parous females transition at an earlier age. The applicability of taking parturition into consideration when assessing the age of females for use in human identification in modern forensic cases will be discussed.



Anthropology Section - 2015

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Forensic Anthropology, Age Estimation, Pelvis



A90 Sex Estimation From the Vertebral Foramen of the Seven Cervical Vertebrae: An Analysis of Greek and Portuguese Skeletal Populations

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After attending this presentation, attendees will recognize the potential value in utilizing the vertebral foramen of the seven cervical vertebrae to estimate sex of unknown individuals. Skeletal remains are commonly exposed to a variety of taphonomic processes, including destructive environmental factors, disarticulation, and scattering due to animal scavenging, which hinder skeletal sex estimation methods. The cervical vertebrae exhibit strong architectural structural integrity resulting in good postmortem preservation that increases the likelihood for successful adult sex estimation when other sexually diagnostic elements of the skeleton are absent or badly preserved.

This presentation will impact the forensic science community by providing alternative means of correctly identifying the sex of unknown individuals from the cervical vertebrae and will be useful in cases such as mass disasters when only fragmented remains are available for examination.

The purpose of this study was to understand the relationship between sex and the cervical vertebral foramen in two White skeletal populations. A total of 295 individuals (157 males and 138 females) were selected from two cadaveric collections: (1) the University of Athens Human Skeletal Reference Collection, Greece (N=135); and, (2) the Luis Lopes Skeletal Collection, National Museum of Natural History, Lisbon, Portugal (N=160). Inclusion criteria consisted of individuals from 20 to 99 years of age, the presence of at least five complete cervical vertebrae, and vertebrae free of skeletal pathologies. The maximum vertebral body height (CHT) and anterior-posterior (CAP) and transverse diameters (CTR) of the vertebral foramen were recorded for each of the seven cervical vertebrae (excluding CHT for C1). Measurements were taken from the superior aspect of the vertebral foramen using a digital Vernier caliper accurate to 0.01mm.

The data were then subjected to statistical analyses; the mean, range, and standard deviation were calculated for each vertebra. Pearson's correlation coefficients were calculated to measure the correlation between CHT, CAP, and CTR measurements and stature. Two-sample *t*-tests were used to test the presence or absence of sexual dimorphism in the Athens and Lopes collections. Two-sample *t*-tests were also used to test whether the mean vertebral measurements for males and females exhibited statistical differences between the two White populations. Canonical discriminant function coefficients were used to develop formulas to estimate sex from the vertebrae that exhibited sexual dimorphism.

The results of this investigation showed that sexual dimorphism is exhibited in the CHT and CTR measurements between males and females with means greater in the male population in all cervical vertebral segments. No statistically significant differences were observed in the CAP measurement between males and females. There were no statistically significant population differences between vertebral measurements in the Athens population compared to the Lopes populations; therefore, males and females from both populations were grouped into one large sample population.

When examining the classification results to assess which vertebrae more accurately estimated sex, a combination of all three measurements (CHT, CAP, CTR) from all seven vertebrae (C1-C7) performed best with 84.9% correct male estimation and 83.3% correct female estimation. Excluding the irregular C1 and C2 vertebrae, classification results correctly estimated males (82.9%) and females (81.7%). When removing the irregular vertebrae and the transitional C7 vertebra, C3-C6 correctly estimated males (80.2%) and females (78.2%). The strongest sexually dimorphic indicator was the CHT measurement followed by CTR in all seven vertebrae with CAP exhibiting minimal statistically significant dimorphism. Utilizing CHT and CTR measurements for vertebrae C3-C7, males and females are correctly classified with accuracies of 84.8% and 80.6%, respectively. Classification using the two measurements of the vertebral foramen (CAP, CTR) results in low accuracies for vertebrae C3-C7 with 71.3% correct male and 67% correct female estimations. The most dimorphic measurements were C1AP, C2HT, C2TR, C3HT, C5HT, C5TR, and C7TR with 79.4% correct male estimation and 84.9% correct female estimation using these seven features. Sex estimation could not be performed on individual vertebrae due to poor male and female classification accuracies that ranged from 58.9% to 74.4%.



Anthropology Section - 2015

This research has shown that sexual dimorphism is present in the vertebral foramen of the seven cervical vertebrae; however, the discriminant functions equations derived from the two vertebral foramen dimensions (CAP, CTR) are of limited use because of the low correct sex estimation accuracies. The CHT measurement must be included in the equations to achieve higher accuracies for sex estimation. Also, including all seven vertebrae in the discriminant functions will result in higher accuracies for sex estimation.

Forensic Anthropology, Sex Estimation, Cervical Vertebrae



Anthropology Section - 2015

A91 The Use of Femoral Neck Axis Length to Estimate Sex and Ancestry

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After attending this presentation, attendees will be familiar with the Femoral Neck Axis Length (FNAL) measurement as well as how FNAL can be used to estimate sex and ancestry from skeletal remains.

This presentation will impact the forensic science community by providing a new, simple, reliable method of sex estimation using the FNAL measurement, including an equation that can be used to estimate sex from FNAL with ~86% accuracy.

Having multiple reliable methods of estimating sex and ancestry from various skeletal features increases the likelihood of identifying an unknown individual from skeletal remains. Measurements of the proximal femur have received some attention in the forensic anthropological literature for sex and ancestry estimation, due in part to the survivability of this region in forensic contexts as well as noted quantifiable variation. FNAL is a measurement of the proximal femur that represents the distance from the base of the greater trochanter (the point directly inferior to the greatest lateral projection of the greater trochanter) to the apex of the femoral head. FNAL and related hip measurements (typically obtained from Dual-Energy X-Ray Absorptiometry (DXA) scans) are often utilized by bone densitometrists and other skeletal health experts in assessing hip fracture risk in living individuals and have been shown to vary by sex and ancestry. This research measured FNAL directly from skeletonized remains and investigated its potential use in forensic anthropological applications to estimate sex and ancestry of unknown individuals.

FNAL was measured on 286 femora from skeletally mature adults, free of visible anomalies potentially affecting the measurement. The sample included female and male American White, American Black, and Native American skeletons. One-way Analysis of Variances (ANOVAs) were used to evaluate the relationships between FNAL and sex and ancestry. Multiple univariate Discriminant Function (uDFA) and Logistic Regression (LR) equations were calculated to test the efficacy of FNAL for sex and ancestry estimation.

A random subset of 50 femora was measured by a second observer for the purpose of inter-observer error assessment. Results showed low inter-observer error in the measurement, with a Technical Error of Measurement of 0.33mm and a Coefficient of Reliability of 0.99, indicating that the FNAL measurement is well defined, easily recognizable, and reproducible.

Significant differences in FNAL were found between females and males, with male FNAL being significantly larger than female FNAL. An LR model using FNAL and sex-pooled samples performed very well. For an individual of unknown sex and ancestry, the following LR equation is applicable: $Sex = -3.89 + (FNAL) * 0.0426$. A negative value indicates that the unknown is female and a positive value indicates that the unknown is male. Using this equation, sex will be correctly classified in ~86% of all cases.

Significant differences in FNAL were found between American Black, American White, and Native American groups, with American Whites having the largest FNAL, followed by American Blacks, then by Native Americans. Post-hoc comparisons showed that American White FNALs are not statistically different from American Black FNALs, but both are significantly larger than Native American FNALs; however, correct classification of ancestry was found to be much lower and more complex than for sex, and the value of FNAL to ancestry estimation is currently considered limited. A uDFA performed moderately well (57% cross-validated correct classification) at classifying the ancestry of males and may therefore have some utility in certain forensic contexts.

FNAL is a measurement that has not been previously utilized by anthropologists, but has been shown to vary significantly by sex and ancestry. This research shows that sex estimation using FNAL measured from skeletonized femora is highly reliable, correctly predicting sex at a rate of ~86%. Further analyses with larger samples of additional ancestral groups may help clarify the relationship between FNAL and ancestry.

These results highlight the benefits of information sharing and collaboration between forensic anthropologists and other scientists and skeletal health experts.

Femoral Neck Axis Length (FNAL), Sex Estimation, Ancestry Estimation



Anthropology Section - 2015

A92 Morphoscopic Trait Expression Within and Among Hispanic Populations

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After attending this presentation, attendees will understand the frequency distribution of morphoscopic traits within two Hispanic populations, the utility of those traits for assessing ancestry at the regional population level, and the necessity of population-level ancestry assessments.

This presentation will impact the forensic science community by providing quantitative data on the distribution of morphoscopic traits for multiple Hispanic populations and a novel method for ancestry assessment using a forensic dataset.

Establishing the ancestry of human remains located along the United States-Mexico border is not a straightforward affair. The Latin American Diaspora is increasing the number of deaths along the United States southern border with Mexico, and consequently, along the major borders between Mexico and its southern neighbors. In the United States, the ancestry of these individuals is generally identified as Hispanic, a term that seemingly covers an intentionally large number of Spanish-speaking (cultural) groups, although doing so without justification in many instances. Previous authors identified craniometric differences among various populations recovered along these borders; however, limited research has focused on the differences in morphoscopic trait expression between Mexican, Guatemalan, and other Latin American groups and the utility of those traits for the assessment of ancestry.

This study evaluated population variation of eight cranial morphoscopic traits using samples of known Southwest Hispanics ($n=72$), Guatemalans ($n=106$), American Blacks ($n=146$), and American Whites ($n=218$). This study applied the Support Vector Machine (SVM) method to build a prediction model based on a sub-sample (20%) of the data; the remainder of the data was used as a test sample. SVMs are supervised learning models for data analysis and pattern recognition, useful for classification purposes. The model searches for a linear boundary (the support vector) between groups using the observations situated between groups, rather than using a centroid value (as in discriminant function analyses). When a linear boundary is not possible — which is often the case with biological data — a kernel trick is used to create non-linear classifiers to best fit the model. SVMs perform well for datasets that are non-linear, sparse, or have high-dimensionality, which are all issues that can occur when working with categorical variables in classification models.

The SVM approach effectively differentiated between the four groups with correct classification rates between 72% (Guatemalan group) and 94% (American Black group). However, when the Guatemalan and Southwest Hispanic samples were pooled, the same model correctly classified all groups with a higher degree of accuracy (American Black=96%; American White=77%; and the pooled Hispanic sample=91%). This study also identified significant differences between the two Hispanic groups in six of the eight traits using univariate statistical tests. These results speak to the unique population histories of these samples and the current use of the term “Hispanic” within forensic anthropology. Finally, it is argued that the SVM can be used as a classification model for ancestry estimation in a forensic context and, as a diagnostic tool, may broaden the application of morphoscopic trait data for the assessment of ancestry.

Morphoscopic trait analysis can be used to assess ancestry, both at the population level and using larger, geographic-based (grouped) ancestries. Although Guatemalan and Hispanic individuals differ significantly in the distribution of character states, combining these two groups under the umbrella-term “Hispanic” is useful in the United States, where ancestry assessments using broad terms are generally sufficient for the identification process.

Ancestry, Morphoscopic Traits, Support Vector Machines



Anthropology Section - 2015

A93 Estimating Ancestry From the Postcrania of Modern South Africans

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After attending this presentation, attendees will understand postcraniometric variation among modern peer-reported Black, White, and Colored South Africans and the statistical techniques employed to identify differences and similarities within and among South African groups.

This presentation will impact the forensic science community by contributing to the knowledge of human variation within a modern South African population and by evaluating the potential of the postcranial skeleton for the estimation of ancestry.

Several successful craniometric approaches have been developed to facilitate the estimation of ancestry; however, the cranium is not always available for analysis, emphasizing the need for postcranial alternatives. The postcranial skeleton is frequently labeled as too variable and unreliable to provide an accurate assessment of ancestry. Yet numerous studies utilize the postcrania for sex and stature estimation, where the a priori knowledge of population affinity results in higher accuracy. Thus, the presence of postcranial differences observed among populations when investigating other biological parameters inherently demonstrates the potential for the estimation of ancestry. The purpose of this study was to quantify postcranial variation among modern, peer-reported Black, White and Colored South Africans. A series of 39 standard measurements were taken from 11 postcranial bones, namely the clavicle, scapula, humerus, radius, ulna, sacrum, pelvis, femur, tibia, fibula, and calcaneus. The sample consisted of 360 modern South African individuals (120 Black, 120 White, and 120 Colored) from the Pretoria Bone and Kirsten Collections housed at the University of Pretoria and the University of Stellenbosch, respectively. Group differences were explored with Analysis of Variance (ANOVA) and Tukey's Honestly Significant Difference (HSD) test. The group means were used to create univariate sectioning points for each variable indicated as significant ($p < 0.05$) with ANOVA. Where two of the three groups had similar mean values, the groups were pooled for the creation of the sectioning points. Multivariate classification models were employed using Linear and Flexible Discriminant Analysis (LDA and FDA, respectively). Classification accuracies were compared to evaluate which model yielded the best results.

The results demonstrated variable patterns of group overlap. Black and Colored South Africans displayed similar means for breadth measurements, and Black and White South Africans showed similar means for the maximum length of distal limb elements. The majority of group variation is attributed to differences in size and robusticity, where White South Africans are overall larger and more robust than Black and Colored South Africans. Classification accuracies for the univariate sectioning points ranged from 43% to 87%, with iliac breadth performing the best; however, as groups were pooled when overlap was observed between two of the three groups, the majority of the sectioning points can only classify individuals into two groups rather than three. Multivariate bone models created using all measurements taken per bone resulted in accuracies ranging from 46% to 62% (LDA) and 41% to 66% (FDA). Multivariate subsets consisting of numerous different measurement combinations from several skeletal elements achieved accuracies as high as 85% (LDA) and 87% (FDA).

Ultimately, the best results were achieved using combinations of different variables from several skeletal elements. Although both Black and Colored South Africans present with narrow diaphyses and pelvis, the longer limbs of Black South Africans distinguish them from the Colored group. Both the Black and Colored groups can be discerned from White South Africans, who have a combination of long limbs, robust diaphyses, and large pelvis. The multivariate models yielded overall better results than the univariate approach, as the inclusion of more variables is generally better for maximizing group differences. Furthermore, FDA achieved higher accuracies than the more traditional approach of LDA. Despite the significant overlap among the groups, the postcranial skeleton has proven to be proficient in distinguishing the three groups. Thus, even in a heterogeneous population, a multivariate postcraniometric approach can be used to estimate ancestry with high accuracy.

Postcraniometric, Discriminant Analysis, Human Variation



A94 Postcranial Assessment of Sexual Dimorphism Among Modern South Africans

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After attending this presentation, attendees will better understand the pattern expression of sexual dimorphism in the postcranial skeleton of modern South Africans and the effect this variation has on correct classification. Different multivariate statistical methods to estimate sex, namely Linear Discriminant Analysis (LDA), Flexible Discriminant Analysis (FDA), and logistic regression, are highlighted.

This presentation will impact the forensic science community by contributing to knowledge on variation in sexual dimorphism in the postcranial skeleton, the types of statistical analyses available to assess sexual dimorphism, and to the improvement of postcranial sex estimations.

Recently, a multivariate approach using the postcrania to estimate sex achieved higher accuracies than the commonly used cranium, thereby recommending postcranial sex estimation in forensic case analysis.¹ In South Africa, sex-estimation techniques using long bones only include univariate or bone-by-bone models, which do not acknowledge sexual dimorphism in other elements and, as expected, represent an outdated statistical approach. The purpose of this study was to evaluate accuracies of sex estimation in the postcrania of modern South Africans using multivariate statistics and to compare pattern expression of sexual dimorphism in Black, White, and Colored groups. Additionally, FDA, a fairly new method to physical anthropology, was compared to two commonly used statistical techniques (LDA and logistic regression).

A total of 360 South African Black, White, and Colored individuals (equal sex and ancestry) were assessed. Colored South Africans are a peer-reported group unique to South Africa that received genetic contributions from a number of different populations from around the world.² All skeletal material was obtained from the Pretoria Bone Collection, University of Pretoria and the Kirsten Collection, University of Stellenbosch, in South Africa. Both collections are cadaveric in origin and are mainly comprised of donated or unclaimed, albeit known, individuals.³

Symmetric percentage differences (sympercents) expressed sexual dimorphism and were compared in the three South African groups.⁴ Three classification methods assessed the 39 standard measurements taken from 11 postcranial bones. The creation of different bone models and a variety of multivariate models revealed the potential of a multivariate technique. Comparisons of LDA, FDA, and logistic regression indicated which model provided the greatest discriminatory power between sex and sex-ancestry groups.

All measurement means, except sacral breadth, were larger in males than females. South African Coloreds showed the greatest differences between the sexes for the most measurement (20 of 39). Black males and females presented with the highest levels of sexual dimorphism for 13 of 39 measurements. South African Whites only showed the highest degree of sexual dimorphism for six of 39 measurements. Overall, the most sexually dimorphic skeletal elements included the anterior-posterior and vertical diameters of the clavicle, the anterior-posterior diameter of the radius, the humeral minimum midshaft diameter, and the dorso-volar and transverse diameters of the ulna.

Multivariate classification accuracies ranged from 75%-90% (LDA) to 75%-91% (FDA and logistic regression) for individual bone models. Overall, the clavicle model classified best for FDA and logistic regression, whereas the radius classified best for LDA. Multivariate subsets (various combinations of measurements) achieved correct classifications that ranged from 85%-98% (LDA) to 88%-97% (FDA and logistic regression). For both FDA and logistic regression the "all-variable" model achieved the highest correct classifications, whereas for LDA, the subset of breadth measurements achieved the highest accuracies. When classifying into sex and ancestry, a multivariate subset using eight measurements achieved classification accuracies of 79% (FDA) and 80% (LDA).

While LDA and logistic regression produced better results for some subsets and bone models, overall FDA achieved greater accuracies. Colored males and females and Black females misclassified most often as the same sex but different ancestry groups, whereas Black males and White males and females misclassified equally into different sex and ancestry groups. Overall, White males and females had the highest correct classification rates for both sex and ancestry. Postcranial bones achieved comparable classification accuracies to morphological analysis of the pelvis and higher accuracies than metric or morphological techniques using the cranium in South Africa. The high correct classifications obtained for LDA also indicate that a custom database of postcranial data can be used with *FORDISC*® 3.0 to improve classification into sex and sex-ancestry groups in forensic case analyses in South Africa.⁵

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Anthropology Section - 2015

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Sexual Dimorphism, Sympercents, Flexible Discriminant Analysis



A95 An Analysis of Butterfly Fracture Propagation

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After attending this presentation, attendees will have a clear understanding of the initial fracture mechanism and subsequent fracture propagation of butterfly fractures (a.k.a. wedge fractures). This presentation will clarify how butterfly fractures form and where, in regard to the applied loading force, this fracture pattern initiates in moderate- to high-speed blunt impacts. Attendees will gain a greater understanding of the fundamental failure mechanisms associated with butterfly fracture patterns. Attendees will also gain knowledge on the potential use of biomechanical bone surrogates in forensic anthropological studies of blunt force trauma.

This presentation will impact the forensic science community by providing clarification on how butterfly fracture patterns are formed using an interdisciplinary approach. Combining biomechanics and forensic anthropology, this study conducted dynamic three-point bending tests on surrogate tibias to determine whether butterfly fracture patterns initiate in tension or compression. The results of the tests and analyses allowed conclusions regarding the initial fracture mechanism and the subsequent fracture propagation. This information can assist pathologists and forensic anthropologists in determining the direction of force when trauma has resulted in the formation of a butterfly fracture pattern.

Purpose: This research addresses the issue of applied loading direction, initial fracture mechanism, and subsequent fracture propagation of butterfly fractures. The purpose of this research is to identify and analyze the propagation of butterfly fractures in lower limbs initiated through blunt force trauma. The scope of this research is limited to ten surrogate tibias.

Materials and Methods: Sawbones® composite tibias were utilized for this study. These models have previously been shown to display similar biomechanical properties (stiffness, fracture toughness, and ultimate strength) as real human tibial bone. All Sawbones® tibias were medium-sized left fourth generation, which have a foam core, 9mm canal, and an overall length of 37.5cm. Tests were conducted using a dynamic three-point bending impactor designed to simulate blunt force trauma applied in a Lateral-Medial (L-M) direction to the surrogate tibias. The applied loading direction of L-M was chosen to simulate a pedestrian vs. vehicle accident, which is a common source of butterfly fractures. Each test performed was documented using a high-speed video camera at 5,000 frames-per-second. Motion analysis was performed using a marker affixed to the impactor to determine dynamic deformation of each specimen. Video was also analyzed to determine where the fracture initiated and the path of fracture propagation. A triaxial accelerometer array was affixed to the impactor for determining impact force using Newton's 2nd law. Accelerometer data were collected at 20,000 Hz and filtered using Channel Frequency Class (CFC) 1000. A photogate was used to measure impactor velocity just prior to impact with the tibia. Photographs and measurements were taken before and after each test to document resulting trauma.

Results and Conclusions: All specimens tested resulted in complete fractures. All fractures initiated on the convex side of the bending bone and were the result of tensile failure of the material. The fracture(s) then propagated toward the concave side of the bending bone on an angle of approximately 45 degrees from the long axis of the bone. This angled fracture pattern is consistent with shear failure of the material within an area of the bone experiencing high compressive stress. Completion of the propagation resulted in the classic "wing shape" seen in butterfly fracture patterns. The current study is in agreement with prior biomechanical studies, which demonstrate the initial bony failure occurs as a result of high tensile stress in the material followed by shear failure on the compressive side of the bending bone. Contrary to some previous studies, none of the butterfly fractures in this study initiated on the compressive side of the bending bone and no compressive wedge fracture patterns were observed.

Fracture Propagation, Butterfly Fractures, Composite Bones



Anthropology Section - 2015

A96 Fracture Characteristics of Fresh Human Femora Under Controlled Three-Point Bending

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After attending this presentation, attendees will better understand the relationship between controlled, experimental loads and fracture outcomes in long bone bending tests on fresh human femora.

This presentation will impact the forensic science community by contributing ground-truth data linking a specific, consistent set of loading conditions under three-point bending with bone behavior and fracture morphology.

Current literature associates long bone bending failure with tension wedge fractures, in which a transverse crack initiates at the tensile surface of a bent bone and branches as it propagates toward the impacted surface of the bone; however, numerous experimental studies of long bone bending have reported variability in gross fracture outcomes. Wedge fragments have not always been presented and in some cases researchers have reported compression wedges with the transverse crack on the impacted surface of the bone.¹⁻³ Martens reported a high frequency of compression wedges under a four-point bending configuration and Kress reported some cases of compression wedges under uncontrolled loading situations.^{1,2}

Fenton et al. performed controlled three-point bending experiments on dry human femora and consistently documented incomplete tension wedge-type fractures.⁴ Within this set of experimental impacts, the orientation of the incomplete wedge could be used to reliably determine the direction of the applied load.

The objectives of the present study were to: (1) execute controlled three-point bending tests of axial loaded, fresh human femora; (2) describe the mechanical behavior of the specimens; and, (3) identify fracture outcomes, including characteristics of the complete and incomplete fractures and gross fracture surfaces.

Six pairs of unembalmed human femora were mounted into a three-point bending fixture in a servohydraulic materials testing machine. Static axial loads simulating standing posture were applied with a spring-mounted fixture. Failure was achieved via controlled displacement of a steel anvil at midshaft, inducing three-point bending. Right femora were loaded on the posterior surface and left femora were loaded anteriorly. Following each failure experiment, specimens were grossly examined for complete and incomplete fractures and fracture surface morphology.

These controlled bending tests demonstrated variability in mechanical behavior between human subjects. Failure load ranged from 4.0 to 9.2kN. The displacement of the bone to failure ranged from 5.8 to 13.2mm and the energy to failure ranged from 15.0 to 66.3J; however, there were no significant differences in failure load, displacement, or energy between the paired anterior and posterior loaded specimens. The variation in mechanical behavior did not appear to affect the consistency of fracture characteristics.

The controlled bending tests produced consistent fracture outcomes across specimens. In each case, a short transverse crack was initiated on the tensile surface of the bone. Thus, the crack occurred on the anterior surface in all posteriorly loaded femora and on the posterior surface in all anteriorly loaded femora. Upon gross examination, the transverse fracture surface was consistently mottled and billowy. Complete and incomplete fractures branched off the transverse crack and angled toward the compressed surface of the bone, producing an incomplete tension wedge. In each case, the branch point occurred on the tensile side of the neutral axis. After the branch point, the fracture surface appeared jagged and sharp. No complete wedges presented. Complete fractures were either primarily oblique (42%) or transverse (58%). All specimens exhibited at least one incomplete fracture that curved from the branch point to parallel with the bone's long axis.

This study demonstrated that bone failure occurred in a predictable manner under controlled, experimental loading conditions. Three-point bending with axial compression produced consistent fracture characteristics, although the mechanical behavior was variable between human subjects. For each specimen, the specific orientation of fracture reliably reflected the direction of the applied load.



Anthropology Section - 2015

This work is in response to the Scientific Working Group for Forensic Anthropology (SWGANTH) Gap Analysis Report, which calls for collaborative work with biomechanists to improve current methods in trauma analysis. To this end, the Michigan State University Forensic Anthropology and Orthopedic Biomechanics Laboratories are pursuing a joint research initiative investigating the effects of specific, experimentally controlled loading conditions on fracture outcome; this presentation communicates the latest work on three-point bending of human femora. The data contributed here will help experts determine the specific loading conditions that lead to a specific set of long bone fracture characteristics.

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Three-Point Bending, Blunt Force Trauma, Long Bone Fracture



Anthropology Section - 2015

A97 Atypical Skull Injuries and the Biomechanical Continuum

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After attending this presentation, attendees will have an understanding of some limitations to the standard object/weapon-centered classification scheme (sharp force, blunt force, gunshot/high velocity) used in classifying bone trauma.

This presentation will impact the forensic science community by providing a broad interpretive framework for trauma analysis of bone through the examination of examples of atypical injuries of known mechanism whose characteristics contradict or overlap with those usually associated with classic trauma classifications of peri-mortem skeletal fractures.

The goal of trauma analysis in forensic anthropology is to determine the mechanism and timing of bone trauma and to address other related medicolegal problems.¹ By applying principles of biomechanics, bone trauma can often be classified as arising from sharp force, blunt force, or gunshot/high-velocity trauma and in so doing serve an important role in guiding medicolegal death investigations; however, a significant deviation from the expected magnitude or combination of extrinsic factors (e.g., acceleration, surface area, force) involved in fracture production from that which is typically associated with a given mechanism of injury can produce confounding or equivocal wounds. In such instances, an over-reliance on weapon-centric classification can lead to misclassification or over-reaching interpretations and may affect the medicolegal determination of cause and manner of death. Therefore, trauma analysis of skeletal material may be better approached by emphasizing the continuous nature of biomechanical factors that influence wound production.^{2,3}

To demonstrate this point, gunshot injuries typically involve high-velocity penetrating injuries with classic entrance/exit defects, beveling, and possibly radiating and concentric fractures; however, in rare circumstances, intermediate targets, unexpected bullet behavior, or similar intervening forces may cause significant deceleration such that a projectile may produce a fracture pattern more typical of blunt force injury. These types of injuries were more commonly seen in the 19th century, but still occur in modern contexts.^{4,5} Similarly, blunt-edged objects traveling at sufficiently high velocity are capable of producing internal beveling defects similar to gunshot wounds.⁶ This presentation provides four cases of documented injuries that exemplify the biomechanical continuum in that the fracture patterns produced are equivocal, overlap more than one category, or are otherwise atypical of the standard classifications.

Overlapping fracture characteristics or features which “transition” between standard classifications may be observed when objects conveying certain combinations of physical (i.e., size and shape) and dynamic (i.e., velocity) characteristics impact bone. Cases that highlight the problematic nature of applying rigid typology/classification in light of the biomechanical continuum underlying wound production will be presented. While a typological and weapon-centered approach to trauma analysis is often necessary to provide useful information to medicolegal authorities, descriptions of trauma are sufficient when a classification is not forthcoming.⁷

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Atypical Wounds, Bone Biomechanics, Trauma Classification

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A98 A Comparison of Radiographic and Osteological Findings in Suspected Infant Abuse Cases

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After attending this presentation, attendees will understand the pros and cons of radiographic and osteological methods of trauma evaluation in suspected infant abuse cases and the benefits of forensic anthropological analyses performed in conjunction with clinical radiographic methods. Examples will also familiarize the audience with key skeletal signatures of infant abuse.

This presentation will impact the forensic science community by supporting the need for more comprehensive osteological analyses to be performed in cases of suspected infant abuse in order to confirm and supplement clinical radiographic findings. By implementing both radiographic and osteological methods, infant skeletal trauma can be more accurately documented and interpreted, thereby contributing to accurate assessments of accidental versus non-accidental trauma and providing justice to the deceased.

Approximately 700,000 children were abused in the United States in 2012, with infants (<1 year) displaying the highest number of deaths due to abuse. Even so, infant abuse cases are rarely received by forensic anthropologists. This is due in part because forensic anthropologists are usually only consulted in the event of a death and only if it is suspected that the death included significant skeletal trauma; however, it may also reflect an assumption that the clinical radiographic analysis performed by the radiologist and autopsy conducted by the medical examiner sufficiently document all necessary trauma, eliminating the need for invasive skeletal processing and osteological analysis. In such scenarios, information vital to the investigation may go undetected.

The goal of this study was to compare clinical radiographic reports of skeletal trauma in suspected infant cases to the skeletal trauma revealed by a comprehensive osteological analysis. Radiographic and osteological findings were compared in four suspected infant abuse cases examined for this study. The limited sample size reflects the scarcity of infant abuse cases received by forensic anthropologists and should not diminish the significance of such cases. The results of the comparison reveal that in many instances, the documented skeletal trauma in the clinical radiographic reports were not as extensive as the injuries observed during the osteological analysis. Errors in radiographic reports of antemortem and peri-mortem trauma were noted (e.g., rib assignments), and smaller healing calluses on the ribs were often overlooked or not apparent from the radiographs, despite being clearly evident after skeletal processing. These inconsistencies are likely due to the imposed two-dimensionality of the radiographs and overlapping anatomical structures that obscure the injuries.

Limb fractures were well documented in the radiographic reports and in one case, the radiologist noted a recently healed fracture in the limb which was not initially visible in the osteological analyses due to the overlying periosteal reaction, but became evident as the processed bones dried out. Generally, the radiographic reports were also more successful at noting metaphyseal fractures than the osteological analyses, due to the fragility of the area and possible disturbances to the metaphyseal surface during processing. In one case, close monitoring of remains during the maceration procedure, with repeated photodocumentation during processing, revealed very distinct metaphyseal ("bucket handle") fractures that were consistent with the radiographic findings. Callus sizes, fracture direction, and evidence of re-fracturing events were also more discernible in the processed remains than radiographs.

The results of this study indicate that both radiographic and osteological analyses should be required in cases of suspected infant abuse. In addition to the clinical radiographs evaluated by the radiologists, forensic anthropologists should perform their own radiographic and photographic documentation prior to any processing, and if possible at different intervals during the maceration process. With such careful procedures, even delicate signatures of infant abuse, such as metaphyseal fractures, can be osteologically documented. It is the forensic anthropologists' responsibility to educate medical examiners in the benefits of performing a full osteological analysis. In addition, because infant abuse cases are rare occurrences in forensic anthropology, it is important for forensic anthropologists to document and share their case experiences in order to create a growing knowledge base of accidental and non-accidental trauma in infant skeletons.

Child Abuse, Infant Abuse, Radiographic



A99 A Forensic Pathology Tool to Predict Pediatric Skull Fracture Patterns: Part V — Controlled Head Drops Onto Shaped Impact Surfaces

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After attending this presentation, attendees will better understand the effects of various shaped impacters on both the biomechanics and pattern of cranial fracturing generated during controlled head-drop experiments using a developing porcine model.

This presentation will impact the forensic science community by providing baseline data regarding some of the effects of cranial impacts against various shaped surfaces and comparisons to impacts against flat surfaces presented in previous studies.

Most current literature on the analysis of blunt force trauma to the cranium cites Gurdjian et al.'s works on fracturing mechanics. Although their experimental work focused on flat surface head drops, they noted that such variables as the size, shape, and energy of the impacting object have an effect on fracturing. They also stated that "a slowly moving object, fairly sharp or pointed in contour, may cause an area of depression more or less patterned after the shape of the object;" however, fatal injuries do not always result in characteristic wounds and systematic studies regarding the morphology and mechanics of injuries resulting from non-flat surface impacts are limited.¹ Thus, there is currently little more than conjecture and anecdotal data regarding the assessment of cranial fractures resulting from impacts with shaped objects. The current study was conducted to understand some of the basic biomechanical mechanisms and cranial fracture patterns from shaped surface impacts using a porcine model.

The hypotheses of the study were: (1) heads dropped against surfaces with a large contact area would have fractures that mimic flat surface experiments with peripherally initiated linear fractures; (2) as the contact area decreased, depressed fracturing would occur; (3) at some intermediate contact size and shape, there would be a combination of depressed and linear fractures; and, (4) the energy and resulting contact force necessary to cause fracture initiation would decrease with the area of the impacting interface.

To document the cranial fracture biomechanics and fracture patterns for various shaped interfaces, heads from pigs between one and 20 days old (n=64) were dropped under controlled conditions onto 14 rigid, shaped impact surfaces.² Four impacters which simulated such objects as a table corner, hammer, etc. were chosen for closer analysis. Biomechanical data were collected during each experiment; thereafter, the heads were visually inspected for fractures, photographed, and the fracture patterns were diagrammed using previously described methods.²

The four impacters were: (1) 2" hemisphere (n=8); (2) 1/16" edge (n=9); (3) 5/8" ball bearing (n=7); and, (4) 1/4" flattop peg (n=8). Peripheral linear fractures were present on 97% of the specimens analyzed (31/32). The 2" hemisphere most resembled results seen in earlier flat surface studies and seems to represent the transition between flat and focalized surfaces as five specimens expressed both peripheral linear and area of impact fractures. As predicted, as the contact area decreased, the presence of depressed fractures increased. Depressed fractures first appeared with the 5/8" ball bearing and the impacter shape also started to become discernible. The 1/16" edge generated linear fractures that extended along the impacter's contact surface and caused creasing of the bone. The 1/4" peg produced punctures through the bone and had the highest frequency of depressed fractures at 88% (7/8).

The peak forces generated at fracture for the four impacters were: (1) 1/16" edge 424±149N; (2) 2" hemisphere 378±148N; (3) 5/8" ball bearing 186±70N; and, (4) 1/4" peg 151±69N. Those in the flat surface studies were 967±350N. These data indicate that the peak force causing cranial fracture decreased with contact area and interface shape. Importantly, the kinetic energy needed to cause fracture followed a similar pattern.

This study using the infant porcine model demonstrated that impact surface shape had an effect on fracture type and pattern. Additionally, it shows the energy and forces causing cranial fracture decrease significantly for impacts against shaped surfaces which reduce area of contact. Such basic biomechanical data may have direct relevance to human pediatric victims as forensic scientists attempt to opine injury causation in cases not involving flat impact surface conditions.

This project was supported by the National Institute of Justice, Office of Justice Programs, United States Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this presentation are those of this study and do not necessarily reflect the views of the Department of Justice.



Anthropology Section - 2015

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Shaped Impacters, Cranial Fracture Patterns, Bone Biomechanics



A100 Experimental and Computational Validations of the Initiation and Propagation of Cranial Fractures in the Adult Skull

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After attending this presentation, attendees will: (1) gain further knowledge of the initiation, propagation, and sequence of cranial fractures in experimental blunt impacts performed on adult heads; and, (2) be presented with high-speed footage confirming peripheral fracture initiation in blunt cranial impacts.

This presentation will impact the forensic science community by helping clarify contrasting data in the current literature and thereby improve the forensic investigator's ability to assess adult cranial injuries.

Gurdjian et al. suggested that impact kinetic energy and implement shape both influence cranial fracture initiation and propagation.¹ Given sufficient energy, those authors reported depressed fractures generated in areas of localized contact; however, for more distributed loads, they reported peripheral cranial fracture initiation in areas of "out-bending." More recently, Kroman et al. used high-speed photography to show cranial fractures initiating at the impact site and propagating outward.² Problematically, the Kroman et al. study was limited in scope and did not reproduce Gurdjian's experiments with respect to energy and implement shape effects on fracture initiation and propagation.

The present study's goal is to help resolve the conflicting data presented above. High-speed video was used to capture fracture initiation and propagation during blunt impacts on unembalmed adult heads. Controlled impact experiments were performed with varying energies and shaped implements to investigate the effects on cranial fracture initiation and propagation. Computational modeling was also implemented to help understand the mechanisms of fracture initiation.

Seven unembalmed adult male cadaver heads were impacted with various shaped aluminum implements attached to a 10kg mass using a custom-built drop tower. Specimens were secured in a plaster of Paris bed and impacted once onto the center of the right parietal. Three heads were impacted at varying energies using a flat, three-inch diameter implement. Two heads were impacted with a flat, one-inch square implement. The remaining three heads were impacted with a two-inch spherical, two-inch spherical, and a one-inch flat implement, respectively. High-speed cameras recorded fracture initiation and propagation at 6,600 and 10,000fps. One specimen was Computed Tomography (CT) scanned. The parietal bone geometry was utilized in a computational model to investigate the distribution of impact-produced stresses.

Video footage clearly showed peripheral initiation of linear fractures in all three impacts with the flat, three-inch circular implement, one impact with the flat one-inch square implement, and the two-inch spherical implement impact.

The results of the flat, three-inch circular impacts reflect variation in location of fracture initiation, propagation, and fracturing sequence with impact energy. In the lowest energy impact (52.5J), a single linear fracture initiated in the pterion region and propagated back to the impact site. The second impact (72.7J) produced a peripherally initiating linear fracture, followed by concentric fracture around the point of impact, and then a linear fracture propagating outward from the impact site. The highest energy impact (120.2J) produced a similar fracturing sequence with more extensive damage.

The results also demonstrated implement shape effects. One impact with the one-inch square implement produced a linear fracture initiating at the impact site and propagating outward. The other produced a peripherally initiating fracture that propagated toward the impact site. The two-inch spherical implement produced a peripherally initiating linear fracture followed by concentric fracture around the impact site. The one-inch spherical implement generated a depressed fracture.

The computational model showed a zone of circumferential-oriented tensile stresses in the parietal bone near the sutures. These stresses may generate radial-oriented fractures initiating peripheral to the impact site in areas of "outbending." With higher loads, the model indicated a zone of large circumferential and radial-oriented stress around the impact site. These stresses may generate concentric and radiating linear fractures initiating at the impact site.

The goal of this study is to address a current issue in our literature involving the location of fracture initiation and direction of fracture propagation in blunt cranial impacts. High-speed photography in the current study clearly showed differences in fracture initiation and propagation depending on impact kinetic energy and the characteristic shape of the impact interface.

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This project was supported by the National Institute of Justice, Office of Justice Programs, United States Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this presentation are those of the authors and do not necessarily reflect the views of the Department of Justice.

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Cranial Fracture, Blunt Force, Trauma



A101 The Fracture Printing Interface: Development of an Automatic Classification System for Cranial Fracture Patterns

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After attending this presentation, attendees will gain knowledge of: (1) the fracture printing interface, a user-friendly computer interface based on classification algorithms capable of automatically categorizing fracture patterns with a high degree of accuracy based on impact energy level, contact surface, and head constraint condition for a porcine model; and, (2) a prototype human fracture printing interface capable of categorizing pediatric human cranial fractures.

This presentation will impact the forensic science community by demonstrating a new system to classify cranial fracture patterns with a high degree of accuracy as validated on an experimental porcine dataset and utilized in a case-based human pediatric dataset. This presentation will introduce the medicolegal community to a fracture printing interface that can aid in the interpretation of pediatric cranial trauma.

Pediatric deaths involving cranial fracture are challenging cases for both forensic pathologists and anthropologists, as little foundational research exists on the interpretation of fracture. That difficulty in interpretation was the impetus for the current study.

A porcine dataset (n=354) has been generated through controlled biomechanical impact experiments. Algorithms have been developed in order to classify fracture patterns by impact energy level, contact interface, and head constraint condition, then incorporated into a fracture printing interface. A subset of these data (n=82) will be highlighted in order to demonstrate the automatic feature extraction and classification methods that will enable the fracture printing interface to determine injury scenarios with high levels of accuracy.

Porcine heads featured in the subset of data were dropped in controlled laboratory experiments onto a rigid aluminum surface at the center of the right parietal bone. Cranial injuries were charted and classified into low- or high-impact energy groups. Data were analyzed by age categories: young porcine specimens (1-9 days old), older porcine specimens (10-18 days old), and both age groups combined. Decision trees, using cross-validation, analyzed 51 automatically extracted features (e.g, fracture length per bone, fractures per bone, and number of initiation points) in a step-wise fashion to classify fracture patterns by impact energy level.

The result of the analysis for the young porcine specimens (n=41) was an 82% correct classification of fracture patterns into energy levels based on the number and length of occipital fractures, number of fracture initiation points, and length of diastatic fractures. The older porcine specimens (n=41) were classified with a 95% accuracy based on the total length of fractures in the right parietal and the number of fractures on the skull. When the two age groups were combined, the dataset (n=82) was classified with an 87% accuracy, based on total length of right parietal fractures and the number of fracture initiation points.

These algorithms, along with others developed on the larger dataset, have also been integrated into a user-friendly fracture printing interface in which fracture pattern features are automatically extracted and are used to predict any or all of the following variables: impact energy level, contact surface, and/or head constraint condition.

The automatic feature extraction algorithms developed from the “ground truth” porcine data have been applied to human cranial fracture data (n=106) collected from death investigation offices across the country. Manually-drawn pediatric cranial fracture diagrams were uploaded into the human-specific interface which automatically extracted fracture features such as fracture length, number of fractures and type of fracture. These features were used to classify an individual case as a homicide or accident; accuracy rates are determined by the autopsy ruling. Currently, the algorithms classified with an 84% accuracy using decision trees. More data is necessary to classify fracture patterns into specific injury categories and improve the classification algorithms.

The overall vision of this work is the dissemination of the fracture printing interface which will provide practitioners with a tool that digitally uploads a pediatric cranial fracture pattern and statistically analyzes the pattern to predict the energy level and the impact surface of injury.



Anthropology Section - 2015

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Pattern Recognition, Cranial Fractures, Fracture Classification



Anthropology Section - 2015

A102 Healing Rates of Antemortem Injuries to Bone

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After attending this presentation, attendees will be knowledgeable concerning guidelines used by radiologists for establishing a time-since-injury in healing and healed wounds, identify how these standards can be adapted for anthropological casework, be aware of discrepancies and issues when using radiological standards in anthropological casework, and understand a need for creating a set of guidelines by anthropologists for use in anthropological casework.

This presentation will impact the forensic science community by presenting the results of a pilot study demonstrating that radiological guidelines for healing rates in bone underestimate and overestimate healing times when the bone is examined macroscopically rather than radiologically.

An estimation of the length of time elapsed since an episode of trauma has occurred has importance within a medicolegal context. This is particularly true in cases where traumatic events may have multiple episodes, commonly seen in cases of domestic abuse, child abuse, and abuse of the elderly, where medical documentation of previous injuries may not exist or be accessible. Radiologically, guidelines established for healing rates are based upon the appearance of the following characteristics: resolution of soft tissues, subperiosteal new bone formation, loss of fracture line definition, soft callus formation, hard callus formation, and bone remodeling. These guidelines were developed using healthy children of various ages and are adjusted based on a practitioner's experience when examining healing or healed trauma in adults. Radiographs may not reveal a fracture clearly, depending upon the exposure, position of the individual, and location of the body. The rate of healing of a particular bone is also dependent upon the age of the individual, health of the individual, extent of injury and re-injury events, in addition to variability in healing rates throughout an individual's skeleton.

These case studies represent a pilot study conducted on individuals autopsied at the Harris County Institute of Forensic Sciences between 2004 and 2014 with medical documentation of antemortem injuries. The case studies represent 20 subadults and adults, ranging in age from one month to 91 years of age, with medical documentation concerning when antemortem injuries occurred and the extent of said injury. Cases of known injury dates in the medical examiner system are few. This presentation will showcase a series of case studies of bone healing with documented dates of injury. The cases provide an overview of healing rate variation observed in subadults and adults. The cases represent a failure in using radiological estimates and a tendency to either underestimate or overestimate time elapsed since occurrence of injury when radiological estimates are applied to dry bone. Anthropologists commonly state that gross evidence of healing is an indication that a fracture is at least two weeks old; however, early response in children is seen as early as three days after an injury occurs. This study demonstrates the need for the creation of guidelines for healing rates using macroscopic examinations of bone tissue. Macroscopic, rather than radiologic, standards will allow anthropologists to make a more complete determination of antemortem injuries.

Antemortem, Healing, Fracture



A103 Sex Differences in Measures of Bone Remodeling

Victoria M. Dominguez, MA*, Ohio State University, Division of Anatomy, 1645 Neil Avenue, Columbus, OH 43210; and Christian Crowder, PhD, Harris County Institute of Forensic Sciences, 1885 Old Spanish Trail, Houston, TX 77054

After attending this presentation, attendees will appreciate the complexity of evaluating sex-related differences in bone remodeling and how identifying these differences may improve methods of histological age estimation. In addition, attendees will learn how variable selection may mask or expose remodeling differences associated with intrinsic factors such as sex and age.

This presentation will impact the forensic science community by demonstrating the need for in-depth analysis of histological variables to determine when and why sex differences in histological variables occur. This will hopefully result in more sex-specific research to improve the accuracy of histological methods.

Estimating adult age is problematic due to biological variability in skeletal age indicators and their differential response to intrinsic and extrinsic factors during life. Histological methods of age estimation are based on bone turnover, which is argued to occur at a predictable rate; however, the literature provides conflicting data. One issue is the existence of sex differences in histological variables and the use of sex-specific age-prediction equations. Considering skeletal physiology and the interplay with biocultural factors, one appreciates the dynamic nature of bone remodeling that may produce or extenuate histological differences between males and females. As remodeling is systemic and not bone specific, it is a fair assumption that sex-specific equations should be used to improve the accuracy of histological methods. The goal of this research is to examine femoral cross-sections for sex-related differences in selected histological variables.

The sample includes femur cross-sections from 321 individuals of known age (167 males, 154 females). The following variables were collected: (1) Surface Area (Sa.Ar.) per mm²; (2) Intact Secondary Osteons (N.On.); (3) Fragmentary Secondary Osteons (N.Fg. On.); (4) Intact Secondary Osteon Density (OPDI) per mm²; (5) Fragmentary Osteon Density (OPD_f) per mm²; (6) Osteon Population Density (OPD): sum of OPD_i and OPD_f; (7) Mean Osteonal Cross-Sectional Area (On.Ar) per mm²; and, (8) Mean Anterior Cortical Width (Ant.Ct.Wi.) per mm².

Histomorphometric data collection protocols developed by Crowder and Dominguez were utilized.¹ Osteon areas and cortical widths were calculated using imaging software. Statistical analyses were performed in a statistical analysis software using variables 4–8.

The variables were tested using a Multiple Analysis of Covariance (MANCOVA), with sex as the independent variable and age as the covariate. Results showed both age and sex are significant overall ($p=0.000$); however, Analysis of Covariances (ANCOVAs) revealed that while age and sex were significant for OPD_f (both $p=0.000$), Ant.Ct.Wi (both $p=0.000$), On.Ar ($p=0.000$ and $p=0.001$, respectively), and OPD (both $p=0.000$), sex was not significant for OPD_i ($p=0.928$). Pairwise comparisons revealed females tended to have significantly higher OPD_f values, as well as significantly lower Ant.Ct.Wi and On.Ar measures than males. Correlation statistics indicate differences in variable strength with age between the sexes.

Overall, the results indicate that the tested variables demonstrate relationships with age; however, age and sex effects between variables are not consistent. Regardless, it is reasonable to conclude that sex-specific regression models are warranted. The ANCOVA results and the difference in correlation strength for OPD_i between males and females, with females showing a weaker correlation to age, suggest that age-related turnover events are best expressed by OPD_f, especially in females. Increasing OPD is coupled with reduced cortical bone thickness over time, with females demonstrating greater loss. Future analysis will include a relative measure of cortical thickness to control for size, which likely amplifies differences. Current literature disagrees about whether sex differences in osteon size exist. This research indicates that On.Ar differs significantly by sex; however, the relationship with age and sex is unclear. Young females tend to have larger mean On.Ar. when compared to males, which may relate to age of parity; however, in older age cohorts, females demonstrate smaller On.Ar compared to males.

Sex differences observed in histological variables likely relate to biological factors involving the endocrine system that affect bone turnover in the female skeleton. This research demonstrates that evaluation of sex differences in bone turnover should be considered in histological studies. Applying concepts of skeletal physiology and remodeling theory to histological observations will hopefully improve the accuracy of estimating age in adults.



Anthropology Section - 2015

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Sex, Remodeling, Histology



Anthropology Section - 2015

A104 The Utility of Radiographs of the Proximal Femur in Positive Identifications: Establishing a Standard and Minimum Number of Concordant Points

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After attending this presentation, attendees will gain a better understanding of the validity and reliability of using antemortem and postmortem radiographs of the proximal femur in positive identifications. This research utilized a novel method in the analysis of radiographs for positive identification: classification decision trees. As a result, associated probabilities and the variance of those probabilities based on the quality and other identifying factors of the individuals and radiographs will be presented. This is of significance to forensic practitioners not only in the field of anthropology but to any forensic practitioners involved in ascertaining the identity of victims.

This presentation will impact the forensic science community by providing a standard for analysis and the minimum number of concordant points with associated probabilities needed to make a positive identification based solely on femoral radiographs in the event that other methods of identification are unavailable.

The 2009 National Academy of Sciences Report, *Strengthening Forensic Science in the United States: A Path Forward*, and the 1993 *Daubert* ruling have set the precedent for a need for more statistically valid and reliable methods for positive identifications. This includes developing testable standards for the use of radiographs for positive identifications. Clinical radiographs of the proximal femur are common, as they are often used to diagnose hip diseases and other disorders. In addition, the femur is a dense bone that preserves well in both archaeological and forensic contexts, and previous research has shown that bone density distributions of the proximal femur are unique, as they are based on musculoskeletal loading. Thus, this skeletal element can be utilized in cases where other skeletal elements have not been preserved and/or are damaged.

At present, there are no standards for the number of concordant points needed for positive identifications using the proximal femur. Therefore, the purpose of this study was to examine the morphology of head, neck, greater trochanter, and lesser trochanter of the proximal femur in a sample of 49 antemortem and postmortem pelvic radiographs of known individuals from the North Carolina (NC) Office of the Chief Medical Examiner to: (1) evaluate the uniqueness of these traits; and (2) establish the minimum number of concordant points necessary to make a positive identification with associated probabilities. Twenty-three identified individuals had both antemortem and postmortem radiographs. To represent the unknown or “no match” sample, either an antemortem or postmortem X-ray was compared to a randomly selected individual from the sample.

Classification decision trees, a robust data mining technique, were used to explore patterns and relationships. For categorical data, a G^2 or the likelihood-ratio chi-square was computed and used for multi-level split of the data. The classification tree results using the femoral head and neck show that if there is one or more femoral head concordant characteristics, the probability of the individual being correctly matched or positively identified is 93%. If there is also a concordant femoral neck trait, the probability of correctly matching or positively identifying an unknown decedent is 97%. Results for the femoral greater and lesser trochanter show that if there is one or more femoral greater trochanter concordant characteristic the probability of the individual being correctly matched or positively identified is 76%. If there are also two or more concordant femoral lesser trochanter traits, the probability of correctly matching or positively identifying an unknown decedent is 93%. Thus, the results indicate that two or more concordant points are needed in order to have a probability of a correct identification above 90%.

This project was sponsored by the National Institute of Justice (2010-DN-BX-K214).

Radiographs, Positive Identification, Femur



Anthropology Section - 2015

A105 Dental Cementum Increment Analysis Can Determine Four Seasons at Death

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After attending this presentation, attendees will have developed an understanding of how dental cementum increment analysis can yield specific season-at-death estimates.

This presentation will impact the forensic science community by a novel forensic method which can be developed and refined with continued investigations.

Forensic anthropologists and bioarchaeologists are increasingly using Dental Cementum Increment Analysis (DCIA) to determine season-at-death. They are using the method of Wedel, which demonstrated that DCIA could correctly sort 112 donated teeth by the season in which they were extracted (a proxy for death) with 99% accuracy.¹ The two broad seasons into which DCIA sorted the teeth were April to September and October to March. Wedel et al. validated the 2007 results, achieving a 94% accuracy rate in correctly determining season-at-death in 467 teeth donated by patients of Creighton University's School of Dentistry.² DCIA has now successfully been used in forensic cases (Wedel et al. 2013) and is being used in various other projects, including one using DCIA to determine whether skeletal remains are evidence of a historical epidemic.

In this project, 100 teeth were chosen from the combined 2007 and 2012 samples to test the hypothesis that the two broad seasons could be separated into four. This study used the same method Wedel used to demonstrate that the width of cementum increments or annulations become thicker from season's beginning to end.^{1,2} One hundred transverse tooth root thin sections that had previously been prepared for cementum increment analysis were randomly chosen and new photomicrographs were taken. These thin sections had been prepared using the following method: each tooth had been cleaned, embedded in epoxy under vacuum pressure, and sectioned at the middle-third of the root. The thin section had been mounted to a petrographic glass slide, then ground and polished to ~100 microns, viewed under a transmitted polarized light microscope, and photographed using a digital camera mounted to the microscope. The photomicrographs were imported into Adobe® Photoshop® where the color of the outermost increment was determined (translucent for spring/summer and opaque for winter/fall) and its pixel width measured. The pixel width of ten other increments of the same color were measured and their widths were averaged. The outermost increment's pixel width was divided by the average pixel width of like increments to derive percent increment completion.

A statistically significant correlation between the number of days a tooth was into its given season and its width ($p > 0.001$) was discovered. The percentage of full-thickness increments range in width from 2% to 98%, and these percentages strongly correlate the number of days into the season. With this solid data, this study rejects its null hypothesis that seasons of death cannot be further narrowed from six-month time periods to smaller ones and support its hypothesis. If a cementum increment is 50% as thick as the average of its like bands, it can be determined during which month(s) 50% of the given season has passed. Fifty-percent completion for a spring/summer band places the date of death halfway between April and September, therefore the end of June/early July. Law enforcement can then be informed that the individual died in the summer, rather than spring/summer as the 2007 study specified.

The full potential of DCIA has not been reached. Science has demonstrated that cementum increments can be counted in pairs and added to the age at which the tooth was known to erupt to yield an age-at-death estimate. The results of this study indicate that the outermost cementum increment can help estimate season-at-death more specifically than the spring/summer and fall/winter seasons. DCIA can be used to correlate cementum increments in a tooth root to which of the four calendar seasons the death occurred. During this presentation, the utility of this method in forensic cases will be demonstrated and a course charted indicating where research on cementum increments will lead.

References:

1. Vicki L. Wedel Ph.D. Determination of Season at Death Using Dental Cementum Increment Analysis, *Journal of Forensic Sciences*; Volume 52, Issue 6, pages 1334–1337, November 2007
2. 2012 Hsieh, SB and VL Wedel. I'd Give My Eye Teeth For Dental Cementum Increment Analysis. *Proceedings of the American Academy of Forensic Sciences*.

Dental, Cementum, Increment



Anthropology Section - 2015

A106 An Updated Validation Test of a Computer-Automated Short-Listing Tool for the Radiographic Identification of Human Remains

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The goal of this presentation is to report on an updated and expanded validation test of the Clavicle Matching Program (CMP), a semi-automated computer capability that employs Elliptical Fourier Analysis (EFA) to generate a short-list of individuals with high-match potential based on quantified clavicle shape.

This presentation will impact the forensic science community by demonstrating the usefulness of this capability for searching large-scale radiograph assemblages such as the collection of chest radiographs held by the Joint POW/MIA Accounting Command-Central Identification Laboratory (JPAC-CIL) for United States soldiers who fought in WWII, Korea, and Vietnam.

The validation test employed in the original study concerned 17 test skeletons and a chest radiograph assemblage of 409 individuals. The current validation test is considerably larger, comprising 30 skeletons and the full 7,362-person assemblage of Korean War-era chest radiographs currently held by the JPAC-CIL.¹

To conduct the test, the clavicle outlines from each of the antemortem images in the reference databank were manually traced using Wacom® touchscreen tablets. The clavicles of each test skeleton were 3D laser-scanned and inputted into the software, which generated four shadowgrams for each clavicle to compare to the chest radiograph dataset using Elliptical Fourier Analysis (40 harmonics x 4 coefficients each=160 Fourier descriptors per clavicle). Using the sum of the squared differences for the Fourier descriptors, the computer then ranked all individuals in the reference databank according to their morphological similarity to the inputted skeleton.

Each search against the reference dataset of 7,362 individuals took less than 70 seconds to complete. Results for the large-scale test indicate that the correct match was included in the short-lists of the top 5% of the antemortem reference sample 57% of the time, and in the top 25% of the lists 80% of the time. This performance is slightly less than that observed for the 409-person database originally reported; however, the utility of the method is still retained and is high given the 18-fold increase in the reference sample size.¹ For cases that lack contextual information, this helps narrow a potentially lengthy candidate list quickly and effectively. Since the original validation study, the CMP has facilitated the identification of several previously unaccounted for individuals from the Korean War and has the potential to further facilitate identification of several hundred still unaccounted-for United States service personnel interred at the National Memorial Cemetery of the Pacific.

Reference:

1. Stephan CN, Amidan B, Trease H, Guyomarc'h P, Pulsipher T, Byrd JE 2014. Morphometric Comparison of Clavicle Outlines from 3D Bone Scans and 2D Chest Radiographs: A Shortlisting Tool to Assist Radiographic Identification of Human Skeletons. *Journal of Forensic Sciences* 59:306-313.

Elliptical Fourier Analysis, Short-Listing Capability, Radiography



Anthropology Section - 2015

A107 Unidentified Border Crosser Deaths in Arizona: Expanding Intra-State Collaborative Efforts in Documentation and Representation

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The goals of this presentation are twofold: first, to implement changes in the data-recording methods currently employed at the Maricopa County Office of Medical Examiner (MCOME) in an attempt to create a more functional approach to identifying Unidentified Border Crossers (UBCs); and, second, to create a working database and facilitate the mapping of UBC deaths in Maricopa County. The present research project was inspired by the 2014 American Academy of Forensic Sciences (AAFS) Scientific Session entitled, “*Medicolegal Investigation of Migrant Deaths.*”

This presentation will impact the forensic science community by supplementing the data presented by the Pima County Medical Examiner’s Office at the 2014 AAFS Meeting. This presentation will illustrate the growing number of UBC deaths occurring north of Pima County and, in so doing, will provide a more comprehensive picture of this important anthropological issue within the state of Arizona.

As previously reported at the 2014 AAFS Annual Scientific Meeting, the issue of UBC deaths in Arizona is a growing concern, largely reinforced by United States border enforcement policies which make crossing increasingly difficult and dangerous.¹ To date, thousands of foreign nationals have died during their attempts to cross the United States-Mexico border. An even greater tragedy is the increasing number of migrants who are not repatriated to their families because personal identifications cannot be made. Currently, the MCOME is in possession of more than 200 unidentified individuals, more than half of whom are presumed to be UBCs.

The primary goal of this study was to evaluate UBC deaths in Maricopa County. Using the PCOME recording system as an example, this study developed criteria for inclusion into the UBC database. These criteria include only individuals found in desert areas and whose biological profiles indicated potential Hispanic ancestral origins. Once cases were classified as potential UBCs, the following data were collected based upon the information requested by the Human Borders Geographic Information Systems (GIS) Project: (1) age at death; (2) sex; (3) date of death/recovery; (4) cause of death; (5) body condition; (6) presumptive identification (e.g., identification cards found on the body); (7) location description including Global Positioning System (GPS) X and Y coordinates; and, (8) any related case numbers (e.g., individuals traveling in a group).

Data were collected for 113 probable UBC cases. All data were extracted from MCOME case files dating from 1981 to 2014. The general trend in UBC cases indicates a gradual increase from 1981 to the present. In 1981, the MCOME analyzed one case of a potential UBC. In 2013, the office analyzed a total of 11 UBC cases. Of the 113 total cases evaluated for the study, four individuals were anthropologically determined to be female and three were probable females. The remaining 106 individuals were males. Age estimates for UBCs were varied but the majority fell between the ages of 20 to 50 years of age with few individuals under 20 or over 50 years old. These cases varied taphonomically from fresh to decomposed or skeletonized/mummified. All individuals included in the study are in the process of being added to The Map of Migrant Mortality, a GIS-based tool containing spatial data related to migrant deaths that was created in conjunction with the Human Borders Project and the PCOME.

In documenting UBC deaths in Maricopa County, the MCOME has extended the northern reaches of migration routes across the state of Arizona, thereby further contributing to the overall awareness of the border crossing issue. It is anticipated that this research will improve the documentation and processing of UBCs at the MCOME and, in turn, will aid in the personal identification of UBCs, allowing for the return of many unknown decedents to their loved ones in foreign countries. By employing the Pima County model, the hope is to demonstrate the importance of intra-state anthropological and pathological collaboration in identification efforts.

Reference:

1. Reineke, Robin. *Lost in the System: Unidentified Bodies on the Border*. North American Congress on Latin America Report on the Americas 2013:46(2):50.

Unidentified Border Crosser, Maricopa County OME, Identification



A108 Odontometric Differentiation Between Southwest Hispanics, Native Americans, and European Americans

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After attending this presentation, attendees will understand the procedures and value of using odontometrics for the purpose of ancestry estimation in a sample of modern American populations.

This presentation will impact the forensic science community by presenting a method of ancestry estimation valuable when cranial morphology and metrics are insufficient for analysis or supplementary techniques must be utilized.

Data were collected from $n=571$ dental casts from a modern North American population from the James K. Economides Collection at the Maxwell Museum at the University of New Mexico. Buccolingual and mesiodistal dimensions of 16 permanent teeth were taken on the left side of the dental arcade. Cervical dimensions were also examined for utility, but many were found to be difficult to obtain due to the replicated soft tissue in the cast and while teeth were still in position. Teeth examined were the maxillary central incisors, canines, third premolar, and first molar and the mandibular lateral incisors, canines, third premolar, and first molar. The teeth chosen follow the morphogenetic field theory of Dahlberg, where these teeth are least variable in size and morphology.¹ Arch dimensions and overall tooth composition were also examined by measuring arch width between antimeres, arch depth, and the proportional make-up of each tooth class in the overall dental arcade. For the purposes of this investigation, only teeth displaying little attrition and no pathological conditions were used for all measurements, and only teeth in correct alignment were used for dental arcade measurements.

Four populations were examined: (1) Southwest Hispanics ($n=202$); (2) Native Americans ($n=161$); (3) European Americans ($n=165$); and, (4) Asian Americans ($n=43$). According to the mean results, Native Americans had the largest dimensions and European Americans had the smallest. Due to the nature of the small Asian American sample, interpretation of the means must be approached with caution. Southwest Hispanic measurements were consistently in the middle, except for the cervical buccolingual diameter of the maxillary canine, the buccolingual diameter of the mandibular incisor, both buccolingual diameters of the mandibular canine and premolar, and the maxillary incisor composition, where they were the largest. Hispanics had the largest arch depth, whereas Native Americans had the smallest. The means for all arch width measurements (width between key teeth antimeres) were largest for Native Americans and smallest for European Americans, except for the mandibular molars, where Hispanics had the largest width and European Americans had the smallest.

Multivariate Analysis of Variance (MANOVA) indicated statistical significance in most variables for sexual dimorphism, except for the cervical and crown buccolingual measurements of the mandibular incisor, the maxillary premolar composition, and the mandibular incisor arch width. For all variables, the means for males ($n=255$) were larger than females ($n=316$). Statistical significance was found in many variables for ancestry, except for the buccolingual cervical and crown measurements of the maxillary incisor, the mesiodistal measurement of the maxillary molar, the cervical buccolingual measurement of the mandibular incisor, the cervical buccolingual measurement of the mandibular canine, the buccolingual measurement of the mandibular premolar, the maxillary molar composition, mandibular premolar and molar composition, and both maxillary and mandibular arch depths. Definite clustering is present of individuals around a centroid, indicating some utility in ancestry estimation. When sex is known, an increased number of clusters occur, differentiating between males and females of each population, indicating an important contribution of sex as a variable in odontometric ancestry estimation.

The findings in the current study indicated possible utility in ancestry estimation techniques in the efforts of undocumented border-crosser identification and other forensic casework. Distinct patterns are visible with respect to tooth size and palate shape. Use of odontometrics may provide supporting evidence for other methods of ancestry estimation and as a valuable method when teeth are the best surviving elements.

Reference:

1. Dahlberg AA. 1945. The changing dentition of Man. *J Am Dent Assoc.* 32:676-690.

Odontometrics, Palate Shape, Ancestry Estimation



Anthropology Section - 2015

A109 The Joint Prisoners of War/Missing In Action (POW/MIA) Accounting Command Solvability and Resolvability Rating Project

Ivett Kovari, PhD, 445 Seaside Avenue, Apt 4302, Honolulu, HI 96815*

After attending this presentation, attendees will better understand the scope, objectives, and applications of the Joint POW/MIA Accounting Command (JPAC) Solvability and Resolvability Rating Project. This system could easily be applied to collections of cold cases in police jurisdictions or universities that remain unsolved and, with relevant modifications, it could also be applicable for other countries' military losses.

This presentation will impact the forensic science community by familiarizing those in the field with the stand-alone multidisciplinary Solvability and Resolvability Rating Project created at JPAC. The advantages of combining anthropological analysis, archaeological research, archival studies, and military history will be presented through the introduction of the Solvability and Resolvability Rating System. The relevance of the rating system lies in the established conjunction of the circumstantial information (circumstances of loss) and the biological information available. The takeaway of the Solvability and Resolvability Rating System is to create a product that is an objective, quantitative assessment of each case. This presentation will put forth the definitions, the structure and elements of the scoring system, and the outcome of the project.

The JPAC Standard Operating Procedures determines that all missing persons are equal: "The importance of any missing person, regardless of the conflict or circumstances of loss, is equal to that of any other missing person." The Solvability and Resolvability Rating Project comprehensive databases, therefore, include all missing persons and unknown remains inclusive of all relevant conflicts. The project established routine procedures to ensure the information in the database is accurate, verifiable, and up to date. The system coordinates and standardizes structure and content with all relevant databases maintained by other members of the accounting community. It ensures that each database is available to relevant members of the accounting community inside and outside of JPAC. The project maintains the database to ensure that the information is not only kept up to date, but that the underlying structure and methodology of the database is tamper-proofed for posterity.

The "Solvability Rating" applies to missing persons only. It is defined as a quantitative expression of the probability that a missing person may be accounted for using existing circumstantial information, scientific evidence, and forensic testing techniques. The Solvability Rating is a quantitative expression of numerous analytical issues, including the probability that a missing person's remains may be located, the potential for a site to yield remains, the quantity and quality of the antemortem records for each missing person, and the probability that remains, if recovered, may be identified using existing forensic testing methods. The rating is based on a scoring system of the accessible information cited above. The Solvability Rating serves three principal purposes: (1) to increase the efficiency and effectiveness of investigation and recovery efforts (by minimizing the risk that JPAC will allocate resources toward recovery of remains associated with missing persons whose antemortem records and site potential lack sufficient probability for the purpose of identification); (2) to pinpoint the sub-optimal informational, evidentiary, or geographical elements that must be acquired or improved for missing persons whose Solvability Rating is insufficient, incomplete, or otherwise non-adequate; and, (3) to consolidate the Solvability Rating for all missing persons.

The "Resolvability Rating" applies to cases believed to be missing persons buried as unknowns in National Memorial Cemeteries in the United States as well as in American Battle Monument Cemeteries overseas. It is defined as the quantitative expression of the probability that the forensic testing of human remains will produce probative information that will contribute to the identification of an unknown person. The Resolvability Rating is an assessment of the postmortem biological evidence associated with each unknown. Besides the determination of the probability, the purpose of the Resolvability Rating is to contribute to the JPAC program of disinterments for the purpose of identification and to consolidate the Resolvability Rating for all unknowns.

The Solvability and Resolvability Rating Project's intent is to support the Accounting Community's mission by supporting the Defense POW/Missing Personnel Office, the JPAC Research & Analysis Group, the JPAC Directorate of Operations, and the analysts of the JPAC Central Identification Lab. The project creates economies of scale and assists to optimize resource allocations as and when required. Dr. Kovari inherited the Solvability and Resolvability Project from Dr. Paul M. Cole. Dr. Cole has given his permission and consent that any text concerning this project written by him may be used by Dr. Kovari as she sees fit, without specific attribution.

Probability Analysis, Missing Persons, Recovery Efforts

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Anthropology Section - 2015

A110 Theory in Forensic Anthropology: A Retrospective and Look Forward

Donna C. Boyd, PhD*, Radford University, Forensic Science Institute, PO Box 6939, Radford, VA 24142; and Charles C. Boyd, PhD, Radford University, Dept of Anthropological Science, Radford, VA 24142

After attending this presentation, attendees will be able to recognize forensic anthropology theory, trace its origins, understand the different levels at which it operates, be aware of its applications in forensic anthropological analyses, and explain how it directs, influences, and stimulates research.

This presentation will impact the forensic science community by illustrating the vibrant but, at times, unrecognized theoretical underpinnings of the science of forensic anthropology and how they direct and influence data collection and analysis in every area of specialty within the discipline.

Theories are basic premises or postulates that can be used to explain and predict empirical phenomena. It is important to explicitly acknowledge and study theory because it provides a foundation and direction for research and the application of knowledge generated by research. It also dictates how a discipline is perceived by insiders, outsiders, and the law. The types of theory applied to research directly influence the results which are obtained. The types of research which are conducted in forensic anthropology enhance its theoretical development and ultimately strengthen the scientific foundation of the discipline.

Although the legitimacy of forensic anthropology as a science and a discipline has recently been questioned due to its perceived lack of a grounding body of theory, theoretical frameworks have clearly been present since publication of the first textbooks in forensic anthropology. Although Krogman and Stewart do not explicitly discuss theory, reliance on it is clearly evident throughout their books.^{1,2} Each credits human variation as the foundation for techniques in forensic anthropology, but neither directly addresses the explanatory nature of a theory of human variation for the human skeleton. They see the lack of precision in explaining human variation as precluding “consideration of forensic anthropology as an exact science.”²

This absence of an explicit acknowledgement of theory has continued. A review of all forensic anthropology research articles, case studies, and technical notes (N=498) from the *Journal of Forensic Sciences* and all major forensic anthropology textbooks published over the past 20 years (1995-2014) was undertaken by this study to examine evidence for and trends in theoretical development and its application in the discipline. Variables scored include presence/absence of a theoretical framework or hypothesis and characterization of theory type and level. Results indicate that while approximately 83% of *Journal of Forensic Sciences* publications relied on some aspect of theory, only 36% of these articles explicitly recognized the theoretical basis for the presented research. Only one article discussed the concept of theory in forensic anthropology as a whole. Likewise, although one forensic anthropology textbook had “theory” in its subtitle, none devoted more than a few paragraphs to the explicit theoretical basis for the discipline.

This theoretical grounding in forensic anthropology was evident on many levels, including high-level (micro and macro-evolutionary theory, including natural selection), middle-range (taphonomic, agency, behavioral theories), and lower-level (methodological, recovery, statistical theories), although these were rarely explicitly discussed. For example, identification of decedents through their biological profile and antemortem conditions relies on the high-level evolutionary principles of human variation as well as statistical (including Bayesian) theories. Trauma analysis borrows from theoretical principles of biomechanical engineering and physics. Recognition and differentiation of postmortem processes derive from middle-range taphonomic theories, while forensic archaeological search and recovery draw from lower-level theoretical principles from geology, geography, agency theory, and abductive reasoning. Trends observed over time include an increased focus on trauma and morphometric analyses, accompanied by discussions of the theoretical basis for these analyses.

Examining the concept of theory in forensic anthropology illustrates the three 2015 American Academy of Forensic Science (AAFS) planks of honoring our mentors and traditions, learning from each other, and stimulating our future. Theory does indeed form a strong foundation in forensic anthropology, albeit often unrecognized. The broadness of this field dictates that this theory is interdisciplinary and multidisciplinary, deriving from a variety of sources and operating at many levels. This eclectic theoretical basis enriches rather than weakens this discipline. Recognition of the ideas, models, and concepts that form the basis of how forensic anthropologists see their subject matter is vital for stimulating future growth of the discipline.



Anthropology Section - 2015

References:

1. Krogman WM. *The human skeleton in forensic medicine*. Springfield: Charles C. Thomas, 1962.
 2. Stewart TD. *Essentials of forensic anthropology*. Springfield: Charles C. Thomas, 1979.
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Theory, Forensic Anthropology, Retrospective



Anthropology Section - 2015

A111 Subjective With a Capital “S”? Issues of Objectivity in Forensic Anthropology

Allysha P. Winburn, MA, C.A. Pound Identification Lab, Cancer/Genetics Research Center, 2033 Mowry Road, Gainesville, FL 32610*

After attending this presentation, attendees will appreciate the presence of subjectivity in forensic anthropology and understand how to constrain this subjectivity by acknowledging sources of bias, developing new research and technologies, and engaging in both intra-disciplinary and inter-disciplinary communication and theory-building.

This presentation will impact the forensic science community by discussing the implications of presenting forensic anthropological science as purely objective and by suggesting realistic ways to constrain scientific subjectivity with data, theory, and technologies that are continuously advancing.

The field of forensic anthropology faces a future of increasing standardization and quantification of error. This presentation proposes that forensic anthropologists cannot continue to present their data as unbiased and purely objective. Like any social science, forensic anthropology is colored by subjectivity — but social science is not necessarily bad science. By evaluating their biases, learning from each other and from other disciplines, and continuously updating and constraining their interpretations of data, forensic anthropologists can achieve a level of mitigated objectivity that more accurately reflects the capabilities of their science, while still presenting accurate and precise data.

In the wake of the post-positivist theoretical turn in archaeology and the theoretical swing toward relativism in cultural anthropology, much of the field of forensic anthropology still maintains the positivist ideal of objective, value-free science. This may be due to the frequent status of forensic anthropologists as expert witnesses, and their desire to portray results as accurate, precise, replicable, and statistically (and legally) defensible. In the post-*Daubert* era, the forensic sciences have been increasingly scrutinized for their standardization and objectivity, and the questions increasingly arise: Is forensic anthropology *scientific* enough? Can it be “science with a capital S”?

Though an unbiased, purely objective forensic anthropological science is an understandable goal, it is difficult, if not impossible, to realize. Science is itself a social process and it is constantly changing. Scientific models necessarily reduce heterogeneity to homogeneity and the values of scientists affect both the re-presentations they create and their subsequent interpretations of them. But in spite of its social nature and inherent reductionism, science works: through sound scientific practice, anthropologists accumulate knowledge that constrains their constructions and interpretations.

While forensic anthropology may not be capable of achieving pure objectivity, anthropologists can aspire to a mitigated objectivity. The key is not purging data of their theory-laden nature, but rather foregrounding and explicitly scrutinizing any external values with which they are infused and committing to evaluating, emending, and updating those data continuously as technologies advance and as theories and interpretations develop and change. The more thoroughly anthropologists understand the types of actors/agents that affect what they observe and present as “objective,” the more accurately they can acknowledge and quantify potential sources of error and bias. Every American Academy of Forensic Sciences (AAFS) meeting represents a step in this direction, showcasing the application of new technologies with the capacity to reduce error, the refinement of methods that better constrain scientific interpretations, and perhaps most importantly, the inclusion of new perspectives in the Anthropology Section with the potential to enrich forensic anthropological practice with cultural and archaeological theory and method.

In this presentation, forensic anthropological casework will be used as a framework for illustrating the process of constraining subjectivity in anthropological science. From recovery through analysis, anthropologists must interpret what they see in the ground and in the laboratory, creating reports, maps, and other documentary re-presentations that are value-laden and necessarily reductionist — presenting certain theories, features, and perspectives while backgrounding others; however, cultural theory enhances the interpretation of context, geological and taphonomic theory constrains these interpretations, and evolutionary theory guides conclusions drawn about the remains.

In their pursuit of sound science — evaluating their own subjective biases, constraining their interpretations with theory, and espousing a sense of mitigated objectivity — forensic anthropologists must commit to a continuous process of constraint, a process which involves learning not only from each other, but also from the other subdisciplines of anthropology and the other disciplines of forensic science.

Anthropological Theory, Subjectivity/Objectivity, Error/Bias



Anthropology Section - 2015

A112 Context and Cognitive Bias: Informed Applied Science vs. Working in the Blind

Michael W. Warren, PhD*, C.A. Pound Human ID Laboratory, Cancer & Genetics Research Complex, 2033 Mowry Road, Rm G-17, Gainesville, FL 32610

After attending this presentation, attendees will join a conversation about the differences between applied science and research science. Practitioners subject themselves to cognitive bias by knowing the context in which an investigation is being conducted or does an informed investigation lead to a more thorough and efficient outcome?

This presentation will impact the forensic science community by opening a dialog between researchers and applied scientists related to how forensic investigations are conducted, if and when practitioners should be provided a priori knowledge of a case, and how that knowledge helps or hinders the field of forensic anthropology.

In 1988, human remains were found that were misidentified as being those of a biological female, when in fact, the remains were those of a male-to-female who had undergone treatment for gender dysphoria (e.g., transsexual surgery and hormonal treatment). The theoretical aspects of this case revolve around a discussions related to “blind analysis” vs. “analysis with context.” In this case, the previous forensic anthropologist knew that law enforcement was looking for a “robust” female. Female gendered clothing was found at the scene as well as two silicone breast implants. To further complicate matters, the remains displayed marked pits of parturition. In 1988, the anthropologist was reliant on the current literature of the day, which attributed dorsal pitting and preauricular sulci as being secondary to birth trauma and, therefore, found only in females. So, did the consulting anthropologist misidentify the sex of the decedent because he harbored a cognitive bias due to a priori knowledge of a putative decedent or was he a victim of the current state of the science?

This presentation will discuss the effects of treatment of gender dysphoria on the skeleton. The case will serve as the backdrop for discussing the question, “Should forensic anthropologists always work in the blind or should they integrate contextual clues into their analyses and conclusions?” Cultural anthropologists consider social context and history in their interpretations of behavior; archaeologists must necessarily be provided some context for their site or else they would not be digging there and, to them, context is critical in terms of interpreting the meaning and significance of the artifacts. What about forensic anthropologists? Do they provide a better service to consulting pathologists, law enforcement agencies, and attorneys when provided with the context surrounding the case, or does that information unduly affect their interpretations? For example, when a pathologist provides a forensic anthropologist with the clinical radiographs of a putative decedent, are the results skewed because of some expectation that a radiographic match will be found? When weather data is received for the area in which a body is found, does that alter the estimation of time since death or facilitate it?

It will be argued that research in biological anthropology must be hypothesis driven and “blind” as to the interactions of variables and performed in a way that does not influence conclusions; however, applied science benefits from knowledge of why specific questions are being asked and focuses efforts on aspects of a case that are most likely to yield results, thus avoiding blind paths that may waste the limited time and resources available to forensic practitioners.

Forensic Anthropology, Cognitive Bias, Theory



A113 From Blumenbach to Howells: The Slow, Painful Emergence of Theory in Forensic Race Estimation

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After attending this presentation, attendees will have a greater appreciation of how a very simple overarching theory of human differences greatly influenced the earlier forensic assessment of race, both in application and research, and that more recent research has provided empirical data to better understand human variation and its relationship to self-identity and ethnicity.

This presentation will impact the forensic science community by reminding us that forensic research and work are influenced by practical goals as well as prevailing theories and technologies.

Forensic anthropology, an applied anthropology, has for the most part been more practical than theoretical, favoring techniques that apparently help identify remains rather than techniques derived from theory, until the lack of theory made application untenable. Early approaches to assessing the ancestry or race of human remains in American forensic anthropology largely followed typological theories of human variation involving discrete races, namely, that there were three to five categories of people in the world (involving the “Mongoloid,” “Caucasoid,” and “Negroid” races, types, or stocks). This view reflected the thinking of the so-called father of anthropology, Johann Friedrich Blumenbach (1752-1840), and the father of taxonomy, Carolus Linnaeus (1707-1778).

The classifications of Blumenbach and Linnaeus, based on observations such as “lazy,” “distrustful,” or “ferocious” dominated physical anthropology despite a lack of scientific testing. Human races were biological, phylogenetic, and tautological. As Caspari noted, typologists would frequently ignore their own results within the same publication if they contradicted the overarching typological theory.¹ Typological views likely dominated American forensic anthropology because the biological taxonomy for mankind corresponded so well with the “folk taxonomy” of the United States. Hypothesis testing involving typology was not deemed necessary. In 1990, Alice Brues emphasized the main goal and practical nature of forensic anthropology by stating, “We need not emphasize any single technique because of its theoretical attractiveness: that may be done when we want to ‘advance the science,’ not when we merely want to know who it was that was dug up in the back forty.”² In the same publication, Brues lauded Hooton for collecting data on non-metric trait expression from many skeletons from around the world. The problem is, no one, including Hooton, bothered to analyze those data beyond a few short descriptive publications. Finnegan published analyses of cranial and postcranial non-metric traits from different populations in 1972 and 1978, but no one followed up on them. Statistics and sampling theory were absent in most publications, including one by Rhine in 1990 in which the sample size of “Negroids” was three.³ Gill invented a metric method for ancestry estimation between American Indians and Whites using facial indices that was fundamentally flawed for several reasons.^{4,5} With the blind acceptance of typology and the aversion to data analysis, ancestry analysis in forensic anthropology was much more “faith based” than theoretical and scientific.

The epitome of the faith-based approach is found in the trait list approach, seen in publications by Rhine and Gill.^{3,6,7} The trait list approach illustrates the problem of confirmation bias because if “typical” traits are provided, and the ancestry of a cranium is known, one can always find some traits that are present, seemingly providing validation. The trait list approach was insidious because it was easy to apparently validate, to teach, and to learn. The inevitable individuals with traits from different races were poorly dealt with because estimating ancestry was considered an art. Subsequent presentations and publications often explained an observed mixture of typical traits as due to racial admixture.

Rather than collecting trait frequencies from groups, non-metric trait variation was simplified to the point that the trait became the race: a post-bregmatic depression meant Negroid, Carabelli’s cusp meant Caucasoid. When Hefner observed traits with large enough sample sizes, and basic frequencies were calculated, the vacuous nature of the trait list and the trait-as-ancestry approaches became quite clear.⁸



Anthropology Section - 2015

More recently, forensic anthropology, always practically oriented, has become more data- and theory-oriented, especially after the establishment of the Forensic Data Bank (FDB) at the University of Tennessee (UT) and the Daubert decision. Sauer pointed out that social races are what forensic anthropologists must work with and made a testable statement that skeletal morphology is associated with American White and Black social races, a statement that was recently validated using the FDB and explained by institutional racism and positive assortative mating.⁹⁻¹¹ Variation within classical races is now much more appreciated, as is variation over time. Forensic data now provide the basis for further theory building and research into human variation and the association of self-identity and ethnicity with morphological features, genetic markers, and Quantitative Trait Loci (QTLs).

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Ancestry Estimation, Scientific Method, Validity



Anthropology Section - 2015

A114 Forensic Applications of Isotope Landscapes (“Isoscapes”): A Tool for Predicting Region-of-Origin in Forensic Anthropology Cases

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After attending this presentation, attendees will gain an understanding of the value of isoscapes for predicting region-of-origin in unidentified decedent cases.

This presentation will impact the forensic science community by highlighting applications of stable isotope forensics to issues of human identification.

The goal of this presentation is to describe a theoretical framework for applying stable isotope analyses in forensic investigations, focusing specifically on the use of isotope landscapes — or “isoscapes” — to determine the source of biological materials, such as human remains. This presentation will describe some fundamental stable isotope relationships and demonstrate with case studies the application of isoscapes.

Stable isotope analyses have been used for decades in research settings, but their use in forensic settings is relatively new. The effectiveness of the analytical method is predicated on the ability to precisely measure differences in the ratios of isotopes within a material. Analyses of stable isotope ratios are particularly effective for relating or distinguishing between two samples that have the exact same chemical composition, but unique isotopic compositions.

Often more beneficial than direct sample comparison is origin prediction using measured stable isotope ratios. In this application, the intrinsic chemistry of a material is used to understand its derivation and history. For the identification of an unknown decedent, stable isotopes can provide answers to two questions: (1) are the remains consistent with a known location; and, (2) what are the possible locations from which the remains could or could not have originated?

Origin prediction is possible due to the theoretical relationships affecting isotope ratios in the environment. As an example, physiochemical processes like the evaporation, transport, and condensation impact the abundances of stable isotopes in water molecules and materials derived from water (e.g., plant and animal tissues) in a systematic manner. It is possible to describe isotope ratio variations caused by the spatial patterns of these processes, leading to the development of predictive isoscapes for geolocation purposes. The application of isoscapes can be a powerful tool for investigating unidentified decedents, as demonstrated in two case examples.

First is the case of Saltair Sally, a set of human remains found by the Great Salt Lake, UT, in 2000. Hydrogen and oxygen stable isotope analyses of her hair revealed Saltair Sally was a frequent traveler in the two years before her death, moving between discrete regions of the western United States. The unknown decedent was identified in 2012 and the origin information predicted from the stable isotope measurements fit the (now known) travel-movement history of the individual.

Second is a case from Siskiyou County, CA, where an isolated mandible was recovered from the North Coast Range in 2012. Morphological and metric assessments suggested the decedent was an adult male, although classification of ancestry was indeterminate. Bone and a molar tooth were extracted for carbon, nitrogen, and oxygen isotope analyses; strontium isotope analysis was also conducted on the molar. Regions-of-origin predicted for the bone and molar were compared to determine if the decedent spent his adulthood before death and childhood in similar locations.

Looking forward, the theoretical framework for applying stable isotope analyses and isoscapes demonstrated in this presentation for human remains can be expanded to include other elements, such as lead, and other materials of interest, such as drugs, foods, and spoils of the illegal wildlife trade. As a consequence, applications of isoscapes are likely to grow in the future and play a greater role in both forensic examinations and criminal prosecution.

Stable Isotope Analysis, Isoscapes, Forensic Anthropology



A115 The Anatomy of Age Estimation

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After attending this presentation, attendees will better understand the theoretical foundations for making inferences about biological age from skeletal characteristics. This presentation will discuss skeletal age estimation in terms of method development, theory, history, and practice.

This presentation will impact the forensic science community by delineating the role of high-level, middle-range, and low-level theory in skeletal age estimation and by discussing criteria for method development that satisfies the scientific rigor required by the medicolegal community and, ultimately, the ethical demands of the criminal justice system.

Evolutionary theory provides the overarching framework for forensic anthropology and serves as the basis by which we understand and interpret human biological variation, largely on a population level.^{1,2} Forensic anthropologists apply these interpretations to the individual level, requiring the use of middle-range and low-level theories to make the inferences required by forensic casework.¹ Forensic anthropologists use middle-range theory to draw inferences about lifestyle and behavior from the human skeleton, much like archaeologists draw inferences about behavior from structures and artifacts in the archaeological record. The task of developing a biological profile from skeletal remains relies heavily on middle-range theory, whereas low-level theory forms the basis of method development in forensic anthropology. Researchers develop hypotheses from inductive data, test these hypotheses, then evaluate results to determine if predictions are supported or falsified.¹ Experienced skeletal biologists recognize morphological patterns and deduce how these patterns may be related to the aging process, then use these data for method development.

Historically, this form of inductive reasoning provided the basis to develop the earliest published age-estimation methods. These methods were based on observations made by anatomists, including researchers now considered to be the founding fathers of skeletal age estimation. For example, T. Wingate Todd was an anatomist who used his extensive observations and knowledge of anatomical variation to describe macroscopic features associated with age-related changes in the pubic symphysis and cranium. Although his methods have been revised, Todd's observations still form the basis for age estimation from these skeletal indicators. Todd applied low-level theory to investigate age-related changes in the human skeleton and to develop age-estimation standards still utilized today.

Science is an iterative process. Age-estimation methods constantly evolve with the addition of more complex statistical approaches and new data. A relevant criticism of age-estimation methods is that they lack a sound statistical framework. One such technique involves seriating samples into arbitrary age categories and then describing common morphologies observed in each category. Other methods have lumped observed morphologies into phases and calculated descriptive statistics associated with each phase, ignoring the effect that reference sample age distribution has on age estimates derived from the method. Bayesian statistics have been suggested as a means of overcoming the limitations and bias of so called "age mimicry," but these methods have been slow to be adopted, perhaps on account of computational intensity.

Skeletal age estimation too often is taught and practiced in the absence of theoretical context. Emphasis tends to be placed on application and deriving age estimates for reporting purposes without encouraging burgeoning academics to think about how the methods were developed initially and, perhaps more importantly, under what biological framework they are appropriate. This presentation advocates that practitioners, researchers, students, and educators incorporate theory and history into application. In addition, estimation of the biological profile requires an understanding of how skeletal and soft tissue morphologies of modern humans reflect both current and past microevolutionary processes. Understanding human evolution demands a working knowledge of the development and function of the skeleton and the related soft tissues. Thus, within the context of research, education, and forensic application, this presentation promotes the idea that formal training in anatomy, evolutionary theory, and statistics as well as a working knowledge of history and theory in forensic anthropology, is imperative to move the discipline forward.

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Biological Profile, Age Estimation, Anatomy

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A116 Theory and Histological Methods

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After attending this presentation, attendees will be introduced to multiple theories that guide research in bone histology. Attendees will understand the importance of examining the theoretical approaches of mentors and peers in order to guide future research.

This presentation will impact the forensic science community by demonstrating that theory is well integrated within forensic anthropology and by reminding researchers to consider theoretical constructs in order to stimulate growth in this field.

It is believed within the broader discipline of anthropology that forensic anthropology lacks a strong theoretical foundation, a perception fueled by the seeming lack of high-level theory development within the field. Forensic anthropology tends to operate within middle-range and low-range theories, which is likely associated with the current focus on method reliability. Furthermore, the multidisciplinary nature of forensic science lends itself to complex theory development making it difficult to devise a comprehensive, unified theory in forensic anthropology. Regardless, when examining a method's development, one readily uncovers the theory from which it was conceived. This certainly applies when reviewing the development of histological methods.

Histological methods follow the principles of bone physiology, which describe bone structure and morphology as being controlled by intrinsic factors and influenced by extrinsic environmental factors. Changes in bone over an organism's life produce tissue level indicators useful in anthropological analyses (e.g., age estimation, differentiating human from non-human). Some theoretical models exploring the reason for bone resorption and formation are discussed.

Early Theories: Bernard and Roux introduced two concepts of bone adaptation: the idea of physiologic homeostasis and the principle of bone functional adaptation based on the dynamic interaction between bone cells and the mechanical environment.¹ Wolff's Law states that bone's mechanical environment determines its final mass and trabecular architecture. While accepted as the foundation for bone's functional adaptation, this theory was based on a static mathematical relationship.

The Mechanostat: In the 1980s, Frost proposed that a mechanical feedback system (dubbed the mechanostat) controls bone mass.² Strain thresholds or set-points called minimum effective strains activate or depress bone modeling and remodeling. The threshold strain ranges determine if, when, where, and how long biologic activity switches on or off and may be altered by hormones and biochemical agents.

Osteocyte Inhibitor Theory: Studies have shown targeted remodeling occurs at the site of microcracks, indicating a cause-and-effect relationship. Frost's original proposal stated that microcracks disrupt canalicular connections between osteocytes, thus providing stimulus to initiate remodeling. The osteocyte inhibitor theory provides a different model, whereby osteocyte-canalicular systems act to inhibit osteoclastic activity. When the osteocyte network is disrupted, the constraining mechanism is released, stimulating resorption activity.

The Principle of Cellular Accommodation Theory: In 1999, Turner reported that the mechanostat theory did not explain why non-weight bearing bones do not resorb away under disuse remodeling or why regulation differs by skeletal sites.³ The principle of cellular accommodation theory states that bone cells learn from their physical and biological environments and that adjusting to a new environment causes set-points to vary from site to site depending on local strain. The set-point will be high in weight bearing bones and lower in non-weight bearing bones.

These theoretical models provide the framework for anthropological research in bone histology and guide data interpretation. For example, researchers may sample non-load-bearing elements to observe more age-related systemic-based remodeling. Others select load-bearing elements, but evaluate areas believed to experience less biomechanical stress (e.g., anterior femur mid-shaft). Some consider biomechanical adaptation as a contributing factor for age-related remodeling and sample cortical regions regardless of the mechanical axes. Similar considerations apply when comparing histological structures across species. Various researchers propose that high mechanical stress/strain results in different osteon structure, shape, and/or density patterns. Others suggest differences result from variation in bone formation rates regardless of stress/strain relationships.

Forensic anthropologists tend to focus on the specific rather than the general, the empirical rather than the theoretical; however, the argument that forensic anthropology lacks theory is nonsensical. As evidenced, researchers subscribe to different theoretical models regarding the factors governing bone histomorphology. Despite differing views, working absent of theory is untenable and leads to results void of any real meaning or interpretive power. Inferences from results should be applied to make population-level interpretations to bridge empirical data and theory.

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Anthropology Section - 2015

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Theory, Histology, Forensic Anthropology



Anthropology Section - 2015

A117 Theoretical Foundation of Child Abuse Recognition

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After attending this presentation, attendees will gain an understanding of the theoretical foundation upon which the recognition of skeletal injuries associated with non-accidental injury and how the knowledge of pediatric bone health is built.

This presentation will impact the forensic science community by demonstrating the sound scientific principles behind bone health evaluation and fracture biomechanics in infants and children.

Anthropologists are playing a larger role in the diagnosis of child abuse in the medical examiner setting. They are asked to assess fracture patterns and bone quality in autopsied infants and young children. Anthropologic interpretation in this area is based on the understanding that inexplicable fractures or fractures inconsistent with the traumatic history, when found in children with normal bone health, are highly suspicious for non-accidental injury (i.e., child abuse). This understanding is supported by strong theoretic evidence derived from biomechanical and bone strength studies.

Biomechanical engineers use computer modeling and validation tests based on biofidelic dolls to define impact forces associated with common household accidents, such as an infant rolling off a bed or couch. Meanwhile, bone strength studies are conducted to establish the failure point of bone. Understanding impact forces associated with common household accidents in conjunction with bone strength studies form the theoretical foundation upon which anthropologists build their interpretations. An understanding of normal, daily impact events associated with infancy and childhood enables anthropologists to deduce skeletal injuries that are contrary to those events.

Bone health and strength are dependent on the material composition, structure, and density. Failure of normal bone development or lower-than-expected bone density increases the likelihood of bone fragility, predisposing it to fracture under forces generated by normal handling. Physicists have developed various modalities that enable the evaluation of the physical properties of bone, including quantitative computer tomography; dual-energy X-ray absorptiometry, and quantitative ultrasound. The theoretical foundation of the clinical evaluation of bone strength is based on current research correlating material and physical properties of bone with bone strength.

Forensic anthropologists are able to utilize these theoretical principles to develop and validate methodologies for evaluating infant bone health and strength. The Harris County Institute of Forensic Sciences Anthropology Division is conducting such a study. The Division is prospectively studying skeletal injuries observed during infant autopsy with the goal of identifying injury patterns that are significantly correlated with physical abuse. Furthermore, it is evaluating quantitative ultrasound as a method to measure bone health of infants in the medical examiner setting. All decedents under the age of one year at the time-of-death are included in the study. The physical properties of the bone of each decedent are measured through three modalities: (1) quantitative ultrasound; (2) X-ray attenuation; and, (3) bone histology. Preliminary results show that specific skeletal injuries are strongly correlated with non-accidental injury. Also, speed of sound as measured by quantitative ultrasound appears to vary depending on infant age and health status.

Non-Accidental Injury, Fractures, Bone Health



Anthropology Section - 2015

A118 Thinking Outside the Box: Theory and Innovation in Sharp Trauma Analysis

John A. Williams, PhD, Western Carolina University, Anthropology & Sociology, 101 McKee Hall, Cullowhee, NC 28723*

After attending this presentation, attendees will have an appreciation for how sharp trauma to bone can be analyzed using a novel approach.

This presentation will impact the forensic science community by demonstrating that pattern analysis on bone can be accomplished using Geographic Information Systems (GIS), a tool not normally associated with forensic applications.

In 1910, Locard was the first to recognize the principle of the transfer of evidence. Since then, pattern analysis has become one of the fundamental tenets of criminalistics and, by inclusion, forensic science. In the forensic sciences, tool mark pattern analysis is an inter-discipline crossover. For the criminalist, the emphasis is on the transfer of tool-specific characteristics to inanimate objects. In forensic anthropology, the transfer is to bone.

Sharp trauma involving toothed instruments or tools is a case in point. Teeth create transfer evidence in the form of patterned marks on bone. The fundamental theory behind the analysis of these marks is a simple one. Each class of toothed instrument creates a cut-mark pattern unique, or nearly so, to that class of tool or instrument. Symes took this theoretical base and created the first comprehensive documentation of these transfer patterns. His work formed the foundation of this and other studies of toothed instrument cut marks.

Once tool-specific patterns have been recognized, interpretation and identification of an unknown pattern is a matter of comparison to known patterns. In most situations, the analysis of class-specific cut-mark patterns relies on morphometric characteristics, like kerf thickness and kerf profile. As Symes has demonstrated, this is a highly effective method. Yet there are times when morphometric analysis is hindered, if not rendered useless, by the absence of observable class-specific characteristics. There is one feature of tooth-instrument transfer that is nearly universally present — striations on the cut surface or kerf wall. As a saw or other toothed instrument cuts through bone, the teeth leave behind striations on the cut surface. Despite their visibility, these striations have limited diagnostic power in pattern analysis using traditional morphometric methods.

GIS is a computer-based process designed to visualize patterns. GIS has been described as a means of analyzing and interpreting relationships, patterns, and trends. Anyone who has used consumer mapping “apps” has used GIS. While the intent of GIS is to recognize patterns on large geographic regions, size should not be a limiting factor. Looking for patterns on an area the size of a state and on the kerf wall of a cut bone is simply a matter of scale.

The inspiration to use GIS to examine striation patterns on bone came from two recent studies. Both were novel approaches to the use of GIS and demonstrated the value of using tools that were not designed for their original purpose. In the first, Powell and colleagues utilized GIS to map out fracture patterns on infant porcine skulls.¹ In the second study, Rose and colleagues moved to the microscopic level in GIS patterns of bone microstructure.² Their success in the use of GIS in their respective studies demonstrated the effectiveness of GIS in the recognition and interpretation of patterns other than geographic ones. Based on these prior studies, Williams and Davis successfully explored the use of GIS in recognizing and differentiating striation patterns on the cut surface of bone.³ In this study, striation patterns were viewed as the equivalent of variations in geographic topology. Two different saw classes were compared, each with uniquely different striation patterns. GIS consistently recognized these as distinctly different patterns of transfer evidence. Using traditional morphometric methods, such a distinction would not be possible.

The theory of transfer evidence on cut bone has followed the traditional route of morphometric analysis. Recent studies have demonstrated that “working outside the box” by using technologies that were not originally designed for bone-specific patterns has yielded encouraging positive results. These studies have shown that GIS has promise to become a tool in trauma analysis and not merely an interesting academic exercise.



Anthropology Section - 2015

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Theory, Cut Marks, GIS



A119 Non-Linear Systems Theory and Its Application to the Assessment of Postmortem Interval

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After attending this presentation, attendees will understand the application of non-linear systems theory and computer simulation to interpreting and resolving the complex, multivariate problem of determining Postmortem Interval (PMI).

This presentation will impact the forensic science community by demonstrating the application of a non-Newtonian scientific model — non-linear systems theory — to forensic interpretations involving the interaction of multiple human and non-human variables.

Since Dr. William Bass' establishment of the "Body Farm" in 1981, a plethora of decomposition studies have emerged from this and other decay facilities with a goal of developing a more complete understanding of PMI through experimentation and actualistic study. These studies have identified a multitude of variables affecting PMI. In spite of these studies, no overall development of precise region-specific PMI models has been proposed.

The goal of this presentation is to explore the utility of non-linear systems theory and computer simulation in enhancing forensic anthropologists' ability to define PMI. Non-linear systems refer to environments with multiple variables, the interaction of which can produce more complex and unforeseen results. Non-linear systems theory is focused on how those systems are understood and analyzed. It is an ideal theoretical model to use for assessment of PMI, given the large number of interacting variables.

Historical contingency (i.e., the effects of unique or random events) and the impact of agents (both human and non-human) are given consideration in non-linear systems theory. This is important, since such events and agents may not produce patterned behavior but would still have considerable impact on the creation of the forensic scene and its interpretation.

Non-linear systems theory is quite different from the traditional reductionist scientific paradigm in that it does not isolate variables for testing but instead focuses on the results of their interaction. One important method often used in non-linear systems analysis is computer simulation which can explicate past events through the analysis of the interaction of a number of variables. Such analyses can also examine the temporal sequence of events by revealing the emergent (new and/or unique) properties periodically resulting from variable interaction. By recording environmental variables for forensic cases and experimental settings and using these in simulation studies, region-specific models for PMI can be developed which can delineate the temporal sequence of decomposition. These models may then be applied to the interpretation of new cases. The application of non-linear systems theory can, therefore, stimulate a new understanding of the PMI for forensic anthropologists and improve future interpretation of this complex problem.

To illustrate the application of this theory to PMI interpretation, a pilot study involving 20 stillborn pigs placed in different environments and examined over a period of 50 days during late summer is presented. Variables examined include pig weight, microenvironment (indoor/outdoor), degree of insect infestation, ambient temperature, and rainfall. A decomposition scale designated the degree of decay at different intervals. Non-linear simulation showed that decay rates varied between outdoor and indoor specimens. Outdoor specimens decomposed more rapidly and at a higher rate during the first five days; subsequent to this, outdoor and indoor decay rates stabilized and were comparable. This simulation created a model for decay rates of specimens over time and explained the stages of decay in relation to the variables noted above, with cumulative temperature being the most significant variable. To calculate PMI, decomposition score is matched to a value for cumulative temperature, then compared to available climate data.

Previous researchers have considered forensic anthropology to be non-theoretical or burdened with so many complex variables that it cannot be "scientific" in its interpretations. Non-linear systems theory can mitigate these problems by providing a broader definition of scientific theory and a methodology (simulation) whereby multiple variables can be examined in a systematic manner. It also can provide the theoretical and methodological tools to develop regional models for PMI. Non-linear systems theory holds the promise of honoring Dr. Bass' original research goal of reaching a more informed understanding of postmortem interval.

Non-Linear Systems Theory, Computer Simulation, Postmortem Interval



Anthropology Section - 2015

A120 The Forensic Anthropologist as Broker for Interdisciplinary Taphonomic Theory

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The goal of this presentation is to provide attendees with an understanding of why it is necessary to develop interdisciplinary theory within the forensic sciences to address questions related to the postmortem interval.

This presentation will impact the forensic science community by outlining how an interdisciplinary, rather than a multidisciplinary, approach is necessary to accurately and precisely estimate the postmortem interval, and by describing why forensic anthropology is ideally suited to broker this interdisciplinary research to build unifying theories. This presentation follows the planks for this year's meeting — honoring our mentors, learning from each other, and stimulating our future — by discussing a complex issue shared by many of the forensic science disciplines: estimating the postmortem interval.

Estimating the postmortem interval is an issue shared by numerous disciplines, including anthropology, medicine, entomology, and microbiology among others. The complexity of decomposition is well understood and those involved in this area strive to uncover the underlying similarities and rules governing how carrion is recycled. In the past, each discipline has attempted to understand this complexity using their own methods and theories; however, new models and approaches are needed, and this cannot be accomplished if the various disciplines approach the topic separately. Complex issues that are driven by multiple forces, such as carrion recycling, require an interdisciplinary approach where multiple disciplines cooperate to develop new methods and theories that transcend each discipline to solve a real-world, multifaceted problem. An integration of the knowledge from various fields, combining data, methodologies, perspectives, and concepts is needed to develop a more unified theory of forensic taphonomy that describes, explains, and predicts all the phenomena observed. It is only through interdisciplinary research that the common problem of estimating the postmortem interval will be satisfactorily addressed.

With the establishment of the first outdoor decomposition facility, Dr. William Bass sought to describe, explain, and even predict the processes of human decomposition. He also encouraged multidisciplinary studies. Under such an approach, forensic anthropologists have commonly addressed questions related to gross morphological changes in the soft and hard tissues of a decomposing body. Entomologists, on the other hand, have mainly been concerned with questions related to developmental stages of arthropods present on carrion, and microbiologists have addressed the diversity and quantity of microorganism on decomposing remains. Using a multidisciplinary approach, a great deal has been learned about the numerous phenomena that occur during decomposition, including how to describe and explain them. Even so, to this date, the postmortem interval still cannot be predicted, especially longer lengths, with the accuracy and precision needed in medicolegal death investigations.

Since the early pioneering work by Dr. Bass and others, forensic anthropologists have adopted methods from other fields, such as the use of accumulated degree days instead of calendar days, to help account for some of the regional and even micro-environmental differences in climate that affect the postmortem interval. Nevertheless, after the examination of over 200 decomposing human remains at Texas State University's Forensic Anthropology Research Facility, for example, it is clear that gross morphological changes do not strongly correlate with accumulated degree days in central Texas.¹ The reason for this is that the necrobiome responsible for carrion recycling is highly dependent on a number of abiotic environmental factors. As vertebrates decompose, microorganisms, arthropods, mammalian, and avian scavengers compete and, in some cases, cooperate to obtain nutrients from the valuable and short-lived carrion resource. In addition, these scavengers must persist and thrive under varying environmental conditions that sometimes involve human intrusions. Unlocking the mystery of the ecology of carrion recycling is key to understanding and interpreting the process and rate of the postmortem interval in various geographical and ecological regions and for establishing methods that accurately and precisely estimate the postmortem interval. Accomplishing this is only going to be possible using an interdisciplinary approach.

Forensic anthropology has traditionally borrowed and utilized theory and methodologies from both the natural and social sciences in research and evidence interpretation. As a result, forensic scientists are in a unique position to act as brokers in the development of interdisciplinary methodologies and theories. What better way to stimulate the future and honor the pioneering efforts of Dr. Bass and others in this discipline than by facilitating interdisciplinary theory that allows an accurate description, explanation, and estimation of the postmortem interval?



Anthropology Section - 2015

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Forensic Anthropology, Interdisciplinary Theory, Postmortem Interval



Anthropology Section - 2015

A121 **Arrows of Influence: The Return Flow of Theory and Method to the Parent Disciplines of Modern Forensic Anthropology**

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After attending this presentation, attendees will have a better understanding of the relationships between the theoretical and methodological foundations of forensic anthropology and those of disciplines which have influenced forensic anthropology historically.

This presentation will impact the forensic science community by stimulating an examination of the evolving relationships between forensic anthropology and its parent disciplines with regard to low-, middle-, and high-level theory. A dialectic “back flow” or return of information and influence is proposed.

Situations in which dominant cultures come into contact with subordinate cultures produce what has been called “contact shock.” This is the overwhelming cultural influence of the dominant culture upon the subordinate culture which may settle over time into a more balanced exchange. Similarly, as forensic anthropology evolved over the last century, it was heavily influenced by other disciplines and may now be reaching a new era in its development, as a more equitable state of exchange with these disciplines appears to be forthcoming. Exponential change in forensic anthropology has come about in recent decades; the contextual framework, perception, and practice of the field have developed under the influence of theoretical and methodological constructs derived from biological sciences, archaeology, geophysics, criminalistics, and molecular biology, among others. This study considers both current and future aspects of informational, theoretical, and methodological “back flow” from contemporary forensic anthropology to several of the disciplines from which it borrowed and adapted theory or methodology in forming a cohesive, evolving discipline.

It will be argued that, as the discipline develops, theories can be offered back to the original disciplines as a means of addressing old questions in new ways. This can be accomplished by linking current and past behaviors via similarities their signatures leave in the environment. Examples of topics from other disciplines to which forensic anthropology can contribute new understanding include: (1) the interpretation of site formation processes in the archaeological record; (2) the interpretation and understanding of sub-surface geophysical signatures and their formative components; (3) the development of systematic training and evaluation of human remains detection dog search methodologies and their potential utility and limitations for archaeological prospection; (4) socio-cultural insights into aberrant behaviors such as homicide, war crimes, and genocide where participants and witnesses are no longer alive; and, (5) studies of modern human variation and their implications for biological anthropology and bio-archaeology. These areas of “back flow influence” have the potential to significantly contribute to new theoretical and methodological approaches to many disciplines that have been important in the development of forensic anthropology. These areas should be fostered by forensic anthropology practitioners as is appropriate to this holistic and dynamic field.

Theory, Archaeology, Method



A122 Strontium Isotope Ratios of Hair for Human Provenancing

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After attending this presentation, attendees will gain an understanding of how the distribution of strontium isotopes in human hair is related to geography and how this information can be used to answer questions regarding human origins and movement. Attendees will gain specific knowledge on how strontium is incorporated into hair, how strontium isotope profiles within hair can be used to reconstruct regions-of-residence and travel movements, and how strontium isotopes can be combined with other isotopes to refine human movement histories.

This presentation will impact the forensic science community by demonstrating that the innate chemical composition of human tissues reflects an individual's origin and travel history.

This presentation focuses on the application of strontium isotope analysis to human hair to aid in the identification of individuals of unknown origin or travel history. Reconstructing the travel-movement history of individuals of unknown origins reveals pertinent information for forensic investigations, spanning from nationwide homeland security issues to investigations of unidentified decedents by local agencies. Analysis of stable isotopes in human scalp hair has shown to be a useful tool for reconstructing the recent geographic-movement histories of individuals because hair proteins (i.e., keratin), and the stable isotopes contained within keratin, are fine-scale recorders of an individual's geographical environment.

As an example, the oxygen (O) isotope values ($\delta^{18}\text{O}$) of human hair keratin can provide travel/geographic origin information and guide criminal investigations. This is due to the well-established relationship between the $\delta^{18}\text{O}$ values of human hair and an individual's drinking water. Since the $\delta^{18}\text{O}$ values of drinking water vary predictably across landscapes, the $\delta^{18}\text{O}$ values of human hair correlate to specific geographic regions. These geographic projections of isotope values across landscapes are termed "isoscapes." While $\delta^{18}\text{O}$ isoscapes provide a valuable tool for describing geographical spaces where an individual may have originated, the estimated geographical regions can be broad. To refine these broad region-of-origin predictions, investigators need a complementary isotopic approach that reflects different geographic information.

Strontium (Sr) isotope ratios ($^{87}\text{Sr}/^{86}\text{Sr}$) of human hair have attracted interest as a complement to $\delta^{18}\text{O}$ values, because geographic variations in $^{87}\text{Sr}/^{86}\text{Sr}$ reflect local geology. While it has been previously established that the $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of human hydroxyapatite tissues (e.g., bones and teeth) relate to geography, the application of Sr isotope analysis to keratinous human tissues (e.g., hair and fingernails) had been avoided to date due to low Sr concentrations within these tissues; however, recent analytical advances have made analysis of keratin-based tissues possible, which have proven useful in reconstructing animal geospatial histories. As human hair is structurally similar to non-human keratinous tissues, $^{87}\text{Sr}/^{86}\text{Sr}$ values of human hair should also provide geospatial information.

To understand the linkages between the Sr in keratinous tissues and an individual's geographical environment, human hair and tap waters were collected from 55 cities throughout the contiguous United States; the $^{87}\text{Sr}/^{86}\text{Sr}$ ratios were measured on paired hair-water samples. Paired tap water and hair $^{87}\text{Sr}/^{86}\text{Sr}$ ratios were significantly correlated and displayed a 1:1 relationship, indicating no Sr isotope fractionation between water and hair. These results indicate Sr isotope signals from water are reflected in the isotope ratios of the individual's hair and, in turn, the $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of human hair provide geographic information relating to the locality where an individual resides. As O and Sr isotope ratios within human hair are both geographically controlled, but reflect different physical and chemical environmental processes, the paired analyses of both O and Sr isotope ratios would allow for a finer resolution reconstruction of the travel-movement history of an individual than analysis of either O or Sr alone.

Geography, Origin, Movement



A123 Refining Hydrogen and Oxygen Isoscapes for the Identification of Human Remains in Mississippi

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After attending this presentation, attendees will understand the importance of conducting regional refinement of hydrogen ($\delta^2\text{H}$) and oxygen ($\delta^{18}\text{O}$) isoscapes for the United States when assessing the variability in stable isotope signatures of tap water.

This presentation will impact the forensic science community by increasing the accuracy of isotope baseline data for the utility of identifying human residential origin and will provide encouragement to conduct regional studies of $\delta^2\text{H}$ and $\delta^{18}\text{O}$ isoscape refinement on a national level.

Isoscapes are maps created to visualize large-scale geographic distributions of isotopes in environmental systems.¹ Baseline isoscapes are generated from the collection of stable isotope data and modeled for large-scale regions using geo-statistical programs. They provide an additional resource for forensic investigators to determine residential origin when assigning human isotope signatures to geolocation. Isoscapes, although accurate, provide imperfect estimates for certain regions with less isotope coverage. For instance, the United States $\delta^2\text{H}$ and $\delta^{18}\text{O}$ tap water isoscapes have insufficient isotope sample coverage for Mississippi (n=4). This research project refines the $\delta^2\text{H}$ and $\delta^{18}\text{O}$ isoscapes for Mississippi to determine whether increased sampling significantly alters the isotope patterns previously reported for the state.

The $\delta^2\text{H}$ and $\delta^{18}\text{O}$ isoscapes for Mississippi were generated from tap water samples collected across the state (n=58). Sample locations were selected by physiographic region and population density to account for diverse water sources. Tap water samples were collected from public restrooms in March and April 2014 and recorded with a Global Positioning System (GPS) unit. $\delta^2\text{H}$ and $\delta^{18}\text{O}$ was measured using a Thermo™ Finnigan™ Isotope Ratio Mass Spectroscopy (IRMS) coupled with an elemental analyzer, and data was normalized to the Vienna Standard Mean Ocean Water-Standard Light Antarctic Precipitation (VSMOW-SLAP) scale. Isoscapes and spatial analyses were conducted with ArcGIS® 10.2, using precipitation and temperature priors from IsoMAP®. $\delta^2\text{H}$ and $\delta^{18}\text{O}$ data were classified into Jenks natural breaks and interpolated using ordinary Kriging. The isoscape data was calibrated using a linear regression model and incorporated into each isoscape using raster math. These isoscapes were compared to the national tap water isoscapes presented in Bowen et al. to evaluate any spatial refinement.²

$\delta^2\text{H}$ values ranged from -18.4‰ to -33.1‰, and the $\delta^{18}\text{O}$ values ranged from -4.10‰ to -5.70‰. These data coincide with those presented in Bowen et al., with the exception of one $\delta^{18}\text{O}$ sample falling between -3.9‰ and -2.0‰ in the coastal region.² Distinct differences were observed between the $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values in east and west Jackson, which may relate to available city water sources. A gradual enrichment of $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values was observed from the northern state boundary toward the Gulf coast suggesting stronger correlation with hydrology rather than physiographic region. Both isoscapes exhibit non-random spatial autocorrelation. The $\delta^{18}\text{O}$ isoscape had significant clustered spatial autocorrelation (Moran's I=0.3568; Z=2.004; p<0.05) with notable patterns in the Mississippi Delta. Isoscapes for various Rare Earth Elements (REEs) will also be presented, which provide a means to cross-reference isotope data for more accurate residential origin estimates.

Increased tap water sampling on a regional scale is warranted for $\delta^2\text{H}$ and $\delta^{18}\text{O}$ isoscape refinement to improve the utility of isotopes for accessing human residential origin. Sample size should be increased in cities with large populations to consider multiple water sources. When utilizing $\delta^2\text{H}$ and $\delta^{18}\text{O}$ isoscapes in forensic anthropology, $\delta^{18}\text{O}$ may be the more reliable indicator due to the increased spatial refinement results presented here. These results will be tested on skeletal remains from Mississippi in the next phase of research using these new assignment models.



Anthropology Section - 2015

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Isoscape, Residential Origin, Spatial Refinement



A124 Finding the Graves of the Missing: A Study of Geo-Anthropological Techniques in Bosnia-Herzegovina

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After attending this presentation, attendees will learn about new geo-anthropological techniques to define search parameters for persons missing and presumed dead from armed conflict. Attendees will learn why the search for the missing in Bosnia, which has relied on witness testimony, has become unproductive and how to use geographic methods to create refined search parameters and discover new sites.

This presentation will impact the forensic science community by showing how geographic techniques can be used by anthropologists to better plan searches as well as to complement or compensate for other, perhaps less reliable, methods. This presentation will demonstrate how geo-anthropological methods enable the more effective prioritization of search areas, perhaps most importantly, when there are no witnesses available to identify burial sites. Attendees will learn how a nearly exclusive reliance on oral testimony in Bosnia-Herzegovina has led to a recent dramatic decline of grave discoveries and missing person identifications despite the fact that there are over 7,000 people still missing from the war. New methods are needed to find the missing for both humanitarian and medicolegal reasons.

Previous research has shown that the movement of victims from the time and place of detention to their burial site is fairly limited. This study uses burial sites as the unit of analysis, using a sample of 274 confirmed burial sites with positively identified victims in Bosnia-Herzegovina. Although many more body disposal sites have been discovered in Bosnia, there are problems with the reliability of information about, among other things, circumstances of disappearance and death as well as victim and/or perpetrator identity. Location precision is another problem with many recovery sites known to date. This study collected and coded data such as victim and perpetrator ethnic affiliation, civil status (e.g., police, military, civilian), combat front lines, site visibility, and other socio-geographic factors. Geospatial and spatial statistical tests were run to examine changes across time and regions throughout the conflict. Tests conducted included spatially weighted regression and Ripley's k-function cluster analysis.

Results show that a 20km buffer around the place of death captures approximately 90% of the victim disposal sites included in this study. Cluster analysis of graves showed their location was not at all random, indicating that the placement of one burial site influences (attracts) subsequent burials. This was especially true for targeted mass killings of civilians where questions of logistics overwhelmed the ability of killers to dispose of all victims at one time and location. Different tests showed a degree of variability in site locations but also allowed the researchers to identify factors that appear to influence offenders in their selection of body disposal sites.

Using these methods to limit and prioritize search areas, investigators can focus their efforts to find new witnesses who are more likely to have direct knowledge of undiscovered sites. Using geo-anthropological methods to understand mortuary behavior in conflict contexts will also enable investigators to better plan for searches in countries where conflict is still ongoing and too dangerous in which to operate, such as Syria, Iraq, and the Ukraine.

Forensic Anthropology, Geospatial Technology, Missing Persons



Anthropology Section - 2015

A125 Multi-Temporal Remote Sensing of Mass Graves in Temperate Environments

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After attending this presentation, attendees will understand how multi-temporal, multi-spectral, fine spatial-resolution satellite imagery and derived vegetation phenological metrics can aid in the location and detection of human mass graves in temperate environments.

This presentation will impact the forensic science community by potentially revolutionizing the way mass graves are detected. If successful, mass graves will be able to be detected efficiently over large areas and independent of physical or political borders. Furthermore, data processing and interpretation can occur remotely without having to deploy forensic teams to the field, thus bypassing major logistical and safety issues.

Mass graves resulting from mass disasters, human rights abuses, and war are worldwide, societal, and humanitarian issues which pose huge geographical issues and challenges to those responsible for their investigation. Existing published scientific literature detailing appropriate techniques is limited and scarce with the methods used often being ad hoc and based on available resources and finances rather than being the most scientifically appropriate.

Recent years have seen mass graves become a stimulant for criminal proceedings and investigations. Consequently, the detection of clandestine mass graves is at the forefront of international forensics and is considered of ever-increasing forensic importance.

This presentation will initially detail results relating to the investigation of phenological differences of a large-scale, proxy, decadal mass grave site resulting from the 2001 foot-and-mouth disease outbreak in the United Kingdom. This provides a pre-operational proof of concept for clandestine human mass grave detection in temperate environments using multi-temporal (18-day repeat period or multiple thereof), multi-spectral (visible and infra-red, .45 to 12.5 μ m), fine spatial resolution (30m ground sample distance) orbital remote sensing. A dense time-series of archive cloud-free Landsat Thematic Mapper (TM) and Enhanced Thematic Mapper Plus (ETM+) satellite imagery (1999-2011) has been used in conjunction with imagery from the Disaster Management Constellation (DMC) imagery (2002-2011) to quantify phenology of the vegetation directly above the grave and the undisturbed vegetation surrounding it.

Results will also be presented which detail the application of the aforementioned imagery and method of the detection of clandestine human mass graves in Bosnia. In this instance, imagery from the early 1990s to the 2000s is used to cover the period of mass grave interment and exhumation resulting from the conflicts in the 1990s. Within-season and inter-annual phenological metrics (for exhumed human mass graves of known location) have been critically evaluated as a means of detection, which can in turn be applied to detection elsewhere.

Mass Grave, Remote Sensing, Phenology



Anthropology Section - 2015

A126 **Twenty-Eight Years of Forensic Anthropological Casework in the Northeastern United States: Trends, Patterns, and Lessons Learned**

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After attending this presentation, attendees will be familiar with forensic casework encountered in the northeastern United States, as the current study is the first long-term analysis of cases in this region.

This presentation will impact the forensic science community by revealing and detailing trends, patterns, and factors that may be encountered in similar regional casework and by providing comparative data for other regionally focused case syntheses.

In the past 20 years, there has been a shift in the field of forensic anthropology from merely a laboratory-based discipline, concerned only with the establishment of a biological profile, to a broad spectrum discipline that also includes forensic archaeology, forensic taphonomy, and trauma analysis.¹ As a result of this expansion in the real and perceived roles of forensic anthropology, forensic anthropologists are being routinely called upon to assist in more cases. Unfortunately, analyses of the trends in casework conducted by forensic anthropologists are rare and are often specific to a particular region of the country; however, these analyses can provide important information about trends in the field that may be used by practitioners and also by educators to re-evaluate the focus and needs of their academic programs as the field shifts and changes. Comprehensive reviews of casework from specific regions can reveal patterns of: (1) taphonomic factors (e.g., types of animal scavenging) to create better Postmortem Interval (PMI) estimates; (2) decedent deposition, including locations and environments; (3) decedent demographics (e.g., sex/age/ancestry differences); and, (3) trauma, all of which can then be used as Komar notes, in the future “to generate research models, support court testimony, and provide comparative data...”²

This presentation focuses on forensic casework from 1986 to 2013, first at the University of Pittsburgh and later from the chair of Mercyhurst University’s Department of Applied Forensic Sciences (DAFS) in Erie, PA. A sample of 543 cases from the DAFS case database were evaluated and information on requesting agencies, specific case location, number/types of cases, depositional environment, recovery season, taphonomic influences, decedent demographics, and skeletal trauma were compiled and analyzed.

Over the period of observation, especially since 1991, caseload steadily increased, which can likely be attributed to: (1) employment of forensic archaeology; (2) education of law enforcement and coroner/medical examiners regarding roles and benefits of forensic anthropology/archaeology; (3) technological advances in timely and accurate evaluations of forensic significance; and, (4) producing high-quality recoveries, analyses, and reports. Cases conducted were documented from 15 states (mostly Pennsylvania, western New York, and eastern Ohio), four countries, and the territory of Guam. Most (79.9%) were modern forensic cases, while others were deemed non-forensically significant upon recovery (historic: 17.0%; prehistoric: 3.9%).

Human cases submitted for laboratory analyses alone were more prevalent (22.1%) than cases involving forensic archaeological recoveries (15.7%) despite the value of visiting the scene and conducting recoveries. Approximately half (42.2%) of the cases were evaluations of forensic significance (human vs. non-human), several of which were consultations for wildlife services and humane societies. Most modern cases involving forensic archaeological recoveries occurred during fall (34.1%) and summer (29.4%). The higher incidence during these months is likely explained by hunting in fall and camping/hiking during summer in remote areas, given that: (1) most of these recoveries occurred in wooded areas (43.7%) or open fields (12.6%); and, (2) surface scatters (50.6%) were more common than burials (32.9%), fatal fires (8.2%), or mass fatalities (8.2%). The remaining case types included searches (10.5%) and reviews of cases (6.8%). In cases with documented trauma, blunt force (28.4%) was most common, followed by ballistic (25.3%), multiple types (18.9%), fire (16.8%), and sharp (4.2%) trauma. In cases with both known and estimated age, adults in their prime (30-55years) were most prevalent. Males and individuals of Caucasian descent were encountered more frequently in both lab analyses and recovery cases. These results correspond with the known demographics of Pennsylvania, given that 83.5% of the population is “White” and that males (48.8%) and females (51.2%) are nearly evenly represented.³

The current research uses the aforementioned data and results as a basis for further interpretations of regional trends in the field, to evaluate how the discipline is progressing, and for future research (e.g., Dirkmaat et al.).⁴



Anthropology Section - 2015

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Forensic Anthropology, Forensic Archaeology, Forensic Casework



A127 Building Sustainable Programs of Recovery and Identification of Human Remains — A Comparative Analysis of Forensic Programs: The Examples of Kosovo and Cyprus

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After attending this presentation, attendees will gain a better understanding of the significance of local forensic capacity building in establishing successful programs of recovery and identification of human remains, as learned by the International Committee of the Red Cross (ICRC).

This presentation will impact the forensic science community as well as those involved in the humanitarian domain by drawing on lessons learned from past and existing forensic programs in order to identify the key elements that contribute to their development.

While acknowledging that success cannot be easily defined or quantified — especially in a context where a plethora of specialists and non-specialists are called to contribute to the process while an even greater number eagerly and apprehensively await for results — the goal of this study is to identify the markers of success as well as the context-specific adversities and socio-economic and socio-political parameters that define the trajectories of forensic programs.

For the purposes of this study, success will be assessed on the basis of: (1) operational continuity; (2) quality of forensic work by adhering to best professional standards; and, (3) activities performed (percentage of persons exhumed and identified).

The presentation will begin with a short historical overview of forensic activities from the 1980s to the present day, providing background from South America, the Balkans, the Middle East, and Asia before turning its focus to the specific examples of Kosovo and Cyprus.

The two European examples were selected based on similarities in context, including the scale and the demographics of the missing population. The island of Cyprus, with a population of 600,000 in 1960, experienced two periods of inter-communal armed conflict, during which approximately 2,000 individuals went missing; in Kosovo — with a population of two million — approximately 6,000 individuals went missing during the armed conflict that broke out in 1998, following the break-up of the former Yugoslavia.

By comparing the two contexts, where the scale is similar yet the approach is completely divergent — Kosovo as an immediate response program with shorter-term legal objectives and Cyprus as a longer-term program with humanitarian objectives — the goal of this study is to extrapolate and consolidate the factors at play and, through statistical analyses and comparison with other contexts, scientifically corroborate the hypothesis that building local capacity is key to success.

In order to achieve the above, the variables that will be looked at include: (1) the nature of the conflict (armed conflict/enforced disappearances); (2) political will; (3) availability of funding; (4) nature of the mandate (legal/humanitarian); (5) involvement of international agencies (external forensic personnel); (6) socio-economic circumstances; (7) staff turnover; and, (8) training and involvement of local forensic practitioners.

Although it has been the long-held rhetoric among forensic experts, international aid agencies, and other stakeholders that investing in local capacity is key to sustainability, there remains a significant gap in the scientific literature — especially for the two contexts upon which this presentation focuses — to scientifically support the argument and to document the long-term practical implications of the (non)involvement of local practitioners and the overreliance on external help. Through both quantitative and qualitative analyses, this study seeks to address this gap, contribute to the historical documentation of the evolution of forensic programs, and provide recommendations for the establishment of future ones.

Data for this study have been collected from ICRC archives, scientific articles and book chapters, websites of international agencies, as well as through interviews with the forensic and non-forensic personnel who provided invaluable and previously unpublished material.

With the on-going conflict in a great part of the world today and 30 years since the application of forensic principles to human rights investigations in Argentina, which saw Clyde Snow's commitment and vision to train a group of local young students, this presentation hopes to build on the collective experience of the forensic community, empower a new generation of forensic scientists, and encourage international organizations to ensure that local authorities assume greater responsibility and local ownership.

Humanitarian Forensics, Forensic Capacity Building, Forensic Strategies



A128 The Impact of Daubert on the Admissibility of Forensic Anthropology Expert Testimony

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After attending this presentation, attendees will understand how forensic anthropological expert testimony has been utilized in both federal and state courts as well as how such testimony has fared in admissibility determinations, both before and after the United States Supreme Court's 1993 *Daubert v. Merrell Dow Pharmaceuticals, Inc.* decision.

This presentation will impact the forensic science community by providing information on the range of topics about which forensic anthropologists are asked to testify and how this testimony is currently being evaluated by judges when the admissibility of the testimony is questioned.

During the 1970s, only a single published judicial opinion made reference to forensic anthropological expert testimony. Since then, there has been a steady and significant increase in the discussion of forensic anthropological testimony in judicial opinions, with nearly 250 references between 2000 and 2014. During this surge in testimony, expert witnesses also faced a change in the standard for the admissibility of their testimony. The 1993 Supreme Court *Daubert* decision shifted the question of admissibility from a relatively low threshold of "general acceptance" to a higher threshold requiring intensive evaluation of the expert's qualifications and the relevance and reliability of their testimony.

The goal of this study is to provide an initial analysis of judicial opinions citing forensic anthropological testimony and determine: (1) the individual identified as the forensic anthropologist; (2) whether this individual was certified by the American Board of Forensic Anthropology; (3) the areas in which this individual provided opinion evidence or testimony; (3) whether any challenges to the admissibility of the testimony were raised; and, (4) if there were any challenges, whether the testimony was admitted or excluded. A comparison of the challenges occurring before *Daubert* to those after *Daubert* provides an indication of whether *Daubert* has actually had a significant effect on forensic anthropological expert testimony. Searches were conducted using two legal databases, encompassing written judicial opinions from all federal and state courts. These databases were searched for the phrases "forensic anthropology" and "forensic anthropologist." Three hundred and thirty-three unique cases were identified and analyzed.

By topic, the most common area of testimony is trauma and/or tool mark analysis (40% of judicial references), followed by biological profile or victim identification (19%), postmortem interval (11%), and a combination of forensic recovery, decompositional processes, movement of remains, footprint analysis, and photographic comparisons accounting for the remaining testimony.

Thirty cases were identified that addressed the admissibility of forensic anthropological expert testimony, including 14 cases prior to *Daubert* and 16 after *Daubert*. Examination of these cases indicates that post-*Daubert* cases do not result in more exclusions. Prior to the *Daubert* decision, in only three cases was forensic anthropological expert testimony excluded, and only a single instance of exclusion was found among the post-*Daubert* cases. Both footprint analysis and photographic comparisons were subjected to a disproportionately higher number of challenges, given the overall low number of judicial references to such testimony.

This study found that *Daubert* does not appear to have actually impacted the outcome of admissibility determinations in the predicted manner. Although many judges cite *Daubert* and follow its framework for analysis of the admissibility of expert testimony, there is no evidence of an increasing number of exclusions of expert testimony in the forensic anthropological context.

Forensic Anthropology, Daubert, Expert Testimony



A129 Getting the Record Straight: Forensic Evidence of the Lurigancho Prison Massacre in 1986

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After attending this presentation, attendees will have a better understanding of the injuries related to cruel, inhuman, and degrading treatment in a prison context as well as of the complexities of death while in custody.

This presentation will impact the forensic scientific community by showing the way in which inmates, after surrendering, were the subject of numerous episodes of trauma that resulted in their deaths while in custody of the Armed Forces.

According to the final report of the Truth and Reconciliation Commission, in the early morning of June 18, 1986, terrorism inmates secluded in three prisons launched a simultaneous mutiny, in some cases taking prison guards and police hostage.¹ Coincidentally, the XVII Congress of the International Socialist was taking place in Lima, Peru. Then-president Alan Garcia Perez entrusted reestablishing order to the Joint Chiefs of Staff, who in turn assigned one prison to each branch of the military (Army, Navy, and Air Force). The next day, June 19, 1986 the human cost of ending the riots was known: at the prison-island of El Fronton, 118 prisoners and four marines died; at the women's penitentiary, one inmate died; and, at Lurigancho prison, once inmates surrendered, 123 of them were summarily executed in the prison yard.

This study presents the results of the examination of 114 bodies corresponding to adult males between 20 and 50 years of age, who were buried secretly and without identity in various cemeteries in and around Lima by the military immediately after taking control of the prison. Under a Supreme Court ruling, graves were located and bodies exhumed in order to identify them and determine the circumstances in which these individuals died.

While official accounts indicate prisoners were shot to death after being extracted from the pavilion in which they were housed, forensic evidence suggests further episodes of trauma prior to the time of death, including, but not limited to, blunt force trauma to the chest, possibly caused by kicks, stomping, or other mechanisms of compression.¹ Additionally, multiple gunshot wounds from high-velocity missiles, the majority of which were located in the head/neck, chest, and upper and lower limbs, were recorded. Considering the good preservation of the skeletal remains and associated artifacts, it has been possible to associate trauma episodes between bone and clothing.

This case study is a unique opportunity to establish the relationship between the "control of fact," by which an organization (the Armed Forces, in this case) controlled a situation through its command structure, functioning, and hierarchy, and the nature of the injuries sustained by the individuals during the time they spent in the organization's custody.

Reference:

1. 2.6_7. Las ejecuciones extrajudiciales del penal de el Fronton y el Lurigancho (1986). pp. 737-768. In: Informe final de la Comision de la Verdad y Reconciliacion. Lima, Peru. Available at: <http://www.cverdad.org.pe/ifinal/>

Extrajudicial Killings, Trauma, Peru



A130 Need a New Headspace? A Semi-Automated Volumetric Approach for Subadult Age Estimation Using the Spheno-Occipital Sychondrosis

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After attending this presentation, attendees will appreciate the advantages and principal processes of applying a semi-automated growth plate measurement protocol based on voxelization and volume calculation to quantify the tissue composition of irregular skeletal regions such as the spheno-occipital sychondrosis.

This presentation will impact the forensic science community by providing an attractive alternative to traditional phase-based age-estimation methods by introducing quantitative methodologies to measure material density distributions embedded in cross-sectional tomography data.

Due to the high probability of recovering the skull in anthropological casework, the spheno-occipital sychondrosis is frequently used as an indicator of subadult age, as it constitutes the last site in the cranium to terminate growth. Disparity regarding the reported age of complete sychondrosis fusion is evident in current literature, possibly attributed to inconsistencies in research design and visualization medium. Consequently, the reliability of ordered age-phase strategies employed in qualitative assessment may be questioned due to: (1) ambiguity in phase descriptions; (2) variation in the number of stages; and, (3) significant overlap in age ranges between consecutive phases.

Volumetric analysis using automated segmentation algorithms constitutes standard practice in medical science for monitoring skeletal toxicity and neurological assessment of intracranial pathologies, where voxelization and volume calculation are imperative for diagnostic and treatment decisions. A semi-automated method for volumetric measurement was developed based on differential voxel intensity values of the Hounsfield scale (HU) that discriminate material composition of the spheno-occipital sychondrosis in Computed Tomography (CT) orthoslices. Manual segmentation was conducted to: (1) create a sub-volume of the original Digital Imaging and Communications in Medicine (DICOM) stack; and, (2) isolate the joint space to create a binary tissue classification mask. MATLAB® commands were written to eliminate voxels exceeding the mask and to produce a histogram of intensity values for each individual based on voxel-clustering of biological material. Intensity values were correlated to specific tissue types (i.e., hyaline vs. calcified cartilage) using Bayesian probabilistic analysis, followed by tissue volume calculations using the voxel count and size. Linear sychondrosal measurements were conducted to eliminate the effect of size. The protocol was applied to cranial CT data from The Skeletal Biology and Forensic Anthropology Research Osteological Database on 169 Australian individuals aged birth to 18 years to generate preliminary density histograms for age estimation using mixture modeling.¹ Cumulative Density Functions (CDF) for key age ranges were calculated using a sub-sample of this data, with age estimates of remaining individuals based on agreement with the conceptualized CDFs using the Kolmogorov-Smirnov test. Non-linear regression with variance modeling was utilized to formulate predictive algorithms, which will be subject to validation on an independent sample acquired from The Royal Children's Hospital, Brisbane and disclosed in February 2015.

Preliminary results demonstrate a gradual decline in standardized cartilaginous volume of the spheno-occipital sychondrosis until 16.3 and 13.8 years in males and females, respectively, which constitutes the age of complete fusion in this population. In the neonate, mean cartilaginous volume in males was $461.67 \pm 49.28 \text{mm}^3$ compared to $299.35 \pm 32.76 \text{mm}^3$ in females; with males exhibiting significantly greater volumes ($P < 0.05$) across all age intervals until 14 years, which correlates with delayed closure of the sychondrosis. Males exhibit a consistent linear decline in volume with age, while the rate of decline accelerates after eight years in females. Height and width variables demonstrate expansion through adolescence, the most prominent increase observed between birth and four years during which time chondrocyte proliferation and matrix production occur rapidly. Proceeding this period, endochondral ossification commences at the superolateral borders, causing a reduction in the gradient of growth. Significantly, voxel cluster distributions successfully discriminate tissue changes with increasing age, with neonates exhibiting a cluster peak corresponding to the density of fibrous tissue. At five years of age, the highest proportion of intensity values denote hyaline cartilage in contrast to cluster peaks at 150-250HU at 14 years



Anthropology Section - 2015

of age, emphasizing significant cartilage calcification prior to complete ossification (+250HU) at 16 years in Queensland males. New mathematical models, validated on a large, modern Australian population, will be introduced as a tool for methodological refinement, providing a robust alternative or adjunct to current subadult aging methods. The utility of the proposed methodological approach to epiphyseal growth plates of the post-cranial skeleton for multi-factorial age estimation will also be discussed.

Reference:

1. Lottering N, MacGregor DM, Barry MD, Reynolds MS, Gregory LS (2014) Introducing standardized protocols for anthropological measurement of virtual subadult crania using computed tomography. *J Forensic Rad Imag* 2(1): 34-8.
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Subadult Age Estimation, Volumetric Analysis, Spheno-Occipital Synchondrosis



Anthropology Section - 2015

A131 Radiographic Age Estimation of the Knee in Young Children

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After attending this presentation, attendees will be offered practical statistics to transform a single skeletal age estimate into a probable chronological age range that is wide enough to reflect the scope of normal variation, yet narrow enough for the estimate to be of value.

This presentation will impact the forensic science community by providing tighter age intervals to assist with radiographic age estimation of young children between the ages of zero to six years. The reported intervals will at the same time be wide enough to reflect normal variation in developmental timings, thus offering a practical resource to forensic practitioners.

One of the more challenging aspects of forensic age estimation is the balance between producing an estimate that is wide enough to cover the full extent of normal human variation, yet narrow enough for the estimate to be of value. While narrow estimates are optimum in theory, an interval that is too small leads to false exclusion of possible ages, which may potentially compromise identification efforts. In an effort to optimize this balance, predicted age ranges often vary in their reported widths depending on the overall life period of the individual. Generally speaking, the younger the individual, the narrower the estimate that can be provided. One of the more useful methods of estimating age in children includes the radiographic atlas technique.¹ This technique considers the morphological changes that epiphyses within a specific joint region undergo from appearance to full maturity, and thus provides a method that has utility throughout the entire juvenile time period. Radiographic atlases have been published for various joint regions; however, these atlases are known for estimating a single skeletal age rather than a range that includes variable rates of developmental timing. Hackman and Black have recently offered a solution to this conundrum for the joint regions of the knee by offering standard deviations documenting variation in developmental timings, then suggesting that two standard deviations can be added and subtracted from an estimated skeletal age to offer an age interval that transforms the method into a robust technique for estimating age.² This provides a single standard deviation for each of the sexes, regardless of the overall maturity level of the juvenile. The standard deviations reported result in an estimate that is nearly four years in width. While there is no doubt that the window of developmental variation is wide-ranging during the teenage years, is it possible that a tighter estimate can be offered for infants and young children? The sample from which the original standard deviations were calculated is extremely limited in individuals younger than seven years, so does not reflect the more cohesive development that occurs during this time period.

This presentation considers the above question by examining rates of development in young American children as assessed through use of Pyle's and Hoerr's radiographic atlas of the knee. Radiographs from 217 children (107 females and 110 males) between the ages of zero to six years were collected via the juvenile radiographic database developed by Mercyhurst University. Standard deviations representing the difference between skeletal age and chronological age were then calculated according to three general time periods, including the first year, second year, and third through sixth year. As expected, variation was observed to decrease with receding maturity, resulting in the creation of significantly narrower ranges when predicting age. Reported standard deviations ranged between 2.7 months and 6.7 months for females and 2.4 months and 8.0 months for males.

References:

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2. Hackman L, Black S (2013) Age Estimation from Radiographic Images of the Knee. *J Forensic Sci* 58 (3):732-737. doi:10.1111/1556-4029.12077

Age Estimation, Developmental Osteology, Radiographs



Anthropology Section - 2015

A132 Interpersonal Violence in Undocumented Border Crossers From Southern Arizona Between 2006 and 2013

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After attending this presentation, attendees will have a better understanding of interpersonal violence in Undocumented Border Crossers (UBCs) who died as a result of homicide in southern Arizona between 2006 and 2013.

This presentation will impact the forensic science community by evaluating peri-mortem trauma, biological variables, and contextual information at an assemblage level in this human rights sample.

While migration across the United States-Mexico border has decreased over the past five years, deaths of UBCs have actually increased. This increase is largely due to the redistribution of migrants along the border resulting from United States immigration control policies, which funnel migrants into less protected, yet more remote and dangerous areas of the border. The Tucson Sector represents one of the most traversed corridors of the border region in the United States. UBCs who die in southern Arizona are examined at the Pima County Office of the Medical Examiner (PCOME) in Tucson, AZ. As such, the PCOME currently handles the greatest number of migrant deaths in the United States.

In order to evaluate patterns of interpersonal violence in UBCs recovered in southern Arizona, biological variables, peri-mortem trauma, and contextual information from crime scenes were collected using investigator reports, autopsy reports, and forensic anthropological examinations. While the majority of UBC deaths between 2006 and 2013 were deemed accidental (42%) or undetermined (48%), approximately 3% represented homicides. In this study, all available homicide cases (n=37) from the PCOME during this period were reviewed, and included both identified and unidentified decedents. The sex and age of decedents were recorded, as were their associated decompositional stage, percentage of body recovered, and degree of trauma. Variables related to peri-mortem trauma were analyzed when possible and included the mechanism of trauma, anatomical location of trauma, and direction of force. Contextual information related to the crime scenes was also evaluated, including recovery site, disposition of the body, recovery cohort, presence of concealment tactics, and material evidence.

Preliminary results indicate that all UBC homicide victims from 2006 to 2013 were male. Decedents' ages were verified and estimated, and ranged from the mid-teens to 57 years at death. Approximately 46% of decedents were classified as young adults (20-35 years), while 38% were middle adults (35-50 years). Approximately half of all decedents exhibited multiple mechanisms of trauma; however, not all mechanisms were related to the cause of death as determined by the pathologist. Gunshot wounds were observed on approximately 84% of decedents, with fewer cases of blunt force trauma (38%), sharp force trauma (5%), and undetermined trauma (19%). Individuals exhibiting projectile trauma were shot between one and four times, with single gunshot wounds (55%) being the most common. Evaluation of contextual information related to the crime scene indicates that the majority of decedents were discovered alone (79%), on the ground surface (81%), and in the open desert (86%) in Pima, Pinal, and Santa Cruz Counties. Attempts to conceal victims' remains by perpetrators were observed in approximately 60% of homicide cases.

Employing a population-based approach, this study reports on patterns of interpersonal violence in hopes of recognizing one of many challenges encountered by this vulnerable population. In general, the majority of UBC deaths in southern Arizona are not violent in nature but rather accidental and related to the harsh environmental conditions of crossing arid and isolated terrain.¹ The low levels of homicidal violence amongst UBCs within the Tucson Sector stand in sharp contrast to reports of large-scale atrocities perpetrated against undocumented migrants traveling through Mexico and Central America.² Evaluation of peri-mortem trauma and the crime scenes from UBC homicides in this study suggests isolated incidents of interpersonal violence against individuals rather than the large-scale, systematic targeting of migrants occurring south of the border. Future research intends to examine the frequency and nature of violence in undocumented migrants from other areas (e.g., northern Arizona, California, and Texas) in order to determine how interpersonal violence varies by border region within the United States.



Anthropology Section - 2015

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1. Martinez DE, Reineke RC, Rubio-Goldsmith R, Anderson BE, Hess GL, Parks BO. A continued humanitarian crisis at the border: Undocumented border crosser deaths recorded by the Pima County Office of the Medical Examiner, 1990-2012. Tucson: Binational Migration Institute, Mexican American Studies Department, University of Arizona, 2013.
 2. Dudley S. Transnational crime in Mexico and Central America: its evolution and role in international migration. Washington, DC: Migration Policy Institute, 2012.
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Interpersonal Violence, Peri-Mortem Trauma, Undocumented Border Crossers



Anthropology Section - 2015

A133 Differentiating Peri-Mortem From Postmortem Blunt Force Trauma by Evaluating Fracture Tension Surface Topography Using Geographic Information Systems

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The goal of this presentation is to present the results of a novel method that uses Geographic Information Systems (GIS) to quantifiably distinguish between peri-mortem and postmortem blunt force trauma fractures. Attendees will learn that microscopic topographic differences are observable on the fracture tension surface between bones fractured near the time of death and those fractured later in the Postmortem Interval (PMI), and that these differences can be detected and quantified from high-resolution digital images of the fracture surface using GIS software.

This presentation will impact the forensic science community by introducing a new quantifiable method for analyzing the fracture tension surface morphology by using GIS software to differentiate peri-mortem from postmortem blunt force trauma in forensic anthropological investigations.

Forensic anthropologists must be able to accurately distinguish between wet and dry bone fractures in cases involving blunt force trauma. While previous research has established that fracture surface morphology can provide clues to differentiate between peri-mortem (wet) and postmortem (dry) fractures, no studies have made attempts to calibrate or measure these changes.¹⁻⁴ The present study explores the use of GIS to assess and quantify changes in the fracture tension surface topography of bones broken throughout the PMI. Because of its ability to evaluate spatial data, GIS software is well-suited to analyzing spatial patterns in the irregular landscape of a fracture surface.⁵ The goal of this study is to establish if fracture surface topography can be consistently used to differentiate between peri-mortem and postmortem bone fractures. The tension surface of the fracture was chosen for analysis because previous research has found that osteons, when placed under tension, will “pull-out” of the fracture surface and create pits and projections on the fracture surface.⁶ These pits and projects are read by GIS software as hills and valleys on the fracture surface.

A total of 144 fracture surfaces from pig long bones broken near the time of death and at weekly periods throughout the postmortem interval were imaged using a NextEngine® 3D scanner. The scans were trimmed and imported into ArcMap™ 10.1 in vector format as coordinate points. The Hotspot Analysis tool was used to identify statistically significant areas of high elevation (hot spots) and low elevation (cold spots) and assessed whether those clusters were statistically significant within the context of neighboring feature values. For each fracture surface, the percentage of the total tension surface area considered a cold spot (valley), hot spot (hill), and intermediate value (flat area) were calculated.

Analysis of variance results indicate there is a significant difference in topographic features (hot, cold, and intermediate spots) and temporal intervals corresponding to early, middle, and late decomposition ($p=0.011$, 0.026 , and 0.025 , respectively). Regression analysis shows low correlation between months in the PMI and the topographic features. The overall trend in the data suggests that bones broken closer to the time of death have a higher percentage of hot and cold spots compared to bones broken later in the PMI. This implies that the bones broken near the time of death exhibit a rougher fracture tension surface with more pits and projections than bones broken later in the PMI. The microscopic roughened appearance of wet bone fracture surfaces is likely the result of torn collagen bundles and osteonal pullouts; however, as the bone dries, the osteons and collagen become less elastic and more brittle, so osteons and collagen bundles will not stretch under tension. Thus, drying results in a smoother fracture surface. The low correlation between PMI and fracture features suggest that time is not a major causal factor, but rather the change in topography is likely the result of the loss of organic material as the bone dries. The findings of this research demonstrate promise for using GIS in forensic anthropological fracture research and cases and that this method warrants further investigation.



Anthropology Section - 2015

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 3. Wieberg DA, Wescott DJ. Estimating the timing of long bone fractures: correlation between the postmortem interval, bone moisture content, and blunt force trauma fracture characteristics. *J Forensic Sci*, 2008;53(5):1028-1034.
 4. Shattuck RE. Perimortem fracture patterns in south-central Texas: a preliminary investigation into the perimortem interval. MA thesis, Texas State University, Department of Anthropology, 2010.
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 6. Hiller LP, Stover SM, Gibson VA, Gibeling JC, Prater CS, Hazelwood SJ, Yeh OC, Martin RB. Osteon pullout in the equine third metacarpal bone: effects of ex vivo fatigue. *J Orthopaed Res*, 2003;481-488.
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Peri-Mortem, Trauma, Geographic Information Systems



A134 Estimating Skeletal Differences Between Contact and Non-Contact Gunshot Wounds to the Head: The Role of Forensic Anthropologists in Understanding Circumstances of Death

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After attending this presentation, attendees will understand some of the skeletal differences observed between contact and non-contact gunshot wounds to the head and will appreciate the potential of skeletal trauma analysis in understanding circumstances of death.

This presentation impacts the forensic science community by contributing to bone trauma analysis and stressing the role of forensic anthropologists as valuable consultants to forensic pathological determination of cause and manner of death.

The analysis of gunshot trauma in medicolegal contexts is relatively straightforward for medical examiners when soft tissues are well preserved. One of the key factors to guide the estimation of manner of death is range-of-fire; however, in skeletonized remains, the task is more difficult because the analysis lacks soft tissue evidence. This project evaluates the contribution of skeletal injury, particularly fracture patterns, to evaluations of gunshot distance. This study was conducted using a case-based analysis approach to determine the presence or absence of skeletal differences between known contact and non-contact gunshot wounds to the head.

Fifteen contact gunshot cases to the head (12 suicides, one homicide, and two undetermined) and 23 non-contact gunshot cases (all homicides) were collected for a total of 38; ten from the McCormick Collection at The University of Tennessee, four from the Office of the Chief Medical Examiner in Knoxville, TN, three from the Wayne County Medical Examiner's Office in Detroit, MI, and 21 from the Modern Skeletal Collection of the National Institute of Legal Medicine and Forensic Sciences in Bogotá, Colombia.

Information on range-of-fire and type of caliber was provided in the autopsy and ballistic reports. All cases were wounded by low-velocity bullets. A total of 24 variables were collected to characterize the overall injury analysis, including quantitative data, such as entrance and exit diameters, number of radiating and concentric fractures associated with entrance and exit defects, number of secondary fractures, and average length of fractures. Qualitative data include anatomic location of entrance and exit defects and types and location of symmetric fractures. Kruskal-Wallis Non-Parametric test was conducted to find statistically significant differences between contact and non-contact gunshot wounds ($p=0.05$).

Results indicate that, compared to non-contact wounds, contact gunshot wounds are associated with higher frequencies of: (1) entrance fractures; (2) exit fractures; (3) average length of exit fractures; (4) total number of secondary fractures; and, (5) average length of secondary fractures.

Qualitative data results suggest statistically significant differences in the anatomic location of entrance defects. Common entrance locations for suicides are in the mouth, the temples, and the forehead. Non-contact gunshot wounds do not follow any pattern because homicidal wounds occur more randomly with less localization.

Two types of secondary symmetric radiating fractures occurred more frequently than others: (1) bilateral diagonal fractures going through the external acoustic meatus and the squamous portion of the temporal bone; and, (2) bilateral diagonal fractures on the maxilla going from the inferior orbital border to the alveolar process. The first type occurred equally often in contact and non-contact gunshot wounds with no specific entrance defect location associated. The second type appeared to be more frequently associated with contact gunshot wounds with no specific entrance location related. Findings indicate that no specific type of fracture is associated with range-of-fire and or specific entrance locations; however, it is suggested that the analysis of these types of fractures needs additional examination.

The results are based on a small sample and must be carefully considered. Further research is important to establish if these findings are consistent and, hopefully, to estimate the presence or absence of craniofacial fracture patterns associated with contact gunshot wounds to the head. Unfortunately, the number of cases in this study does not support evidence of any frequent type and/or location of fractures in relation to range-of-fire. Additionally, while the establishment of manner of death cannot be solely based on scientific analysis of the victim's remains — due to the significance of an interdisciplinary investigation of each case — the importance of the expertise of forensic anthropologists in the analysis of bone trauma and their contributions in the understanding of circumstances of death is emphasized.

Skeletal Gunshot Trauma, Bilateral Symmetric Fractures, Manner of Death



Anthropology Section - 2015

A135 Identification of Peri-Mortem Cranial Trauma After Cremation: How Easy Is It?

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After attending this presentation, attendees will have learned how much of a cranial peri-mortem fracture really survives after cremation and where the pitfalls in distinguishing it from a heat-related fracture may be.

This presentation will impact the forensic science community by demonstrating how traumatic fractures survive cremation, how they may change, and how they may differ from heat-related fractures even after the cremation process through the analysis of 32 cadavers subjected to autopsy, then cremated.

Twenty male and 12 female cadavers between 22 and 90 years of age, were followed and analyzed from the autopsy room to the crematorium. The mean age of the male sample was 58.6 years and of the female sample was 65.9 years. All subject died of head trauma: 30% from traffic accidents; 52% from falls from a height; and, 18% from gunshot injuries. This study focused only on the skull. The advantages of this study were the standardization of all the samples due to the fixed oven temperature and time of cremation (1,200°C for 90 minutes), known demographic (age, sex, weight, and height), clinical information, availability of detailed autopsy reports (injuries and cause of death), and photographs.

Moreover, both during the autopsy and after the cremation, photos of the bone fragments and their edges were accurately taken from different angles with a metric reference. In each case, all fragments were counted both during autopsy (from zero to 25 fragments, with a mean of 6) and after the cremation (from 44 to 106 fragments, with a mean of 78). After a recomposition of the skull fragments according to the bone of origin (parietal, frontal, etc.), the fracture visible at the autopsy, which had been photographed, was searched for among the burned fragments. If the original fracture could not be found, it was classified as “a;” if it could be recognized but had been greatly altered, it was classified as “b;” if it was found to be superimposable in its shape, it was classified as “c.”

Results showed that only 20.8% of peri-mortem fractures were of type “c” (i.e., recognizable and of the same shape); 25% were of type “b;” and 54.2% were classified as “a.” One interesting finding was that if beveling due to the trauma was present during autopsy, it preserved its general shape even after the cremation but was smoother at the edges. As already reported in literature, saw marks were always clearly visible, confirming that the saw action on the bone is demonstrable even after the cremation at extreme temperatures. It also seems that fire-induced fractures generally produce straight, sharp, and acute angles at fracture edges whereas pre-existing traumatic fractures are modeled by fire, creating generally smoother and rounder edges.

In conclusion, this study has shown that in more than half of the cases, the original peri-mortem fracture could no longer be seen. Where the fracture can be detected, in 25% of the cases it is visible but severely altered, and in the remaining cases it is very similar in shape but not superimposable since the margins are rounder and smoother.

Forensic Anthropology, Trauma, Cremation



Anthropology Section - 2015

A136 Computerized Reconstruction of Fragmentary Skeletal Remains

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After attending this presentation, attendees will have gained an understanding about the process of fragmentary remains reconstruction using computerized methods and the use of Computed Tomography (CT) and 3D models to sort commingled fragmentary remains.

This presentation will impact the forensic science community by sharing results of the developed software program to enable the determination of the number of individuals present and make metric assessments of sex, ancestry, and stature based on statistically sound bone reconstructions.

Within the larger medicolegal system, forensic anthropologists perform the essential task of creating a biological profile to aid law enforcement in identifying unknown human remains — an important first step in the criminal apprehension and conviction process. In cases of mass disasters or commingled remains, the determination of individual biological profile parameters is complicated by the presence of multiple unassociated elements. The ability to make biological profile assessments on isolated bones or bone fragments is critical. Although developed independently, the 3D approach to the quantification of commingled remains is a logical extension of coding and 2D methods developed in zooarchaeology and bioarchaeology.¹⁻³ Recent work quantified small fragmented remains into an Osteological Information System (OIS) using Geographic Information System (GIS) software to derive Minimum Number of Elements (MNE) values and Minimum Number of Individuals (MNI) estimates.³ These systems are time-consuming and are dependent on the observer to manually digitize each fragment into the OIS application. The resulting image provides an MNE estimate for the element under investigation.

Methods: In order to enable the computerized reconstruction of fragmentary remains, a new method was developed to match fragmentary remains with 3D template bones for the pelvis, humerus, femur, and cranium. These template bones are average bones generated from a training set with homologues points on the 3D surfaces. In order to generate such homologues points for all training sets, the 3D models had to be added to a statistical atlas that redistributes the points on the bone surface to ensure correspondence among landmarks.⁴ Fragmentary remains are matched to each template bone using surface descriptors. Outputs of this process are fragmentary pieces that are registered together in space. The next step involves reconstruction of a full bone by interpolating missing data between registered pieces. This step is enabled by optimizing the principal components calculated from the training set. In order to develop and test the system, a highly fragmentary commingled sample was used as a proxy for a mass disaster: the Morton Shell Mound osteological collection. The Morton sample represents over 25,000 human bone fragments from approximately 125 individuals.

Results: During this study, 22,400 bones have been CT scanned — of those 17,700 have been sorted and coded, and 170 bone fragments have been 3D scanned. Using the developed software, the innominate reconstruction had a mean Root Mean Square (RMS) error of 0.6mm with a maximum of 3.29mm error, whereas the skull reconstruction had mean error of 1.1mm. The developed application allows all scanned skeletal remains from each scene to be reviewable within the user interface. An estimate of MNE of the scanned material is provided following osteological protocols developed in forensic anthropology and bioarchaeology.³ Finally, the application calculates a combination of traditional anthropological measurements and frequently used biomedical measurements. The traditional measurements can be used in a program such as FORDISC® 3.0 or manually plugged into equations for the metric assessment of three elements of the biological profile: sex, ancestry, and stature.

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Anthropology Section - 2015

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Fragmentary Remain, Computerized, Commingled



B1 Screening and Confirmation of Nitramine, Nitroaromatic, and Nitrate Ester Explosives in Soil

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The goal of this presentation is to inform attendees regarding Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS) screening and confirmation methods on explosives.

This presentation will impact the forensic science community by providing attendees with information about a new and original method with low Limit Of Detection (LOD) and Limit Of Quantitation (LOQ) values on explosive determination using LC/MS/MS.

Sensitive, rapid, and inexpensive methods for explosive determination in samples of explosive debris and environmental samples (water, soil, etc.) are important in order to determine the type of explosive substances that are present. In this study, Liquid Chromatography/Atmospheric Pressure Chemical Ionization with Tandem Mass Spectrometry (LC/APCI-MS/MS) was used in order to determine trace amounts of widely used explosives and plasticizers in soil, such as 2,4,6-Trinitrotoluene (TNT), Research Department Explosive (RDX), octogen (HMX), Pentaerythritol Tetranitrate (PETN), 2,4,6-Trinitrophenylmethylnitramine (TETRYL), 2,6-Dinitrotoluene (2,6-DNT), Polyamide (PA), and Trimethylolethane Trinitrate (TMETN). The method was developed for qualitative determination purposes. No previous methods were encountered in the literature regarding the simultaneous determination of these substances in a soil matrix.

The cleavage products for 2,6-DNT, TNT, picric acid, TETRYL, TMETN, RDX, HMX, PETN, and phenytoin (which is used as an Internal Standard (I.S.)) were investigated in MS/MS and the signals of mass-to-charge (m/z) ratios were optimized. MS/MS parameters and chromatographic methods were optimized by using a gradient ammonium nitrate: methanol mobile system and a C^{18} column. The samples were analyzed in LC/MS/MS with APCI negative ionization mode after a 30min single-step extraction procedure. The multiple reaction monitoring ions used were: 226.10 \rightarrow 88.20 for TNT, 377.90 \rightarrow 62.00 for PETN, 357.90 \rightarrow 61.80 for HMX, 181.20 \rightarrow 151.30 for 2,6-DNT, 240.70 \rightarrow 212.50 for TETRYL, 317.00 \rightarrow 61.60 for TMETN, 227.70 \rightarrow 197.50 for picric acid, and 283.40 \rightarrow 61.70 for RDX.

Selectivity-specificity, accuracy (recovery), linearity, and LOD and LOQ values were investigated. The chromatographic peaks were not affected by matrix and the other peaks. The intra-day precision limit was chosen as 15%. Samples including TNT, PA, HMX, TMETN, PETN, TETRYL, 2,6-DNT, RDX, and Phenytoin (I.S.) were eluted from the system within ten minutes without interfering with each other's channels. Average recovery obtained from the analyses of the soil samples, including the explosive 250.00, 500.00, 1,000.00ngg-1 explosive mix, both before and after extraction, were between 93.01% and 104.20% ($n \geq 6$). The method was linear in the concentration ranges of 58.00-1,000.00ngg-1 for PA, 241.50-1,000.00 ngg-1 for TNT, 13.20-1,000.00 ngg-1 for RDX, 22.00-1,000.00 ngg-1 for HMX, 100.00-1,000.00 ngg-1 for 2,6-DNT, 114.00-1,000.00ngg-1 for TETRYL, 97.00-1,000.00ngg-1 for PETN, and 190.00-1,000.00ngg-1 for TMETN. Regression coefficients for all calibration graphs were ≥ 0.99 . LOD and LOQ values obtained from the analyses of the soil samples including the explosive mix were between 8.92-161.22ngg-1 and 13.20-241.50 ngg-1, respectively.

As a result, an economical, fast, easy, repeatable, and selective method with low detection and quantification limit and high recovery was developed for the analysis of TNT, PA, HMX, TMETN, PETN, TETRYL, 2,6-DNT, and RDX in soil by using LC/MS/MS with APCI ionization.

LC/APCI-MS/MS, Explosive, Determination in Soil



B2 Raman Microspectroscopy of Body Fluid Traces: Intrinsic Method Selectivity

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After attending this presentation, attendees will have a better understanding of the recent advancements of a particular application of Raman spectroscopy. The implementation of advanced statistics for automatic analysis of spectroscopic data and the evaluation of the accuracy and reliability of the conclusions made will be discussed.

This presentation will impact the forensic science community by potentially having a great effect on the accuracy and effectiveness of biological stain analysis for forensic purposes.

The identification of traces of body fluids discovered at a crime scene is a major part of forensic investigation today. The three most common fluids found are blood, semen, and saliva, and there are several methods used currently to distinguish one from another. Blood can be presumptively tested by using different color spot tests, but these tests are destructive to the sample and can also result in false positives. Semen is similar in that there are destructive presumptive tests as well as confirmatory tests; however, saliva has no confirmatory tests. Most presumptive tests can be performed in the field, but some sample preparation such as extraction is often necessary. Most confirmatory tests must be done in the laboratory. The main problem with these tests is the destruction of the sample. The forensic community is in great need of a reliable, non-destructive, on-field method for identification of all common body fluids.

Raman spectroscopy is a technique that is increasing in popularity among the different disciplines of forensic science. Some examples of its use today involve the identification of drugs, lipsticks, and fibers, as well as paint and ink analysis. The theory behind Raman spectroscopy is based on the inelastic scattering of low-intensity, non-destructive laser light by a solid, liquid, or gas sample. Very little or no sample preparation is needed and the required amount of material tested with a Raman microscope can be as low as several picograms or femtoliters. A typical Raman spectrum consists of several narrow bands and provides a unique vibrational signature of the material. Typically, non-resonance Raman spectroscopic measurements do not damage the sample. The stain could be tested in the field and still be available for further use in the laboratory for DNA analysis. A portable Raman spectrometer is a reality that should now allow for identification at the crime scene.

The latest development of a new method for identification of body fluid traces using Raman spectroscopy combined with advanced statistics is reported in this study. Multidimensional Raman spectroscopic signatures of dry traces of sweat, vaginal fluid, semen, saliva, and blood have been reported earlier. The dry blood signature has been upgraded to eliminate possible photodamage.¹ The method was expanded for the application to semen stains on common substrates.² The differentiation of menstrual and peripheral blood with high confidence was demonstrated.³ Raman spectroscopy has also been shown to be effectively applied as a non-destructive technique for differentiating human blood from a wide survey of animal blood.⁴ A Partial Least Squares Discriminant Analysis (PLSDA) model was built from a training set of the near infrared Raman spectra from 11 species. Various performance measures, including a blind test and external validation, confirmed the discriminatory performance of the chemometric model, which demonstrated 100% differentiation. Most importantly, a satisfactory differentiation between 11 individual animal classes was demonstrated.⁵

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Criminalistics Section - 2015

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Raman Spectroscopy, Chemometrics, Biological Stain



B3 Individual Identification for Bones More Than 60 Years Old Using Autosomal SNPs on an Affymetrix® Chip

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After attending this presentation, attendees will understand the usability of Single Nucleotide Polymorphisms (SNPs) for individual identification in cases with aged bone samples as well as the basic principles involved in the use of resequencing arrays through practical analysis of case work using an Affymetrix® chip.

This presentation will impact the forensic science community in cases such as mass disasters by introducing a promising DNA typing tool. Short Tandem Repeat (STR) typing is typically performed to estimate genetic relationships, such as paternity tests; however, the limited number of STR markers, even though expanded more than previously before, is not sufficient enough in some cases. For example, it is hard to get full STR alleles in degraded samples such as bone or enough differentiation in very closed genetic relationships like those in an isolated population where the STR markers are only now being analyzed. In these cases, a supplementary tool is necessary to increase the power of matching probability with additional markers. SNP analysis is promising in this respect as it can be designed to be short for easy amplification, even in degraded samples, and can easily be multiplexed.

A resequencing array containing 169 autosomal SNP markers, which are distributed throughout the autosome except for chromosome 21, was used in this study on an Affymetrix® platform. The DNA chip analysis was applied to 14 identification cases involving bones that had been buried when the Jeju 4.3 incident occurred in South Korea from 1948 to 1954. Each case was comprised of recovered bone from a putative father and blood from an individual believed to be their child or sibling. For each analysis, the 169 SNP markers were applied to multiplex Polymerase Chain Reaction in a tube, then hybridized to probes on a chip. The statistical parameters, such as cumulative matching probability or cumulative identity index, were represented on an Identity by Descent (IBD) basis.

When comparing markers among the typed SNPs, the resulting data showed approximately 50 to 125 SNPs were shared in pairs. The genetic relationship was confirmed in all of the identification case pairs with over 99.99% of cumulative matching probability. In addition, no instances of discordance of STR alleles in a pair were found.

This study demonstrated a successful evaluation of genetic relationships was possible on challenging bone samples using autosomal SNP marker DNA chip resequencing results. Given the outcome of this study, it is expected that this test could be applicable to forensic samples as a complementary tool.

Resequencing, Affymetrix® Chip, Single Nucleotide Polymorphism



B4 Development of Multiplexed Autosomal STR, Y-STR, and mtDNA Systems for Forensic Identification Using Next Generation Sequencing

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After attending this presentation, attendees will understand how next generation sequencing can impact their workflow and provide an increased ability to deconvolute mixed samples.

This presentation will impact the forensic science community by providing more information from crime scene samples to enhance criminal investigations.

Next Generation Sequencing (NGS) is capturing significant interest in the forensic community because of its promise of greater mixture resolution and increased capacity for multiplexing. Three systems have been developed to enable forensic identification of samples using NGS. The PowerSeq® Auto System includes all loci in “Section A” of the proposed expanded Combined DNA Index System (CODIS) core, as well as two loci from “Section B” (TPOX and D22S1045) and two highly polymorphic pentanucleotide loci (Penta E and Penta D).¹ The combination of these 23 Short Tandem Repeat (STR) loci and amelogenin makes this multiplex an effective tool for human identification using NGS while maintaining compatibility with existing databases worldwide. The PowerSeq® Mito System includes reagents to produce a set of small amplicons for sequencing the mitochondrial control region. NGS allows laboratories access to mitochondrial DNA (mtDNA) analysis using a simpler, yet potentially high-throughput workflow. Increased mixture deconvolution and heteroplasmy resolution are achieved by deep sequencing coverage and digital read counts, compared to traditional sequencing methods. Additionally, the use of small amplicons to sequence the mitochondrial control region improves sequencing results from degraded samples.

A multiplexed system of autosomal STRs, Y-chromosomal Short Tandem Repeats (Y-STRs), and mtDNA will render more information from crime samples in a single assay. The developed multiplex system described here includes all loci from the PowerSeq® Auto System and the amplicons from the PowerSeq® Mito System combined into one multiplex, as well as 23 Y-STR loci for enhanced interpretation of mixed samples. This system is used in conjunction with the Illumina® MiSeq® System to generate a complete sequence analysis (STR genotype, STR repeat structure, mitochondrial haplotype, and SNP information) from a single amplification and sequencing reaction. Four different DNA samples for a total of 18 samples were typed using PowerSeq® Auto, resulting in 100% full profiles that are fully concordant with the profiles obtained through capillary electrophoresis. Two different DNA samples were typed using the PowerSeq® Mito System for a total of 14 samples, resulting in full concordance with profiles obtained through Sanger sequencing. Five libraries were analyzed with the multiplex of autosomal STRs, Y-STRs, and mtDNA and were found to be fully concordant.

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Next-Gen Sequencing, DNA Mixtures, STRs and mtDNA



B5 Efficiency of Human DNA Isolation and Short Tandem Repeat (STR) Profiling From Burnt Teeth

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After attending this presentation, attendees will have deeper insight concerning the efficiency of DNA profiling in burnt teeth.

This presentation will impact the forensic science community by providing a quantitative analysis for obtaining complete or partial DNA profiles from burnt teeth under controlled conditions of time and temperature.

Identification of a deceased individual is important from the relatives' viewpoint and is a vital factor in facilitating criminal investigations, inquests, and other tribunals. Mass disasters, aircraft or car accidents, and accidental deaths frequently involve the presence of fire.

The identification of human remains subjected to incineration depends on the degree of destruction of the remains, which is affected by the intensity and duration of the fire. One of the approaches for identification of burnt remains is genetic analysis. The genetic approach is usually unproblematic in cases of fire victims with conserved internal organs; however, extremely charred bodies frequently render highly degraded DNA, hampering Short Tandem Repeat (STR) analysis. Also, few studies focus on the possibility of amplifying authentic DNA from burnt remains. No consensus has been reached regarding the degree of cremation at which bone will still yield authentic DNA signals. Although previous studies have used similar temperature ranges (between 100°C and 1,000°C), the duration of fire exposure differs among these studies. In the studies developed on whole teeth and pulp DNA, the same problem is encountered: the temperatures were similar to those used in the bone studies and the durations of exposure varied. Therefore, more research is needed to evaluate the possibility of extracting DNA from burnt remains.

Since teeth are the hardest tissue of the human body and one of the most abundant types of biological remains available in forensic cases, the present study focused on the evaluation of the efficiency of DNA isolation from burnt teeth and the achievement of obtaining a DNA profile at different conditions of temperature and time exposure.

Twenty-eight healthy erupted third molars, aged 20 to 70 years, were collected from dental clinics. The Smithsonian Institution's ethical committee approved all procedures related to experimentation with human subjects. The teeth were divided into seven groups treated at different temperatures: 100°C, 200°C, 300°C, 400°C, 500°C, 600°C, and 700°C. The teeth in each group were treated at their assigned temperature for 1 minute, 5 minutes, 10 minutes, and 15 minutes, removing one tooth after each time period. Two non-burnt teeth were used as controls.

Control and burnt teeth were then mechanically ground and submitted to DNA extraction and quantification. Based on the quantification data, it was not possible to obtain DNA from the teeth subjected to 400°C for both 10 and 15 minutes, 500°C for 15 minutes, 600°C for 5 minutes and 700°C for 5, 10, and 15 minutes. To study the efficiency of obtaining DNA profiles, the following STRs were chosen: D7S820, D13S317, D5S818, CSF1PO, TPOX, TH01, vWA, D16S539, and FES/FPS, along with amelogenin. These regions were analyzed by SYBR® Green Real Time PCR. Each sample was tested in duplicate. The analysis of relative gene expression data was calculated using $2^{\Delta CT}$ method.

Quantitative PCR results were similar for all STRs tested. In the first temperatures and times, 100°C and 200°C, one and five minutes, it was possible to get amplification similar to the controls; however, in the majority of STRs, the amplification was very low from 300°C for one or five minutes onward. This DNA amplification was nearly undetectable, specifically in STRs located in an intron region, like TPOX, CSF1PO, TH01, and vWA. In contrast, the analysis of the amplification of the housekeeping gene used for the Quantitative Polymerase Chain Reaction (QPCR) quantification, Glyceraldehyde 3-Phosphate Dehydrogenase (GAPDH), showed that DNA for this region was amplified in all combinations of temperatures and times, finding highest Ct (meaning the least amplification) with the increase in temperatures and times. This indicates that even in burnt teeth subjected to high temperatures, it is possible to amplify DNA, at least housekeeping DNA; however, the data also shows that an STR profile would be difficult to obtain, probably due to the size of these regions which makes them more prone to degradation.



Criminalistics Section - 2015

The findings from this research provide a quantitative study for the achievement of obtaining a DNA profile from burnt teeth. Future research may be able to expand on these results, analyzing other potential DNA regions for identification under the same conditions.

Burnt Teeth, DNA Isolation, STR Profiling



B6 Age Estimation in Living Individuals Based on Photographs: Relevance in Cases of Pedopornography

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After attending this presentation, attendees will better understand how to test the accuracy of age estimation on the basis of facial morphology.

This presentation will impact the forensic science community by providing results from an experiment in an area with very little previous research. This presentation, accomplished through forensic anthropology, will alert professionals responsible for age estimation in cases of child pornography to be careful in the methodologies used, since such conduct may cause judicial errors.

Age estimation of child pornography victims has played an essential role in forensic practice, due to the high number of pedopornography cases, for decades. In order to address this problem, experts are often called upon to testify in court. The goal of this study is to test the accuracy of age estimation on the basis of facial morphology.

In this study, 12 evaluators (three forensic anthropologists, three forensic pathologists, three pediatricians, and three “non-experts”) analyzed 128 photographs of the faces of minors between the ages of one and 17 years (83 females and 45 males) to identify the age of each child. Each evaluator completed four observations, days apart, which were organized into two groups. In the first two observations, evaluators were asked to identify the age of each child and, in the last two, were asked to additionally indicate the most relevant facial characteristic for determining the age estimation for the child in each photograph.

The results confirmed that the best values obtained (87.92%) were associated with age estimation of younger individuals (<12 years old). When assessing the reliability of evaluators’ answers, reliable results (79.51%) were only observed after the age range of ± 2 years. After examining the influence of some parameters in the estimation obtained (sex, number of children, professional status of the evaluators, and sex of the study subjects), it was observed that, for real age, only the evaluator’s professional status and the sex of the study subjects did not influence age estimation. On the other hand, for age groups, the results showed that professional status was the only parameter influencing the correct estimates (highlighting the better results of the pediatricians). With respect to what the evaluators felt was the most relevant facial characteristic for age estimation of the children observed in each of the photographs, the “general appearance of the face” stood out, although it was more often associated with incorrect estimates (75.39%).

Overall, even though Portugal has no software that allows the analysis of age estimation based on photographs, new criteria should be implemented to improve this type of methodology in order to avoid judicial error.

Age Estimation, Child Pornography, Facial Images



B7 Maximize Information From Your Mixture Samples Using a Combined Autosomal STR and Y-STR Multiplex System

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After attending this presentation, attendees will be able to evaluate the potential benefit of using a combined autosomal Short Tandem Repeat (STR) and Y-chromosomal Short Tandem Repeat (Y-STR) marker set for analyzing mixture samples.

This presentation will impact the forensic science community by describing a less expensive and quicker STR method for analyzing casework samples.

The PowerPlex® Fusion™ 6C System is a six-color STR system for simultaneously amplifying 23 autosomal loci, three Y-STR loci, and amelogenin. The 20 required (amelogenin, D18S51, FGA, D21S11, D8S1179, vWA, D13S317, D16S539, D7S820, TH01, D3S1358, D5S818, CSF1PO, D2S1338, D19S433, D1S1656, D12S391, D2S441, D10S1248, and DYS391) and three recommended (TPOX, D22S1045, and SE33) proposed expanded Combined DNA Index System (CODIS) core loci are combined with Penta D, Penta E, DYS570, and DYS576 to give this system a discriminatory power ($PI=1.80 \times 10^{-32}$) that is more than 10^8 -fold higher than that for the 20 required expanded CODIS core loci ($PI=9.35 \times 10^{-24}$). With DYS391 and nine autosomal loci being less than 250bp, the additional genetic information obtained with this 27-loci STR system will be extremely useful for analyzing degraded samples, where even a partial profile would be informative.

The DYS391 locus is included in the proposed expanded CODIS core loci for verification of gender in amelogenin null samples; however, it has one of the lowest locus variability values and does not significantly increase discriminatory power. In contrast, DYS570 and DYS576 have two of the highest locus variability values within American subpopulations and contribute more to the system's discriminatory power than DYS391. Additionally, because they are rapidly mutating Y-STRs, DYS570 and DYS576 provide the potential for separating close male relatives and further improving useful information from a single STR analysis. These three Y-STR loci will allow more confident determination of the number of male contributors in complex mixtures without the need for a separate Y-STR analysis, thus saving time and money.

This system is designed for 1ng of optimal input DNA template. The average peak height ratio is over 90% with one ng DNA template and remains high (80%) with as low as 100pg DNA template. It is very sensitive and is capable of calling $98 \pm 21\%$ (average \pm SD) of alleles with 100pg DNA template. Even with as low as 50pg DNA template, $77 \pm 17\%$ (average \pm SD) of alleles are called. The system is also resistant to very high concentrations of PCR inhibitors. In reactions containing up to 0.5mM hematin, 100% of alleles are called with this system. Improved resistance to humic acid and tannic acid is also observed.

To improve laboratory workflow efficiency, this system is designed for use with both casework samples as well as with reference and database samples. Direct amplification of blood or buccal samples on multiple substrate types such as FTA® card, non-FTA® cards, and swabs eliminates the extraction process, which saves time and money. To further save time and improve efficiency, automation methods are available for multiple liquid-handling platforms which minimize potential cross-contamination and result in more than a 95% first-pass success rate.

STR, Y-STR, Casework



Criminalistics Section - 2015

B8 Self-Generating Robot Worklists and Complete Sample Traceability Through Laboratory Information Management Systems (LIMS) Integration and Barcodes

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After attending this presentation, attendees will be familiar with a customized robotic liquid handling system which is capable of self-generating worklists through the use of barcodes and LIMS integration.

This presentation will impact the forensic science community by informing forensic DNA laboratories about an automated system which has integrated the LIMS database and utilizes barcodes to improve sample traceability and reduce analyst input errors.

The Texas Department of Public Safety (TX DPS) Crime Laboratory in Houston, TX, recently procured a Hamilton AutoLys STARplus for the purpose of automating the entire DNA workflow, including lysis and substrate removal, on a single platform. Sample traceability is of utmost importance to the TX DPS Crime Laboratory system; therefore, the use of barcodes and LIMS integration was essential to this project. The instrument was integrated with the DPS LIMS database which enabled self-generating worklists with built-in data verifications and complete sample traceability through the use of 2D barcodes.

The STARplus system was modified by Hamilton to accommodate both 2D and 1D barcode reading systems. The 2D barcode on the bottom of the AutoLys tube is registered in the LIMS system to a specific item of evidence while another 2D barcode is generated for the label on the 1.5mL tube that will be used for the final storage of the purified DNA extract. Both barcodes are associated with the same sample, so the sample's movements are recorded in its chain of custody as it makes its way through the DNA process. To work around limitations of the current LIMS system, samples are batched together using a barcoded mobile container.

Through the use of barcodes, data verification measures, and custom programming by the Hamilton Automation Applications engineer, the instrument has the ability to ensure the proper number of reagent blanks, distinguish "known" and "questioned" samples to prevent co-extraction, determine the length of the lysis buffer incubation and elution volume during extraction based on sample type, verify user input for extraction kit information, and create self-generating worklists. These measures reduce both the amount of input needed from the analyst and the potential for transcription errors.

In order to store and access quantification results, a custom-built quant value database was developed by Hamilton for TX DPS Houston. When the robot creates a worklist for the normalization/amplification method, it queries the LIMS database for sample information, and queries the quant value database for quant values. The method then combines the data to generate a worklist.

Finally, TX DPS Houston has developed a separate spreadsheet workbook for each method that uses output files generated by the robot to create casefile worksheets. All sample information is imported into the spreadsheet workbook, requiring the analyst to input only the occasional date and run identifier. The worksheets also offer extra functionality including highlighting samples from a specific case, printing, and selecting capillary electrophoresis instruments and injection times.

Overall, the Hamilton AutoLys STARplus has met expectations and has proven to be reliable in terms of ease of use for workflow and sample traceability.

Automated DNA Workflow, Barcode Traceability, LIMS



B9 Obtaining DNA-Short Tandem Repeat (STR) Profiles From Evidentiary Samples With Extremely Limited Amounts of DNA

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After attending this presentation, attendees will have learned about a systematic approach to obtaining DNA-Short Tandem Repeat (STR) profiles from samples with very limited amounts of DNA. Forensic science practitioners will have learned about an optimized method for: (1) the collection of small biological samples; (2) the extraction of DNA from these samples; (3) the purification of DNA from such samples; and, (4) the post-Polymerase Chain Reaction (PCR) purification and concentration of amplicons. The final goal of this sequence of techniques is to obtain DNA profiles from the smallest possible forensic samples; this study has fully optimized the individual steps and introduced a method that utilizes the entire PCR reaction for electrokinetic injection on Capillary Electrophoresis (CE) instrumentation.

This presentation will impact the forensic science community by describing the procedure that can be used to successfully obtain DNA-STR profiles from very challenging forensic samples (sometimes referred to as “touch DNA” samples) like single fingerprints, single bullets, and small areas of worn clothing.

For a variety of reasons, both legal and criminalistic, forensic practice increasingly demands DNA profiles from ever-smaller (in terms of DNA content) samples. This trend includes testing “gun swabs,” swabs from bullets (fired and unfired), exploded bomb parts (including Improvised Explosive Devices (IEDs)), zipper pulls, door knobs, hammer handles, and even latent ridge impressions (fingerprints). Many of these kinds of samples fail to provide useful DNA profiles using current methods. This is primarily due to losses of biological material during the collection, DNA extraction, and purification steps. Another contributing factor in failing to obtain a useful DNA profile is that only a fraction (typically <6%) of the multiplex STR PCR reaction is used for CE analysis. The procedure discussed here corrects all of these deficiencies.

Specifically, the protocol includes the following materials and methods. Larger objects (e.g., aluminum cans) and absorbing surfaces (e.g., clothing) are swabbed with regular-sized swabs. Small objects (e.g., individual fingerprints, bullets, etc.) are swabbed with user-produced mini-swabs. Mini-swabs are made by deliberately truncating the swab head to approximately ¼-½ of the regular cotton swab. Regular cotton swabs were wet with 50µL and mini-swabs with 10-20µL of collection buffer containing a low concentration of detergent. Following collection, regular swabs were saturated with 70µL and mini-swabs with 40µL of lysis buffer that includes detergent and Proteinase K. Swabs were incubated at 56°C for one hour. The lysate was collected by centrifugation in a spin-basket and loaded onto a column filled with polyamide resin. DNA was purified on the column at 4,000 Relative Centrifugal Force (RCF) for two minutes. Purified DNA was either amplified directly or concentrated three- to four-fold under vacuum centrifugation. Following PCR amplification using a commercial forensic multiplex kit (the use of additional PCR cycles is not recommended), an AmpliconRx™ kit was used for post-PCR purification and concentration of the entire PCR reaction, as this further increases (by up to approximately 20 times) the final CE signal.

The current study shows that when combined into a complete protocol, this integrated procedure for collection, extraction, purification, and post-PCR clean-up, concentration significantly increases the sensitivity of obtaining DNA profiles from low-template (touch DNA) samples. Specifically, the limit of detection of this method was tested by applying progressively smaller amounts of a control DNA solution to a non-absorbent surface, allowing the deposition to dry, and then collecting and processing as described. These experiments demonstrated that full profiles (26 alleles) could be reproducibly obtained from as little as 62.5pg of starting DNA material on a non-absorbent surface.

Testing of this method has continued using developed and undeveloped fingerprints on a variety of surfaces. Examples of some successfully profiled items that will be presented are: (1) three poorly developed fingerprints deposited on an aluminum can — full profile (28 alleles); (2) a single well-developed fingerprint on a plastic surface — 26 out of 27 expected alleles; and, (3) an unfired bullet, loaded into a magazine, retrieved, and tested — 25 out of 28 expected alleles.

Touch DNA, Sample Collection, DNA Purification



B10 Validation and Incorporation of RapidHIT™ Technology Into Routine Forensic DNA Casework

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After attending this presentation, attendees will understand that the RapidHIT™ instrument can be used for forensic DNA evidence in addition to buccal swabs for routine forensic analysis.

This presentation will impact the forensic science community by educating analysts about the ability of this system to be used for routine forensic DNA casework.

In December of 2013, the Richland County Sheriff's Department (RCSD) acquired a RapidHIT® (IntegenX®) instrument for fully automated sample-to-answer Short Tandem Repeat (STR) -based human identification. This system produces a DNA profile from swabs or cuttings in approximately two hours. The focus of this system in the scientific field has been on its use for DNA profiling of buccal swabs for a myriad of applications. The laboratory validated and implemented this instrument for full integration into routine forensic DNA casework to include buccal swabs from suspects as well as evidence swabs and cuttings. During validation, a study was performed to assess usable sensitivity (ability to produce a profile for exclusion or inclusion) of the system for evidence samples. Of the 28 different samples evaluated (drinking straw, gum, pulled hair, cigarette butt paper, drink can, steering wheel, door handle, foam cup, bitemark, jacket collar, cell phone, etc.), 22 produced full (16 of 16 loci) profiles, four produced partial profiles (11 to 15/16 loci), and two produced no profiles (whole cigarette butts were placed into the sample cartridge which resulted in interference of the extraction). Upon completion of validation, RapidHIT® was approved for casework and has been successfully utilized for several different case types.

A serial burglary (three related burglary cases), a criminal sexual-conduct case, an aggravated armed robbery/attempted murder case, and multiple suspect standards for the local database have been analyzed with RapidHIT®. The burglary cases consisted of two blood swabs and a bloodstained cutting from a leather pouch from the crime scenes as well as a buccal swab from a suspect. These were analyzed on a RapidHIT® utilizing PowerPlex® 16 HS chemistry using the "Other" protocol. Complete (16 of 16 loci) profiles were generated from the evidence and the suspect's buccal swab. Resulting DNA profiles were interpreted after the two-hour run and the results were communicated to the investigator after technical review. The profiles generated from the three different crime scenes matched the suspect. As a result, the suspect was arrested and charged with 2nd-degree burglary and larceny. Evidence from the attempted murder case consisted of swabs from the suspect's pants, shoes, and a victim's buccal swab. These were run on the RapidHIT® utilizing GlobalFiler® Express chemistry with the "Other" protocol. Complete (22 of 22 STR loci) profiles were generated from the evidence and victim's standard. Profiles generated from the suspect's clothing matched the victim. After technical review, findings were communicated to the investigator and the suspect was arrested for attempted murder and armed robbery.

The RCSD successfully renewed its American Society of Crime Laboratory Directors/Laboratory Accreditation Board (ASCLD/LAB) -International accreditation in June of 2014. RapidHIT® technology was in place, reviewed, and memorialized during this assessment. Currently, the results generated from this system are used for investigative information. If the customer requires a laboratory report, samples are re-analyzed utilizing the conventional DNA profiling method. Once the quality standard requiring human quantitation is modified to accommodate this technology, any profile generated from this instrument could be considered eligible for the Combined DNA Indexing System. The RCSD DNA laboratory views this instrument as an evolution in DNA typing technology.

Rapid DNA, Forensic DNA, Casework



B11 A “Whodunit” Solved Utilizing Mixture Interpretation Software With Quantitative Genotyping

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After attending this presentation, attendees will appreciate how useful touch DNA samples can be and gain an understanding of how mixture interpretation tools can be pivotal in deconvoluting difficult mixtures. The algorithms used in the ArmedXpert™ software will also be introduced.

This presentation will impact the forensic science community by demonstrating how a complex DNA mixture from a perplexing case was able to be interpreted, using quantitative genotyping, and led to a useful investigative lead.

Touch DNA is DNA which is deposited when someone touches or comes in contact with an item and leaves sloughed off skin cells behind. It is usually present in very low quantities and full DNA profiles are often difficult to obtain due to the low number of cells which are usually deposited. Because of the nature of touch DNA samples, it's very rare that a Combined DNA Index System (CODIS) -worthy profile is developed; however, with the help of mixture interpretation software, difficult mixtures, such as those obtained when swabbing for touch DNA, can more readily be interpreted and can result in useful investigative information.

In 2012, the United States Army Criminal Investigation Laboratory (USACIL) received a case involving the abduction of a young child. A little girl was playing on a playground when she was approached by an unknown male who proceeded to kidnap her. Shortly after being captured, the child was able to flee to safety; however, even after she gave a description of the man, investigators had no idea who the abductor was. The child's pants were subsequently submitted to the laboratory and, during analysis, the pockets were swabbed for touch DNA since it was alleged that the assailant touched that area.

The resulting DNA profile was analyzed using ArmedXpert™, a mixture interpretation software tool that was developed in-house and for which USACIL currently holds a patent. It uses a simple deconvolution strategy based on three rules: (1) shared alleles are shared in proportion to the unshared alleles; (2) where possible, peak height ratios are defined as 100%; and, (3) minimum allele heights are maintained. This software has been invaluable in streamlining the analysis and interpretation process. It has also provided a relatively high level of consistency in final results as determined by the statistical analysis of mixed DNA profiles used during internal competency testing.

With the help of the mixture interpretation software, the DNA profile obtained from the pants' pockets was able to be interpreted as a mixture of three individuals where the owner could be assumed, a family member could be identified, and a full unknown male DNA profile could be teased out for CODIS submission. In the months following case completion, approximately 150 standards were submitted for comparison; however, no matches were made. Finally, months after the unknown profile was uploaded to CODIS, it hit on an arrestee who has since accepted a plea deal and is currently serving 25 years.

Touch DNA, Complex Mixture, Mixture Software



B12 Database Samples Warranting a Closer Look and Examination of the D8S1179 Locus

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After attending this presentation, attendees will have closely observed two samples that originally produced profiles requiring additional examination for questionable allele calls at the D8S1179 locus. Attendees will see that in addition to standard practices employed at the Marshall University Forensic Science Center (MUFSC), steps were needed to determine the true genotypes for each of these samples in question.

This presentation will impact the forensic science community by enlightening attendees that analysts should be very aware that profiles produced may require additional examination due to inconsistencies or questionable calls. It is imperative to ensure that the true DNA profiles for each sample will be uploaded into local, state, and federal databases.

The original samples were previously spotted on Whatman® FTA® blood cards at the West Virginia State Police Forensic Laboratory (WVSPFL) and processed using the direct amplification method with PowerPlex® 16 HS employed at the MUFSC. These two database samples required more lab work due to problems with an allele call, a peak height ratio, and dropout at the D8S1179 locus.

Sample A and Sample B (generic sample names used in place of identifying the barcodes used in processing) were extracted and amplified using PowerPlex® 16 HS, PowerPlex® 16, and AmpFISTR® Identifiler® Plus. Sample A was initially direct amplified using the Powerplex® 16 HS kit resulting in an Off Ladder (OL) peak at the D8S1179 locus with a height of 704 RFU, a size of 225.08, and a calculated call of a 12.3. When comparing the sample with the ladder, the math came out to: $225.79 - 225.08 = 0.71$ on one side of the ladder and $225.08 - 221.77 = 3.31$ on the other side of the ladder, resulting in the call of a 12.3. This microvariant has been seen at the MUFSC six times and on STR base three times, leading an analyst to believe this could be a true call. The calculations resulting in the 12.3 call did seem a little far off from the normal OL/microvariant calculation normally seen. Normally, when calculated, the OL/microvariants are closer to a “1” nucleotide difference; however, based on the analysis, the sample appeared to have an allele call of 8,12.3 at D8S1179. Similar results were obtained from a cutting that was extracted on the EZ1® robot resulting in the 8 and 12.3 at D8S1179.

Sample A was subsequently amplified with Identifiler® Plus with a 1.0µl load of the neat extract, with a target of 1.933ng. With this kit, the allele in question fell into the 13 bin, resulting in a 8,13 call for the D8S1179 locus. In this testing, the 13 allele had a height of 1688 RFU and a size of 143.84. These Identifiler® Plus kit results led to the previous Powerplex® 16 HS kit results being questioned. Additional processing of Sample A was completed by the assistance of the National Institute of Standards and Technology (NIST) to obtain a profile that could be submitted to the West Virginia State Police for upload into the Combined DNA Index System (CODIS). The final and true allele calls for Sample A at the D8S1179 locus were determined to be an 8 and 13.

Sample B originally appeared to be homozygous at the D8S1179 locus with a 12,12 allele call, but close examination of the data caused the analyst to question whether there was possible allelic dropout. This sample would normally pass because the homozygous allele passed the stochastic threshold at the MUFSC. More laboratory work concluded that this sample did in fact have a sister allele of a 14 at this locus. This sample had to be extracted and amplified using PowerPlex® 16 HS, PowerPlex® 16, and AmpFISTR® Identifiler® Plus to obtain the true genotype.

This study will present the steps and processes taken to obtain successful and true profiles and the initial reasons for further examination of each sample. Each profile produced will be highlighted and explained for each amplification of the two samples. It is recommended that persons in the field are familiar with DNA analysis of single-source reference samples, direct amplification, and traditional methods of extraction and amplification, as well as peak height ratios and dropout.

DNA, Database, Microvariant



B13 Separation of Epithelial Cell Mixtures Using Fluorescently Labeled Antibodies and Flow Cytometry

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The goal of this presentation is to introduce attendees to a novel molecular technique that utilizes fluorescent antibody probes that target antigens within the Major Histocompatibility Complex (MHC) on epithelial cell surfaces. Attendees will also learn how antibody hybridization can be coupled to flow cytometry to provide rapid separation of targeted cell populations from a forensic mixture.

This presentation will impact the forensic science community by introducing a high throughput, non-destructive method for generating single-source DNA profiles corresponding to each contributor of an epithelial cell mixture. The technique can assist forensic caseworking units by providing an alternative to complex profile interpretation procedures, thereby reducing analytical bottlenecks and loss of evidence.

Previous research has shown the application of flow cytometry coupled to antibody probes targeting the Human Leukocyte Antigen (HLA) system for selectively isolating individual cell populations from a blood mixture to generate single-source Short Tandem Repeat (STR) profiles. The goal of this research was to test this technique against various types of epithelial cell mixtures for resolving individual contributor profiles. Due to its efficacy in earlier studies, an antibody probe complementary to the A*02 allele was chosen for all experiments. Hybridization experiments were conducted on both buccal cell mixtures and contact epithelial cell mixtures. Results from buccal cell mixture hybridization demonstrated that this cell type has intrinsically high levels of fluorescence which can interfere with the signal originating from specific probe-antigen interactions on the cell surface; however, detailed statistical analysis of fluorescence histograms did indicate greater shifts in cell fluorescence when donor cell populations were either heterozygous or homozygous for the A02 allele. This suggests that separation of labeled cell populations is still possible even if antibody reactivity is not strictly a function of HLA genotype.

In the second experiment, hybridization of the A02 probe was tested against contact epithelial cells. Cells were sampled from the stratum corneum of the epidermal layer from four different individuals representing a range of HLA types. Hybridization results showed little to no interaction with the cell surface across all donors tested. Preliminary experiments using enzymatic digestion of cell surface prior to probe hybridization, as well as novel types of surface antibodies, indicate that these may be effective strategies for enhancing differential labeling and separation of individual cell populations from contact epithelial mixtures. Because epidermal cells and buccal cells showed drastically different reactivity to A02 probe, mixtures containing these two cell types were analyzed. Two-person mixtures containing buccal cells from one contributor and epidermal cells from a second contributor produced two distinct cell populations after antibody hybridization that were easily resolved by flow cytometry. When the labeled cell population (i.e., buccal cells) was separated by Fluorescence Activated Cell Sorting (FACS), the STR profile was identical to its single-source profile across all six STR loci tested. This suggests that HLA antibody probes have the potential to resolve complex mixtures containing different sources of epithelial cells (buccal cells from skin) which may aid in the analysis of certain types of forensic cases.

Mixture, Flow Cytometry, STR Profiling



B14 Additional Criteria for Identification of Gasoline in Fire Debris Samples

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After attending this presentation, attendees will understand new characteristics of gasoline which may support the identification of residues of gasoline in fire debris casework.

This presentation will impact the forensic science community by adding criteria, to the minimum criteria described in literature, for the identification of gasoline in fire debris casework.

Gasoline is a complex refinery blend product of crude oil. Due to its low flash point, it is extremely flammable and therefore a favorite liquid for arsonists.

The identification of residues of gasoline in fire debris samples can be challenging. The composition of gasoline is defined and is controlled by physical properties, while its presence in fire debris is based on the identification of its chemical composition. External factors may add to the complexity. Part of the gasoline may be lost due to evaporation, the release of background components from the burnt/pyrolyzed debris materials may interfere and either alter the composition of the gasoline residues present or may show resemblances in composition with gasoline, and the presence of bacteria and fungi may partially decompose the composition of the gasoline residues present.

Fire debris experts must have a broad knowledge on both the (variation in) the chemical composition of gasoline and on the factors that may affect this composition or may show resemblances with this composition. The latter is extremely important as a way to avoid false positive identifications.

Different guidelines can be found in literature on the identification of gasoline in fire debris samples. These include minimum criteria that should be met for a positive identification and warnings in order to avoid a false positive conclusion. These minimum criteria focus on the main compounds in gasoline including the aromatics and the more volatile alkanes with the necessity of both being present with aromatics usually more abundant than alkanes. It is important to note that their Gas Chromatographic (GC) patterns may vary from batch to batch. Due to this variation, a comparison to reference gasoline(s) is recommended for a positive identification. These minimum criteria are still somewhat general. Additional criteria that may support a positive identification without having to compare to the compositions of reference gasoline(s) on a case-to-case basis would therefore be most welcome and can — by opinion — be found by looking carefully at the way gasoline is produced.

Some gasoline blends are primarily added or produced as blend-product for gasoline: these blends are the octane enhancers. These can be oxygenates and/or the alkylate product from the alkylation refinery process. Typical oxygenates that may be encountered in gasoline are ethanol, Methyl Tert-Butyl Ether (MTBE), Ethyl Tert-Butyl Ether (ETBE), Tertiary Amyl Methyl Ether (TAME), and/or Tertiary Amyl Ethyl Ether (TAEE). The combination of oxygenate(s) in gasoline may vary from blend to blend and their use may be restricted by country legislations. The alkylate product usually consists of branched C₈-alkanes from the 4/4-alkylation (including iso-octane, 2,2,4-trimethylpentane) and/or branched C₇-alkanes from the 3/4-alkylation, and is different in composition from the C₇- and C₈-alkanes in crude oil and found in “straight-run” light petroleum distillates. The alkylate alkanes (in particular the C₈-alkanes) can therefore be considered characteristic for gasoline, despite some variation in their pattern from gasoline blend to blend.

Based on the national reference collection of gasoline, the impression is that most, if not all, gasolines today contain either one or more oxygenates or an alkylate fraction as octane enhancer. To test whether this also applies to gasolines in other countries, a total of 48 gasolines from 11 different European countries (Netherlands not included) were collected with help from European forensic colleagues and analyzed. Overall, 47 out of the 48 were observed to contain an octane enhancer. One gasoline from Scotland did not; this gasoline contained a naphtha fraction instead.

In the identification of gasoline in fire debris samples, adding the presence of an octane enhancer as minimum criteria for a positive identification is recommended: either 4/4-alkylate (C₈-isomers, including iso-octane) and/or oxygenates (e.g., MTBE, ETBE, TAEE), whereas the C₈-pattern and oxygenate combination may vary from batch to batch. Depending on the laboratory method(s) employed, they can still be recovered from fire debris samples, even when the gasoline residues show significant weathering.

Gasoline, Identification Criteria, Fire Debris Analysis



B15 A New Headspace-Mass Spectrometry Method for the Identification of Ignitable Liquids in Fire Debris Analysis

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After attending this presentation, attendees will understand the importance of developing new analytical techniques for the identification of accelerants in arsons. They will realize how Headspace/Mass Spectrometry (HS/MS) technique can help in fire debris investigation which does not require sample preparation.

This presentation will impact the forensic science community by providing a green analytical technique for the identification of Ignitable Liquid Residues (ILRs) in fire debris. Apart from the speed of the analysis and the fact that the sample does not require sample pre-concentration, this technique also has good accuracy, is low cost, is easy to handle for routine analysis, and does not produce any residues as solvents are not used.

In arsons, accelerants such as ignitable liquids are commonly used to initiate or accelerate a fire. Therefore, the detection of ILRs at fire scenes is a key step in the investigation.¹ The most commonly used ignitable liquids are petroleum-based products like gasoline, diesel fuel, or kerosene as they are easy to obtain and easy to ignite.² In some cases, traces of ignitable liquids may remain at the fire scene and these could be matched to samples that are associated with the suspect. The identification of the type of ignitable liquid is very useful information for investigators when there is a suspected arsonist.

Gas Chromatography/Mass Spectrometry (GC/MS) is the most used analytical technique for the analysis of ILRs in fire debris. Indeed, American Society for Testing and Materials (ASTM) International provides guidelines for the identification and classification of ILRs from fire debris by using GC/MS.³ Before chromatographic analysis, it is necessary that the ILR be in a vapor or volatile form, thus a prior suitable sample preparation step of the ILR from fire debris samples is required. Different sample preparation standard practices have been approved by ASTM International for isolating the ILR. Passive headspace concentration with adsorption on Activated Carbon Strips (ACS) is currently the most commonly used method for isolating ILRs from fire debris because of its sensitivity, ease of handling, and because it is not non-destructive.^{4,6} The drawback of this method is the need of a solvent such as Carbon Disulfide (CS₂) for the desorption of the compounds from the ACS, which is extremely hazardous.

In this study, a new analytical method based on a Headspace/Mass Spectrometry (HS/MS), also known as electronic nose, for the analysis of ILRs is presented. The working conditions for the HS/MS analytical procedure were optimized using different fire debris (wood burned with gasoline, diesel, and citronella oil). The variables optimized included incubation temperature and incubation time. The optimal conditions were as follows: 115°C and 15 minutes. The optimized method was applied to a set of fire debris. To simulate a post burn, samples of several ILs (gasoline, diesel, citronella oil, kerosene, and paraffin oil) were used to ignite different substrate (wood, cotton, cork, paper, and paperboard). Chemometric methods (Hierarchical Cluster Analysis (HCA) and Linear Discriminant Analysis (LDA)) were applied to the MS data (45-100m/z). At first instance, HCA was enough to perform a correct classification of different ILRs.

Compared to the current methods, HS/MS does have specific advantages. Apart from the speed of the analysis and the fact that the sample does not require sample pre-concentration, this technique also has good accuracy, low cost, is easy to handle for routine analysis, and does not produce any residues because solvents are not used. This technique can be considered as a green technique for fire debris analyses.



Criminalistics Section - 2015

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Fire Debris, Ignitable Liquids, Headspace-Mass Spectrometry



B16 The Use of Isotope Dilution Inductively Coupled Plasma/Mass Spectrometry (ICP/MS) for Precise and Accurate Determination of Elemental Concentration of Trace Elements in Float Glass Standards (FGS1 and FGS2)

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After attending this presentation, attendees will understand basic principles concerning the application of Isotope Dilution-Inductively Coupled Plasma/Mass Spectrometry (ID-ICP/MS) as a method of ultra-precise analyses. Issues such as the required materials and the working procedure will be addressed.

This presentation will impact the forensic science community by raising awareness on the activity of the re-evaluation of the concentration values of Float Glass Standards (FGS) glasses as calibration material for forensic glass analysis by Laser Ablation (LA) ICP/MS.

Over the last two decades, LA-ICP/MS has been proven to be a reliable and powerful technique in forensic glass analysis, especially for the comparative analyses of questioned and control glass samples. Harmonized measurement parameters have been established over time, leading to methods commonly used by the majority of forensic laboratories employing LA-ICP/MS.¹

In order to improve the accuracy of the quantitative analysis of float glasses by LA-ICP/MS, two matrix-matched standards (*doped with 20 elements of high discrimination power*) were produced in 2002 as part of a Bundeskriminalamt (BKA) research project. These FGS resemble soda-lime glass composition, but vary in their concentration of doped elements by a factor of 5. For more than a decade, FGS1 and FGS2 have been in use by several laboratories serving as matrix-matched calibration standards for the quantitative analysis of float glasses.

Information values for the FGS, validated by different analytical techniques (Atomic Absorption Spectrophotometry (AAS), Inductively Coupled Plasma/Optical Emission Spectrometer (ICP/OES), ICP/MS, LA-ICP/MS, Scanning Electron Microscopy with Energy-Dispersive X-ray Spectroscopy (SEM/EDS), and X-Ray Fluorescence (XRF)) were published in 2005.¹ Due to the great capability of isotope dilution ICP/MS in offering results of highest analytical quality and as the most important reference method for elemental analysis, this study will demonstrate the use of Isotope Dilution-Inductively Coupled Plasma/Mass Spectrometry (ID-ICP/MS) for three elements (neodymium, hafnium, and lead) resulting in more accurate and more precise values.²

In ID-ICP/MS, a known amount of isotope-enriched (spike) material of the analyte element is added to a sample with known isotopic composition of the analyte element, but with unknown elemental content. This completely mixed isotope diluted sample (blend) contains a new isotope ratio of the analyte element, which directly reflects the analyte concentration in the sample.²

The determined isotope dilution values of neodymium, hafnium, and lead for the glasses FGS1 and FGS2 will be compared to already-published data for these glasses. As for these three elements, results of isotope dilution ICP/MS are in good accordance with the neodymium, hafnium, and lead of isotope dilution resulting in a higher precision (lower uncertainty values) of the information values of FGS1 and FGS2. Therefore, this work should serve as a first step for a wider re-evaluation of the concentration values for main, minor, and trace elements in the Float Glass Standards FGS1 and FGS2.

Particular issues regarding the homogeneity of the FGS1 and FGS2 will be presented. Previous analyses have shown that particular areas of the glass (rim) exhibit elevated concentration of Cerium (Ce) and Lanthanum (La). This has been caused by the material applied by the final cutting process of the glass.

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Isotope Dilution, FGS, Quantification

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B17 Rapid Identification of Designer Drugs With Nuclear Magnetic Resonance (NMR) Spectroscopy

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After attending this presentation, attendees will understand the principles of Nuclear Magnetic Resonance (NMR) spectroscopy and how it can be applied to the rapid characterization and identification of designer drugs. Attendees will also learn about the power of combining multiple NMR techniques, such as proton spectroscopy, Correlation Spectroscopy (COSY), Total Correlation Spectroscopy (TOCSY), and Diffusion Ordered Spectroscopy (DOSY) in the analysis of designer drug samples. TOCSY can provide information about long-range proton-proton interactions, allowing for accurate identification with extra 2D molecular “fingerprints” beyond COSY. DOSY has the potential to separate the components of a mixture based on their diffusion coefficients. Consequently, signals from solvents and other materials can be separated from synthetic cannabinoid signals without chromatographic separations.

This presentation will impact the forensic science community by introducing a simple but highly discriminatory NMR procedure that can be used to quickly identify a wide variety of designer drugs such as “Spice” (synthetic cannabinoids), “Molly” (phenethylamines), and “Bath Salts” (cathinones).

Designer drugs are a persistent problem for forensic laboratories. As the government bans synthetic cannabinoids, phenethylamines, and cathinones, those who produce these substances create new substances to maintain the legality of their business. The ever-changing nature of this market can be a burden to forensic laboratories that receive large amounts of seized materials containing unfamiliar substances.

The proposed methodology expands upon the laboratory’s previous work in which proton and COSY NMR methods were developed to quickly identify and quantify synthetic cannabinoids in herbal samples. The whole analytical process took less than one hour after cannabinoids were extracted from 50mg of herbal incense with an NMR solvent such as CDCl_3 . The same simple pre-NMR sample preparation technique is preserved to rapidly obtain NMR results. The current expansion includes incorporating more NMR techniques such as TOCSY and DOSY. TOCSY experiments were performed on herbal samples with previously known synthetic cannabinoids. These experiments revealed that signals coming from the alkyl region of the cannabinoid molecules can be elucidated further, as this is a region where signals tend to overlap or may be hard to assign with proton NMR and COSY. DOSY experiments with herbal samples successfully separated solvent and other materials from cannabinoid signals in samples that contain only one cannabinoid. Due to signal overlap, separation based on diffusion coefficient is extremely difficult in samples containing multiple cannabinoids. As of now, signals from two different cannabinoids cannot be separated efficiently using DOSY.

The original methodology is further expanded to include additional designer drugs typically found in “Molly” and “Bath Salt” mixtures. Eleven phenethylamine and cathinone standards were characterized using proton-NMR and COSY with similar parameters compared to the synthetic cannabinoid experiments, with the exception of D_2O as the NMR solvent. Potential signature peaks were identified that could be utilized to quickly identify the components of a street sample. Compared to the results from liquid chromatographic separation with Diode Array Detection (DAD) on the same standards, NMR proves to be a more reliable process at the identification because many of the substances are fairly similar, often isomers of each other, and therefore elute at similar times.

In conclusion, the addition of TOCSY and DOSY NMR methods can improve the discriminatory power of NMR for the identification of synthetic cannabinoids in herbal incense blends. The NMR parameters can also be successfully applied to other designer drugs. These methodologies could be valuable screening tools for backlog reduction, allowing analysts to quickly obtain “fingerprints” of the substance while preserving the original sample for further testing.

Designer Drugs, NMR, TOCSY



B18 Differentiation of Cosmetic Foundations Using Liquid Chromatography/Tandem Mass Spectrometry

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After attending this presentation, attendees will have a better understanding of how cosmetic foundation can be analyzed as a form of trace evidence.

This presentation will impact the forensic science community by providing a simple and sensitive Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS) method to differentiate different cosmetic foundations through the analysis of specific preservatives.

Preservatives are natural or synthetic ingredients that are commonly added to products in order to prevent spoilage, including but not limited to microbial growth or undesirable chemical changes, ultimately extending the products' shelf life. Without the addition of preservatives, the foundation has the ability to easily become contaminated, leading to product degradation, and increasing the risk of irritation or infection. In the United States, the Food and Drug Administration (FDA) regulates the use of preservatives under the cosmetic provisions of the law and manufacturers to determine at what levels the preservatives are considered "safe" for consumer use. The most widely used preservatives in cosmetic products are parabens.

This study has developed an LC/MS/MS method for differentiating different brands of cosmetic foundations by observing the absence and/or presence of specific preservatives, including six different parabens. The analyte preservatives used in this study were methylparaben, ethylparaben, n-propylparaben, isopropylparaben, butylparaben, benzylparaben, tocopheryl acetate, and 3,5-di-tert-Butyl-4-Hydroxytoluene (BHT). The method is capable of separating and identifying all eight preservatives in less than seven minutes including the separation of n-propylparaben and isopropylparaben which has not been accomplished and reported prior to this method. LC/MS/MS data was acquired using an ABI® Sciex 3200 QTRAP® triple quadrupole mass spectrometer interfaced with a Shimadzu® LC system. The instrument utilized Electrospray Ionization (ESI) and all samples were run in positive-ion mode monitoring. Chromatography was performed on a 5.0cm x 3.0mm x 2.7µm Ultra® biphenyl column. The strong mobile phase used was 0.1% formic acid in 2-propanol and the weak mobile phase used was 0.1% formic acid in High-Performance Liquid Chromatography (HPLC) -grade methanol. A Shimadzu® SIL-20AC Prominence auto sampler injected 2.0µL of sample and the column oven temperature was set isothermally at 25°C throughout the run with a flow rate of 0.300µL/min. A retention time optimization study provided preeminent separation conditions.

Foundation samples were prepared by adding approximately 100mg of each foundation to 5mL of methanol:acetonitrile (1:1) and sonicating for ten minutes. After sonication, the solution was placed into centrifuge tubes and centrifuged for 5 minutes at 3,000rpm. After centrifugation, the supernatant was carefully removed using disposable pipettes and filtered using a 0.2µm Millipore® filter. One mL of the supernatant was added to a vial along with 60µL of the internal standard. Lastly, 2.0µL of sample was injected onto the LC column.

Separating and identifying the six parabens as well as the other preservatives proved to be simple and quick. The method is capable of identifying which preservatives are present in a cosmetic sample with a limit of detection of 0.5µg/mL. Twelve different brands of cosmetic foundations were tested and all were easily differentiated by analysis of the preservatives in the samples using the LC/MS/MS method.

Preservatives, Cosmetic Foundation, LC/MS/MS



B19 Latent Fingermarks Revelation on Human Skin With the Lumicyano™ One-Step Fluorescent Revelation Process

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After attending this presentation, attendees will have an enhanced understanding of a new method of fingerprints revelation on human skin.

This presentation will impact the forensic science community by serving as a key aspect of crime scene investigation as it can augment traditional means of investigation with a fast latent fingerprint detection method on human skin.

To detect latent fingerprints on smooth surfaces like metals, plastic, glass, etc., the treatment with glues containing Cyanoacrylate Ester (CA), also known as super glue, is a commonly used technique worldwide. In fuming chambers, the CA is vaporized and polymerizes as a white residue on all surfaces of the possible fingerprint carrier. This makes every fingerprint easily detectable and fixes them at the same time. Additionally, the polymerized CA fixes all other possible biological traces on the carrier that could be of interest for subsequent genetic analysis (e.g., blood, saliva, or sperm stains). Even though this process is widely known, no such revelation method exists when considering human skin surfaces.

Lumicyano™ is a one-step fluorescent cyanoacrylate able to develop fingermarks on various surfaces. It appears much easier to use in a forensic laboratory than the other fluorescent cyanoacrylate described in the literature, especially as it has been designed for use under standard fumigation conditions (120°C, 80% humidity). It allows investigators to develop fingermarks with a quality equivalent or better compared to the conventional, non-fluorescent cyanoacrylate.

In this study, three donors (three males of different ages) were chosen for the intervariability of their fingerprint quality. Sebaceous and eccrine marks were deposited on four 10cm x 10cm human skin samples of the back. A 3x5 square grid was represented on each of the skin samples and donors were asked to let their fingers contact the samples for two seconds on each of the five squares of the corresponding line (i.e., line one for donor one, etc.). Between each contact, hands were washed and dried and donors were asked to wipe the wings of their noses.

Time and ageing conditions have an effect on cyanoacrylate fuming efficiency. To check the Lumicyano™ ability to detect fresh and old marks, three ageing times were chosen (i.e., immediately after deposition, one hour after, and one day after). Cyanoacrylate fuming with Lumicyano™ was then performed in a fuming cabinet MVC 5000 (2003 model version 6.2b). After being processed, each fingerprint was gathered and photographed under white light, then lit under ultraviolet light (312nm), and photos were taken within four hours of fumigation.

For each series, technicians were provided with an instruction sheet and were asked to assess fingerprints according to three evaluation criteria: (1) contrast (meaning the ability of the fingerprint ridges to part with the background); (2) ridge continuity (meaning the possibility of watching unbroken ridges and the ridges flow); and, (3) level of detail (meaning the possibility of watching second level (minutiae) and third level (pores and ridge edges)) details. Results were then compared to fingerprints deposited on microscope slides.

The detailed comparative examinations presented in this study show that Lumicyano™ makes it possible to accurately develop fingermarks on human skin with a high-quality contrast. The luminescent signal provided by the Lumicyano™ allows obtaining readily fluorescent marks; however, the signal is sometimes less intense than the one obtained on microscope slides, especially in the case of poorly developed fingermarks. Nevertheless, this study has shown that Lumicyano™ allows the development of fluorescent fingermarks on human skin for which other two-step sequences methods are often problematic.

Further studies are in progress to improve the fluorescence signal, either by modification of the fluorophore or by making several developments on one item without overdevelopment of the fingerprint.

Latent Fingermarks, Fluorescent Cyanoacrylate, Human Skin



B20 Characterization of Electrical Tapes by Laser Ablation-Inductively Coupled Plasma/Mass Spectrometry (LA-ICP/MS) and Scanning Electron Microscopy With Energy-Dispersive Spectroscopy (SEM/EDS)

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After attending this presentation, attendees will better understand the versatility and utility of LA-ICP/MS for the characterization and comparison of tape evidence and its performance compared to SEM/EDS.

This presentation will impact the forensic science community by providing an assessment and validation of an LA-ICP/MS method for the forensic analysis of electrical tape evidence commonly found in murders, kidnappings, and improvised explosives, to mention a few. This technology has demonstrated its utility in other matrices such as glass, paint, soil, document examinations, and chemical taggants. The validation of this method for tape analysis will now expand the variety of forensic evidence that is currently characterized and analyzed by LA-ICP/MS, providing a more cost-effective solution that can be used across a large number of subdisciplines and materials, hence facilitating the incorporation of this useful tool into forensic laboratories.

Forensic examiners make use of chemical characterizations of both the organic and inorganic components in tapes to support other examinations including physical properties and even an apparent physical match. Current protocols used for electrical tape examinations may include the elemental analysis of backings and adhesives by SEM/EDS and/or micro X-ray fluorescence. Although these techniques have been shown to provide valuable elemental characterization of tapes, they have limited selectivity and sensitivity. The current study evaluated the forensic utility of LA-ICP/MS as an alternative method for the inorganic characterization of tape evidence.

The hypothesis of this research was that the superior sensitivity and selectivity of LA-ICP/MS methods could provide enhanced chemical characterization and improved discrimination between electrical tapes originating from different sources. To test that hypothesis, this study compared the performance of LA-ICP/MS to SEM/EDS data previously collected by another group on a collection set of 90 black electrical tapes. The overall discrimination for the 90 samples was found to be 93.8%, which was superior to the discrimination found using SEM/EDS (87.3%). Moreover, LA-ICP/MS detected a larger number of elements offering complementary information to the chemical characterization obtained by SEM/EDS. A second hypothesis was that the analytical performance provided by LA-ICP/MS was appropriate for the inherent chemical heterogeneity and the subsequent characterization of tape evidence. For this purpose, studies on the variation of the elemental composition within a roll were conducted for seven rolls. Each roll was split into six sections and each section was characterized in six replicates at different locations. Results demonstrated that the amount of material removed during analysis provided a representative characterization of the elemental composition of a number of tape rolls. Inter-roll variations were found to exceed intra-roll variations in all cases, making this technique viable as a comparison tool.

A set of 45 duplicate-control blind samples, each one originating from different locations of the same roll, were analyzed on different days and by different analysts to evaluate instrumental variations, inter-day variations, and false exclusion rates. A set of 100 electrical tapes originating from different sources was analyzed in four to six replicates to evaluate the false inclusion rates and the discrimination capability of the proposed method. False exclusion rates lower than 5% and false inclusion rates lower than 6% were observed for the selected collection sets, respectively. These results were evaluated to make informed decisions about sampling, chemical analysis, and interpretation of the data collected. A variety of statistical tools were evaluated to determine which one(s) are suitable to the particular sensitivity and precision level of the proposed method.

This work will also describe the optimization and examinations performed on electrical tapes using LA-ICP/MS to achieve ideal penetration into the tapes' backings and adhesives with minimal destruction to the evidence.

Electrical Tape, Trace Evidence, Elemental Analysis



B21 Body Fluid Identification by Mass Spectrometry From Sexual Assault Evidence

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After attending this presentation, attendees will be aware of a novel workflow for screening of biological material originating from sexual assault kits through the use of targeted mass spectrometry.

This presentation will impact the forensic science community by illustrating how this work has the potential to significantly improve serological screening of forensic evidence. Not only will practitioners be able to obtain unambiguous test results for saliva and seminal fluid, but the multiplex design will eliminate the need to perform separate tests on an unknown stain. In short, the successful completion and implementation of this technology will provide a powerful tool for screening and prioritization of sexual assault-type samples for laboratory processing and criminal investigation.

While DNA profiling makes it possible to individualize biological stains, the accurate characterization of the biological material present can provide critical information to prioritize samples for subsequent microscopic and DNA analyses. Currently, the technologies used for the confirmation of seminal fluid and saliva (critical fluids for screening sexual assault evidence) are primarily based on antibody and enzyme activity-based assays.

Contrary to the marketing claims made by some commercial providers, the identification of seminal fluid and saliva by these methods is based on a presumptive indication of the presence of a body fluid. Positive results with non-target body fluids, false positives with non-biological materials, and antibody cross-reactivity with non-human sources have been well documented in the forensic literature.

Thus, it is recognized that there is clear value in developing alternative approaches that are both sensitive and accurate for the identification of human biological stains. To date, a proteomics-based analysis of human body fluid proteomes have identified multiple protein biomarkers for six forensically relevant body fluids — including seminal fluid and saliva. The specificity of each biomarker, the reliability with which it can be detected, and the degree of inter-individual variability in expression has already been demonstrated across a large sample population of human subjects.

These biomarkers have been incorporated into a targeted mass spectrometry assay for the identification of human seminal fluid and saliva. The work described here employs simulated sexual assault swabs in order to compare the sensitivities of the mass spectrometry approach relative to several commercial immunochromatographic tests for the detection of seminal fluid and saliva. To assess this, simulated sexual assault swabs were prepared by spotting varying quantities of semen and saliva onto blank semen-free vaginal swabs. Each dilution was assayed using commercial immunochromatographic systems for saliva (RSID™ Saliva) and semen (RSID™ Semen and Abacus Diagnostics® ABACard® p30) as well as by targeted mass spectrometry on a triple quadrupole mass spectrometer. All targeted biomarkers for both seminal fluid and saliva were reliably detected (based on response ratios, retention times, peak shape, and symmetry as compared to a known positive control) at all dilution levels by the mass spectrometry method. Conversely, several immunochromatographic systems produced erroneous weak positive results due to the hook effect while yielding only weakly positive results at the lowest quantities of spotted body fluid.

Based on these results, the mass spectrometry-based approach offers a superior detection platform while consuming less evidentiary material. In addition, the mass spectrometry-based approach is well suited for simultaneous fluid identification, is compatible with batch analyses in multi-well plate formats, and offers automated data processing and reporting of true confirmatory results.

Serology, Mass Spectrometry, Proteomics



B22 The Occurrence of Forcibly Removed Hairs in Combs and Hair Brushes

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After attending this presentation, attendees will learn about the occurrence of forcibly removed hairs found in combs and hair brushes.

This presentation will impact the forensic science community by accounting for one alternate explanation as to why forcibly removed hairs could be found at a crime scene.

A hair examiner is occasionally asked whether or not a hair has been forcibly removed, as indicated by a hair possessing an anagen root. In one particular instance, a hair examiner was asked during testimony in a child abuse case if a large group of hairs possessing anagen roots was pulled out or could have come from brushing. This study was designed to observe the growth stages of hairs present in hair brushes and combs to determine whether or not it is likely for a large group of forcibly removed hairs to have come from a person simply brushing their hair.

Subjects were asked to clean out their hair brushes or combs prior to obtaining the sample for this study. They were then asked to comb or brush their hair as normal and collect the comb or brush in a ziplock bag. The hairs were then removed from the comb or brush, mounted on slides with permount, and proximal ends were categorized into one of three groups: anagen, catagen/telogen, or broken.

For each individual, a number of factors were recorded to include race, sex, age (or approximate age when the subject would not reveal their age), and type of comb or hair brush used. Combs and brushes were categorized into seven different general types.

Thirty-nine individuals participated in this study and a total of 1,216 hairs were examined from these individuals. Of these hairs, 26 had anagen roots (2%), 306 were broken at the proximal end (25%), and 884 had catagen/telogen roots (73%). Thirty-one of the samples examined did not contain any anagen roots. The highest number (and percentage) of anagen roots found in any one sample was six out of 26 hairs (23%) from a 13-year-old White male using a comb.

The three Black samples examined are of note, as they contained predominantly broken hairs: 61 out of 73 hairs (84%), 51 out of 68 hairs (75%), and 74 out of 137 hairs (54%). High numbers of broken proximal ends were also seen in a White female with heavily treated hair: 21 out of 22 hairs (95%) and a 6-year-old White female: 36 out of 53 hairs (68%). The Black samples and the color-treated White sample did not contain any anagen roots, while the 6-year-old sample contained four anagen roots (8%).

The results of this study show that it is not common to find large numbers of anagen roots on hairs recovered from brushes or combs after a single brushing. This finding should be considered when evaluating possible explanations for the occurrence of forcibly removed hairs.

Hair, Roots, Force



B23 Utilization of Commercial Portable Instruments for Screening Hand Swabs for the Presence of Firearms Discharge Residue (FDR): Validation of Commercial IMS and XRF Instruments to Screen for FDR

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After attending this presentation, attendees will understand the potential merit of screening for FDR using commercially available portable instruments and hand swabs.

This presentation will impact the forensic science community by providing method validation results and figures of merit for the portable instruments used to screen hand swabs for the presence of FDR.

Gunshot Residue (GSR) as traditionally defined consists of particulates containing lead, antimony, and barium (principally) that are formed from primer components that are vaporized during the firing of a weapon. The primary analytical technique used to characterize GSR is Scanning Electron Microscopy (SEM) coupled to X-ray spectroscopy. While conceptually and analytically sound, the drawbacks to this procedure are known and include loss due to secondary transfer and the challenge of interpretation of results.¹⁻⁴ Consequently, the forensic analysis of samples collected from the skin of potential shooters is no longer commonplace.

This practice is regrettable given that the firing of a weapon yields a wealth of useful physical and chemical evidence. GSR is only one type of evidence produced when a weapon is discharged; organic compounds are also produced and encompass residual energetics, stabilizers and additives, and combustion products. This chemical residue is referred to as Organic Gunshot Residue (OGSR) and has been addressed in recent literature reports.^{2,5-7} The organic and inorganic components combined are referred to as Firearms Discharge Residue (FDR). The focus of this study is the description of the validation of three Ion Mobility Spectrometers (IMS) and one X-Ray Fluorescence (XRF) spectrometer to screen hand swab samples for presence of FDR. A significant effort was directed toward identifying the ideal swabbing media and protocols that yielded samples compatible with all instruments as well as with Scanning Electron Microscopy with Energy-Dispersive X-ray Spectroscopy (SEM/EDS) for confirmation purposes; these results will be summarized. Overall, muslin and Nomex[®] substrates were found to be optimal and, using high-vacuum SEM, it is possible to identify GSR particulates on these swabs as well.

Method validation followed acceptable international standards (**International Organization for Standardization (ISO)** and Scientific Working Groups (SWGs)) and the relevant figures of merit will be presented for each instrument. Instruments were used in alarm mode (present/absent) with thresholds rigorously determined using signal/noise measurements and determination of limits of detection for several organic compounds. Repeatability, reproducibility, ruggedness, and robustness were characterized and daily performance was monitored using control charts. For the OGSR, diphenylamine, dimethyl phthalate, ethyl centralite, and methyl centralite were the primary target compounds and for XRF, lead was the focus. Sample stability and holding time data will be presented. It was determined that samples stored at -20°C in darkness were stable for at least six months. Because only portions of the swab surfaces were subject to thermal desorption for IMS sample introduction, sufficient sample remained for further analysis. Results showed that overall, the best performance was achieved using a Smith's Detection Sabre 4000 portable IMS; the limitation to this instrument was the inability to analyze samples that ionize in the negative mode such as nitroglycerin and other compounds that yield a nitrate response.



Criminalistics Section - 2015

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FDR, IMS, Validation



B24 The Development of Paper Microfluidic Devices for the Presumptive Determination of Seized Drugs

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The goal of this presentation is to demonstrate the application of paper microfluidic devices and colorimetric tests in the presumptive determination of seized drugs. Information will be presented on the design of paper-based multiplexed colorimetric detection, how to optimize detection of drugs on paper microfluidic devices, and will demonstrate a variety of applications of the multichannel paper chips.

This presentation will impact the forensic science community by demonstrating the application of paper microfluidic devices as a useful tool for detecting seized drugs in solution. The new method is rapid, inexpensive, and applicable to a variety of seized drugs.

Currently, a wide variety of illicit drugs can be screened using presumptive colorimetric test solutions. These colorimetric reagents have been used to detect seized drugs for many years; however, the storage of these solutions not only occupies space but also involves the use of toxic and corrosive chemicals. Use of these tests in the field can be awkward and may not always be possible in challenging environments. This study has been working on an alternative platform for colorimetric detection based on paper microfluidic devices. Using wax printing and chromatographic paper, four lane chips have been created that adapt the colorimetric reagents to a ready-to-use format. Sample and solvent are applied to the paper just prior to analysis and each lane performs a different test. These devices can be used at crime scenes, in laboratories, and at any other location where seized drugs need identification. The preparation of paper microfluidic devices is simple and inexpensive and they can be conveniently stored for later use.

The paper microfluidic devices are designed as a four-channel multiplexed system. Preparation of the devices requires a wax-ink printer, chromatography paper, and colorimetric reagents. A series of hydrophilic channels are created and outlined in wax on the paper using the wax-ink printer and a laminator. Next, a set of different colorimetric reagents are spotted in each channel to create the detection zone. Drugs dissolved in solutions are then transferred to the chips where they move to the detection zone via capillary action. Sequences of different reagents can be applied to each channel to produce a series of reactions and the color changes finally appear at the end of each channel. The entire process generally takes less than five minutes. Because each drug can produce specific color changes in different channels, it becomes possible to presumptively determine the type of drugs in solutions.

One important aspect of this project is the selection of potential reagents for the device. Traditional colorimetric reagents, such as the Mandeline and Frohdes reagents, use concentrated sulfuric acids. Acids such as sulfuric acid and nitric acid can burn and digest chromatographic paper. As a result, a variety of chemical tests have been performed to modify these reagents to make them more compatible with the paper-based format. For example, potassium manganate (VII), copper (II) sulfate, and iron (III) oxide have been utilized as alternative reagents. The adjusted colorimetric reagents produce specific color changes for seized drugs on paper microfluidic devices. Procedures have been developed for the detection of cocaine, ketamine, codeine, and ephedrine and have been tested against a variety of potential interferences.

Overall, the use of paper microfluidic devices permits the development of rapid, easily stored test beds for a variety of seized drugs. They present a quick presumptive tool for samples which can be used in the field, prior to confirmatory laboratory analysis.

Paper Microfluidic Devices, Seized Drugs, Colorimetric Test



B25 Utilization of Commercial Portable Instruments for Screening Hand Swabs for the Presence of Firearms Discharge Residue (FDR): Collection Efficiency Using Commercial and In-House Media

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After attending this presentation, attendees will understand what types of hand-swabbing materials are available for collection of FDR from the hands of potential shooters for subsequent analysis using a variety of analytical instruments.

The presentation will impact the forensic science community by providing data on methodology and efficacy of hand swabbing for the detection of FDR.

Gunshot Residue (GSR) consists of particulates containing lead, antimony, and barium (principally) that are formed from primer components that are vaporized during the firing of a weapon. The primary analytical technique used to characterize GSR is Scanning Electron Microscopy (SEM) coupled to X-ray spectroscopy. While conceptually and analytically sound, the drawbacks to this procedure are known and include loss due to secondary transfer and the challenge of interpretation of results.¹⁻⁴ Consequently, the forensic analysis of samples collected from the skin of potential shooters is no longer commonplace.

This is regrettable given that the firing of a weapon yields a wealth of useful physical and chemical evidence. GSR is only one type of physical evidence available; organic compounds are also produced when a weapon is discharged. This chemical residue is referred to as Organic Gunshot Residue (OGSR) which has been addressed in recent literature reports.^{2,5-7} The organic and inorganic components combined are referred to as FDR. The focus of this study is to discuss research and validation studies undertaken using a variety of hand swabbing methods with the goal of using the collected samples in both presumptive and confirmatory analyses for organic and inorganic compounds.

The criteria for selection of media were: (1) wettability using isopropanol; (2) stability under thermal desorption conditions; (3) compatibility with several portable instruments for presumptive testing (X-ray fluorescence and ion mobility spectrometry); (4) extractability; and, (5) compatibility with Scanning Electron Microscopy with Energy-Dispersive X-ray Spectroscopy (SEM/EDS) methodology. Materials analyzed including clean room wipes, a raw fabric of Kevlar[®]/Nomex[®], and commercially prepared Nomex[®] and muslin swabs. The combination of these materials and isopropanol as the wetting agent yields a simple, non-invasive sampling technique that has been approved by the West Virginia University Institutional Review Board (WVU IRB). The advantages and disadvantages of each media will be presented in terms of recovery efficiency from a skin surrogate, instrumental compatibility and performance, extractability, and stability of samples under typical storage conditions.



Criminalistics Section - 2015

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Hand Swabbing, GSR, IMS



B26 High-Resolution MS/MS Spectral Library for Synthetic Cannabinoids by Direct Analysis in Real-Time Quadrupole Time-of-Flight Mass Spectrometry

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After attending this presentation, attendees will learn how Direct Analysis in Real-Time Quadrupole Time-of-Flight Mass Spectrometry (DART[®]-Q-TOF/MS) can be applied for the identification of synthetic cannabinoids and the usefulness of a spectral library for these compounds. This presentation will highlight the advantages and limitations of DART[®]-Q-TOF/MS over traditional Gas Chromatography/Mass Spectrometry (GC/MS) and Liquid Chromatography/Mass Spectrometry (LC/MS) drug analysis as well as show the development of a spectral library for synthetic cannabinoids using this technique.

This presentation will impact the forensic science community by demonstrating how DART[®]-Q-TOF/MS can be used to rapidly produce high-resolution data for synthetic cannabinoids that can then be compiled into a spectral library to allow for faster identification of these compounds in seized products.

Abuse of synthetic cannabinoids has increased dramatically since the first report in Europe in 2004 and first detection in the United States in 2008. Typically sprayed on plant leaves, flowers, or stems, and packaged as herbal incense mixtures under the names “Spice” and “K2” among others, these compounds are marketed as “legal highs.” Although legislation has been passed such as the Synthetic Drug Abuse Prevention Act in 2012, the number of synthetic cannabinoids has continued to increase, many of which are analogs of other compounds developed to circumvent this legislation. This complicates identification of synthetic cannabinoids due to the high level of similarities in structure. DART[®] is an ambient ionization technique that requires little-to-no sample preparation and utilizes lower energy ionization than electron ionization, typically used with GC/MS, meaning less fragmentation and the observance of molecular ions. The DART[®] and Q-TOF/MS parameters were optimized and are reported. When paired with a mass spectrometer, DART[®] allows for rapid analysis and identification as the sample only needs to be introduced into the gas stream for a few seconds in order to produce spectra. Over 100 synthetic cannabinoid standards were analyzed by DART[®]-Q-TOF using the optimized method to generate molecular ions and fragmented spectra and searched on an existing database of Electrospray Ionization (ESI) -generated spectral database with excellent results. The DART[®]-Q-TOF method is a fast and effective tool that provides rapid analysis and identification of seized synthetic cannabinoid products.

This presentation will describe the capabilities of DART[®]-Q-TOF to quickly and accurately identify synthetic cannabinoids and their analogs while also describing the spectral library development. DART[®]-Q-TOF provides an alternative technique for the more commonly used GC/MS and LC/MS which are slower, increasing the amount of analysis time required in drug seizure cases.

Direct Analysis in Real Time, Synthetic Cannabinoids, Spectral Library



B27 Nanoparticle-Assisted Laser Desorption Ionization of Drugs

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After attending this presentation, attendees will better understand the operation and capabilities of Laser Desorption/Ionization (LDI) mass spectrometry, as well as being aware of a broader range of possible analytes and applications.

This presentation will impact the forensic science community by exposing them to the capabilities of a technique traditionally used for biological purposes, expanding its use to forensic samples such as drugs.

Matrix-Assisted Laser Desorption Ionization-Mass Spectrometry (MALDI-MS) is a technique commonly used for the analysis of large biological molecules. Matrices are added to the system as a means of aiding in desorption and ionization of relatively stable analytes, transferring the required energy from the laser. As a soft ionization technique, these thermally unstable samples are able to be analyzed via MS with minimal fragmentation. This means more molecular ions are produced and analysis of results is simpler. Other advantages of this technique include minimal sample preparation as well as high selectivity and sensitivity. Having MALDI available for the analysis of drugs would be highly useful; however, the low mass nature of the drugs results in matrix interference due to peaks from the matrix itself being in the low mass region, making analysis more difficult. This has led to the use of a variety of alternative matrices and surfaces to facilitate the analysis of low mass entities. One of these alternatives is nanoparticles, which absorb the heat from the laser and pass this energy on. A range of different nanoparticles have been used in the analysis of peptides and other biological analytes.

This research uses nanoparticles as an alternative matrix in Laser Desorption Ionization-Mass Spectrometry (LDI-MS) to analyze a range of licit and illicit drugs in liquid and solid forms and from soaked fibers in the low mass (less than 400 Dalton) range.

In this project, gold and silicon nanoparticles were used, either mixed or layered with the drug of interest. The drug samples examined in this manner included opiates, such as codeine and morphine, and amphetamines including methamphetamine and other illicit drugs. These samples were analyzed at concentrations of approximately 1mg/mL. Samples were analyzed both directly on the MALDI plate and from drugs on paper samples mounted to the MALDI plate. Fibers from the loaded paper were removed and mounted on tapes of varying conductivity. Analysis took place in low mass, positive, reflectron mode using a 337nm N₂ laser. The laser strength required for analysis decreased from 100% without assisting materials to 50% or less when analyzed with small amounts of nanoparticles.

Nanoparticles have been previously found to be a viable matrix alternative for biological samples. The results from this study provide insight into the possibility of using these same materials for rapid and unequivocal drug analysis as well. Investigations continue into the analysis of other materials such as inks, paints, bodily fluids, energetic materials, and other compounds of forensic interest using this technique.

LDI, Drugs, Nanoparticles



B28 Exploration of the Electrochemical Enhancement of Latent Fingerprints

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After attending this presentation, attendees will better understand the materials and processes involved in electrochemically depositing polymers onto latent fingerprints on metal surfaces to enhance their visibility. Deposition of intensely colored electrically conducting polymer in the valleys of the fingermarks is shown to result in a reverse image.

This presentation will impact the forensic science community by demonstrating the ability of select monomers, solvents, and electrochemical conditions to obtain excellent enhancement, including third-level detail of sweat pores, on a variety of metallic surfaces (doorknobs, spoons, coffee mugs, etc.). Exploratory studies also uncovered limitations of the method for base metals oxidized at potentials lower than the organic monomer.

While enhancement of latent fingerprints is well-established, significant challenges still exist. Among these is imaging prints on metallic surfaces including ammunition casings. Superglue fuming is widely used, partially due to the multiplicative effect of the polymerization reaction to produce a large amount of material from a smaller amount of fingerprint residue; however, cyanoacrylate is fairly clear in color and provides relatively poor contrast on metal surfaces. Several studies have suggested that oxidation of organic monomers to form intensely colored, electrically conducting polymers may be a viable approach to improving the poor success rate of enhancement on metal surfaces.¹⁻³ The present work was undertaken to systematically study the effect of the choice of organic monomer and solvent, as well as study controlled current and controlled potential systems. The controlled current studies were thought to allow the best repeatability of the amount of polymer deposited. The controlled potential system is most amenable to translation to a hand-held battery device for field use. Three electrode laboratory-based systems were used to understand important parameters including the oxidation of metal specimens and the effect of changing the monomer concentration. Two electrode systems were evaluated with an electrochemical potentiostat and the results used to design and test a portable battery-based system.

Initial screening results of five organic monomers (thiophene, 3-methyl thiophene, 3,4-Ethylenedioxythiophene (EDOT), pyrrole, and aniline) showed that the best enhancement was obtained with EDOT. Screening of five solvents of various polarities showed that better enhancement was obtained in more polar solvents, presumably since non-polar solvents dissolve the sebaceous fingerprint residue (fatty acids, wax esters, squalene, cholesterol, etc.) to a greater extent. Controlled current studies showed that charge densities in the range of 275-375 coulombs per square centimeter applied over a period of 20 seconds generally resulted in sufficient polymer to enhance the image so that it was judged suitable for identification; that is, either a three or four on the Bandy scale.⁴ The average thickness of the polymer is estimated as 0.23-0.30 micrometers based on 50% area coverage and 2.7 electrons per monomer unit polymerized. Additional controlled current studies (chronopotentiometry) demonstrated that progressively lower potentials were reached as the concentration of EDOT monomer was increased. Lower potentials reduce the amount of undesired side-reactions (oxidation of the metal substrate and oxidation of the solvent). At an EDOT concentration of 177mM, the potential generally remained below approximately 1.4 volts (vs. Ag/AgCl) at the optimal charge densities previously noted. In a two-electrode system, the optimal charge densities resulted in a voltage drop close to 3.4 volts across the cell.

Two electrode cells were constructed using 8mm-thick ethyl vinyl acetate foam from a craft shop sealed with silicon and applied over the latent fingerprint using double-sided duct tape and a stainless steel plate as a counter electrode. Various sizes of foam were easily constructed to conform to irregular shaped objects (doorknobs, spoons, coffee mugs). A typical cell with an area of 5.4 cm² required 4.2mL of EDOT solution. When the print was overdeveloped by deposition of excess polymer, application of tape was successful in improving the Brady score. Photography of the print on irregular metal objects was difficult, so various tapes were evaluated to identify if they could lift the polymer. In an evaluation of 12 brands of tape, D-SQUAME[®] performed the best.

In conclusion, significant fundamental work has been performed to identify materials and conditions for the formation of intensely colored conducting polymers to enhance latent fingerprints on metal. Good third-level detail prints have been obtained using short treatments with an easily constructed hand-held battery device.



Criminalistics Section - 2015

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Fingerprint, Tape Lift, Latent Fingerprint



B29 Effect of Reusing Swipe Materials for Particle Collection

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The goal of this presentation is to investigate the number of times a collection wipe can be used before the Particle Collection Efficiency (PCE) decreases or the Ion Mobility Spectrometry (IMS) response is negatively affected. Trace collection is an important part of forensic work and knowing the effects of heat and age of the collection media can help in understanding optimal methods for trace collection.

This presentation will impact the forensic science community by offering a different perspective on swipe-based sampling media and how they can be reused multiple times.

When trace evidence is collected via wipe sampling, the collection material is rarely reused after it is analyzed; however, in the trace contraband detection community, it is sometimes common practice to reuse sampling media several times, especially when no contraband material is previously detected from it. The number of times a wipe can be used before it gets too dirty or the PCE becomes too poor for it to be useful for IMS-based trace detection is currently unknown within the trace detection community. There have been various suggestions, such as using a wipe until it is visibly dirty or discarding it after 25 uses. But there is a need for a method that measures the collection efficiency performance of a wipe material as a function of swipe use or age. The primary purpose of this study is to investigate the number of times a collection wipe can be used before the PCE decreases or the IMS response is negatively affected.

In order to “age” the wipe in a quick and repeatable fashion, a wipe-aging device was fabricated. The setup includes using a movable platen with an automated wand head, where three wipes can be attached at an ideal angle for swiping the “sweet spot” of the wipe. Air pressure is used to force the wand onto a surface and swipe with a repeatable distance and velocity. The air pressure was adjusted such that the downward force exerted by the wipe material was seven Newtons. In an earlier study, this force was found to be the average force a user would apply to a surface when asked to use “firm” force.¹ A variety of materials can be placed on the platen under the wand head to be swiped, such as canvas, vinyl, or cardboard. A robotic arm moves the collection wipe to a thermal desorber where it is heated for seven seconds (the same as a typical IMS analysis cycle), and cools for ten seconds before returning to the platen for the next swiping cycle.

To date, two types of wipes (Teflon[®]-coated fiberglass and Nomex[®]) have been aged by swiping them over various surfaces, including canvas and dirty cardboard, which represent materials that are likely to be swiped in a screening environment. They were swiped between 10 and 1,000 times, then PCEs were determined using previously discussed methods.² Next, known masses of explosives were inkjet-printed on the aged wipes to investigate whether the aging had an effect on IMS response. Finally, SEM images were captured to reveal and compare possible surface degradation of the wipes on a microscale level. Results will be discussed during the presentation.

It is understood that simply aging a wipe by swiping it across a surface does not capture all of the variables and environmental contaminants, a wider variety of surfaces, and contact with other materials such as disposable gloves can influence realistic aging of wipe materials in an operational setting. This experiment should help answer just a piece of the much larger puzzle that is the effect of wipe age as a function of wipe performance.

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Trace Detection, Ion Mobility Spectrometry, Particle Collection



B30 Development of Paper Microfluidic Devices for the Detection of Organic and Inorganic Gunshot Residue

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After attending this presentation, attendees will better understand current research on the development of paper microfluidic devices as a simple and inexpensive alternative to existing presumptive tests for Gunshot Residue (GSR). Minimal training is required to operate these devices and they are ideal for use in the field by military and law enforcement entities. Attendees will also gain a basic understanding of the analysis of lead-based and lead-free ammunition.

This presentation will impact the forensic science community by providing insight into the possibility of inexpensive, user-friendly, presumptive testing devices for GSR.

Colorimetric tests implemented on paper microfluidic devices permits residue from both inorganic and organic GSR to be detected in the field. Paper microfluidic devices are typically prepared from chromatographic paper creating hydrophilic channels through the use of wax printing and lamination at elevated temperatures. Capillary action is then used to mobilize liquids containing dissolved analytes through the wax ink channels of the device. Colorimetric reagents are placed at the terminal end of each channel for detection of the individual analytes. These devices were initially designed for application in medicinal and disease testing in remote areas where the lack of refrigeration limits the ability to store expensive reagents. Because reagents are dried on the device prior to use, shelf lives are increased when compared to liquid reagents. Various forensic applications of this technology have been explored. In this project, a paper microfluidic chip has been developed that involves presumptive, colorimetric tests for multiple different compounds contained in GSRs. Colorimetric tests have been designed for a variety of components in GSR. These tests were first prepared in solution and then optimized for use on paper.

GSR consists of organic and inorganic components that are left behind following the discharge of a firearm. Results are typically detected by atomic spectroscopy, mass spectrometry, or Scanning Electron Microscopy with Energy-Dispersive X-ray Spectroscopy (SEM/EDX) in the laboratory. Paper microfluidic devices have thus been developed for lead, barium, antimony, manganese, and zinc. Lead is detected via potassium iodide as well as through the use of a buffered mixture of sodium rhodizonate, which yields a yellow color and a pink color, respectively, upon reaction. Barium is also detected via the sodium rhodizonate mixture, yielding an orange color upon reaction. Antimony is confirmed via sodium sulfide which yields an orange/brown color upon reaction. Manganese is detected via potassium periodate which yields a brown color upon reaction. Lastly, zinc is detected with dithizone which yields a bright pink/purple color upon reaction. Work is also underway on a similar device to detect organic GSR including organic nitrates, nitrate esters, and diphenylamine.

This device, when optimized for reproducibility, stability, and sensitivity, should prove useful in the analysis of GSR, as the chip is not much larger than a postage stamp and minimal time is needed to produce results. The ultimate goal of the project is to design and test a set of these devices for the presumptive detection of lead-based and lead-free GSR in the field.

Gunshot Residue, Paper Microfluidics, Smokeless Powder



B31 New Psychoactive Substances Abused in South Korea: Drug Monitoring on the Seized Materials by National Forensic Service From 2009 to April 2014

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After attending this presentation, attendees will be informed about the recent trends of New Psychoactive Substances (NPS) seized during drug trafficking in South Korea.

This presentation will impact the forensic science community by providing information about the regional NPS trends in South Korea. International cooperation and information-sharing would be necessary to efficiently regulate the NPSs without a balloon effect.

Recent increases in the use of NPS have become a remarkable worldwide trend. These NPS are traded as “a legal high,” “herbal incense,” or “research chemicals.” Some of the NPS imported to South Korea were first interdicted by customs, while others have been seized by the police agency and the prosecutor’s office during drug trafficking. The National Forensic Service (NFS) identifies psychoactive substances seized mainly by the police agency and part of the prosecutor’s office; this collection may reflect the regional status of NPS abuse in South Korea.

According to drug statistics from the NFS, from 2009 to April 2014, the most frequently identified NPSs were synthetic cannabinoids (38 species) and synthetic cathinones (16 species). Recent trends in synthetic cannabinoids may be summarized as an increase in halogenated derivatives and new substances, including UR-144 and A-836,339, developed as analgesics by Abbot™ Laboratories. The N-pentyl fluorinated analog of UR-144 (XLR-11) has become the most frequently found synthetic cannabinoid in 2013 since its first appearance in 2012, whereas abuse of A-836,339 analogs has been little reported despite its abuse potential. Until early 2011, nicotine was the most frequently found active co-ingredient in synthetic cannabinoids; however, various psychoactive substances such as Δ^9 -tetrahydrocannabinol, α -PBP, α -PVT, and 5-MeO-DALT have often been found as co-ingredients in herbal highs since late 2011. On the other hand, increase of phenethylamine derivatives, including synthetic cathinones and amphetamine analogs, has recently become a new trend. It might be induced by the regional and global regulation on the herbal highs containing synthetic cannabinoids. The phenethylamine derivatives have been found in various types of materials including herbal incenses or dietary supplements.

Trade and abuse of NPS are highly restricted in South Korea by the Narcotics Control Act, which includes the temporary drug designation act and regulation on the analogs of major NPS. The numbers of chemical species and abuse cases of NPS have increased rapidly since 2010, and the Korean Food and Drug Administration (KFDA) added analogs of major NPS to the list of narcotics controlled by law in February 2011 based on the Canadian analog system; however, new compounds have continuously appeared. As a result, the KFDA applied a temporary drug designation act in June 2011 in order to reduce the interval required to legislate the drug regulation act; however, evolution of synthetic cannabinoids has become much faster than before. The most efficient ways to regulate the NPSs may include a rapid legislation system and extensive regulations of the analogs of possible abuse. For these reasons, regulatory systems should be improved continuously to cope with the endless evolution of NPS.

New Psychoactive Substance, Trend, South Korea



B32 Gunshot Residue on Evidence Packaging

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After attending this presentation, attendees will better understand a source of Gunshot Residue (GSR) contamination and methods to minimize GSR contamination prior to analysis.

This presentation will impact the forensic science community by raising the awareness of law enforcement personnel, evidence technicians, and analysts to GSR packaging contamination so that personnel handling GSR-evidence packaging can take steps to prevent it from affecting GSR analyses.

GSR analysis is a valuable tool commonly used in forensic science to associate a suspect with the discharge of a firearm. Associations may result from firing a weapon, being in close proximity to a discharge, handling a weapon or fired cartridge, or handling some other surface bearing GSR. The presence of GSR particles does not prove that a person fired a weapon, but in a criminal investigation it can place an individual in the proximity of the firing of a weapon. As few as three GSR particles are sufficient to provide an association.

GSR particles can contaminate a surface by unintended transfer during collection. Since areas frequented by law enforcement personnel are known to contain GSR, effective efforts must be made throughout the evidence collection process to reduce the possibility of contamination. Proper hygiene and the use of Personal Protective Equipment (PPE) can help prevent contamination. Precautionary practices such as control sampling of a collector's hands before evidence collection can demonstrate the absence of GSR particles that could be a source of contamination.

In order to evaluate the potential for packaging to be a source of GSR contamination, the exterior of 100 GSR collection kit packages of varying types was sampled for GSR. Samples were blindly tested using Scanning Electron Microscopy/Energy Dispersive X-ray Spectroscopy (SEM/EDX) with automated software to detect particles characteristic of GSR by standard operating procedures.

Results showed that 12 of the 100 sampled packages yielded characteristic GSR particles. Of the twelve positive exteriors, three had more than one particle. The source of the GSR could not be determined but the packages were purchased by and in the control of the law enforcement agency prior to submission.

These results show that GSR evidence packaging should be treated as if it were contaminated. The laboratory should maintain separate package handling and analytical testing areas. Good practices should include wiping GSR packaging at evidence intake, regular cleaning of dedicated laboratory packaging area and analytical areas, and the use of PPG and good hygiene practices.

In conclusion, this presentation will illustrate a potential source of GSR laboratory contamination and will provide suggested actions that may be taken to mitigate its occurrence.

GSR, SEM, Contamination



B33 Fourier-Transform Infrared Spectroscopy Investigations of Smokeless Powders

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The goal of this presentation is to determine whether novel smokeless powder classes can be identified beyond the single-base and double-base designations by statistical analysis of Fourier-Transform Infrared Spectroscopy (FTIR) data.

This presentation will impact the forensic science community by providing a statistically valid classification method for powders exclusive of the classic division of single-base and double-base designations.

In 2009, the National Center for Forensic Science (NCFS) developed a smokeless powders database in conjunction with the Technical/Scientific Working Group for Fire and Explosives Analysis (T/SWGFEX) regarding the analysis of smokeless reloading powders. Smokeless powders are low explosives that contain energetic materials and function as propellants in reloading ammunition and they are frequently found in Improvised Explosive Devices (IEDs) such as pipe bombs. The three main types of smokeless powders are single base, double base, and triple base, with all three types containing nitrocellulose. In addition to nitrocellulose, double-base powders contain nitroglycerin while triple-base powders contain nitroglycerin and nitroguanidine. The smokeless powders database housed by NCFS contains analytical data for a large number of commercially available single-base and double-base powders that have been analyzed through analytical methods including Gas Chromatography/Mass Spectrometry (GC/MS), stereomicroscopy, and FTIR.

For this research, FTIR data for 87 smokeless powder samples analyzed at NCFS were utilized. The percent transmittance (%T) was converted to absorbance and the intensity values for wavenumbers 400-4,000 cm^{-1} were normalized to scale 0-1. Since no prior information was known regarding potential grouping of the data, an unsupervised technique, Agglomerative Hierarchical Clustering (AHC) was used to generate dendrograms showing the clustering groups of powders based on FTIR. Hierarchical clustering utilizes a distance metric (i.e., Euclidean, maximum, etc.) to calculate the distance between samples and a linkage-metric (i.e., single, complete, etc.) to cluster data.

Further studies included defining the appropriate distance and linkage by creating dendrograms using Euclidean distance and single, complete, and ward linkage-methods to determine the best clustering. It was determined that the distance and linkage combination of Euclidean and Ward, respectively, gave partially overlapping clusters. The similarity data was then used to generate a heat map to visualize the groupings.

Preliminary results from cluster analysis identified four groups among single-base and double-base powders that do not cluster based on the presence or absence of nitroglycerin, indicating that there are additional factors influencing cluster formation. Additional research will seek to determine the chemical factors contributing to the clustering. In addition, 2D mapping studies of smokeless powder cross-sections will be performed to investigate heterogeneity throughout the kernel.

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Cluster Analysis, Smokeless Powders, Statistics



B34 Creating a Bayesian Network Using Normalized IBIS Scores of .357 Magnum® and .38 Special Cartridge Cases

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After attending this presentation, attendees will understand that transformations through normalization of the correlation scores of the firing pin and breech face influence the likelihood ratio. The Receiver Operator Curves (ROCs) of the firing pin and breech face ranks, including the product of the two, are different from the normalized ROCs of the firing pin, breech face, and their product. A firearms examiner can make a more informed decision by using normalized Integrated Ballistic Identification System® (IBIS®) scores.

This presentation will impact the forensic science community by demonstrating that transforming the IBIS® correlation scores through normalization affects the accuracy of the results. The generation of ROCs from both non-normalized and normalized data sets will show the influence that the variation in data has on the likelihood ratios.

The IBIS® developed by Forensic Technology International, serves as the backbone of the National Integrated Ballistic Information Network (NIBIN) system.¹ This system allows for the databasing of images of cartridge cases and bullets. For each cartridge case, two areas are imaged: the firing pin impression and the breech face impression. All of the cartridge cases were entered into the 2D IBIS® system in order to generate the match data. The acquisition method followed was established in a previous study.² The data was mined to evaluate the variance between relationships involving the following variables: firing pin rank, breech face rank, make and model of the firearm, the ammunition used, caliber, and type of primer reload (if any).

The research involved the acquisition of .357 Magnum® and .38 Special revolver cartridge cases from 15 .357 Magnum® firearms and eight .38 Special firearms. The .357 Magnum® revolvers were used to fire both .38 Special and .357 Magnum® cartridges. The IBIS® compares unknown .38 Special cartridge cases to known (in the database) .38 Special and .357 Magnum® cartridge cases, but only compares unknown .357 Magnum® cases to known .357 Magnum® cases.

A ROC can be used to determine the crossovers between match and non-match. ROCs demonstrate the discriminating power of the method. Normalized and non-normalized ROCs were generated for each individual .38 Special and .357 Magnum® firearm used.

Normalization originates from statistics and eliminates the unit of measurement by transforming the data into new scores (Z-scores) with a mean of zero and a standard deviation of one.³ Normalizing a set of scores involves subtracting the sample mean from the score and then dividing by the standard deviation of the sample. For the purpose of this research, the mean and standard deviation of a variety of sampling percentages of non-match scores for each firearm was found and then used to convert each cartridge case fired from that firearm to a Z-score. This was performed for firing pin, breech face, and their product.

A Bayesian network was used to determine the relationship between the IBIS® scores from the cartridge casings that were collected from the firearms in this study. Scores from IBIS®, such as Firing Pin (FP), Breech Face (BF), and rank (BF/FP) scores were included, as well as factors controlled by the analysts, such as the make/model of the firearms of a chosen caliber. Four new concepts were added as part of the network. They included: breech face rank, normalized firing pin and breech face scores, and the normalized multiplication of the two. The network includes a node titled “Match” and depending on what is instantiated in the network, the probability of a match fluctuates (this will be demonstrated using a computer while presenting). These match and non-match probabilities can then be used to calculate a likelihood ratio based on assigned prior odds. The match/non-match probabilities act in support of $P(H_p|E)$ (probability of the prosecutorial hypothesis being true, given the evidence) or $P(H_d|E)$ (probability of the defense hypothesis being true, given the evidence), respectively.⁴

Transformation of the scores through normalization can lead to improved accuracy of results.



Criminalistics Section - 2015

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Firearms, Bayesian Networks, Normalization



B35 Weathering and Microbial Degradation of Ignitable Liquids

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The goal of this presentation is to help attendees understand the purpose of the updated Ignitable Liquid Reference Collection database, pattern recognition of a reference ignitable liquid when weathered and/or degraded, and see an example that ties together weathering and microbial degradation of a simple mixture.

This presentation will impact the forensic science community by presenting the updated database in regard to weathering and degradation and by demonstrating these mechanisms quantitatively on a simple mixture.

Fire debris evidence is important in arson investigations in regard to finding the origin and cause of a fire. Most ignitable liquids are complex mixtures composed of many organic compounds that are exposed to weathering and/or microbial degradation. With both of these mechanisms either simultaneously or individually affecting the ignitable liquid, it may be difficult for an analyst to assign class and subclass based on the criteria established by the American Society for Testing and Materials (ASTM) E1618. In this research, a simple mixture was made from 14 known compounds which are commonly found in different ASTM classes and was made to undergo both weathering and microbial degradation. Each compound was degraded separately to see if any by-products that formed would coincide with compounds that may normally be present in a complex ignitable liquid. Additionally, a quantitative assessment was performed to observe and record the relative rates of degradation of the compounds found in the simple mixture. The purpose of this research was to measure the effects on a simple liquid mixture as a result of introducing soil containing bacteria. This research further quantifies the rate of compound degradation compared to the use of complex liquids which utilize pattern recognition that only indicates the presence or absence of a given compound or compound type.

Databases are designed and available, both in in-house laboratories and nationally, to aid investigators in connecting fire debris evidence to a potential source by comparing the total ion chromatograms and extracted ion profiles. The Ignitable Liquid Reference Collection (ILRC) has been extended to include examples of weathered and biologically degraded liquids from each ASTM class. The results of the weathering and biological degradation database will help investigators understand what components of the ignitable liquids are being recovered and which components are lost.

Passive-headspace adsorption/elution and gas chromatography/mass spectrometry methods were used in accordance with ASTM E1618. For weathering, 10 milliliters of an ignitable liquid were evaporated to the percentages 25%, 50%, 75%, 90%, and 95% using nitrogen gas. Twenty microliters of the weathered liquid were placed into a vial with one milliliter of carbon disulfide and analyzed. For the purpose of degradation, potting soil was spiked with an ignitable liquid in a quart-sized paint can, which was then sealed for a specified period of time (zero, two, seven, or 14 days). An activated charcoal strip was then placed in the can's headspace and heated in an oven at approximately 85°C for approximately four hours. Once removed and cooled to room temperature, the strip was eluted using 500 microliters of carbon disulfide and analyzed.

The results of the weathered ignitable liquids were compared to the neat chromatograms in regard to what compounds were still present and if the ASTM classification was the same. Microbial degradation results revealed what compounds the bacteria prefer and comparison of the relative rates at which different compounds degrade. The results have been compiled into the ILRC to assist the analyst in visualizing the pattern of weathered and degraded samples based on evaporation percentages and time period of soil exposure. Results from the microbial degradation of a simple mixture reveal that no by-products were formed when the 14 components were tested separately for zero, seven, and 14 days. Experimental investigation of the quantitative losses of the components of a simple, but representative mixture of hydrocarbons through biological degradation is ongoing. This presentation will provide data representing the enhanced utility of the ILRC and quantitative results from the biological degradation of a simple mixture of hydrocarbons.

This work was supported in part by the National Institute of Justice, Office of Justice Programs, award 2011-DN-BX-K539. The content of this publication does not necessarily reflect the position or the policy of the government, and no official endorsement should be inferred. Support is also acknowledged from the University of Central Florida, National Center of Forensic Science, a State of Florida Type II Research Center.

Ignitable Liquids, Weathering, Microbial Degradation



B36 Analysis of Anthraquinone Dyes by Electrospray Ionization Quadrupole-Time-of-Flight Tandem Mass Spectrometry (ESI-QTOF-MS/MS)

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After attending this presentation, attendees will understand how tandem mass spectrometry on a Quadrupole Time-of-Flight (QTOF) can provide structural elucidation of acid dyes extracted from fibers by specific key fragmentation patterns and how these data can be used to unambiguously identify commercial textile dyes.

This presentation will impact the forensic science community by providing an improved and faster analysis of dyed fiber by imparting a better understanding of the dye fragmentation pathways on a QTOF mass spectrometer for better identification compared to current methods.

In forensic analysis, textile fibers are frequently encountered as evidences in forensic crime scenes. Unambiguous identification and characterization of the dyes present in the fibers are of significance for forensic analysis. Several analytical methods such as Fourier Transform Infrared (FTIR), Ultraviolet/Visible (UV-Vis) spectroscopy, and Nuclear Magnetic Resonance (NMR) can be used for the identification of dyes; nevertheless, the low quantities of trace evidence found at crime scenes can hinder their analysis by these methodologies. Mass spectrometry offers an excellent option for analysis of trace evidence of dyes, due to its high sensitivity and versatility. In this sense, ESI-QTOF-MS/MS can be used for efficient identification and characterization of dyes.

This study is the first stage on the building of a mass spectrometry dye database from the recently donated Max Weaver dye library from Eastman® Chemicals, with approximately 100,000 dyes, to the College of Textiles in North Carolina State University. In this study, several sulfonated anthraquinone acid dyes containing the structure of 1-amino anthraquinone-2-sulfonate acid were analyzed by ESI-QTOF-MS/MS. A loss of 64amu was observed for all dyes and model compounds. This loss was confirmed to be SO₂ by high-resolution analysis. The unimolecular rearrangement and mechanisms of fragmentation triggered by Collision-Induced Disassociation (CID) were investigated by tracing the sulfur isotopes, ³²S and ³⁴S, as well as by exploring the functions of the ortho-amino group during the rearrangement. Also, it was found that the presence of different group functionalities (e.g., secondary or tertiary amines and thiols) attached to the anthraquinone structure have specific fragmentation pathways under similar CID conditions that can be used to identify them. For example, an anthraquinone having a secondary amine with an aromatic group attached to it (e.g., acid blue 25), can be distinguished from an anthraquinone having a secondary amine with an alkyl group attached to it (e.g., acid blue 62). The resultant fragmentation patterns could contribute to a dye database for identifying and fingerprinting unknown dyes with similar chemical structures and can be used for trace fiber evidence in forensic analysis.

Mass Spectrometry, Dyed Fibers, Trace Evidence



B37 Expert Witness Testimony and DNA Evidence in South Africa

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After attending this presentation, attendees will understand that the diligence and manner in which DNA evidence is processed and interpreted in the South African justice system should indeed serve justice. The contributing factors that prevent this from happening, as well as relevant solutions, will be discussed.

This presentation will impact the forensic science community by explaining how contesting DNA evidence in South African courts is not the norm. In addition, there are few independent DNA experts to assist either the prosecution or the defense and bias is rife. The forensic science profession is not regulated and the national laboratory of the state is not accredited. All of the above factors have contributed to a less-than-ideal situation in terms of the criminal justice system in South Africa and, indeed, justice itself.

Even though the status quo does not always serve justice, it does maintain a high conviction rate — which is sometimes the only metric employed to measure “success” in terms of forensic science in South Africa.

Over the past 16 years, South Africa has observed that bias testimony is prevalent and mostly accepted by the courts. Since the first case where DNA evidence was contested in depth, *State v. Maqhina*, there have been some improvements in how DNA evidence is presented in court. There have also been improvements in the discovery process, albeit that both these improvements came about after judgments that were not in favor of the State in significant/key cases.

The level of science is not high when testimony is delivered in court. Statements of identity are common and the bias is perpetuated throughout the system in case after case. Specific examples will be discussed to illustrate what the level of science is in the current status quo. Is there a solution to this for the South African justice system?

Although the solution seems logical, its implementation will require a totally new frame of mind at various levels, including science, law, and policy, in order to truly serve justice. At a minimum, the following will be required in order to address the above challenges: (1) regulation of the forensic science profession in South Africa; (2) accreditation of forensic science laboratories; (3) correct interpretation of DNA evidence — a major factor; (4) addressing the lack of scientific knowledge by forensic scientists; (5) interaction with other forensic science professionals and peers at the national and international levels; (6) transparency with regard to processes followed in the forensic community; (7) eliminating the emphasis on the prosecuting perspective; and, (8) trained legal professionals that can competently handle DNA evidence in court.

Regulation of the profession is key since it will put measures in place to allow the ethical practice of science. Without this aspect, the bias that currently prevails in the system will not be eradicated and justice cannot be served. It will also serve to lift the level of science in the courts, as testimony should be scientifically correct and not mere assumption.

No perfect system exists in forensic science globally; however, if the above can be addressed in South Africa, regardless of how steep the climb to achieve it is, at least justice will be served — as promised to the people of South Africa in the Constitution.

To achieve this goal, both the scientific and legal professionals in South Africa will have to work together in order to bring about the change, without which justice in the forensic science context will remain elusive in South Africa.

DNA Evidence, Interpretation, Ethics



B38 Forensic DNA Collection at Death Scenes

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After attending this presentation, attendees will have a better understanding of the operations of a team of forensic DNA analysts called to select death scenes to collect trace DNA evidence. Case examples will be presented to outline successes from recovering foreign DNA from decedents.

This presentation will impact the forensic science community by informing attendees of the importance of collecting and preserving trace evidence from decedents at death scenes and the impact such evidence has in providing valuable investigative information.

The Trace DNA Evidence Collection Team at the Harris County Institute of Forensic Sciences (HCIFS) is a group of qualified DNA analysts that respond to death scenes for the purpose of collecting trace and DNA evidence from decedents' bodies prior to transport to the morgue. Team members from the Forensic Genetics laboratory provide on-call coverage 24/7. They respond to homicides where close contact is suspected to have occurred between the perpetrator and victim. Evidence such as hairs, fibers, body fluids, and potential areas of touch DNA are collected from the body at the scene to prevent possible loss during transport to the morgue.

A guide is provided to HCIFS medicolegal death investigators to aid them in determining whether to dispatch a Trace DNA Evidence Collection Team member to collect evidence at a scene. Evidence of close contact between the decedent and a perpetrator, such as signs of a struggle, blunt force trauma, or possible evidence of sexual assault, as well as cases where the body has been transferred or dumped, will lead to a call-out. The Trace Team would not be called to collect from highly decomposed or skeletonized bodies or bodies submerged in water. If the scenario suggests evidence may be present but it is not likely to be lost during transport of the body, the decedent may be sealed in a body bag for trace evidence collection at the morgue just prior to autopsy. This might be the case, for example, for a body that is dry or has minimal blood on it.

A surprising number of positive DNA results from victims of gunshot wounds have been noted. DNA foreign to the victim has been found in at least 20 gunshot wound cases from the decedent's pockets that were turned inside out and on facial bruises. The turned-out pockets and bruises indicate close contact, so these cases meet the call-out policy despite use of a firearm.

Between 2009 and 2013, the team responded to 345 scenes. Forty-six of the cases yielded DNA foreign to the decedent. Of the 46 cases, 31% yielded one to ten foreign DNA alleles, while 69% yielded more than ten alleles. In 18 of the cases (35%), the DNA was found to be consistent with a known suspect named in the case. In addition, two DNA profiles obtained from evidence collected by the Trace Team resulted in Combined DNA Index System (CODIS) hits. Both CODIS hits involved unclothed female decedents found outside. In the first case, a pair of hand cuffs used to bind the decedent were swabbed and the resulting profile hit on a suspect in CODIS. For the second case, a stain on the decedent's calf was swabbed and the resulting profile developed a suspect in CODIS, which ultimately led to a confession by the individual.

The Trace DNA Evidence Collection Team is one of the only organized teams associated with a medical examiner's office that regularly collects evidence from decedents at death scenes. This team has become an integral part of the HCIFS medical examiner service. The HCIFS hopes the unexpectedly high success rate of recovering foreign DNA from decedents will encourage others to establish this type of program.

Touch DNA, Death Scenes, Evidence Collection



B39 The Making of the Criminalistics Maestro — On the Skills, Knowledge, and Abilities to Work Proficiently on Non-Routine and Complex Cases

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After attending this presentation, attendees will better understand which skills, knowledge, and abilities a criminalist should acquire to work proficiently on non-routine and complex cases as opposed to routine-based laboratory tasks.

This presentation will impact the forensic science community by stimulating discussions about the education and training of forensic science practitioners and on how one becomes an effective generalist in defining the problems to be investigated and in coordinating criminalistic investigations.

In this age of increasing scientific specialization, some have argued that time and scientific advances have passed the generalist scientist by. They argue that one cannot be a generalist or that the generalist is an outdated concept with no value in this day and age. Conversely, others have argued for the need for generalists as well as specialists. The debate has been especially apparent in criminalistics. A committed proponent of one side or the other typically has little understanding of the other's position. Each may be attracted to absurdist descriptions of the other's position. The generalist may be described as someone who knows less and less about more and more until ultimately he/she knows virtually nothing about everything. On the other hand, the specialist is characterized as learning more and more about an increasingly narrow area until he/she knows everything about nothing. Clearly, the truth lies between these two extremes. A useful analogy is that of the symphony conductor or maestro and the musicians who make up the orchestra. The maestro may be unable to master a particular musical instrument to the world-class level expected of a musician in the orchestra but is a master of understanding how the contributions of all of the instruments and musicians contribute to the success of the orchestra. Of course, there is nothing to prevent a conductor from being a virtuoso on one or a limited number of instruments but such an individual cannot be a master of all. The maestro would not be expected to be. The strength and purpose of the maestro lies elsewhere.

Previous discussions advanced the concept that criminalists should be involved at the outset of the investigation of non-routine and complex cases. Further, the criminalists will be the ones to define and circumscribe the scope of the scientific investigation of the physical evidence record. Unfortunately, in most jurisdictions, criminalists are limited to examining some preselected portion of this record. They are constrained by established customs and practices. They are prevented from seeing the "big picture" and having a major role in defining the physical evidence problem to be investigated. To some extent, this issue is starting to be addressed through efforts to provide quality assurance in the form of International Organization for Standardization (ISO) accreditation of crime scene work as well as the (re)discovery of possible generalist criminalist functions such as forensic case managers or case assessors/evaluators; however, care needs to be taken to ensure that the people chosen, educated, and trained for this key role possess the necessary skills, knowledge, and abilities to tackle their responsibilities, especially in non-routine and complex cases. It is imperative that these individuals are scientists/criminalists rather than technicians.

It is not banal to reiterate that every case and crime scene is different. Each presents challenging scientific problems. What scientific and non-scientific skills would, or should, a criminalist bring to this task? How can these skills, knowledge, and abilities be developed? Can they be taught?

This presentation is not going to take up the generalist/specialist debate. It is going to attempt to deal with the question of how one becomes an effective generalist in defining the problems to be investigated and in coordinating investigations involving physical evidence. How can this be done? This presentation will pose as many questions as solutions offered.

Generalist Development, Complex Cases, Case Management Skills



B40 The Failure of Forensic Science Academia to Address Perceived Scientific Shortcomings

Victor W. Weedn, MD, JD, George Washington University, 2100 Foxhall Road, NW, Somers Hall, Lower Level, L-12, Washington, DC 20007*

After attending this presentation, attendees will be aware of limitations in traditional forensic science education programs to generate adequate foundational scientific underpinnings for forensic science analysis of impression/pattern evidence.

This presentation will impact the forensic science community by bringing to light the limitations of traditional forensic science educational programs to address criticism lodged against the forensic science community in the area of patterned evidence.

Forensic science educational programs have mushroomed since *CSI* aired on television. In fact, a surplus of students has resulted. The quality of the educational programs has increased. Forensic science educational programs are moving to faculties with more than a single member; in fact, all Forensic Science Education Programs Accreditation Commission (FEPAC) -accredited programs have multiple full-time forensic science faculty members. FEPAC has been launched. Nonetheless, despite significant progress, some problems in forensic science education, discussed in the 2004 Technical Working Group on Education (TWGED) report, have in some ways deepened. There is still too little hands-on laboratory analysis in many programs. But what has received less attention is a faculty knowledge gap. Over and over, forensic science programs are bringing chemists and molecular biologists into their faculties, but other forensic science disciplines are generally getting short shrift. There are virtually no questioned document examiners, firearms and tool marks examiners, latent print examiners, or bloodstain pattern analysts as full-time faculty members. University accreditation commissions require a terminal degree for faculty positions — in particular, a PhD degree is sought. Of course, there are no PhD programs in questioned documents examination, firearms and tool marks examination, tire marks examination, etc. Thus, there is a chicken-and-egg situation that results in the continuation of this gap in forensic science academia. Universities do not seem to know how to address this gap. The result is that research in the underlying foundational science that has been called for in the patterned and impression evidence area is lacking.

The 2009 National Academy of Sciences (NAS) Report, *Strengthening Forensic Science in the United States: A Path Forward*, declares, "...the major forensic science disciplines...those that are used most commonly for investigations and trials...have been cause for concern in court or elsewhere because their reliability has not been sufficiently established in a systematic (scientific) manner..." *Chemical and Engineering News* recently reported, "Five years ago, the National Academy of Sciences put out a report condemning the state of forensic science. It concluded that many common forensic techniques — the analysis of fingerprints, bite marks [sic], blood splatter [sic], and ballistics, for example — lack sufficient scientific underpinnings. Thousands of convictions were thrown into question. But in the years since, little has been done to shore up the discipline's scientific base..." There is, of course, some research in these areas, but not as much as there should be. Some of this research is performed in collaboration with true expert practitioners, but some research is being conducted by academicians without deep understandings. Furthermore, the absence of doctoral students hampers research in the field. The presentation will end by discussing a concept to fill in this expertise and research gap by creating an innovative new PhD program, in which the professors are non-experts and the students are experts.

Forensic Science Education, Research Gaps, Patterned Evidence



B41 Implementing Independent Research Projects in a Graduate Forensic Science Degree Program

Walter F. Rowe, PhD, George Washington University, Dept of Forensic Science, 2100 Foxhall Road, NW, Washington, DC 20007*

After attending this presentation, attendees will understand the problems involved in implementing a program of independent research projects as required by the Forensic Science Education Programs Accreditation Commission (FEPAC). These problems include obtaining adequate resources (such as instrumentation, reagents, and samples) and recruiting external members of research committees.

This presentation will impact the forensic science community by showing the value to the forensic science field of independent research projects conducted by graduate students in forensic science degree programs and by encouraging forensic science practitioners to participate in such research projects as principal investigators or as members of research committees.

One of the accreditation requirements of FEPAC for forensic science graduate degree programs is that each student successfully complete an independent research project. Successful completion entails writing a publishable manuscript reporting the student's research results and a public presentation of the results before the student's research committee. The research committee has to have a minimum of three members, one of whom must be from outside the forensic degree program. Studies validating existing analytical methods for use in a particular laboratory are excluded as acceptable research projects.

While few forensic science laboratories conduct research (the overwhelming majority being casework laboratories), future forensic analysts benefit from conducting successful research projects. First of all, research projects provide opportunities for students to gain greater experience in the operation of analytical instruments and in the interpretation of results. Research projects also hone problem-solving skills. Finally, the required manuscript and oral presentation before a committee of experienced scientists gives the students further experience with communicating scientific ideas, experience which is directly applicable to report writing and courtroom testimony.

At The George Washington University, the Department of Forensic Sciences has had to deal with a number of issues in establishing a viable master's-level research program as a part of its Master of Forensic Science (MFS) degree program. First, it was realized that students need to start on a research project early in their degree programs. This allows time for the student to recover from delays resulting from such events as instrument breakdowns or failure of a series of experiments. One particularly vexing delay has proven to be obtaining Institutional Review Board (IRB) approval for projects involving human research (which means most forensic molecular biology projects). Students conducting research in federal government laboratories have also been shut out of these laboratories during government shutdowns.

To insure that projects move along in a timely manner, the department has developed a form on which the MFS student outlines the proposed research project, detailing the experiments to be performed, proposed data analysis, a timeline for conducting the experiments and reporting the results to the student's research committee, and a list of assets required to complete the project (such as instruments, reagents, and samples). This proposal is submitted to the student's research committee for revision and final approval. The department has been able to recruit research committee members from other departments within the University and also from state and federal laboratories in the Washington, DC, area.

Because each year the department has a large number of MFS students who require research projects, it has been difficult to find enough viable projects and sufficient resources for conducting them. Some students have been able to conduct their research in one of the federal government laboratories in the Washington, DC, area. Senior scientists in these laboratories have been willing to serve as principal investigators/mentors for the students. Their laboratories benefit, of course, by having students carry out research that the laboratories otherwise lacked the manpower to accomplish. Departmental resources have also had to be devoted to support student research projects. Departmental faculty members are actively seeking external funding for research; however, under the best of circumstances only a fraction of the MFS students will have external support for their research. The large number of MFS students conducting research has also required full-time faculty of the department to serve on multiple research committees.

As discussed above, FEPAC requirements that graduate forensic science students conduct independent research projects enhance the educations of the students, but present the directors of the graduate degree programs with a number of ongoing challenges involving availability of resources and personnel.

FEPAC, Forensic Science Research, Forensic Science Education



B42 Strengthening Forensic Science in the United States: An Update on National Efforts in Research and Development

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After attending this presentation, attendees will have a greater understanding of how the forensic science community has progressed in the area of research and development. Attendees will also be aware of findings from studies that are having an impact on the forensic science community and potential areas that require additional research.

This presentation will impact the forensic science community by providing an update on national efforts to bolster the quantity and quality of forensic science research studies since the 2009 National Academy of Sciences (NAS) Report, *Strengthening Forensic Science in the United States: A Path Forward*. This presentation will provide an overview of some studies that are beginning to have a positive impact on the perception that forensic science has been lacking in research.

Since 2009, the National Institute of Justice (NIJ) has provided more than \$120 million to fund more than 300 research and development projects related to forensic science, resulting in more than 600 scientific publications, presentations, and final technical reports. In March of 2009, NIJ immediately began addressing recommendations in the NAS Report. There were two major recommendations made in the 2009 NAS Report related to scientific research. Recommendation three called for more research to address issues of accuracy, reliability, and validity in the forensic science disciplines. Recommendation six was to encourage research programs on human observer bias and sources of human error in forensic examinations. More specifically, there was an emphasis on the impression and pattern evidence disciplines such as friction ridge analysis, firearms and tool mark examinations, shoeprint and tire tread evidence, questioned documents, and bloodstain pattern analysis. Therefore, this presentation will focus specifically on progress in the impression and pattern evidence disciplines. NIJ's efforts have earned the support of many respected leaders in the scientific community, including members of the American Academy of Forensic Sciences (AAFS). Douglas H. Ubelaker, former President of the AAFS, wrote in an article to the membership, "A recent major boon to research in forensic science has been the National Institute of Justice's Office of Investigative and Forensic Sciences (OIFS) whose sole goal is to strengthen the quality and practice of forensic science." Most importantly, the presentation will clearly demonstrate that research and innovation are the core requirements needed to continue the progress that has been attained and to strengthen the science in forensic science.

Research and Development, NAS Report, Strengthening Forensic Science



B43 Mentorship to Colleague Inspired by Service: An Evolution to a Brother- and Sister-in-Law Forensic Family Relationship

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After attending this presentation, attendees will be shown how a mentoring relationship can evolve to a peer relationship and sometimes may also lead to a family connection.

This presentation will impact the forensic science community by showing how a “forensic science family” relationship was shaped by two individuals who rose to leadership positions in forensic science.

Larry Quarino and Desiree Reid met in 1984 at John Jay College of Criminal Justice in New York City. Desiree was an undergraduate working toward her Bachelor of Science degree in Criminalistics while Larry began work toward a Master of Science in Forensic Science. Both studied under Dr. Peter De Forest who always emphasized the importance of service to the forensic science community. John Jay students regularly attended professional conferences and Larry and Desiree understood what was expected of them as future criminalists and professionals.

Larry and Desiree always had a close friendship and Larry’s fiancée, Denise, always tried to find a nice boyfriend for Desiree. Larry also kept his eyes and ears open to help Desiree get the proverbial foot in the forensic door. That door was opened one day when Desiree was afforded the opportunity to pursue a summer internship at the New Jersey State Police North Regional Lab, where Larry worked in what was referred to in those days as the Serology Unit. Larry was assigned stewardship of the new intern and became her mentor. Larry had such confidence in Desiree’s abilities to perform tasks that he occasionally got in trouble for giving Desiree an excessive amount of responsibility. Larry believed so strongly in Desiree’s future potential as a criminalist that he convinced a coworker to persuade his wife to hire Desiree in her laboratory. After sojourning a few years at the Union County, NJ, Prosecutor’s Office Forensic Science Laboratory, Desiree was offered a drug chemist position at the North Regional Lab in 1989; however, Larry’s and Desiree’s reunion was short-lived, since within a few months of Desiree’s start date, Larry left for a supervisory position with the brand new Department of Forensic Biology at the Office of Chief Medical Examiner in New York City.

Although Larry and Desiree no longer worked together, they maintained a friendship. Larry and Desiree were among the inaugural group who took the American Board of Criminalistics (ABC) General Knowledge Examination at the 1993 American Academy of Forensic Sciences (AAFS) meeting in Boston and, happily, both passed. Both Larry and Desiree are huge supporters of certification and both have served on the Board of Directors of the ABC. Desiree even had the honor of serving as president of the organization for several years.

Desiree’s commitment was never more evident than in the weeks and months after the attacks of September 11, 2001. For several months, Desiree (as well as many others) gave up weekends to work as an unpaid volunteer at the New York City Office of Chief Medical Examiner on the World Trade Center Identification project. Larry, on the other hand, earned a doctorate in 2000 and embarked on a career as a forensic science academician. He has served as a Commissioner and Chair of the Forensic Science Education Program Accreditation Commission and currently serves as the Chair of the Criminalistics Section of the AAFS and is President-Elect of the Northeastern Association of Forensic Scientists.

Denise, now Larry’s wife, finally did find a special someone for Desiree: her brother Anthony. Desiree and Anthony were married in 2006. Even though Larry’s and Desiree’s professional relationship has changed from one of mentorship to colleagues and their personal relationship has changed from friends to brother- and sister-in-law, they continue to be supportive of each other’s careers. A few years ago, Desiree gave a talk on drug chemistry to the students at Cedar Crest College where Larry serves as program director and recently Desiree reached out to Larry for help with a drug chemistry project. Nobody from their John Jay days could have ever imagined how Desiree and Larry would one day find themselves in a “forensic family.”

Forensic Family, Reid, Quarino



Criminalistics Section - 2015

B44 Scientific and Technical Support of Dangerous Mail Investigations

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After attending this presentation, attendees will understand some of the ways that scientific and technical support has been extended to the law enforcement professionals comprising the United States Postal Inspection Service's Dangerous Mail Investigations (DMI) Program. This program has fundamentals in science, but is staffed and administered by law enforcement personnel, typically without such backgrounds. This model of scientific support may be beneficial for other agencies.

This presentation will impact the forensic science community and various law enforcement and/or "first responder" communities by demonstrating the value of applying the knowledge, skills, and abilities of an experienced forensic chemist, including in ways that are non-traditional.

The law enforcement and "first-responder" communities are often unaware of the myriad ways that scientists can support their mission beyond the obvious examination of evidentiary materials. Similarly, forensic scientists are often precluded from extending their services beyond more traditional roles. It is the position of this presentation that there is great value in reliance upon scientific personnel when matters of scientific importance are encountered.

This study will introduce attendees to the United States Postal Inspection Service's DMI Program, including the events precipitating the creation of the specialized program along with its unique mission. The presentation will include the policies and procedures of the program from the fitness and training requirements for qualified specialists, to threat assessment practices, deployment, and competent/safe use of specialized equipment and instruments. Typical casework will be used to exemplify the manner in which these policies are implemented.

Attendees will learn about the duties and responsibilities established for the Scientific and Technical Advisor (STA) to the DMI Program, the underlying knowledge skills and abilities expected to execute the requirements of the position, and the strong correlation to those qualifications that might be expected of an experienced forensic chemist. The STA is expected to: (1) provide specialized expertise and leadership to evaluate and recommend practices that will optimize the assessment of threats to the United States mail; (2) conduct innovative and complex research; (3) serve as an authoritative source and subject matter expert by liaising between program leadership and the larger scientific and law enforcement communities; (4) plan, coordinate, and conduct training; and, (5) manage a robust quality assurance and safety program. To perform these duties, the STA is expected to possess: (1) the knowledge of forensic science theories, techniques, protocols, and methodologies; (2) the ability to identify and solve complex problems and develop sound recommendations; (3) the ability to communicate orally and in writing, especially with respect to presenting scientific information to a non-scientific audience; (4) the ability to develop and implement a program founded on best practices; (5) the knowledge of proper evidence practices, including the ability to support major field investigations; and, (6) the ability to represent the organization to peer groups, professional organizations, government officials, and industry representatives.

This study will provide examples of scientific and technical work completed in support of the DMI program, demonstrating how the successes of this model may translate to local, state, and federal laboratories and organizations, as well as within private entities.

Collaboration between forensic scientists and law enforcement professionals is prudent when the latter will rely upon complex technical equipment and protocols to conduct their mission. Such collaboration can facilitate the creation or advancement of a stronger program possessing a foundation employing quality assurance concepts and optimized practices. It is recommended that the forensic science community become familiar with the many ways its members can support the law enforcement and/or first responder community. Likewise, it is recommended that those communities become familiar with the abilities of their colleagues in the forensic science community.

Dangerous Mail, Scientific & Technical Advisor, Forensic Chemist



B45 Use of Lean Six Sigma Methodology to Improve Laboratory Productivity and Reduce Backlog

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After attending this presentation, attendees will better understand how a laboratory can increase productivity and reduce case backlogs by implementing a workflow optimization method called Lean Six Sigma (LSS).

This presentation will impact the forensic science community by improving the experience and potential for success for laboratories considering implementing LSS concepts within their laboratory.

In 2012, the Massachusetts State Police Forensic Services Group (MSPFSG) secured grant funding to address existing case backlogs in the forensic biology section. As part of this initiative, the MSPFSG completed a LSS project that evaluated the laboratory's workflow and implemented changes to increase productivity and efficiency. The project team included two facilitators from the consulting agency and 12 MSPFSG members representing the Evidence Control (ECU), Criminalistics, Case Management (CMU), and DNA sections. The ultimate goal was to create a highly productive, quality-driven environment capable of meeting the demand for forensic biology examinations and eliminating the current backlog.

Employing the systematic process Define, Measure, Analyze, Improve, Control (DMAIC), the team evaluated the laboratory's current state and developed baseline metrics to monitor the progress of the project. Using data collected from the Laboratory Information Management System, current case demand and output were calculated and a project charter was constructed outlining specific, measurable goals and a timeline to achieve them. The forensic biology section spent approximately six weeks on the measurement phase eliminating all work in progress, mapping current processes to identify sources of process waste, and summarizing the current work and information flow. This data was analyzed to determine what changes could most significantly improve the overall process. A focused problem statement was developed by each unit with ideas for improvements to address these identified areas.

In Criminalistics, the improvement phase addressed extensive wait times between process steps and a lengthy review process. Data indicated that approximately 80% of errors identified during case review were administrative in nature. Consequently, standardized worksheets for evidence examination were created in place of "free-form" note taking, and ECU intake procedures were updated to address sources of administrative errors prior to case submission. The case assignment process was restructured to create smaller, more frequent case assignments and a standardized workflow schedule was designed to maximize the capacity of work completed in a five-day cycle; however, successfully implementing this schedule proved to be a challenge due to analysts' frequent court testimony and crime scene response. The unit is continuing to evaluate and modify the work flow and, despite these challenges, as of July 2014, the analysts' turn-around time decreased by 74% and the case review time decreased by 21%.

CMU focused on the case activation process for DNA testing and the method for obtaining authorization to consume samples of limited quantity. Many cases remained on the backlog indefinitely because they required critical documentation from the requesting agencies. Several redundancies were also identified in the case assignment process. The improved procedures streamline the activation of cases for DNA analysis and place specific timelines on the requesting agencies to provide the documentation required for analysis to proceed. Using these improved procedures, as of July 2014, the CMU case backlog decreased by approximately 30%.

In the DNA unit, the improvement phase focused on designing a schedule that condensed the workflow from 12 weeks to two weeks. This new schedule introduced standardized work practices, implemented a team-based approach, and defined expected daily tasks. Though the DNA unit needs to make further adjustments to meet the new schedule, as of July 2014, the average case turn-around time decreased from 202 to 102 days and the average analyst turn-around time decreased from 122 to 35 days. The control phase provided the unit with tools to monitor progress and a means for supervisors and management to predict the working capacity of the unit and the status of individual cases. This phase also introduced a significant shift in supervisory approach, with supervisors accountable for distributing work based on the DNA unit's daily needs and priorities.



Criminalistics Section - 2015

This study will present the journey of a large state laboratory implementation of LSS concepts. Though implementing these principles is not without operational challenges for a laboratory to consider, LSS provides effective tools for a laboratory striving to improve productivity and reduce backlog.

Productivity, Backlog, Quality



B46 Demystifying the American Board of Criminalistics Examination

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After attending this presentation, attendees will understand how factors such as hours of study, educational background, and years of professional experience impact performance on the American Board of Criminalistics (ABC) certification examinations.

This presentation will impact the forensic science community by offering information that will help individuals understand the purpose of the ABC, the history of the examinations, their success rate, and their performance trends.

In light of the 2009 National Academy of Sciences Report, *Strengthening Forensic Science in the United States: A Path Forward*, and the subsequent creation of the National Commission on Forensic Science, many forensic scientists are considering pursuing certification. The ABC at present provides a path to professional certification to forensic scientists seeking peer recognition of competency in six areas of criminalistics (Comprehensive Criminalistics, Drug Analysis, Fire Debris, Molecular Biology, Hairs & Fibers, and Paints & Polymers) as well as maintenance of certification in the disciplines of General Criminalistics and Biochemistry. At present, the ABC certifies over 1,000 scientists among these disciplines. Prospective applicants must first pass a credential review and then successfully challenge a written examination that assesses knowledge, skills, and abilities within the discipline. A more thorough description of this process is available at www.criminalistics.com.

This presentation describes post-examination reviews that the ABC Examination Committee uses to assess the efficacy of the examination process. Data collected in the examination process includes self-reported demographic information (e.g., education major, degree, years of experience, and hours devoted to study), examinee critiques, and examination scores. Examination scores are first correlated against the demographic information. Separately, test scores are used to monitor the performance of each examination over time and to compare the performance of different examinations.

Analysis of the demographic information clearly shows a positive correlation between the hours of study and a successful outcome for the test applicant. This review shows that within the independent examinations, the success rate is stable through time, while also demonstrating varying levels of difficulty between the individual examinations. For example, whereas each examination is based on a maximum score of 200, the Drug Analysis median test scores varied only two points (148 to 150) over the course of three versions of this examination over a seven-year period, while the difficulty between examinations is demonstrated by median test score values ranging from 133 for the Comprehensive Criminalistics Examination to 165 for the Paints & Polymers Examination.

The ABC Examination Committee reviews all of this data to monitor success rates of the applicants and performance of the examinations.

Certification, Criminalistics, ABC



B47 A Forensic Odyssey: When Doing the Right Thing Doesn't Always Lead to the Desired Result or Good Deeds Can Be Punished!

Barry A.J. Fisher, MS, MBA, 81620 Avenida Estuco, Indio, CA 92203*

After attending this presentation, attendees will better understand unintended consequences of ethical conduct in a crime lab setting.

This presentation will impact the forensic science community by providing attendees with a possible reaction when an employee stands up to do what is right and faces the consequences. It will also suggest ways to both act and to not be punished for reporting the deed.

In the early 1990s, it was determined that a drug chemist was “dry-labbing” drug testing results in the laboratory. The criminalist would run an analysis at the start of her shift and subsequently select cases that contained the same drug as determined by a presumptive test run by officers. She then used the same Infrared (IR) spectrum for each subsequent analysis but changed the annotation on the printout to correspond to each case she reported out. She ultimately claimed that she followed procedure and ran each case separately; however, running IR spectra ½ minute apart seemed to belie that explanation.

The analyst's supervisor discovered the ruse. He happened to have her day's work piled on his desk with each of the IR spectra visible. He was surprised to see how closely they matched up and, out of curiosity, held the printed spectra over a light box. They all registered identically. The analyst's supervisor immediately proceeded with his direct supervisor to discuss what he had discovered with the laboratory director.

Knowing this might become a criminal matter, laboratory management went to the sheriff's department's Internal Affairs Bureau to report the matter and did not interview the employee. The employee was relieved of duty, with pay, and ordered to remain at home during the investigation. The matter was referred to the district attorney's office for further investigation and possible prosecution. The employee, who had an MS degree in chemistry, claimed that she had not been properly trained on the lab equipment. Eventually the district attorney decided not to prosecute (they thought the matter was too arcane and a jury would not understand the issues). The analyst was subsequently discharged from the sheriff's department.

Once laboratory management determined that this situation existed, the evidence from all cases the analyst had examined was ordered back and a neighboring crime laboratory was asked to reexamine the cases. At the same time, laboratory management also recalled a sampling of other criminalists' drug testing cases. All reported results, without exception, turned out to be correct.

The laboratory reported the incident and the remediation to their accrediting body, the American Society of Crime Laboratory Directors/Laboratory Accreditation Board (ASCLD/LAB). In addition, the district attorney's office notified the local defense bar of the matter. At the conclusion of this, the manager felt the laboratory had done the right thing and the matter had sorted itself out.

To much surprise, a department investigation was ordered and the manager's behavior became its focus. The theory was that this whole thing should never have happened if the manager had been more attentive. After months of interviews with every employee at the lab and an Internal Affairs investigation with the manager as the subject, he was found wanting and demoted. The sheriff's department allowed the manager to keep his job title and salary; however, the manager now reported to a captain who was the defacto crime lab director, with the manager as the figurehead. The manager was also given ten days leave without pay. In addition, the manager was told that if he chose to fight these sanctions, he would be terminated. He decided to take his punishment. Naturally, the manager was pretty depressed over the whole business. The depression took him five years to get over!

The lessons the manager learned from this incident were: (1) if you honestly feel you are in the “right,” you should fight back and use all the remedies at your disposal, including hiring a lawyer to represent you and, if necessary, civil litigation; (2) keep detailed records of everything leading up to the event and, for that matter, all you do as a manager or supervisor. Remember the admonition: if it's not in writing, it never happened; (3) be wary about being a civilian in a police environment. The manager's experience was that civilians were treated differently. He saw this firsthand a few years later when one of the lab's firearms examiners failed a proficiency test. Initially the laboratory recalled some of the firearms examiner's work and found other mistakes. Ultimately, the laboratory had to



Criminalistics Section - 2015

review some 200 of the firearms examiner's cases, mostly homicides. The captain in charge of the lab at the time was never chastised in the slightest for this event that occurred on his watch; (4) there is an unfortunate tendency to sometimes "throw someone under the bus" as penance for an act. A word to the wise: make sure it's not you!; and, (5) if it happened again, the he would still do the right thing and report the misdeed as he did.

Ethics, Whistle Blowing, Ethical Payback



B48 Deconvolution: An Automated Means of Unknown Identification in the Criminalistics Lab

Sarah A. Keeling, MS, 3855 Blair Mill Road, Apt 235-N, Horsham, PA 19044*

After attending this presentation, attendees will have a fundamental understanding of the benefits of using deconvolution.

This presentation will impact the forensic science community by allowing for more objective and efficient analysis of data. It will incorporate retention time, providing better matches to compounds in the target database.

Traditional means of identification in a forensic chemistry laboratory has been Gas Chromatography/Mass Spectrometry (GC/MS) using routine library search methods. The task of identifying drugs of abuse has become more challenging due to their increased diversity. There has been a proliferation of new psychoactive substances including the “bath-salts” and synthetic cannabinoids. In order to circumvent **Drug Enforcement Administration** (DEA) scheduling, illicit manufacturers of these compounds continually alter the molecules slightly, giving rise to a class of compounds with many structural similarities. The mass spectral similarity of these molecules can lead to misidentification using traditional library searching procedures. In addition to the growing list of drugs, many samples are complex mixtures containing active and inactive constituents (i.e., synthetic cannabinoid mixtures sprayed onto a botanical matrix). The chromatograms require additional manual manipulation to confirm the presence of these drugs, which can be subjective and time consuming. Trace amounts in these complex mixtures can be missed. Chemists must properly identify isomers and analogs of new compounds. In order to properly distinguish the closely related forms of these molecules, precise retention time measurements are required.

To maintain this precise control over retention time of the drugs, Retention Time Locking (RTL) is used. Deconvolution Reporting Software (DRS) is an automated and objective means of analyzing chromatographic data using the Automated Mass Spectral Deconvolution Identification System (AMDIS) -searching algorithm and Agilent® Chemstation®. The software analyzes extracted ion chromatograms for each mass throughout the entire chromatogram. Using the shape and apex of the chromatographic peak, masses are assigned to a specific component. This produces superior cleaned spectra compared to data processing using traditional searching algorithms. Coupled with retention time locking, DRS is able to generate match factors based not only on the cleaned mass spectrum, but also the expected retention time of analytes from the target compound library. Even with interferences and coelution, the process is capable of identifying trace components.

A database was built in-house under retention time locked conditions containing both traditional drugs of abuse and newer novel psychoactive substances. DRS was applied to multiple chromatographic situations encountered in real case work. Comparison data was generated for 550 drug identification cases, encompassing 1,757 findings and representing 131 individual drugs. These samples were processed using DRS and traditional data processing methods. These cases included five different scenarios: simple single component data, column overloads, trace amounts, samples with interferences/coelutions, and compounds with similar mass spectra. After the comparison study, DRS was implemented as the primary means of data analysis in the laboratory. Cases processed included samples that contain traces of fentanyl in heroin, coeluting peaks that share many of the same fragments (THC and hydrocodone), psilocin in mushroom samples, and specific isomeric determinations (2-Methylethcathinone (MEC), 3-MEC, and 4-MEC).

Because of retention time locking, the data generated from multiple instruments had virtually identical retention times, eliminating the need to run standards along with the case samples suspected to contain isomers and allowing the in-house library to be used on all instruments in the laboratory and at remote sites. DRS consistently identified components in multiple chromatographic scenarios. In the majority of cases, manual data processing yielded the same results as DRS; however, the time and level of experience necessary to make the identification via traditional data processing was far greater than that needed for DRS. In approximately 40 instances, traditional means failed to identify trace components in a sample whereas DRS properly identified these analytes. DRS is an automated, objective tool that was found to be more efficient and reliable for processing GC/MS data.

Deconvolution, GC/MS Data Processing, Retention Time Locking



B49 Chemometrics Applied to Spectral Comparison in 2D Raman Mapping

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After attending this presentation, attendees will understand this demonstration of applying multivariate statistical methods available in Renishaw® WiRE™ 3.41 to interpret and validate Raman mapping data.

This presentation will impact the forensic science community by demonstrating the importance of the use of orthogonal statistical methodologies to corroborate analytical conclusions.

Chemometrics concerns the extraction of relevant information from chemical data by mathematical and statistical tools. Chemometric methods rely on a wide variety of multivariate operations that are applied to chemical spectral data to separate noise from information, compare and classify spectra, and validate both methodology and conclusions. Although routinely applied in academic and research settings, crime laboratories do not routinely use chemometrics.

This study uses different statistical methods that are part of the instrument software to quickly verify and validate conclusions without using separate statistical software. Such orthogonal crosschecks add strength to analytical decisions with minimal additional effort.

Maps are 2D arrays composed of rows of locations called cells. The process is much the same as digital photography in which each cell is called a pixel. In both, each location contains information for that location and in the case of the mapped cell, the information is both photographic and spectral. After data collection, the spectrum in each cell is identified by the statistical operations below and is assigned a false color for identification. The map is then redrawn using the false colors showing the location of the chemical components.

Mapping is particularly important in trace material analysis where the distribution and size of individual components are important identifying characteristics that can only be assessed by mapping the sample. Polymer identification, counterfeit pharmaceutical products, and ink location in altered documents are conventional forensic applications. Non-mapping analysis determines only a single or a small number of locations that, due to micro-heterogeneity, can lead to false identification. Mapping analysis corrects that deficiency.

Raman spectra were determined with a Renishaw® inVia Raman microscope equipped with a motorized stage and the data were analyzed using Direct Classical Least Squares component analysis (DCLS), Principle Component Analysis (PCA) and Multivariate Curve Resolution (MCR) techniques available in Renishaw's® WiRE® 3.4 instrument software.¹ The mapped data were evaluated by the three statistical methods with the goals of correct spectral assignment and validation of one method by agreement with another.

The key to mapping analysis is the ability to accurately identify the spectral information in each cell. DCLS relies on reference spectra for comparison and applies least squares analysis to compare the unknown spectrum to the reference spectra. MCR/Alternate Light Source (ALS) does not rely on reference spectra for comparison but is able to extract component spectra from the data of a mixture. Usually, at least a single component of the mixture must produce a strong enough response to be unambiguously identified for this method to work. PCA does not produce spectral data but reorganizes the data into groups (principle components) that account for the greatest variation. As such, PCA is often used to eliminate noise and sort spectra into groups containing the same properties.

The presentation will include tables and colored maps that compare the statistical results.

Reference:

1. Chemometric analysis and processing methods in Raman spectroscopy, Renishaw® apply innovation PP108, Chemometrics1. pdf, Renishaw® Inc, Hoffman Estates, IL, 60192.

Raman Mapping, Chemometric, Spectral Comparison



B50 Effectiveness of Zar-Pro™ Fluorescent Blood Lifting Strips

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After attending this presentation, attendees will be better informed about Zar-Pro™ fluorescent blood lifting strips and which surfaces obtained an effective and detailed lift of a bloody fingerprint impression. Attendees will also learn if the amount of dilution affects the degree of fluorescence produced by the lifting strip when visualized with an Alternate Light Source (ALS).

This presentation will impact the forensic science community by introducing a simple, cost-effective tool to lift bloody fingerprints, especially from immovable objects at a scene. Also, the fluorescence enhances ridge details of the lifted print to provide a permanent identification comparison.

Many times at a crime scene, objects with bloody impressions on them have to be removed and brought back to the laboratory and processed using chemical techniques. This can sometimes be onerous and difficult if the object is large and immovable. Jessica Zarate's invention of the fluorescent blood-lifting strips allow for bloody impressions to be lifted and preserved from virtually any surface. Zarate developed a chemical formula which has a high binding power with proteins, added a photo fixative, and bound it to a nylon transfer membrane. The photo fixative allows the prints to be preserved without being altered or smudged. Titanium dioxide has only been tested for the last ten years but has been found to be a non-toxic alternative that can be used on fingerprint impressions. These lifting strips provide a non-toxic fluorogenic method for lifting bloody impression evidence. The fluorogenic properties are a product of metal-enhanced fluorescence and don't require additional chemicals. The fluorescence is produced by blood proteins and other proteinaceous secretions, which contain intrinsic fluorophores when excited with an ALS. These strips were developed to create an easy-to-use, portable, non-toxic method for lifting, enhancing, and preserving bloody impressions, with the intent of being universally accepted. They are both durable and affordable for law enforcement agencies with minimal training needed. The entire process only takes minutes, cutting out numerous chemicals and time-consuming multi-step procedures. The major advantage is that they are preserved in a non-perishable form until ready to be used.¹

In this study, the materials being tested were hardwood floors, 2x4s (unfinished wood), brick, granite counter top, and unpainted drywall. This study used human blood and pig blood to test if there is a difference in the results of ridge detail or fluorescence with the lifting strips. In addition, this study tested dilutions of the blood to determine if there are limitations of visualization on the lifting strips (undiluted, 1/10, 1/100, and 1/1,000). For deposition, the subject's finger was held in a horizontal position with approximately ten microliters of blood placed on the thumb. The dry time on the subject's finger before deposition on a test surface ranged from 20 to 50 seconds, timed using a digital stopwatch. After deposition on the substrate, the bloody impression was left to dry for one hour before use of a lifting strip. The strips were then activated according to manufacturer's instructions. After the lifting strip was used, it was viewed with the CrimeScope® ALS using the 455nm filter with an orange barrier filter. Photographs were taken using a Canon® EOS® Rebel T5i EF-S after deposition, after lifting, and after fluorescence to ensure proper documentation of the process.

Data shows that the lifting strips produced a full print with significant identifying ridge details. The experiment also showed that fluorescence occurs with undiluted blood, although the blood did quench the fluorescence in some cases. The dilution series was used to test the limit of detection of the strips. For treated hardwood samples, fluorescence occurred down to the 1:10 dilution for both human and pig blood, whereas for untreated pine, fluorescence occurred down to the 1:10 dilution for both blood sources, but it did not fluoresce for undiluted human blood. In comparison, for lifting strips used on polished granite, fluorescence was seen down to the 1:10 dilution for both blood sources. Lifting strips used on masonry bricks showed fluorescence at the 1:10 dilution but not for undiluted blood. For lifting strips used on unpainted drywall, undiluted human blood did not fluoresce but fluorescence was noted for all other blood sources down to the 1:10 dilution. Overall, there was no difference or change in fluorescence by source of blood tested.

Reference:

1. Zarate, J. (2011). A Method for Lifting Bloody Impressions Using a Lifting Strip Containing Titanium Dioxide. *IAI Manuscript*, 1-60.

Blood, Fingerprints, Fluorescence



B51 An Analytical Profile of 2-[(2,6-dichlorophenyl)-amino]phenylacetoxyacetic Acid (Aceclofenac)

Michael White, BS, 99 10th Avenue, New York, NY 10011*

After attending this presentation, attendees will be able to identify aceclofenac encountered in both illicit and licit preparations.

This presentation will impact the forensic science community by explaining how, in this time of tight budgets and increasing turnaround times, this data can assist in the identification of aceclofenac in both licit and illicit samples in lieu of purchasing a standard, thus saving the forensic community not only time but also money.

Structurally, aceclofenac is a phenylacetic acid derivative and classified as a Non-Steroidal Anti-Inflammatory Drug (NSAID) with analgesic properties.¹ According to Saraf, aceclofenac is a potent prostaglandin inhibitor through the inhibition of the enzyme cyclooxygenase.² The analysis and identification of 2-[(2,6-dichlorophenyl)-amino]phenylacetoxyacetic acid commonly referred to as aceclofenac is discussed. Through the course of routine analysis, it became apparent that the Electron Ionization (EI) mass spectrum of aceclofenac was similar to diclofenac and could be difficult to differentiate using Gas Chromatography/Mass Spectrometry (GC/MS) alone. After an exhaustive search, it was determined that there was a lack of forensically relevant data available to make an identification. This analytical profile is intended to fill this need. The following techniques were chosen to represent a wide range of instrumental techniques utilized in forensic laboratories to include Ultra High-Performance Liquid Chromatography/Quadrupole-Time-of-Flight/Mass Spectrometry (UHPLC/QTOF/MS), Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS), Liquid Chromatography/Mass Spectrometry (LC/MS), Gas Chromatography/Mass Spectrometry (GC/MS), Fourier Transform Infrared/Attenuated Total Reflectance (FTIR/ATR) spectroscopy, and Nuclear Magnetic Resonance (NMR) spectroscopy.

Through the use of the various instrumental techniques mentioned above, it was determined that an effective analytical approach for the identification of aceclofenac in both licit and illicit samples is LC/MS/MS, LC/MS, and NMR. Depending on the purity of the sample, FTIR/ATR is also a viable test for identifying aceclofenac. Through the use of these instrumental techniques, it is possible to confirm the presence of aceclofenac in unknown drug samples.

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1. Dooley, M., Spancer, C.M., and Dunn, C.J. "Aceclofenac: A Reappraisal of Its Use in the Management of Pain and Rheumatic Diseases." *Adis International Limited* 19.9 (2001): 1351-378. www.Pubmed.com. Web 25 Sept. 2013.
2. Saraf, S. "The Pain Management Is Always a Problem for a Physician and the Search for a Safe and Effective Option Is Still On." *Aceclofenac: A potent Non-Steroidal Anti-Inflammatory Drug*. N.p., n.d. <http://www.pharmainfo.net/reviews/aceclofenac-potent-non-steroidal-anit-inflammatory-drug>. Web 25 Sept. 2013.

Aceclofenac, Forensic Analysis, Aceclofenac Identification



B52 Rapid Loop-Mediated Isothermal Amplification (LAMP) of RNA Biomarkers for Forensic Identification of Semen and Saliva

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After attending this presentation, attendees will learn how a new isothermal amplification method can be applied to mRNA profiling for the identification of forensically relevant biological fluids and tissues.

This presentation will impact the forensic science community by presenting possible alternatives to how mRNA profiling assays are performed and how these new methods may be more amenable to on-site analysis at crime scenes.

Conventional body fluid identification methods are often costly, not only in terms of time and labor required for their completion but also in the amount of sample consumed. Additionally, while methods are available for the identification of human blood and semen, none of the routinely used serological and immunological tests can definitively identify the presence of human saliva, vaginal secretions, menstrual blood, or skin. Thus, attempts to develop molecular-based approaches to body fluid identification have been made in order to provide operational crime laboratories with significantly improved specificity and sensitivity. The use of messenger RNA (mRNA) profiling has been proposed to supplant conventional methods for body fluid identification. It is based on the premise that each single tissue type is comprised of cells that have a unique transcriptome or gene expression profile. Currently, numerous biomarkers have been identified and validated for the identification of forensically relevant body fluids and tissues (blood, semen, saliva, vaginal secretions, and skin). Standard mRNA body fluid assays require laboratory equipment that is not meant to be used at crime scenes and requires hours to generate data. Thus, there is a need for the development of more rapid, simple, and inexpensive methods for mRNA profiling.

A potential alternative mRNA profiling strategy involves the use of LAMP. LAMP's advantages are primarily derived from its unique reaction mechanism. The amplification starts at 60°C to 65°C with primers annealing the target DNA strand and self-priming to form dumbbell-shaped structures that will be used in the strand displacement amplification. In this way, the primers can continuously prime, extend, and displace on their own amplicons. For the detection of RNA targets, LAMP can be combined with Reverse Transcriptase (RT-LAMP) in a single mix. The reaction can produce large quantities of DNA (up to 10⁹ copies in under an hour), enabling naked-eye detection of amplicons with suitable colorimetric or turbidometric sensors. Product detection without aperture of the reaction tube diminishes concerns about amplified product contamination of laboratory environments, a matter of constant vigilance with current detection schemes based on the Polymerase Chain Reaction (PCR).

Here a prototype rapid LAMP of mRNA biomarkers method for the identification of semen (PRM2, TGM4) and saliva (STATH, HTN3) is described. Based on initial testing, the developed 90-minute assays (one-step closed reaction from RT to detection) permit the identification of the body fluid origin of dried semen and saliva stains and demonstrate a high degree of reproducibility (technical and biological replicates). For semen, it was determined that PRM2 was the most suitable candidate. Semen was correctly identified in 8/10 known semen samples (one false negative, one vasectomized male donor so PRM2 was not expected or observed). Specificity was demonstrated with a failure to detect PRM2 (semen) in blood, saliva, vaginal secretions, menstrual blood, or skin samples (N=15) (separation in detection time of ~40 to 55 minutes). Reproducibility was assessed through an evaluation of ten donors (three technical replicates per donor). Semen was correctly identified in nine donors (10th donor was a vasectomized male and the observed standard deviation among individual donors was as little as 0.2-3.4 minutes). While more variability was observed for saliva due to the nature of the body fluid itself, saliva (STATH) was successfully identified in samples stored at room temperature for one to two years (N=4) and samples exposed to environmental insults (heat, light, humidity, and rain) for days to up to one month (N=17), with results comparable to a Capillary Electrophoresis (CE)-based mRNA profiling multiplex. Successful detection of saliva was achieved for 38% of the compromised and environmental samples using the STATH-LAMP assays compared to 31% successful detection of STATH using CE-based detection.



Criminalistics Section - 2015

The results from these initial studies indicate a possible utility of isothermal RT-LAMP assays for body fluid identification. The potential exists (and is the subject of current work) for the development of these assays on an integrated microfluidic device which would permit confirmatory body fluid identification by mRNA profiling at its point-of-use (crime scenes or in the biology screening laboratory).

Isothermal Amplification, RNA Profiling, Body Fluid Identification



B53 Determination of Gunshot Residue (GSR) in Vehicle Head Liners Using Scanning Electron Microscope (SEM)

Heather M. Hammond, BS, 3344 Lakeside Place, Hermitage, TN 37076*

After attending this presentation, attendees will understand some principles of Gunshot Residue (GSR) collection, the use of a Scanning Electron Microscope equipped with Energy Dispersive X-ray (SEM/EDX) for gunshot residue analysis, the potential for using a High-Efficiency Particulate Absorption (HEPA) filter/hand-held vacuum approach to collect GSR from vehicle head liners, and the feasibility of using this alternate collection method.

This presentation will impact the forensic science community by offering the possibility of augmenting current GSR collection from vehicles with the addition of a HEPA filter/hand-held vacuum methodology to collect GSR from any part of a vehicle. Identification of a practical approach for collecting GSR from vehicles could allow for faster and more accurate collection.

This study began as a continuation of a previous project that used the same collection methodology to recover GSR from hair. It was determined from this earlier project that this collection methodology could be a useful approach for collecting GSR evidence elsewhere. This experiment reinforces the methodology presented in this research as a means of reducing the amount of time spent processing surfaces for the presence of GSR.

The experiment consisted of the collection of GSR from ten different vehicles — five with the firearm discharged inside the vehicle and five with the firearm discharged one foot distance outside the vehicle from the passenger-side window. The firearm in all instances was discharged perpendicular to the long axis of the open car window. Each vehicle was divided into eight sections for collection. The eight sections were the Driver-Side Front Head Liner (DFH), Driver-Side Front (DF) that consisted of everything except the head liner, Passenger-Side Front Head Liner (PFH), Passenger-Side Front (PF) that consisted of everything except the head liner, Driver-Side Rear Head Liner (DRH), Driver-Side Rear (DR) that consisted of everything except the head liner, Passenger-Side Rear Head Liner (PRH), and Passenger-Side Rear (PR) that consisted of everything except the head liner. For this project, the sections of the vehicles labeled PFH were analyzed. The collection method included a hand-held vacuum with a HEPA filter and GSR stubs. The GSR stubs were manually analyzed at 500x using a horizontal method that covered the width of the stub. This was done in a single pass using SEM/EDX.

Analysis of the passenger head liner from firing inside the vehicle showed a success rate of greater than 20% of the particles scanned and from firing outside the vehicle, a success rate of 10%-15%. The success rate was based on a particular particle containing lead, barium, and antimony (Pb-Ba-Sb). Pb-Ba-Sb is such a unique particle that, when found, can directly be connected to GSR. The collected results suggest that using this alternate collection method could aid greatly in the time spent on collecting evidence from vehicles. Firing from completely inside the vehicle resulted in more GSR being recovered than from firearms discharged outside the vehicle.

The results further indicate that it is feasible to detect gunshot residue from vehicle head liners using a HEPA filter and a vacuum. The HEPA filter must screen for particles as small as 1-10 μ m. The filters used in this experiment screened for particles as small as 0.3 μ m. Because portability is a necessary requirement for crime scene equipment, the vacuum used was a rechargeable hand-held model that had been proven previously to have enough power to gather GSR particles from hair. The significance of these results show that this alternate collection methodology can be a useful tool for crime scene investigators.

Gunshot Primer Residue, Vehicles, SEM/EDX



B54 Forensic Examination of Oriented Polymer Films: Polarized Light Examinations of Packaging and Shipping Tapes

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After attending this presentation, attendees will understand the nature of mon-axially and bi-axially oriented polypropylene polymer films, which are widely used in the manufacture of packaging, shipping, mailing, and moving tapes. Attendees will also understand the role that polarized light microscopy can play in the forensic analysis of such polymer films. Attendees will understand the potential for the application of digital photomicrography and digital image analysis to the forensic analysis of polymer films in commercial products such as packaging, shipping, mailing, or moving tapes.

This presentation will impact the forensic science community by emphasizing the continuing value of simple analytical tools such as polarized light microscopy, particularly when this tool is enhanced with digital photography and digital image analysis.

Because polymer films produced commercially are typically composed of polyethylene, polypropylene, or polyesters, Infrared Spectrometry (IR) has limited utility for making brand identifications. The physical characteristics of these films may be more useful in making such identifications. Amorphous polymer films are optically isotropic and appear dark at all orientations between crossed polarizing filters. On the other hand, crystalline films may have up to three indices of refraction: one parallel to the direction of extrusion of the film (sometimes called the machine direction), one perpendicular to the machine direction in the plane of the film, and a third perpendicular to the film plane. Crystalline films are birefringent; they will also show classic bi-axial interference figures.

Commercial tape products variously designated packaging, shipping, mailing or moving tapes are frequently made of polypropylene films. During manufacture, the film may be stretched only in the machine direction, resulting in a Mono-Axially Oriented Polypropylene (MOPP) film; if the film is also stretched perpendicular to the machine direction, the result is a Bi-Axially Oriented Polypropylene (BOPP) film. Both MOPP and BOPP tapes are birefringent; however, when specimens of BOPP tapes are examined with a polarizing microscope and are rotated slightly away from extinction, they display crosshatched patterns.

This research explored the application of digital photomicrography and digital image processing to the forensic examination of packaging tapes. Seventeen samples of 5cm (two inch) nominal width clear packaging tape, representing seven different brands, were examined. Small strips were cut from each tape sample and mounted on microscope slides directly, using the tapes' adhesives. Care was taken to retain at least one machine edge. Slide mounts were also prepared by stripping the adhesive from the tape backing using a mixture of xylenes. Again, small strips were cut from each tape sample, care being taken again to keep one machine edge intact. The adhesive layers were removed to facilitate thickness measurements of the tape backing. Thickness measurements were made in triplicate on each cleaned strip of tape using a calibrated digital caliper. Finally, the cleaned strips of tape backing were mounted in a medium having $n_D=1.54$. Each slide was viewed under a polarized light microscope equipped with a ten-megapixel digital camera. Digital photomicrographs of the tape samples were taken: (1) at maximum birefringence; (2) at extinction; and, (3) just past extinction to display the crosshatched patterns characteristic of BOPP polymer films. The tapes' extinction angles (defined as the smallest angle between the machine edge of the tape when it is at extinction and an analyzer or polarizer direction) were measured using the camera's software. The discrete 2D Fourier transform was applied to the digital images of the crosshatched patterns of the BOPP tapes.

The thicknesses of the tape backings provided little discrimination among samples; however, different brands of tape often showed different interference colors, even when the thicknesses of the backings were the same. Rolls of the same brand and type of tape could sometimes be differentiated by differences in interference colors. The extinction angles of the tapes varied from 0° (parallel extinction) to 13.5° . There were differences between brands but also between rolls of the same brand and type of tape. The crosshatched patterns of the BOPP tapes consisted of mosaics of diamond shapes. The diamonds differed in size and in the angles made by their sides. Several different types of crosshatched patterns were found. The discrete 2D Fourier transforms of the crosshatched patterns provided a simple method for comparing the crosshatched patterns.

Polarized Light Microscopy, Polymer Films, Fourier Transform

B55 Weight Measurements in the Forensic Chemistry Laboratory: A Surrogate Weight Study

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After attending this presentation, attendees will better understand the importance of weighing processes in the laboratory.

This presentation will impact the forensic science community by demonstrating the advantages of implementing a surrogate weight program for monitoring the performance of laboratory balances.

Analysis of controlled substances in the forensic chemistry laboratory involves both qualitative and quantitative measurements. Although final identification of the main psychoactive component(s) is often the final goal of many analytical schemes, some laboratories also provide customers with purity determinations, as well as adulterant and salt-form identifications, if needed based on jurisdictional requirements; however, there is one very relevant quantitative test that is performed by every law enforcement laboratory, regardless of its jurisdiction: the determination of the net weight of a substance. That is, the measurement of the amount of powder, plant material, liquid, etc. present in a particular piece of evidence.

Seized-drug weight determinations are important laboratory measurements that have direct effect on the judicial process. The net weight of material seized is often the very factor that determines a controlled substance felony level. Therefore, forensic chemistry laboratories should have well-established procedures for the appropriate procurement, maintenance, verification, and utilization of laboratory scales and balances. These procedures should include, at minimum, annual calibration by an external entity and a documented program for the routine maintenance and performance verification of balances. The use of weight standards (reference weights) must also be part of a balance verification program as it provides the traceability necessary for all weight measurements performed.

However, monitoring the performance of a weight measuring apparatus using standard reference weights may not provide a full picture of the weight measurement process. Standard weights are ideal for monitoring balance properties such as linearity, accuracy, and eccentricity; however, they are not expected to represent routine laboratory weighing processes where the buoyancy and density of the materials being tested can drastically differ from those of a standard reference weight. Controlled substance laboratory submissions are often irregularly shaped and submitted in plastic bags, buckets, bottles, etc. Weight measurements for these materials are therefore expected to be affected by multiple factors that may not be accounted for using standard reference weights.

This study presents the use of surrogate weights to monitor the variability of laboratory weight measurements over an extended period of time. Surrogate weights are made of plastic beads in flexible, clear plastic, heat-sealed bags that were barcoded for identification purposes. Surrogate weights were prepared (representing four different sizes) and are currently being used to monitor a total of 33 laboratory balances. Balances with readability of 0.1g, 0.01g, 0.001g, and 0.00001g are monitored using surrogate weights of 1,000g, 500g, 100g, and 40g, respectively. Surrogate weight measurements are performed once a day and have been on-going since December 2013. The data is collected and statistically analyzed to obtain the standard deviation of measurements for each balance being monitored. This provides a quantitative measure of the variability of weight results over an extended time period, supplementing the balance verification data obtained using standard reference weights.





Criminalistics Section - 2015

This presentation will summarize the results of this surrogate weight study. Calculated standard deviation values will be used to calculate minimum weight thresholds and to suggest acceptance performance criteria for each type of balance. This study will also provide a better understanding of the uncertainty of measurement associated with surrogate weights as representatives of routine weight measurements in the forensic laboratory.

Weight Measurements, Controlled Substances, Measurement Uncertainty



B56 Infrared Microspectroscopy for the Analysis of Nail Polish

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After attending this presentation, attendees will be able to discuss and evaluate the application of infrared microspectroscopy for the analysis of various types of nail polish.

This presentation will impact the forensic science community by informing examiners of: (1) the microscopic and spectroscopic differences between two visibly similar nail polish chips and other cosmetic smears that may be encountered as trace evidence; (2) the spectroscopic differences between classic and novel nail polish formulations; and, (3) the changes that impact comparative analysis of nail polish chips on exposure to various environmental conditions.

Coloring products are the mainstay of the cosmetic industry. These include products such as lipsticks, foundations, eye products, and nail polishes. Market research reports indicate that nail polish products have had record-breaking growth in recent years, spurred by celebrity endorsements. With the ubiquity of nail polish, it is highly likely that it would be encountered as evidence in a crime scene. As trace evidence, it may be encountered in the form of small chips, smears, and as coatings on a broken nail.

Nail polishes are pigmented coatings that are used to cosmetically enhance the appearance of toenails and fingernails. A typical nail polish is composed of film-formers, adhesive polymers, plasticizers, pigments, and other additives to add desired properties to the finished product. Nitrocellulose is the most common film-forming polymer used in the nail polish industry. Other film-formers include polycondensates of adipic acid, neopentyl glycol, and trimellitic anhydride or a styrene-acrylate copolymer. Tosylamide formaldehyde resin is a common adhesive polymer that is used. Plasticizers such as dibutyl phthalate, camphor, and citric acid resins are added to enhance the stability and flexibility of the polymers. Pigments could be either inorganic or organic pigments. Glitter particles are also added to some formulations to improve the shine of the cured product. All of these components and other curing and Ultraviolet (UV)-stabilizing additives are suspended in solvent with good drying properties. Examples of solvents include toluene, ethyl acetate, isopropyl acetate, isopropyl alcohol, methyl, and ethylacetone. Newer nail polish formulations are moving away from phthalates, toluene, formaldehyde, and other traditional components due to their toxic health effects. Three categories of novel formulations have invaded the market in recent years: water-based nail polish, 5-free nail polish, and 3-free nail polish. The water-based nail polish formulations use water instead of the above-listed solvents and sometimes use styrene-acrylate copolymers instead of nitrocellulose. The 5-free products do not include formaldehyde, formaldehyde resin, dibutyl phthalate, toluene, or camphor. The 3-free products eliminate dibutylphthalate, toluene, and formaldehyde.

The goal of this study was to characterize nail polish based on its microscopic and spectroscopic features using an infrared microscope. The development of an analytical method to conduct comparative analysis of nail polish chips is described. The method uses a germanium crystal Attenuated Total Reflectance (ATR) accessory for analysis of the samples. The surface area analyzed is 20x20 μ m and the sensitivity is greatly enhanced using a cooled Mercury Cadmium Telluride (MCT) detector. This study includes 12 brands of classic nail polish formulations. Each brand further includes multiple finishes. The microscope images are analyzed for particle size in products that have glitter and this information is combined with the spectroscopic information across the surface of the chip. Minor spectroscopic changes that occurred during storage of the smears and chips led to further study of environmental effects. In the classic formulations, the absorption peaks for nitrocellulose and tosylamide/formaldehyde resin dominate. The spectroscopic properties of the three novel formulations of nail polish described above have been studied. A color theme was selected and two products from each of the three categories were studied. This presentation includes Principal Component Analysis (PCA) and Discriminant Analysis (DA) of the data obtained for the various nail polish brands.

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Infrared Microspectroscopy, Nail Polish, Micro-FTIR

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B57 Mathematical Modeling of Evaporated Petroleum Distillate Standards

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After attending this presentation, attendees will be familiar with a mathematical model that can be used to generate chromatograms corresponding to different evaporation levels of petroleum distillate standards. These chromatograms can be included in reference collections that are currently used in the analysis of fire debris to determine the presence of an ignitable liquid in a submitted sample.

This presentation will impact the forensic science community by mathematically modeling chromatograms for different evaporation levels of petroleum distillate standards based on the original, unevaporated standard, with no additional experimental measurement necessary.

Fire debris samples are typically analyzed by Gas Chromatography/Mass Spectrometry (GC/MS) and the resulting chromatograms are compared to a reference collection of ignitable liquid standards to identify any liquid in the debris samples. Reference collections include chromatograms of ignitable liquids representative of the chemical classes defined by American Society for Testing and Materials (ASTM) International, as well as chromatograms of the liquid standards evaporated to different levels; however, evaporating liquids to a range of different volumes or masses can be time consuming and it may not be practical to generate evaporated counterparts for every liquid in the reference collection.

In this research, a mathematical model was used to generate chromatograms corresponding to different evaporation levels for a range of petroleum distillate reference standards. The utility of the model was investigated by comparing chromatograms of experimentally obtained evaporated petroleum distillate reference standards to chromatograms derived theoretically using the model.

For the experimentally obtained data, several petroleum distillate reference standards, including torch fuel, lamp oil, and kerosene, were evaporated to various levels under nitrogen, and the volume and mass remaining at each evaporation level was recorded. All unevaporated and evaporated liquids were subsequently analyzed by GC/MS and retention indices were calculated for all compounds in each chromatogram.

The mathematical model was then applied to the chromatographic data for the unevaporated petroleum distillate standards. The model predicts evaporation rate constants for compounds in each standard as a function of retention index. The determined rate constants were used to predict the fraction of individual compounds remaining, which ranges from one to zero, where one indicates no evaporation and zero indicates complete evaporation. The distribution of compounds determined using the model was then plotted versus retention index to generate chromatograms corresponding to the different evaporation levels.

Chromatograms derived theoretically using the model were compared to the experimentally derived chromatograms using Pearson Product-Moment Correlation (PPMC) coefficients, which offer a side-by-side comparison of all variables in the two chromatograms. Coefficients range from ± 1 to 0, with coefficients greater than ± 0.8 indicating strong correlation. For these data, PPMC coefficients indicated strong correlation ($r > 0.95$) between the theoretically derived chromatogram and the corresponding chromatogram obtained experimentally for each evaporation level.

This presentation will demonstrate the utility of the mathematical model to generate chromatograms representative of evaporated petroleum distillate standards based only on the unevaporated standard. Modification of the model to improve correlation between the theoretically derived and experimentally obtained chromatograms will be discussed, as well as further applications of the model.

Fire Debris Analysis, Evaporation, Mathematical Model



B58 Use and Detection of Ribosomal Inactivating Proteins (RIPs) as Surrogates for Active Toxins Via Immuno-Precipitation and Matrix-Assisted Laser Desorption-Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF/MS)

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After attending this presentation, attendees will understand the forensic issues associated with Ribosomal Inactivating Proteins (RIP), the purpose of a surrogate for validation of detection assays, and the significance of detection using immune-precipitation and Matrix-Assisted Laser Desorption/Ionization-Time-Of-Flight/Mass Spectrometry (MALDI-TOF/MS).

This presentation will impact the forensic science community by providing a rapid, cost-effective, and sensitive method for the detection of RIP in various food matrices thought to be targets during a biocrime. Due to RIP toxins' lethality, availability, ease of isolation, and lack of treatment, there is a critical need for continued development of RIP countermeasures.

Naturally produced RIPs, such as ricin and saporin, can be highly lethal when ingested. Due to the availability or ease of production of these toxins, their use is attractive in the commissioning of a biocrime or use as a tactical bioweapon. Therefore, it is necessary to test complex matrices, such as food or drink, for protein toxins in a rapid, cost-effective manner. Researchers have developed an MS-based assay that involves capture of ricin from milk, apple juice, serum, and saliva using magnetic beads and testing of the toxin's enzymatic activity; however, many protein toxins are tightly regulated select agents making it difficult for many laboratories to test new methodologies or perform basic research. A surrogate, non-toxic protein that works in the immuno-capture step and mimics ricin's activity should be capable of use in validating the detection assay with additional matrices in order to test the effects of pH and viscosity. If successful with ricin, this approach could be used to safely test variety of toxins for adulteration of different food stuffs and drinks.

The first goal of this study was to demonstrate the efficacy of a surrogate protein as a toxin substitute for validation and matrix testing. This study used a MALDI-TOF/MS assay developed by the Centers of Disease Control and Prevention (CDC) to test and optimize detection of a surrogate RIP protein, Uracil DNA Glycosylase (UNG). UNG, which removes a single uracil from an AUAT stem-loop, yields a similar DNA product as a RIP-II toxin, while by-passing the safety concerns of active, purified toxins. Next, the validity of this assay concept as a detection and activity method was confirmed by testing complex matrices with a wide pH range and texture complexity, such as a sports drink, ketchup, salad dressing, dry coffee creamer, mustard, and sweet potato and banana baby foods. Lastly, this study determined the lower Limit Of Detection (LOD) of this assay by reducing the UNG concentration in water.

AmpErase® UNG and its target substrate, ric12aUtA, proved suitable surrogates for ricin and its substrate target since this enzyme has similar RIP activity and DNA target as ricin. The cleaved substrate resulted in a loss of 94 Da, which was measured with MALDI-TOF/MS. Ratios of the cleaved product, corresponding to the m/z 3,535 peak, and the uncleaved product, seen at m/z 3,630, were used to estimate the efficiency of the process in the various matrices. A cleavage efficiency of $\geq 90\%$ was measured for water, the sports drink, ketchup, salad dressing, and coffee creamer. Very viscous matrices (mustard, sweet potato baby food, and banana baby food) had very low to zero cleavage efficiencies ($46.8 \pm 31.4\%$, $16.6 \pm 8.3\%$, and 0.0% , respectively). The LOD for this assay was reduced from 1-5 pmol (CDC's method) to 0.18-1.8 pmol of the surrogate per reaction. Various incubation times were also tested in an effort to optimize the assay when protein concentrations were very small. Overnight incubation of water was $32.1 \pm 0.01\%$ efficient, while three- to four-hour incubation was $10.7 \pm 0.01\%$ efficient. This indicates that a longer incubation time could be used when surrogate concentration is very low.

Ribosomal Inactivating Protein, MALDI-TOF, Validation



B59 A Static Collection Approach for Target Explosive Analog Odors

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After attending this presentation, attendees will understand: (1) some critical aspects of employing a passive collection method for the creation of potential canine training aids using real explosive as the odor source; (2) a longevity study of target explosive odorants from such training aids; and, (3) practical field testing using certified canine explosive teams.

This presentation will impact the forensic science community by serving as a feasibility study of efficient canine training-aid creation through novel non-contact passive procedures that can augment the practical applications of enhanced canine training and use in military operations.

Currently, there are many training aids being used for explosives detection canines; however, to date, a superior form of an explosive training aid has not been created that effectively mimics the evolving variety or ordnance encountered in the battlefield. For this reason, the development of a training aid suitable for the explosive encountered in daily military operations is necessary to provide effective canine explosive detection, as the use of sniffer dogs has proven to be a reliable tool for the rapid detection of volatile explosive vapors. In practical field operations, an imperative need is to be able to collect target explosive material in a rapid and safe manner. The creation of such aids is not only useful during criminal investigations at pre- and post-blast sites but also critical for further optimized training of explosive canine teams who need to be exposed to the evolving range of explosive material being used in the military arena. The goal of this research is to present a detailed evaluation of the static collection of target explosive analog odors for the creation of useful canine training aids from real explosive materials. Even though currently available training aids utilize synthetic chemicals mimicking the odors emanating from real explosives, this study investigates the feasibility of creating training aids using a passive collection methodology of target odorants using the real explosive as the odor source.

In this study, experiments were based on previously identified signature explosive odors such as 2-ethyl-1-hexanol, derived from plasticized explosives, 2,4-dinitrotoluene from single-based smokeless powder, and 2,3-Dimethyl-Dinitrobutane (DMNB), derived from taggants added to explosives. C-4 explosive material was used for the static collection of the 2-ethyl-1-hexanol and DMNB target odor signatures, as well as single-based smokeless powder for the collection of 2,4-dinitrotoluene. The samples were collected indoors (23.8°C with a relative humidity of 77.2%) with a 4"x4" cotton gauze pad as the collection material. All samples were stored in silanized 40ml glass vials. In order to obtain odorants profile, vials collected from C-4 explosive material were injected via Solid-Phase Microextraction (SPME) for 30 minutes at approximately 56°C and eventually analyzed by GC/MS and vials collected from single-base smokeless powder were injected via SPME for 21 hours at approximately 56°C and eventually analyzed by Gas Chromatograph/**Electron Capture Detector** (GC/ECD). A metal holder device was used to clamp the gauze pad at a distance of approximately two to three inches from the odor source (explosive material). A time optimization analysis was conducted for all three target odor signatures in time intervals of 0.5, 1, 5, 15, 30, 45, 60, 90, and 120 minutes. Samples were taken in triplicate for each of the selected time intervals and each trial was conducted on different days with a corresponding control sample to monitor for any possible background/contamination. According to the generated results, it has been shown that odor signatures such as 2-ethyl-1-hexanol can be collected at an optimal time of 15 minutes, with a collected mass of 82.7±7.75ng for the target odorant. The collection for the DMNB and 2,4-dinitrotoluene target odors showed an enhanced collection at 30 minutes of static exposure of the gauze pad, with a collected mass of 125±10.1ng and 5.05±0.51ng, respectively.

The results demonstrate the successful collection of target odor signature Volatile Organic Compounds (VOCs) from the real explosive as the odor source onto an absorbent material such as a gauze pad, which can then be consequently sealed and stored as a canine training aid. Other factors to be presented include how long the collected odor lasts after being stored and the efficacy of these stored static collected training aids for field use with certified explosive detection canines. Overall, the results demonstrate that it is possible to collect representative explosive odorants using a completely passive static collection procedure for the creation of reliable training aids via this non-contact passive approach which can aid in the development of effective canine team training.

Static Collection, Training Aid, Canine Detection



B60 Illicit Substance Volatile Organic Compounds (VOCs) Analysis for Canine Detection

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After attending this presentation, attendees will have a better understanding of an optimized analytical approach for the determination of the Volatile Organic Compounds' (VOCs') profile of a frequently trafficked illicit substance. The development of such a method will aid in improving the quality of information currently being used for canine training purposes.

This presentation will impact the forensic science community by providing a detailed overview of potential compounds that might serve as key constituents in the development of training aids. The development will likely ensure an improvement on the capability of detection of one of the most popular illicit substances by providing a reliable, cost-effective, and non-hazardous system.

Law enforcement agencies have put forth great effort toward stopping drug trafficking. Regardless, the trafficking and use of illicit substances continues to be a national problem. Marijuana is the most widely available and commonly abused illicit drug in the United States. According to the 2013 National Drug Threat Survey (NDTS), 88.2% of responding agencies reported that marijuana availability was high in their jurisdictions.¹ Therefore, the detection of this substance, especially during transportation and storage, is of importance. The current state of the art in concealed contraband detection is the use of a canine. To maintain the operational readiness and reliability of these canines, routine training using training aids must be conducted. Though some law enforcement agencies have been able to satisfy the strict guidelines set forth by the Drug Enforcement Administration (DEA), most agencies are unable to satisfy these requirements and therefore have restricted access to narcotic training aids. This has limited the ability of many canine handlers to perform routine training resulting in shortcomings in a canine team's performance. To alleviate this problem, many canine teams have resorted to the use of odor mimics as a substitute for the use of actual narcotics.

Detector canine teams are trained to detect most commonly found illicit substances. The premise for the detection of illicit narcotic substances is based upon the fact that these substances, though hidden, will emit VOCs. These VOCs are not often the parent drug; they are essentially a chemical associated with the source and provide a reliable indication of the illicit substance. Ongoing research has identified dominant active odor signatures of other major drugs such as cocaine, 3,4-Methylenedioxy-Methamphetamine (MDMA), and methamphetamine, to include methyl benzoate, 3 piperonal, and benzaldehyde, respectively, and effective odor mimics have been created using the identified active odor chemicals.²⁻⁴ Currently, limited information about the volatile components for other popular illicit substances can be found.

The purpose of this study is to identify the odor profile of marijuana for canine detection. For this purpose, headspace analysis of the illicit substance from different batches of marijuana was conducted by Solid-Phase Microextraction-Gas Chromatography/Mass Spectrometry (SPME-GC/MS) for the identification and comparison of similarities and dissimilarities of VOCs present in the each sample. The laboratory's current research has revealed terpene compounds such as caryophyllene, pinene, myrcene, and limonene as common VOCs found in the headspace of the illicit marijuana samples tested. Preliminary canine field tests using isolated samples of these VOCs with certified law enforcement canines have resulted in changes in behavior by canines toward some of these VOCs. Upon completion of this field test, the VOCs that were suspected to produce an alert were combined and again presented to certified canine teams to develop reliable odor mimics for field calibration of detection canines to marijuana. The results of the developed canine odor mimics and their efficacy will be presented as this will result in an overall improvement in seizure rates of these substances by canines.



Criminalistics Section - 2015

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Illicit Substance, Volatile Organic Compounds, Detector Canines



B61 Using Likelihood Ratios for Source Attribution of Glock® Model 21 Fired Cartridge Cases

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After attending this presentation, attendees will understand how likelihood ratios are used to interpret impression evidence of pistol cartridge cases.

This presentation will impact the forensic science community by guiding future researchers and firearm examiners to analyze firearm data from the Integrated Ballistic Identification System® (IBIS) (Ultra Electronics Forensic Technology® (UEFT)). Using statistical analysis, the examiner will be able to infer source determinations in a more logical manner.

The purpose of this study was to determine the effectiveness of using likelihood ratios to evaluate the accuracy of source attribution using IBIS® scores. This was achieved by evaluating the error rates of the method when using Bayesian networks to assess the correctness of the attribution using the IBIS® scores for the firing pin and breech face comparisons.

Firearm examiners are prone to criticisms due to the purported lack of consistency when making source determinations.¹ For example, examiners with different educational backgrounds and experiences may draw different conclusions from the examination of the same samples. In particular, vague terminology may contribute to incorrect conclusions of same source attributions. To decrease potential error of terminological inconsistencies, standards should be implemented and universally utilized by firearm examiners.² Additionally, erroneous source determinations may be decreased by eliminating unnecessary, outside information pertaining to the case.³

Researchers commonly use pristine, consecutively manufactured firearms (or parts thereof) to evaluate methodologies. Although this approach reduces variability, pristine conditions may also diminish the generalization of results to real-world conditions.⁴

This research hypothesized that this method will: (1) provide a statistical basis for the interpretation of firearms evidence; and, (2) increase reliability and validity of determining source attributions.

The IBIS® system was used in this research to provide scores of known cartridge cases against a database of both known and unknown cartridge cases. This system measures each case and generates breech face and firing pin scores based on the comparison of a pair of cartridge cases. IBIS® consists of two fully automated comparison systems known as BULLETPROOF®, which compares projectiles, and BRASSCATCHER®, which compares cartridge cases.⁵

This research used a quantitative correlational study design to examine impression evidence on cartridge cases fired by various Glock™ pistols. Twelve Glock™ .45ACP firearms were used for this study. Each gun fired 30 cartridges (reloads: Magtech® Ammunition large pistol primers and Accurate® #2 powder). The firearms were from a local law enforcement agency. Although the firearms are used, the exact number of shots fired through each is unknown.

The firearms were fired at an outdoor shooting range and all cartridge cases were collected from each test fire. The casings were then entered into IBIS® using standard protocols and the candidate matches were organized into Microsoft® Excel®, RStudio® and R® were used to process the data and Netica™ was used to generate a Bayesian network. The Bayesian networks contain the conditional probability tables and density distributions required to interpret new data. Such networks intrinsically incorporate likelihood ratios in the odds form of Bayes' Theorem.

The firing pin score, firing pin rank, breech face score, and breech face rank generated by the IBIS® system were used together with the model, caliber, and firearm identifier of the sample and database firearms to build the network.



Criminalistics Section - 2015

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Firing Pin, IBIS[®], Glock[®] Pistol



B62 Gas Chromatography/Mass Spectrometry (GC/MS) Measurement of Gasoline Vapor Absorption on Clothing in a Confined Space

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After attending this presentation, attendees will better understand gasoline vapor absorption on clothing in a confined space.

This presentation will impact the forensic science community by providing experimental data related to the absorption of gasoline vapors by cotton clothing. This has potential ramifications in arson investigations.

A recent case produced a question regarding the possible deposition of gasoline vapors on clothing as a suspect walked across a room in which the vapors were present. In the course of testimony, the defense council noted that gasoline was present on the defendant's footwear, yet there was no determination of gasoline on the defendant's clothing.

Research by Folkman et al., Coulson et al., and Erita et al. (under review) primarily deals with splashing or spilling of ignitable liquids onto clothing and the persistence of sufficient concentrations of ignitable liquids for identification.¹⁻³

The experimental process was as follows. New, never-laundered, same-sourced cotton T-shirts were purchased along with new, one-United States-gallon paint cans. An unventilated, steel flammable cabinet, 1.6m x 0.8m x 0.5m, containing approximately 30L of 87-octane gasoline in standard one-United States-gallon gasoline containers was used as the source of gasoline fumes. Vapors were allowed to accumulate in the cabinet for three months prior to the experiment. Samples were taken in triplicate of cotton T-shirts stored inside the cabinet for six hours, one hour, and 15 minutes. In addition, one experimental condition measured the amount of absorbed gasoline vapor after 15 minutes exposure, followed by a one-hour ventilation in a fume hood. Subsequently, an additional experiment was performed on three T-shirts left hanging one meter from the open doors of the cabinet. Each T-shirt was placed in a clean, new, one-United States-gallon paint can. An activated charcoal strip was suspended in the can which was then heated at 70°C for 16 hours. The activated charcoal strips were washed with carbon disulfide and analyzed by GC/MS.

Results of the experiment show detectable amounts of gasoline (BTEX, alkylbenzenes, and naphthalenes) for the T-shirts exposed to gasoline vapors in the cabinet for periods of six and one hour; however, even in the extremely confined and unventilated space, the T-shirts exposed for 15 minutes did not display sufficient naphthalene concentrations for an identification even prior to the one-hour, in-hood ventilation. The conclusion is that the necessary concentration for gasoline vapors to absorb into cotton T-shirts and be detected would likely lead to the incapacitation or death of the individual wearing the clothing.⁴ The absorption and persistence of solely gasoline vapors onto cotton clothing of a suspect who has left the crime scene appears very unlikely according to the results of these experiments.

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Gasoline, Ignitable Liquid, Vapor



B63 Trace Chemical Signatures of Calcium Hypochlorite: Implications for the Attribution of Hypergolic Mixtures

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After attending this presentation, attendees will understand the basis of chemical variation among sources of inorganic chlorine-based incendiary mixtures and the potential for this variation to support forensic investigations.

This presentation will impact the forensic science community by introducing a new signature system that can determine the source of unknown chemical residues from a crime scene which may be used to establish investigative leads.

Criminal use of self-igniting, or hypergolic, mixtures is a significant issue for law enforcement and forensic laboratories. Many of the possible reactants are available as inexpensive commercial products and chemical ignition of the mixture is easy to execute. As such, hypergolic reactions have been encountered in a range of illicit activities including arson, person-on-person crime, and deployment of improvised explosive devices. One of the more common recipes involves combining inorganic pool chlorine, specifically calcium hypochlorite, with ethylene glycol-based products (e.g., automotive brake fluid). Despite their prevalence, there remain few techniques that can be used to analyze chemical evidence from hypergolic reactions for the purposes of attribution.

The goal of this research was to investigate variation in trace metal composition across different sources of calcium hypochlorite both as precursor compounds and within the post-reaction residues. Metals are likely to differ across sources owing to differences in synthesis routes, trace mineral additives, and levels of purity. Four different types of calcium hypochlorite, three household commercial products, and one laboratory-grade sample were prepared in nitric acid for analysis with Inductively Coupled Plasma/Optical Emission Spectroscopy (ICP/OES). Small-scale reactions with two grams of hypochlorite and one milliliter of polyethylene glycol were made and the residues also analyzed with ICP/OES. Results showed that each hypochlorite source exhibited distinct variation in the presence and relative abundance of certain elements that was consistent in both pre- and post-reaction residues. For example, aluminum was enriched in one sample group (~40ppm) compared to the other sources which were all less than 7ppm while boron was enriched in another sample group (~2ppm) compared to the other sources which were all below the limit of detection. Strontium concentrations were slightly elevated in the laboratory-grade samples versus two High Test Hypochlorite (HTH) commercial brands by 1-2-ppm. Similarly, concentrations of iron were enriched in two sample sources, in comparison to the other commercial source (~8-9ppm vs ~6ppm). Error rates for all measured elements were less than 0.01ppm.

To enhance signature detection and differentiation among reaction residues, ICP/OES data across all 13 elements examined was analyzed with Discriminant Function Analysis (DFA). Results showed robust separation among all three reaction sample groups along the first two functions. Function coefficients revealed that Aluminum (Al), Chromium (Cr), Copper (Cu), Potassium (K), Nickel (Ni), and Strontium (Sr) contributed the most variation among the groups. These may be promising candidates for stand-alone chemical markers for comparing residue samples. Overall, this work suggests that trace metal variation can be used to differentiate each source of calcium hypochlorite and that multivariate metal profiles may be a useful forensic signature for the attribution of hypergolic mixtures involving this type of reactant.

Hypergolic Mixtures, ICP/OES, Trace Metal Signatures



B64 Enantiomeric Identification of Pregabalin Via Methylation and Chiral Derivatization, Utilizing Gas Chromatography/Mass Spectrometry (GC/MS)

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After attending the presentation, attendees will understand the need for a viable method to identify pregabalin, or (S)-3-(aminomethyl)-5-methylhexanoic acid, utilizing traditional capillary GC/MS. Attendees will also be exposed to the methylation and chiral derivatization procedure used to prepare pregabalin for enantiomeric identification by GC/MS. Identification of the correct enantiomer of pregabalin by GC/MS will be demonstrated.

The presentation will impact the forensic science community by demonstrating an alternative method of identifying pregabalin through enantiomeric determination utilizing GC/MS, an instrument commonly found in forensic laboratories.

Many available methods for the identification of pregabalin involve Liquid Chromatographic separation with electrospray ionization Mass Spectrometry (LC/MS). LC/MS is not as common in forensic laboratories as the traditional GC/MS. Alternatively, Fourier Transform Infrared (FTIR) spectrophotometry is an effective way to elucidate the structure of pregabalin; however, FTIR cannot distinguish between optical isomers. Therefore, it would be beneficial to provide a method of enantio-selective identification of pregabalin using GC/MS.

Pregabalin, or (S)-3-(aminomethyl)-5-methylhexanoic acid, marketed under the brand name Lyrica®, became a Schedule V controlled substance in the United States in July of 2005 due to its depressant activity. It is used legitimately to treat neuropathy in diabetic patients as well as for fibromyalgia. Pregabalin is an amino acid and an amphoteric compound, which makes it difficult to analyze using traditional GC/MS instrumentation. Problems associated with GC/MS analysis of pregabalin include: (1) ring closure to the corresponding lactam in the injection port or in the transfer line; as well as, (2) difficulty with chiral derivatization due to lack of reactivity of the derivatization agent in the presence of a carboxylic acid moiety. The ring closure and chiral derivatization problems are overcome by methylating (capping) the carboxylic acid portion of the pregabalin molecule and converting it to its corresponding methyl ester.

The simple procedure used to cap the carboxylic acid portion of pregabalin involves small amounts of thionyl chloride and dry methanol using cold temperatures achieved with dry ice/acetone in a typical laboratory fume hood. Once methylation occurs, the carboxylic acid is deactivated and the attachment of the chiral derivatization reagent (S) Trifluoroacetylpropyl chloride, or (S)-TPC, is straightforward. Once the S-TPC is attached, the correct (S) enantiomer of pregabalin can be identified via GC/MS.

Data will be presented showing the corresponding methyl ester of pregabalin as well as the separation of the (S) and (R) enantiomers via GC/MS. This method will allow for the proper identification of pregabalin utilizing GC/MS, the most common instrumentation found in a forensic drug laboratory.

Pregabalin, Chiral Separation, GC/MS



B65 Analysis of Cannabinoids Found in Seized Marijuana Using Automated Headspace Solid-Phase Microextraction Coupled With Gas Chromatography/Mass Spectrometry

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After attending this presentation, attendees will better understand automatic headspace solid-phase microextraction methodology and its application to the analysis of *Cannabis sativa* L. plant material.

This presentation will impact the forensic science community by providing an automated method for the direct headspace sampling of cannabinoids from solid samples of suspected marijuana. This presentation will enhance the applicability of Headspace Solid/Phase Microextraction coupled with Gas Chromatography/Mass Spectrometry (HS/SPME/GC/MS) to controlled substance analysis.

After attending this presentation, attendees will gain an understanding of the use of HS-SPME methodology to identify and quantify cannabinoids in marijuana samples.

The term marijuana refers to the plant material of *Cannabis sativa* L. There are more than 60 natural cannabinoids found in marijuana. The primary psychoactive component is Δ^9 -tetrahydrocannabinol (Δ^9 -THC). Current analytical methods for the detection of cannabinoids and other natural constituents of marijuana include solvent extractions followed by gas or liquid chromatography. Such methods have several limitations, including the use of hazardous solvents, the expense of said solvents, disposal of the waste generated from solvent use, and the time needed to perform such extractions. A solution that may eliminate such limitations for the detection of cannabinoids in marijuana samples is the use of HS/SPME/GC/MS.

In this research, an optimal automated HS/SPME/GC/MS method has been developed using cannabinoid standard reference materials and actual marijuana material samples. Internal standards and any standard reference samples used were placed in a vial and the solvent evaporated before analysis. The plant material was ground and sieved before being weighed out into sample vials. Unlike previous methods that would require the sample to be extracted with solvents before analysis, the HS/SPME/GC/MS method required the sample to be sealed in the sample vial and placed on a GC/MS autosampler that would carry out the HS/SPME extraction using a Polydimethylsiloxane (PDMS) 23-gauge, 100 μ m absorbent fiber and inject the extracted sample into the GC/MS. The optimized extraction temperature for cannabinoids was found to be 150°C and the optimal extraction time was found to be five minutes. Regeneration of the PDMS fiber was achieved by heating the fiber to 250°C in the autosampler conditioning chamber during the run after the fiber was exposed to the inlet of the GC/MS.

Results from the optimized HS/SPME/GC/MS method show the method to be comparable to the common liquid extraction method. The same cannabinoids can be seen with both methods and in some cases the HS/SPME/GC/MS method shows more cannabinoids than the liquid extraction suggesting it has a higher sensitivity. Chemical constituents of marijuana other than cannabinoids could also be extracted and detected with the optimal HS/SPME/GC/MS method. Future research will include statistical analysis of the data collected from marijuana plant samples using this method with the goal being to determine whether or not two samples share a common origin.

Marijuana, HS Solid-Phase Microextraction, Drug Analysis



B66 Identifying Emerging Drugs of Abuse

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After attending this presentation, attendees will understand the process of identifying emerging drugs of abuse discovered through internet searches of drug forums and vendors of legal highs. This will include: (1) the misinformation offered by vendors of legal highs; (2) how to identify potential structural isomers and diastereomers; (3) using Nuclear Magnetic Resonance (NMR) to distinguish between isomers with similar mass spectra but different retention Gas Chromatographic (GC) retention times; and, (4) combo drugs.

This presentation will impact the forensic science community by raising awareness about identifying emerging drugs of abuse, identifying misinformation offered by the vendors, and discussing issues to be considered in developing a method for analytical analysis.

Legal highs are analogs of controlled substances that retain the psychoactive and addictive properties of the parent drug. The most recent influx of legal highs has been cannabimimetics and cathinones marketed as “Spice” and “bath salts,” respectively. They can be purchased in gas stations, head shops, or online, often by minors. As the components in the different “Spice” and “bath salts” products have been scheduled, new analogs have been introduced to take their place. Their manufacture is not regulated and best practices cannot be ensured. Because legal highs are not developed as potential pharmaceuticals, there is little or no clinical data available so emergency medical personnel have no guidelines for treatment in the case of overdose.

The focus will be on three new legal highs: (2-aminopropyl)-benzofuran (APB), methiopropamine (MPA), and 4-methyl pentedrone (4-MPD), each purchased from online vendors of research chemicals. There are four potential positional isomers of APB, 4-, 5-, 6-, and 7-APB. Both 5- and 6-APB are offered for purchase as legal highs. The isomers have distinct GC retention times that are confirmed by NMR. Analysis shows that both the 5- and 6-APB are being provided as claimed.

Methiopropamine is a thiophene analog of methamphetamine. It is often offered as part of a combo-drug, where two mildly psychoactive drugs are combined in an effort to mimic a controlled substance. Two samples of M&M were analyzed and contained MPA and 5,6-Methylenedioxy-2-Aminoindane (MDAI) as advertised. A sample of synthacaine, purported to be a mixture of MPA and dimethocaine, was actually a mixture of MPA and benzocaine.

Finally, the analysis of a product labeled 4-MPD is offered as an example of a legal high where the name and structure posted on the vendor’s web page did not match the drug. According to the name, it is pentedrone with a methyl group attached at the para position on the benzene ring. The structure that was posted on several vendor web pages was 2-(methylamino)-1-phenylbutan-1-one (buphedrone). When analyzed, the substance was actually 2-(ethylamino)-1-(4-methylphenyl)pentan-1-one (MEAP).

Legal High, Drug Analysis, Analog



B67 The Development of New Enzymes for 20-Minute Rapid Direct Amplification of Crime Scene Samples and Its Application in Presumptive DNA Screening

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After attending this presentation, attendees will understand how new inhibitor-resistant, rapid polymerases may be used to type blood, saliva, and semen.

This presentation will impact the forensic science community by showing how ultimately the development of direct, rapid Polymerase Chain Reaction (PCR) -based systems will greatly expand the capabilities of police, custom agents, and first responders to process crime scene samples and mass disasters, providing information on a timely basis that otherwise might take hours to obtain. Because these procedures involve existing commercial systems, issues such as injection, loading, and reliability have already been worked out through extensive marketplace testing. The analytical systems have very small footprints and the chips and PCR tubes are disposable and easy to transport. These systems can be implemented immediately and incorporated into current forensic labs with only the addition of a fast thermal cycler and no complex training or engineering design. The overall result will be a massive improvement in the current processing of submitted DNA samples and in the capability to perform remote testing and screening.

Current DNA typing methods provide the best biometric information yielding potential identity, kinship, and geographical origin, but they are not sufficiently fast to permit rapid identity of a suspect. There are situations in which it is very important to rapidly screen crime scene samples and unknown individuals who may have been involved in a crime. Examples include seized evidence potentially linked to a suspect, commingled bone samples buried in a mass grave, or the determination of which bloodstains present at a crime scene may be probative. In these situations, many samples may need to be run in order to create probable cause for detaining a suspect. The goal of this project is to develop a rapid and direct method for determining DNA from a wide variety of crime scene samples. Rape kit screening will also be possible.

In the first phase of this study, a rapid thermal cycling procedure combined with a direct amplification was tested and optimized on control DNA standards, K562, 9948, and 9947a cell lines, and paper saliva punch samples from 20 individuals. Optimization included testing different polymerases, buffer compositions, salt concentration, pH, and varying concentrations of magnesium and dNTPs, as well as thermal cycling parameters. It was demonstrated that the rapid direct PCR using rapid DNA polymerases (Z-Taq™ Polymerase) and direct PCR buffer (AnyDirect™ F buffer with 30 cycles at 98°C for five seconds, 60°C for ten seconds, and 72°C for 15 seconds) permitted a high-speed amplification of a 7-locus multiplex that required no extraction step. A 1.2mm punch of samples from FTA® paper was directly added to the rapid direct master mix and amplification performed in 15 minutes and 54 seconds on rapid thermal cyclers. This was then coupled with a short 1.8cm microfluidic electrophoresis system and it was shown that the entire procedure from paper punch to genotype can be completed in under 25 minutes.

In the second phase of this study, mutant taq polymerases that are inhibitor resistant were tested on replicate samples spiked with hematin or phenol chloroform. Different volumes of the OmniTaq™ polymerases were tested on control DNA with varying amounts of inhibitors. The new enzymes were capable of partially overcoming the inhibition induced by hematin (15µM) and phenol chloroform (0.4µl).

In the third phase of this project, the proven high-speed thermal cyclers and rapid enzymes capable of performing 28 cycles of amplification in 14 minutes or less, in combination with the new inhibitor resistant enzymes on different biological fluids, will be tested. This procedure will be coupled with high-speed genotyping using microfluidic chips or standard capillary-based systems modified for faster run times.

Rapid direct PCR procedures can greatly speed the processing time but they primarily work with saliva.^{1,2} The goal is to perform high-speed DNA detection for a wider variety of sample types including blood, bone, and sexual assault kits. Specially engineered enzymes will be used, high-speed thermal cyclers, and chip-based electrophoresis, as well as inhibitor-resistant DNA polymerases to achieve sample genotypes in less than 20 minutes for a wide variety of sample types.³⁻⁵ The goal will be to provide methods for any crime laboratory to obtain genotypes in less than 40 minutes using off-the-shelf, high-speed thermal cyclers and novel polymerases and in less than 20 minutes using a commercial microchip prototype.¹

This project is interdisciplinary involving the efforts of instrument manufacturers, biochemistry research, and law enforcement.



Criminalistics Section - 2015

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Rapid, Direct PCR, Inhibition, DNA Polymerase



B68 Examination of Factors That Affect the Recovery and Analysis of DNA From Spent Cartridge Casings

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After attending this presentation, attendees will understand the effects a number of variables, including pre-processing for fingerprints, DNA isolation methods, and cartridge size, have on the retrieval and analysis of DNA from spent cartridge casings.

This presentation will impact the forensic science community by providing instruction on which DNA retrieval methods and other variables have the greatest influence on recovering and analyzing DNA from spent cartridge casings. The findings have the potential to influence methods used by practitioners and will aid in prioritizing samples based on the likelihood of recovering genetic information on the loader of a firearm.

Hundreds of thousands of crimes involving firearms are committed annually in the United States; therefore, it is extremely important to identify the person who fired a weapon.¹ The weapon itself is rarely found at a crime scene; however, spent cartridge casings are, which could potentially link the shooter to both the firearm and the crime. Some forensic laboratories have attempted to isolate and analyze DNA from spent cartridge casings, though this has met with little success. Recent research in this study's laboratory has shown that optimized DNA extraction methods can improve the number of Short Tandem Repeat (STR) alleles generated from spent casings, yet there are many other factors that have the potential to influence the recovery of genetic information, several of which were examined in this research.

The effect of cyanoacrylate fuming was studied by having volunteers load nine cartridges into the magazine of a firearm at the Michigan State Police Lansing Laboratory, firing the cartridges, and collecting the casings. One-third of the spent casings were fumed with cyanoacrylate on site, one-third were transported to the Michigan State University laboratory and fumed, and the remaining third were not fumed. DNA was recovered using a double-swab method, utilizing one wet and one dry swab, followed by an organic extraction procedure previously optimized in the laboratory. DNA yields were determined using an Alu-based real-time Polymerase Chain Reaction (PCR) assay, and STRs were analyzed using the Promega® PowerPlex® Fusion Kit. The number of alleles consistent with the handler (established via a buccal swab) and the percent profile recovered were calculated. Statistical differences among methods were determined using the Mann-Whitney test at a significance level of 0.05.

Swabbing strategies and the influence of cartridge size were also examined simultaneously. Volunteers loaded twelve rounds each of 0.22 and 0.45 caliber cartridges into magazines, the cartridges were fired, and the casings collected. Three of the casings were swabbed individually, while the remaining casings were cumulatively swabbed in sets of three. DNA was extracted, quantified, and STR profiles were produced as above.

Cyanoacrylate fuming had a negative effect on DNA yields, as non-fumed casings had a significantly higher DNA yield than fumed casings. This was confirmed by STR results, wherein non-fumed casings generated an average of 27 loader alleles, while casings fumed both on-site and in the laboratory produced a third as many. Cumulative swabbing recovered an average of 2.6pg/μL of DNA from the 0.45 caliber casings, which was significantly higher than the average 0.9pg/μL recovered through individual swabbing, thus allowing for more DNA to be added to PCR reactions. As a result, cumulative swabbing recovered a higher percentage of the loader's profile, averaging 25 loader alleles compared to 14 recovered through individual swabbing. Similarly, an average of 0.75pg/μL and 12 alleles were recovered from cumulatively swabbed 0.22 casings, while 0.4pg/μL and 6 alleles were recovered from individually swabbed casings. Regardless of swabbing strategy, 0.45 casings produced more DNA and more STR alleles than did 0.22 casings. Overall, each tested variable was found to have a substantial effect on DNA yields and STR profiles, and therefore needs to be strongly considered when DNA analysis from spent casings is undertaken.

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Criminalistics Section - 2015

Reference:

1. National Institute of Justice. Gun violence. <<http://www.nij.gov/topics/crime/gun-violence/Pages/welcome.aspx>> (accessed 07.16.2014).
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DNA Recovery from Casings, Cartridge Casings, Touch DNA



B69 Maximizing DNA Recovery and Short Tandem Repeat (STR) Data From Spent Cartridge Casings

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After attending this presentation, attendees will understand the highly improved performance of optimized methods for touch DNA recovery and analysis from spent cartridge casings, which are commonly retrieved from shooting incidents.

This presentation will impact the forensic science community by providing improved procedures for recovering and amplifying DNA from spent cartridge casings in order to increase DNA yields and STR data. The findings have the potential to influence the techniques scientists utilize when analyzing DNA from spent casings, ultimately connecting an individual to a crime based on the presence of their genetic material on casing evidence.

Firearms, particularly pistols, are commonly used in violent crimes, though the weapon is rarely recovered. Nevertheless, spent cartridge casings ejected during firing are often recovered by law enforcement and may contain DNA deposited by the loader of the firearm, who could potentially be identified via STR analysis. DNA recovered from spent casings; however, is often degraded and present in low copy numbers. Owing to this, crime laboratories have had limited STR typing success from casings; thus, it is essential that effective methods for DNA recovery and analysis are determined.

Previous research in this study's laboratory examined multiple variables in cell/DNA isolation to improve DNA yields and typing results from spent casings, including swabbing versus soaking casings, the duration of digestion, shaking during digestion, etc. Based on the results, in the current study, DNA yields were examined and STR profiles were generated using five optimized methods: swabbing or soaking with an organic extraction, swabbing or soaking with a silica-based extraction (QIAamp® DNA Investigator Kit), or swabbing with a non-binding DNA extraction (Fingerprint DNA Finder® Kit). Volunteers loaded uncleaned cartridges into the magazine of a pistol, the cartridges were fired, and the casings were collected. The first method consisted of swabbing casings with a wetted swab followed by a dry swab. The second method involved fully submerging casings in digestion buffer for 30 minutes, transferring the buffer to microcentrifuge tubes, then swabbing the casings with a dry swab, and incubating the tubes containing the soaking solution and swabs at 85°C for ten minutes. Proteinase K was added to all samples, which were digested for one hour with concurrent shaking at 900rpm. Organic or QIAamp® extractions were then conducted, with the former followed by purification using an Amicon® column pre-treated with yeast RNA. FDF® processing followed the manufacturer's instructions. DNAs were quantified and amplified with AmpFISTR® MiniFiler™ and/or PowerPlex® Fusion kits. Statistical analysis of DNA yields, the percent of loaders' STR profiles recovered, and allelic consistency with the loader were performed.

Significantly more loader alleles were amplified using Fusion than MiniFiler™, while the average number of non-loader alleles did not differ. Therefore, Fusion was used for subsequent analyses. Overall, organic extractions of swabbings or soakings yielded significantly more DNA than QIAamp® or FDF®. Further, double swabbing and organic extraction resulted in significantly higher DNA yields than the other four methods and generated, on average, 30% of loaders' profiles (~12 alleles), including some full 24 locus profiles. Organic extraction of soaked samples produced the second-highest amount of DNA, and an average of 10 loader alleles. QIAamp® purification of swabbed or soaked samples recovered significantly less DNA and resulted in fewer STR alleles than those organically extracted. The non-binding DNA extraction, which was specifically designed for touch samples, generated minimal or no loader alleles (an average of only 2.7% loaders' profiles); however, the method that produced the most DNA (double swabbing and organic extraction) also resulted in the most non-loader/drop-in alleles, although their prevalence was concentrated in a small percentage of samples and were generally of undeterminable origin. Taken together, these findings indicate that genetic analysis can become a viable tool for identifying the loader of a firearm, increasing the probative value of this type of evidence.

Cartridge Casings, DNA From Touched Objects, STR Analysis



B70 Internal Validation and Comparative Analysis of the Promega® PowerPlex® Fusion and the Applied Biosystems® GlobalFiler™ Express Amplification Kits for Direct Amplification

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After attending this presentation, attendees will better understand the key differences between the Promega® PowerPlex® Fusion and the Applied Biosystems® GlobalFiler™ Express Amplification Kits based on comparison and internal validation studies completed at the Department of Forensic Sciences. The evaluation focuses on the ability to amplify blood and saliva samples from FTA cards, as well as buccal swabs, in the same 96-well plate using a single cycle number and one optimal injection time.

This presentation will impact the forensic science community by explaining how the internal validation and comparison of Promega® PowerPlex® Fusion and the Applied Biosystems® GlobalFiler™ Express Amplification Kits will educate attendees on the performance of both Polymerase Chain Reaction (PCR) amplification kits. This comparison highlights the key differences in the kit protocols and overall results. The optimization of three different types of reference samples into a single cycle number and one optimal injection time will assist the laboratory with manual sample throughput until a fully automated workflow can be established.

In 2010, the Federal Bureau of Investigation (FBI) Combined DNA Index System (CODIS) Core Loci Working Group identified reasons for expanding the CODIS Core Loci in the United States. Among the reasons is a need to increase international data compatibility and the power of discrimination in missing person cases.

Both the Promega® PowerPlex® Fusion and the Applied Biosystems® GlobalFiler™ Express PCR systems have adapted to the extension of the CODIS Core Loci by increasing their multiplex reactions to 24 loci. The PowerPlex® Fusion Amplification Kit is a five-dye system that allows for the amplification and fluorescent detection of the 13 core CODIS (United States) loci and the 12 core European Standard Set loci. The GlobalFiler™ Express Amplification Kit is a six-dye assay that targets 21 autosomal STR loci, one Y-STR locus, and one Y insertion/deletion locus. Both the PowerPlex® Fusion and the GlobalFiler™ Express Amplification Kits have been approved for the National DNA Index System (NDIS). Therefore, the evaluation of both kits is important in determining the best fit for each individual laboratory as these are implemented for DNA testing.

Sensitivity, precision, concordance, reproducibility, and contamination studies were completed as part of the internal validation of the PowerPlex® Fusion and the GlobalFiler™ Express Amplification Kits using the Applied Biosystems® 3500 Genetic Analyzer. In addition, sample preparation, injection time, analytical threshold, and stochastic threshold were determined to optimize the protocol for analysis of blood and saliva on FTA® cards and buccal samples. The goal of the validation was not only to ensure that both kits produced reliable and robust results, but to also optimize a single thermal cycling parameter and one optimal injection time for all three types of samples.

The internal validation determined that for the PowerPlex® Fusion Amplification Kit, optimal results for blood and saliva on FTA® cards, as well as buccal samples, can be obtained with 26 amplification cycles and 12 second injections on the Applied Biosystems® 3500 Genetic Analyzer. Alternatively, for the GlobalFiler™ Express Amplification Kit, optimal results for these three types of samples can be obtained using 25 amplification cycles and 15 second injections on the Applied Biosystems® 3500 Genetic Analyzer. The ability to amplify and inject blood and saliva from FTA® cards and buccal reference samples on the same 96-well reaction plate will assist the laboratory with manual sample throughput until a fully automated workflow can be established.

GlobalFiler™ Express, PowerPlex® Fusion, Direct Amplification



B71 High-Throughput Spermatozoa Detection Using the Proximity Ligation Real-Time PCR (PLiRT-PCR) Method

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After attending this presentation, attendees will be informed of how the PLiRT-PCR method has been improved for high throughput spermatozoa detection in forensic applications.

This presentation will impact the forensic science community by demonstrating that the PLiRT-PCR method can confirm the presence of spermatozoa from large quantities of samples simultaneously in a streamlined process, demonstrating potential to replace the time-intensive microscopic detection method currently in use.

In the processing of sexual assault evidence, mass media often places emphasis on obtaining a suspect DNA profile; however, equally important but rarely acknowledged is the need for confirmatory identification of spermatozoa, which is often a critical step in determining whether a sexual act occurred or not.

Currently, the only universally accepted method to confirm the presence of spermatozoa is to visualize it microscopically; however, the method has low efficiency as each sample has to be examined individually. Automated fluorescence-based microscopic detection can decrease the time spent per sample, but still processes one sample at a time and requires expensive equipment. Therefore, an alternate method that is capable of processing multiple samples at a time with high accuracy would be a strong candidate for replacing the microscopic method.

The PLiRT-PCR assay combines the specificity of an immunological reaction with the sensitivity of PCR to detect sperm-specific proteins in samples. Specific antibody probes bind simultaneously to target proteins to achieve proximity ligation of conjugated oligonucleotides and the resulting DNA product is detected via real-time PCR. The amount of signal corresponds to the amount of ligated product, which in turn indicates the amount of protein in the sample. The assay only requires a real-time PCR instrument, which is commonly used in forensic laboratories.

The assay has been tested using antibodies targeting two sperm-specific proteins, SP-10 and CRISP-2. Both targets were selected for their localization inside the acrosome of the spermatozoa, which protects them from environmental damage until lysis, increasing the chance of successful detection even with aged samples.

Previously, the assay reported high variability between reactions running aliquots from the same sample. This not only affected the reproducibility of results but also significantly diminished the sensitivity of the assay, as the threshold value for spermatozoa detection is based on three times the standard deviation of Cycle threshold (Ct) values from a set of five No Protein Controls (NPCs). Through protocol and reagent optimization, variability issues have been reduced significantly, with NPCs standard deviations often reaching as low as 0.5 Ct. Testing of both antibodies after optimization places their limit of detection at a 1:100 semen dilution for SP-10 and a 1:1000 semen dilution for CRISP-2, which are conservatively equivalent to 600 and 60 spermatozoa in the assay, respectively. High throughput experiments were also carried out with the Applied Biosystems® 7500 Real-Time PCR System using samples eluted from cotton swabs to simulate actual forensic evidence. In this set-up, three semen swab sample eluates from 2012, 2013, and 2014, as well as a positive control of a 1:100 semen dilution, were run in duplicate along with a set of eight NPCs, in which semen swabs prepared in 2012 and 2014 were successfully detected by the assay.

While these results show promise, several issues have been found. For instance, the probe development process requires further improvement, particularly in the antibody biotinylation step. Also, the new SP-10 antibodies exhibited much less sensitivity than before (reported to be at a 1:5000 semen dilution), whereas CRISP-2 antibodies were found to cross-react with CRISP-3, a protein expressed in salivary glands. As assay development continues, other antibodies that target similar semen-specific proteins will be tested in search of one that provides good balance between sensitivity and specificity. This presentation will discuss the experiments conducted using the optimized PLiRT-PCR protocol, covering the setup, procedure, and results from high throughput runs. The difficulties and potential for further development will also be discussed.



Criminalistics Section - 2015

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Spermatozoa, Proximity, Ligation



B72 Developing a Dynamic Model of the DNA Laboratory Process to Characterize the Sources of Uncertainty in DNA Signal: Applications to Forensic DNA Education, Training, and Validation

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After attending this presentation, attendees will understand the ways in which the DNA laboratory process can be dynamically modeled using a systems-thinking approach. Attendees will also be made aware of how the model can be modified by the user in order to test the impacts of various systems and their errors.

This presentation will impact the forensic science community by demonstrating that a dynamic model can be utilized to train students and practitioners in the nuances associated with the forensic laboratory process. This presentation will demonstrate that modifying the Polymerase Chain Reaction (PCR) efficiency and the error in PCR can substantially impact the peak heights and peak height ratios. Further, it will be demonstrated that dilution of a concentrated stock solution during validation results in additional peak height variability and may need to be considered a process which introduces additional stochastic effects not typically introduced during evidence processing.

The model mimics the generation of a random 16-locus profile obtained by following these steps: extraction→quantification→dilution→amplification→electrophoresis. The input values that can be modified by the user are shown in the Table below.

	Unit	Range	Description
Nominal Target based on qPCR	ng	0-10,000	The target value obtained in the DNA extract as per qPCR.
% RSD in qPCR	%	0-100	Relative standard deviation associated with qPCR results.
Buffer 1 switch	μL	0-1,000	Volume of buffer utilized when the DNA extract is diluted.
Buffer Pipette 1 Stdev	μL	0-10	Standard deviation of the pipette volume during the first dilution.
Buffer 2 switch	μL	0-1,000	Volume of buffer utilized when the DNA is serially diluted twice.
Buffer Pipette 2 Stdev	μL	0-10	Standard deviation of the pipette volume utilized during the second serial dilution.
Buffer 3 switch	μL	0-1,000	Volume of buffer utilized when the DNA extract is serially diluted three times.
Buffer Pipette 3 Stdev	μL	0-10	Standard deviation of the pipette volume utilized during the third serial dilution.
Number of PCR Cycles		28-29	The number of PCR cycles.
Deviation in PCR Efficiency		0-0.2	The PCR efficiency is different at each cycle and is normally distributed about 1. It is the standard deviation of the PCR efficiency. It is set such that the efficiency decreases from 1 as the number of amplicons increase, as per $1 - e^{-(4.5 \times 10^{-10} \cdot [DNA]^c)}$, where c is the cycle number.
Stutter Ratio Information		0-1	Mean and standard deviation of the stutter ratios for each locus.
Cumulative Population Statistics		0-1	Cumulative allele frequencies for 15 STR loci.
CE Sensitivity	RFU		The increase in average RFU signal with amplicon. It can be approximated by determining the slope of a the linear portion of a graph which plots the average peak signal ($\frac{RFU}{RFU}$) for heterozygous loci versus $2C_{tot}[DNA]_0$, where C_{tot} is the total number of cycles (i.e., 28 or 29) and $[DNA]_0$ is the template mass.
RSD of CE		0-1	Relative standard deviation of the capillary electrophoresis set-up and injection.



Criminalistics Section - 2015

The dynamic model treats each copy of DNA as an independent molecule (i.e., no molecular interaction) without systematic preference for one allele over another. The number of copies varies as per the Poisson distribution for each aliquot. For example, if there is an average of 16 copies of DNA in an aliquot, the aliquot that is actually taken may contain 15 copies of allele 1 and 16 of allele 2. The PCR efficiency is different for each allele amplified and varies at each cycle. Thus, the PCR efficiency of cycle 27 may be 0.976 for one allele and 0.983 for another allele. During cycle 28 the PCR efficiency may be 0.979 and 0.980 for allele 1 and 2, respectively. Stutter ratios and CE sensitivity are treated similarly.

The output of the dynamic models gives the user information on: (1) the actual number of alleles/molecules added to the PCR; (2) the known genotypes; and, (3) the electropherogram.

Testing shows that decreasing the deviation in PCR efficiency decreases the differences in peak heights between alleles. Further, it has been determined that peak height differences increase when samples are serially diluted.

DNA Laboratory Validation, System Thinking, Dynamic Model DNA Laboratory



B73 Room Temperature DNA Preservation and Rapid Purification of Decomposing Human Tissue Samples: An Alternative DVI Approach

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After attending this presentation, attendees will be provided with information regarding the efficiency of various solutions to preserve DNA in decomposing skin and muscle samples when stored in tropical ambient temperatures for up to three months. In addition to the benefits of storing samples at room temperature, this presentation will also describe the results of combining these preservatives with various purification methods in order to more rapidly process a high volume of tissue samples for DNA identification.

This presentation will impact the forensic science community by examining how these technologies may have an important impact in the field of forensic genetics and for Disaster Victim Identification (DVI) protocols regarding DNA identification in particular. The application of enhanced liquid tissue preservatives coupled with a reduced DNA extraction procedure may improve the sample processing throughput during a mass fatality incident. Therefore, the implementation of these techniques in future mass disaster responses may help preserve the quality of DNA and improve Short Tandem Repeat (STR) results from decomposing human tissues by improving sample preservation methods and providing a more direct and streamlined high-throughput strategy for processing those samples.

After a mass fatality incident, adequate freezer facilities to house victims may not be available and, therefore, rapid decomposition of bodies will be seen in hostile climates. In order to minimize the DNA degradation, and maximize throughput of samples for identification, an ideal preservative would protect the DNA within tissues while also leaching DNA into the surrounding solution. By maximizing the quantity and quality of “free” DNA in the preservative solution, the time-consuming steps of tissue digestion and DNA extraction may be eliminated.

Skin and muscle samples were harvested over a two-week time period in early fall from three human cadavers placed in an open field at the Southeast Texas Applied Forensic Science facility in Huntsville, TX. Five liquid preservatives (LST, DESS, modified TENT, DNAgard® Tissue, and RNAlater®) were evaluated at 35°C and 60% to 70% humidity for up to three months of storage. DNA was extracted from tissues using the QIAamp® DNA Investigator kit on the QIAcube® platform. The efficiency of isolating the “free” DNA directly from the liquid preservative was tested using the QIAamp® DNA Investigator kit, QIAquick® PCR purification kit, modified ethanol precipitation followed by Microcon® filtration, Agencourt® AMPure® XP PCR Purification system, and the Fingerprint DNA Finder (FDF) kit.

All solutions except the LST buffer adequately preserved the DNA in fresh and decomposed skin and muscle. RNAlater® consistently generated the highest DNA yields (up to 250ng/μL); however, DNAgard® and the modified TENT buffer were the only two preservatives which consistently leached high amounts (0.06-40ng/μL) of good quality DNA into solution for DNA isolation and successful genotyping using the AmpFISTR® IdentiFiler® Plus STR Amplification kit.

The QIAquick® PCR purification kit was the best method tested. It isolated adequate amounts of DNA (0.5-4ng/μL) from all tissues to produce complete profiles in less than 25 minutes. Results suggest that DNAgard® and the modified TENT buffer are better at leaching and simultaneously protecting the “free” DNA in solution than the other methods tested. Therefore, extracting DNA directly from the DNAgard® or the modified TENT buffer preservative using the QIAquick® purification kit may be the best options for room-temperature storage and rapid sample processing in cases of mass disasters.

DNA Preservation, DNA Extraction, DVI



B74 Further Development of an Short Tandem Repeat (STR) Multiplex Reaction for Teaching and Research Purposes

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After attending this presentation, attendees will be familiar with a Short Tandem Repeat (STR) multiplex reaction which is cost-effective for educational and research purposes and will understand how multiple variables affect the validation of novel STR kits.

This presentation will impact the forensic science community by allowing more students to gain hands-on experience with affordable STR amplification reactions while reducing the cost of performing multiplex reactions for academic research programs.

The commercial STR multiplex kits utilized in forensic DNA laboratories are capable of amplifying the 13 core STR loci, amelogenin, and additional loci to provide high discriminatory power when identifying an individual. Producing a rare genotype specific to an individual may not be necessary when STR multiplex reactions are used for teaching in forensic biology laboratory courses. Amplification of fewer loci may also be sufficient for analysis of research samples. Developing a reliable multiplex amplification reaction that costs less than reactions using current commercial kits would be useful to teaching and research labs.

Previous work at the University of Alabama at Birmingham developed a multiplex reaction capable of amplifying three loci: D3S1358, TH01, and vWA.¹ Split peaks for vWA alleles made the interpretation of data difficult, so a new reaction was developed replacing vWA with D13S317. This presentation focuses on the development of a ladder and a sensitivity study for a multiplex reaction using D3S1358, TH01, and D13S317 loci.

An allelic ladder was prepared from amplified volunteer DNA. Chelex extractions of buccal swabs from 13 volunteers were quantitated before amplification. A primer mix of fluorescently labeled primers for D3S1358, TH01, and D13S317 was prepared and combined with a commercial Polymerase Chain Reaction (PCR) amplification mix for all amplifications. Amplified product was separated and analyzed using an Applied Biosystem® 310 Genetic Analyzer and Genemapper® ID software. The peak heights among the three loci were not balanced in initial electropherograms. This imbalance was corrected by adding additional TH01 primers to the prepared primer mix and re-amplifying samples. Amplified product from ten selected samples was combined to form a working allelic ladder for the multiplex reaction. New panel and bin text files were created based on the alleles present in the allelic ladder.

A sensitivity study was performed using the D3S1358, TH01, and D13S317 multiplex reaction. A set of serial dilutions was prepared from two volunteer samples and one control DNA sample. Each sample was amplified in triplicate using the primer mix and PCR amplification mix described above, with either 10ng, 1ng, 500pg, 100pg, 50pg, or 20pg of DNA in each reaction. Overall, few alleles failed to exceed a 70rfu threshold in 1ng samples, less than half the alleles failed to cross this threshold in 500pg samples, and alleles did not amplify in 100pg samples.

In conclusion, this STR multiplex reaction is efficient for use in teaching and research laboratories. The low cost of approximately \$0.50 per reaction makes this an attractive option for labs when producing a rare profile specific to an individual is not necessary. Peak imbalances may occur with individual preparations of primer mix, which can be corrected by adding additional primers for the low locus. Also, this reaction is not as sensitive as reactions using a commercial kit.

Reference:

1. Grefer EE, Aldrich E, Linville JG. Development and Validation of a PCR Amplification Kit: An STR Multiplex System for Teaching and Research Purposes. Proceedings of the American Academy of Forensic Sciences, 65th Annual Scientific Meeting, Seattle, WA. 2013.

STR Multiplex, Education, DNA



B75 Whole Genome Amplification as a Potential Means for Sample Immortalization

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After attending this presentation, attendees will better understand the evaluation of Whole Genome Amplification (WGA) for DNA sample archiving.

This presentation will impact the forensic science community by highlighting the pros and cons of using WGA to generate abundant quantities of DNA from samples that are currently in limited amounts. Specifically, this study was designed for a database of samples that have been collected as part of the National Institute of Justice-funded research, with ancestry and phenotypic information of the donors. The *immortalization* of these samples will allow sharing them with other researchers to improve ancestry and phenotype prediction models.

The availability of high-quality DNA from the aforementioned samples is limited, as only three cheek swabs were collected per individual. A potential solution to this problem is the use of WGA to increase the concentration of DNA extract. To determine if WGA is a viable solution for maintaining reference samples of limited quantity, the most important parameter is whether WGA can yield a complete and balanced representation of the markers to be genotyped, be it Short-Tandem Repeats (STRs) or Single Nucleotide Polymorphisms (SNPs).

In this study, the REPLI-g® Mini Kit (a strand displacement-based amplification incorporating ϕ 29 polymerase) was used to amplify DNA extracted from buccal swabs. Five samples were amplified in triplicate with an input of 2ng while 23 samples were amplified singly with an input of 10ng (the amount recommended by the manufacturer). The amplified product was quantitated using multiple methods: Quantifiler® Human; Quantifiler® Trio; and, an in-house TaqMan® assay targeting a single copy region on Chromosome 5. Quantitation results varied significantly with the three methods, suggesting that the WGA process amplifies the targets of the three TaqMan® assays with varying efficiency. Based on Quantifiler® Human results, WGA yielded an average of 250ng of DNA from a 10ng input (ranging from 28ng to 900ng), whereas with a 2ng input, the reaction yielded an average of 54ng (ranging from 4ng to 208ng). Two samples with an input of 10ng and one sample with an input of 2ng failed to amplify.

The WGA product was then amplified with AmpfSTR® Identifiler® Plus using 0.3ng of input DNA (based on Quantifiler® Human results) in a 5 μ L reaction volume (conditions optimized for reference samples at George Washington University (GWU)). The WGA product was also genotyped at 50 SNP loci, using SNaPshot® assays developed at GWU with 2ng of input DNA (based on Quantifiler® Human results). Off-scale peak heights and split peaks were obtained from the Identifiler® Plus amplification products, suggesting a possible Quantifiler® Human underestimation of the WGA yield in respect to the STR regions targeted in the assay. This issue is being further investigated.

For WGA-DNA input levels of both 10ng and 2ng, one allele dropped out per profile on average. All profiles showed locus-to-locus imbalance. The average peak height ratio between sister alleles in the 10ng samples was higher than in the 2ng samples. The 2ng triplicates yielded a full consensus profile although each replicate contained one instance of drop out. Of the tested SNPs, 49/50 were successfully genotyped; the failed SNP also failed in the original (non-WGA) DNA extract, possibly due to PCR primer efficiency issues. All peaks were present and distinguishable from artifacts/noise and genotypes were consistent with those obtained from the original DNA extracts.

According to the results obtained, WGA is a promising solution for sample archiving, although caution must be exercised. In this experiment, SNPs appeared more robust than STRs. Further studies evaluating a larger panel of markers with Next Generation Sequencing (NGS) technology, and evaluating higher levels of input DNA, will better assess the potential of WGA for sample immortalization.

As technology rapidly progresses, it is likely that in the foreseeable future new and comprehensive methods for high-level multiplexing of multiple marker types will be available to the forensic community. Thus, future availability of these well-characterized samples will be increasingly important in the development of methods and interpretation guidelines for ancestry and phenotype prediction.

WGA, SNPs, Sample Archiving



B76 Highly Multiplexed Analysis of STRs and SNPs Using Massively Parallel Sequencing: Concordance With Current Methodologies

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After attending this presentation, attendees will be informed about next generation sequencing applied to forensic DNA analysis and whether data generated from sequencing is concordant with capillary electrophoresis data.

This presentation will impact the forensic science community by exploring the advantages of Next Generation Sequencing (NGS) to forensic DNA analysis as well as its concordant results with current methodologies.

Capillary Electrophoresis (CE) is the predominant method for DNA analysis currently employed by forensic laboratories and has been regarded as the gold standard for many years. Data from this technique has been admitted into court since 1996. Although this is an effective technique, it is limited in the amount and type of information that can be generated efficiently. Although Short Tandem Repeats (STRs) can give sufficient information for human identification, it is often useful in forensic cases to be able to analyze other variants including Single Nucleotide Polymorphisms (SNPs) and Y-chromosomal Short Tandem Repeats (Y-STRs), which can provide valuable information to law enforcement. Analyzing SNPs using CE is difficult due to multiplexing constraints and is not often performed in forensic laboratories.

Whole Genome Sequencing (WGS) is now more widely available with the advent of NGS techniques. WGS allows analysts to analyze STRs as well as other informative variants in the DNA sequence simultaneously. With NGS systems, scientists are able to obtain an accurate sequence of a genome in a relatively short amount of time due to the massively parallel sequencing that is utilized by NGS platforms. Each base is sequenced 30 or more times to ensure accurate calls are being made. NGS can also be used for targeted sequencing to study specific genetic regions of interest according to application.

As technology continues to expand, it would be useful to consider if these advanced technologies could help forensic laboratories gain more information from their often difficult and limited samples. Illumina's® Prototype ForenSeq™ DNA Signature Prep Kit is an NGS kit targeted for forensics. With this system, one workflow is required to analyze a multitude of variants including autosomal STRs, SNPs (identity, phenotype, and ancestry informative), Y-STRs, and X-chromosomal Short Tandem Repeat (X-STRs). The kit targets all STRs required for the Combined DNA Index System (CODIS) and European Standard Set (ESS) of STR loci, as well as many more. Because of the system's ability to distinguish indexed samples, 32 to 96 samples also can be run concurrently with NGS on a single run. This approach could tremendously impact existing backlogs in forensic laboratories. Sequencing by NGS has the advantage of being able to analyze more loci at a time, thereby generating more information for the analyst.

This study was performed to check concordance of sequencing data performed on Illumina's® MiSeq™ FGx using the Prototype ForenSeq™ DNA Signature Prep Kit against data obtained using traditional CE methods. Concordance was also checked between SNP data from the Prototype ForenSeq™ kit against Illumina's® WGS data from the Platinum Genome Project. This study focused on concordance between the 95 identity informative SNPs, 29 autosomal STRs, 9 X-STRs, 24 Y-STRs, 22 phenotypic informative SNPs, and 56 ancestry informative SNPs that are included in the Illumina's® Prototype ForenSeq™ DNA Signature Prep Kit. Full profiles were generated for all 23 samples run simultaneously with the Prototype ForenSeq™ DNA Signature Prep Kit. A total discordance of 0.001% was observed for this study. Showing concordance between this new technology and the existing technologies would confirm the possibility of implementation of the ForenSeq™ DNA Signature Prep Kit into forensic laboratories.

Next Generation Sequencing, Concordance, Forensic DNA Analysis



B77 Evaluation of a Novel Material, Diomics X-Swab™, for Collection of DNA

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After attending this presentation, attendees will better understand the current issues associated with DNA collection and recovery and the exacerbation of these issues in low copy number DNA analysis. Attendees will be provided with information on a new collection device for DNA and the results of an experimental study to gain an appreciation of the critical role collection devices play in forensics.

This presentation will impact the forensic science community by making attendees aware of critical issues in DNA collection and recovery and by providing information on a novel collection device which offers a potential solution.

Success of DNA typing is related to the amount of target material recovered from an evidentiary item. Generally, the more DNA that is recovered, the better the chance of obtaining a typing result that will be robust and reliable. One method of collecting stain materials is by swabbing. Recovery of DNA from a number of commercially-available swabs is not an efficient process. The X-Swab™ is a unique bio-specimen collection material which can be dissolved during certain extraction conditions. Therefore, more DNA may be collected from a substrate and be released from the swab matrix than other swabs. The ability to recover DNA from the X-Swab™ and success in Short Tandem Repeat (STR) typing were compared with the Copan 4N6FLOQSwab™, a device which utilizes a proprietary flocked-swab technology to maximize DNA collection and elution efficiency. Both types of swabs were impregnated with known amounts of DNA and body fluids (replicates of 10) and allowed to air dry. In addition, blood was placed onto glass slides (replicates of 10), allowed to dry, and collected using both types of swabs. DNA recovery was assessed by DNA quantitation and by STR typing. X-Swab™ material had a significantly higher DNA recovery at the 1:10 (p value=0.008) and 1:50 dilutions of whole blood (p value= 2.1×10^{-7}). For dilutions of saliva, a significantly higher DNA yield was observed for X-Swab™ material for the 1:5 (p value= 1.1×10^{-7}) and 1:10 dilutions (p value=0.002) compared with DNA recovery from the 4N6FLOQSwab™. For the 1:10 dilution bloodstains, the X-Swab™ and the 4N6FLOQSwab™ had very similar average DNA yields ($62\text{ng} \pm 17$ and $73\text{ng} \pm 27$, respectively; p value=0.82), even though the X-Swab™ surface area and volume were considerably smaller. For the 1:100 dilution stains, the X-Swab™ yielded nearly twice as much DNA ($6.6\text{ng} \pm 1.5$) as the 4N6FLOQSwab™ ($3.6\text{ng} \pm 1$), but the difference in yield was not significant (p value=0.30). Partial or full STR profiles were obtained for all samples for both swab types at both dilutions using the PowerPlex® ESI 17 Pro System. For both the 1:10 and 1:100 dilutions, the X-Swab™ yielded higher Relative Fluorescence Units (RFU) values at all loci. The results demonstrated that the X-Swab™ material yielded greater DNA recovery, particularly of low quantity samples, compared with the 4N6FLOQSwab™. Results also indicated that the X-Swab™ material itself enhanced yield of PCR products.

DNA Collection, DNA Recovery, Diomics X-Swab™



B78 Assessing the Forensic Potential of Small Arms Propellant Micromorphometry as an Aid in the Investigation of Improvised Explosive Devices (IEDs): A Pilot Study

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After attending this presentation, participants will have a better understanding of the strengths and limitations of morphometry as a method for the characterization and discrimination of small arms propellants.

This presentation will impact the forensic science community by demonstrating the application of automated image analysis for the measurement of small arms morphometry as a quantitative method for the characterization of explosives.

Pipe bombs are the most common IED in the United States, with the majority containing small arms propellant (Smokeless Powder (SP) and Black Powder (BP)) as the main explosive charge.¹ In addition, United States and coalition war fighters combat IEDs at an unprecedented scale.² Thus there is a need to develop robust metrics for the characterization of propellants that are used as explosives as well as for comparisons between exemplar and recovered explosive residues. SP micrometry (length and width measurements) has been shown to help reduce the number of possible manufactured brands, thus providing fast, valuable investigative information.¹

In this pilot study, the potential to use automated image analysis software to characterize small arms propellants was investigated. Approximately 50 samples of SP and BP were acquired. These samples consist of eight propellant distributors and span 37 brands. In addition, there are several sample brand replicates that have either different production dates or different distributors. For each sample, at least 100 kernels of powder were placed on transparent adhesive backings. Transmitted light was used to produce high-contrast “masks” of the individual kernels. Photomicrographs were captured at 10-30x magnification using stereomicroscopy. Open-source image analysis software was used to process the propellant kernel images, thus allowing thousands of particles to be quickly characterized. The following parameters were measured for all the particles in each captured image: area, perimeter, major and minor axis of best fit ellipse, circularity, porosity, aspect ratio, and solidity. Canonical Discriminant Analysis (CDA) was used to separate the sample classes. These classes were treated as a database of known standards. Next, an independent set of particles was processed (from the original stock propellant samples). These were treated as “unknown” samples. These “unknowns” were assigned to the class (database entry) that had the smallest Mahalanobis distance. Seven samples were incorrectly assigned to the specific database entry; however, two of these were simply assigned to replicate entries (same brand, different lot or distributor). In addition, two of the incorrect samples were assigned to database entries that have been reported to be the same propellant but sold under different brand names. The remaining three samples were truly incorrect assignments. The results from this study show that there is potential for using automated image analysis for the characterization of small arms propellants. By using automated methods, the time required for particle measurements is dramatically reduced in comparison to manual methods.

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 2. Countering Improvised Explosive Devices, 2013. http://www.whitehouse.gov/sites/default/files/docs/cied_1.pdf
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Image Analysis, Smokeless Powders, Explosives



B79 Integration of the QIAcube® Into the Laboratory Workflow for Efficient Processing of Sexual Assault Casework

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After attending this presentation, attendees will learn how the Qiagen® QIAcube® was implemented into the workflow for processing of sexual assault cases and how they may be able to modify their own laboratory's workflow to gain efficiency.

This presentation will impact the forensic science community by demonstrating how many laboratories struggle with DNA backlogs and showing how to increase throughput without an increase in staffing. The changes detailed in this presentation may assist laboratories in addressing their own DNA backlogs.

The Alaska Scientific Crime Detection Laboratory has historically separated processing of forensic casework into two distinct services of biological screening and DNA analysis. Biological screeners would examine evidence to identify samples/stains that were appropriate for DNA testing based on the results of presumptive chemical tests and microscopic examinations for spermatozoa. A DNA analyst would then resample an item and begin work at the DNA extraction phase, repeating microscopic examinations to assess the efficacy of the differential DNA extraction for sexual assault evidence. This workflow has been inefficient in that two people are independently opening, documenting, sampling, and sometimes performing microscopic exams of the same item.

The laboratory has recently completed validation of the QIAcube® and modified the workflow for sexual assault evidence to eliminate redundancy and maximize the utility of the information provided by the presumptive chemical tests and the DNA quantification. These changes will lead to many samples being extracted and quantified with no prior screening and significantly fewer microscopic examinations being performed. Additionally, the biological screener will be performing the extraction and quantification. A DNA analyst will pick up the case at the amplification stage and carry it through the remainder of the processing.

Several positive outcomes are expected as a result of the new instrumentation and workflow for sexual assault cases. These changes will decrease the turnaround time for DNA cases and increase the throughput of the laboratory. By eliminating the redundancy of tasks between the biological screener and the DNA analyst, the time required to prepare samples for DNA extraction will be cut in half. The DNA extraction process itself, formerly a three-day process that included two overnight soak/digest steps, can now be completed in one day.

Historically, DNA analysts were selecting samples based on the forensic history provided by the alleged victim, the results of presumptive chemical tests, and microscopic examinations; however, these test results are not as predictive of successful typing results as human and male quantification results. Furthermore, it has been observed that even after an aggressive digestion of the cellular material on casework, a significant number of sperm cells remain on the substrate, often greater than what was removed during the extraction protocol. Analysts are now routinely performing a Dithiothreitol (DTT) digest of substrates in addition to the sperm fractions generated on the QIAcube® and quantifying all three samples (epithelial, sperm, and substrate). Armed with quantification results, DNA analysts are able to make better informed choices when selecting samples for amplification and typing, minimizing the need to perform additional testing when the initially processed samples do not yield probative DNA typing results.

Although the laboratory has only recently gone online with the modified differential workflow, the initial results are promising. Compared to the previous (manual) differential method, the QIAcube®-facilitated extractions generally yield more single-source sperm (and/or substrate) fractions and the yield of male DNA in these fractions has been observed to be higher with the QIAcube® than with the manual method. Furthermore, samples processed with the QIAcube® have been observed to yield interpretable sperm fractions even when the microscopic exams did not result in the observation of spermatozoa. It is anticipated that the laboratory will now obtain probative male DNA profiles in cases which may not have proceeded to DNA when the laboratory relied on microscopic exam results for DNA sample selection.

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QIAcube®, Differential Extraction, Workflow

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B80 The Evaluation of the Qubit® 2.0 Fluorometer Quantitation System and Comparison to Real-Time Quantitative PCR

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After attending this presentation, attendees will understand the basic functionality of the Qubit® 2.0 Fluorometer and how the instrument compares to current real-time Polymerase Chain Reaction (PCR) technology in terms of cost, time required, and accuracy of the results.

This presentation will impact the forensic science community by providing a comparison between quantitation methods currently employed by forensic laboratories and a newer fluorescence-based method, the Qubit® 2.0 Fluorometer, which is currently being used in the next generation sequencing workflow.

Real-Time quantitative PCR (RT-qPCR) is the current forensic method to determine the concentration of human DNA and is accurate, reliable, human-specific, and has a high degree of sensitivity; however, the method is time consuming, typically requiring two hours, and relies heavily on the accuracy of the serially diluted standards. As the field of DNA quantification advances, new methods that can accurately quantify the DNA more efficiently and without using a large amount of the sample could potentially replace RT-qPCR in forensic laboratories. The Qubit® 2.0 Fluorometer is a fluorescence-based quantitation system that measures the concentration of double stranded DNA (dsDNA) with a detection range between 10pg/μL and 1,000ng/μL; however, the assays are currently not human-specific and measure the total amount of dsDNA that is present in a sample.¹

This study compares the quantitation values of a sample when measured by the Qubit® and the Plexor® HY kit with an Applied Biosystems® 7500 Real-Time PCR System. Fifteen anonymous donors provided buccal swabs for use in the study. DNA from each sample was extracted using the “tip dance” protocol of the EZ1 Advanced robot and an initial quantitation value of the samples was determined using the Qubit® instrumentation. A dilution was made based on the resulting concentration to target 5ng/μL or 2ng/μL, depending on the starting concentration of the extracted DNA sample. This was followed by a serial dilution to produce a range of concentrations in order to test the limit of detection of the two Qubit® Assay kits: High Sensitivity (HS) and Broad Range (BR). The HS Assay kit is accurate for initial concentrations of 10pg/μL to 100ng/μL, while the BR Assay kit is accurate for 100pg/μL to 1,000ng/μL. The same dilutions were used with both the HS and BR kits. For comparison, the same samples were quantified using the Plexor® HY system. The results indicated that the Qubit® BR Assay kit is not ideal for forensic samples as it is unable to read samples with concentrations below 1ng/μL when using 2μL of sample to quantify the sample. The HS kit was much more consistent reading the samples that had concentrations below 1ng/μL. When the quantitation values generated by the Qubit® were compared to those of RT-qPCR, it was evident that RT-qPCR was more sensitive when small amounts of DNA were present in the sample. After capillary electrophoresis, the peak heights on the electropherograms were analyzed for the amplification reactions targeting 1ng (or 1,500 Relative Fluorescence Units (RFU) per allele) based on the Qubit® and RT-qPCR quantitation values. The average RFU value for the samples quantified using the Qubit® was 1,588±313 and the average RFU value for the RT-qPCR was 1,080±434 RFU per allele and Qubit® variance was 20% while the RT-qPCR variance was 40%, thus indicating that the Qubit® yielded results as consistently as RT-qPCR-derived profiles and more closely to the desired 1,500 RFU per allele.

The short amount of time needed to quantify a sample, along with the low cost, would make the Qubit® useful in forensic laboratories. The Qubit® takes less than five seconds to determine the concentration of a sample, thereby allowing analysts to quantify a large number of samples in a relatively short amount of time. It costs less than \$1.00 to run a sample on the instrument and is cheaper than the reagents currently required for use with the Applied Biosystems® 7500 (~\$3.00 per reaction); however, based on the results of this study, RT-qPCR is still more sensitive when directly compared to the Qubit®, and specificity for human DNA is required for implementation in forensic laboratories according to the Quality Assurance Standards for Forensic DNA Testing Laboratories published by the Federal Bureau of Investigation.

The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense. Names of commercial manufacturers or products included are incidental only, and inclusion does not imply endorsement by the authors, DFSC, OPMG, DA, or DoD.



Criminalistics Section - 2015

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Qubit®, DNA Quantitation, Comparison



B81 White-Light Versus Shortwave-Ultraviolet Illumination for Visualizing Fingerprints Developed With Columnar Thin Films of Alq3

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After attending this presentation, attendees will understand how to appropriately illuminate fingerprints developed with Columnar Thin Films (CTFs) of Tris(8-hydroxyquinolino) aluminum, commonly known as Alq3, for visualization.

This presentation will impact the forensic science community by alerting forensic scientists to a currently emerging technique of fingerprint development that may be more valuable than conventional techniques. It will also act as a guide to clarify the appropriate illumination procedures in documenting and analyzing fingerprints that have been developed with Alq3 CTFs. It has previously been believed that shortwave-ultraviolet illumination was best suited to obtain the most detail in fingerprints developed with Alq3 CTFs, but it is hypothesized that white-light illumination will provide as good, if not better, detail in photographs of the developed fingerprints.

The CTF technique preserves the topology of the fingerprint. A CTF is a collection of parallel nanoscale columns. These columns are grown upright atop the fingerprint residue.

In this study, CTFs were deposited on partial bloody fingerprints laid on brass substrates. Brass is a forensically relevant material, being used in such items as cartridge casings and household items. The CTF material used was Alq3, which has an absorption band centered at 259nm, and two fluorescence bands centered at 390nm and 519nm. According to Muhlberger et al., a fingerprint developed by the deposition of an Alq3 CTF is best visualized with illumination by shortwave-ultraviolet light.¹ The goal of this study is to determine which type of illumination is better in order to image the best detail of the fingerprint: white-light or shortwave-ultraviolet illumination.

To accomplish this goal, the partial bloody fingerprints on brass were developed by the CTFs of Alq3, then photographed using different sources of illumination. The quality of the fingerprints after deposition was graded objectively and subjectively. The objective grading was performed using a combination of three software programs that ultimately assigns a percentage score to the fingerprint based on the amount of definitive minutiae. The subjective grading was performed by visual examination and assignment of a score based on the clarity of ridge flow and minutiae.

Initial observations suggested that both the white-light and shortwave-ultraviolet illumination resulted in good-quality photographs of the partial bloody fingerprints. After objective grading of the photographs, white-light illumination is significantly better than shortwave-ultraviolet illumination. Subjective grading also shows a similar trend.

Thus, experiments have shown that contrary to previous knowledge, fingerprints developed by Alq3 CTFs can be better visualized through the use of white-light illumination than shortwave-ultraviolet illumination. Use of the correct type of lighting will allow for better-quality photographs of the developed fingerprints, which is important for identification.

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Columnar Thin Film (CTF), Fingerprint Development, Alq3



B82 The Effect of Walking on the Evidentiary Value of Soil Taken From Footwear

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After attending this presentation, attendees will understand how soil analysis from footwear is impacted by distance traveled from the point of origin of the soil.

This presentation will impact the forensic science community by demonstrating the validity of comparisons between soil samples taken at a point of origin and that found on footwear that has been walked on up to 1.5 miles after transfer.

Evidentiary 3D footwear indentations are often casted using a form of dental stone. The cast can then be compared to the exemplar footwear using identifying and individualizing characteristics; however, if the cast does not provide sufficient detail to make it useful in an investigation, scientists may examine adhered soil to the cast in the event that evidentiary footwear is found. Examination of soil from both the cast and the collected footwear may be performed using several methodologies. These analyses include pH, organic loss on ignition, palynology, particle size distribution, mineralogy, and elemental composition. Particle size distribution is particularly probative because it directly relates to the mineral content which in turn directly relates to the elemental composition. In order for a comparison between soil in the footwear and soil adhering to the cast (at least at the point of origin) to be valid, the composition of the soil in the footwear must not be changed as a result of the impact of the footwear with surfaces after transfer. To date, no research has been performed to see if the characteristics of soil in footwear change between transfer and collection. This study examines this question by determining whether the particle size distribution of soil in footwear is altered while walking on an asphalt surface as a function of distance.

Soil was collected along a tree line, mixed, and homogenized. Particle size distribution was performed on five samples of the soil using a previously published method.¹ Cumulative weight graphs were generated from size fractions weighed after sieving with mesh sizes measuring 2,000 μm , 500 μm , 250 μm , 125 μm , and 63 μm . These results served as the zero-mile trial, representative of the point of origin or site of casting. Soil from the same sample was moistened and applied to the grooves of sneakers (by stepping into the soil) from two volunteers weighing 110 and 130 pounds, respectively, and collected after each of five trials at four walked distances (0.5, 1, 1.5, and 2 miles) on dry asphalt. The same type of sneaker was worn by both volunteers. After each trial, soil remaining on the treads of the sneakers was collected from four regions — right heel, right toe, left heel, and left toe — and approximately 1.2g of the soil was analyzed using the particle size distribution method and compared against the zero-mile trial. Mean cumulative weight graphs were converted to semi-log graphs from soil collected from each region of the sneaker from both volunteers at each distance and compared to mean semi-log graphs generated from the zero-mile trial. The Kolmogorov-Smirnov test, a non-parametric statistical test, was used at the 95% confidence interval to determine differences between the semi-log graphs from the zero-mile samples and the distance trials. This test showed that all (ten out of ten) of the 0.5-mile trials, eight out of ten one-mile trials, and nine out of ten 1.5-mile trials were indistinguishable from the zero-mile samples. Of the two differing one-mile trials, only one region out of eight was found to be indistinguishable from the zero-mile samples. Of the single differing 1.5-mile trial, one-half of the regions were found to be indistinguishable from the zero-mile samples. The two-mile trials did not yield enough soil for analysis.

Although several other parameters need to be examined (different weights of volunteers, running, different surfaces), results of the present study show that false exclusions are not likely to occur when comparing soil from footwear from a point source after walking a distance of up to 1.5 miles.

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False Exclusions in Soil, Footwear, Particle Size Distribution



B83 Characterization of Solder by Trace Metals Using Atomic Absorption Spectroscopy (AAS)

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After attending this presentation, attendees will understand: (1) an available method for analyzing solder using AAS; (2) some of the information that may be obtained from trace elemental analysis of solder; and, (3) potential issues in using solder elemental profiles for investigative purposes and as prosecutorial evidence.

This presentation will impact the forensic science community by providing insight into the use of solder as a potential investigative tool in criminal cases that involve improvised explosive devices and/or handmade electronic circuits and fuses.

Trace elemental analysis is capable of characterizing materials found during an investigation and identifying a potential source. This includes potentially linking the evidence to a known brand, lot, or sample of a given product. As a consequence, this technique can provide investigators with valuable knowledge about evidence present at a crime scene. Solder is a relatively common and easy-to-obtain material and is likely to be used in the construction of improvised explosive devices. Rapid analysis of solder found on either exploded shrapnel or undetonated devices may provide investigators with early leads as to the origins of the device and possible primary suspects as well as evidence against the suspect in the case of a trace profile match.

This study employs a method for rapidly digesting and analyzing samples via AAS. Twelve distinct brands of 60/40 tin/lead solder were digested in hydrochloric and nitric acids. Analysis of sample digestions was targeted at identifying differences in trace element concentrations between samples. To examine consistencies within brands, three separate spools each of several brands were analyzed. While other methods for analyzing solder using inductively-coupled plasma sampling techniques have been proposed, AAS is currently more commonly found in forensic laboratories, which would allow for a more facilitated processing of solder samples. A rapid response and dissemination of information pertinent to identifying suspects is critical in cases involving explosives evidence.

This study will present measured concentrations for copper, silver, and bismuth in several commercially available solder samples. Discriminant analysis of these trace profiles suggest that differentiation of solder samples by as little as three elements is possible using AAS according to the proposed method. Sample identification based on trace element concentrations generally had an accuracy of >90%; however, identifying the brand of solder from a given trace element profile is much less accurate. Overall, this study indicates that AAS identification of solder by a direct comparison of an unknown sample to an exemplar may be performed with a high level of accuracy.

Trace Elements, Solder, AAS



B84 Surface-Enhanced Raman Spectroscopy for Forensic Analysis of Human Semen

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The goal of this presentation is to provide attendees with a general understanding of a common vibrational spectroscopic technique known as Surface-Enhanced Raman Spectroscopy (SERS) and how it can be used to identify an unknown stain such as semen and mixtures of semen with other body fluids. The protocols established in order to obtain reproducible results and the reasoning behind variations present between liquid and dried samples will be presented.

This presentation will impact the forensic science community by discussing how these preliminary studies and methodology development for semen identification via SERS demonstrate a potential new tool for the analysis of stains relevant to sexual assault cases. As research in this field progresses, reliable identification of additional body fluid stains is highly likely. This could greatly reduce the effort expended to analyze unknown samples, save the forensic community time and money, and establish a single confirmatory identification method for all human body fluids.

Identification of an unknown stain encountered at a crime scene, especially where the context of the case does not provide an indication of the identity of the stain, currently requires a number of time-consuming and costly presumptive and confirmatory tests be performed. SERS is a vibrational spectroscopic method that could allow crime scene analysts to rapidly identify unknown stains both in the laboratory and in the field. The SERS technique utilizes a laser which interacts with molecules applied to a gold nanoparticle chip (SERS substrate) that enhances the normal Raman signal, producing a shift in energy characteristic of the vibrational modes present. These shifts are detected as peaks and the combination of peaks provides the analyst with a unique spectral fingerprint of the molecular components of the sample. The advantages of this method include its high sensitivity, speed, non-destructive nature, ease-of-use, minimal sample preparation requirement, portability, and multiplexing capabilities.

In contrast to conventional Raman spectroscopy, SERS offers higher sensitivity resulting in small sample volumes (~1 μ L or less) being required for sample identification and the ability to process dilute solutions. This allows for the remaining sample to be utilized for other forensic tests, making the SERS technique an ideal analytical method for use at a crime scene.

It is hypothesized that SERS can be coupled with multivariate statistical methods and established as a confirmatory technique in the analysis of human body fluids encountered at a crime scene. While SERS has been explored for other applications such as analysis of drugs, bacterial diagnostics, and detection of single molecules, little research has been conducted to apply it to forensic analysis of human body fluids.¹⁻³ This investigation identified and characterized semen from a single donor: neat and neat stained on cotton swatches and swabs. All samples were measured in triplicate to ensure reproducibility and the SERS spectra were acquired using a 785nm laser excitation exposed for 10 seconds at 0.6mW. Ten spectra of each chip were taken, averaged, and then compared to one another. Analysis revealed two components of the semen spectra as hypoxanthine and xanthine. Using ordinary least squares analysis, the abundance of each was determined to be 0.34 \pm 0.10 and 0.76 \pm 0.16, respectively; residuals revealed the possibility of an unaccounted-for component(s).

A protocol was designed for the extraction of dried semen stains and application to the SERS chip, in which approximately 150 μ L of semen was pipetted onto a cotton swatch and allowed to dry for 24 hours. The semen was then extracted under various conditions by adjusting both the volume of water and the time elapsed of the cutting's exposure to water. This resulted in extractions using 5 μ L of water sitting for one, two, five, and ten minutes, and 10 μ L of water sitting for five and ten minutes. Optimal results were obtained when a small cutting of the stain was extracted in 5 μ L of water for five minutes.

Additionally, 1:1 mixtures of semen in combination with blood, saliva, urine, and vaginal fluid were evaluated. Resulting spectra were determined to be combinations of the body fluids present which can be procedurally pulled apart using ordinary least squares analysis. Overall, it was concluded that semen produces a spectral pattern that is consistent and readily distinct from blood, saliva, urine, and vaginal fluid.



Criminalistics Section - 2015

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Raman Spectroscopy, SERS, Semen Identification



B85 An Improved Method for Extraction of DNA From Envelopes

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After attending this presentation, attendees will have a basic understanding of how to approach envelope DNA extractions in order to maximize DNA yield.

This presentation will impact the forensic science community by offering a more robust method for extracting DNA from historic envelopes with commonly found laboratory reagents.

To identify the best protocol for obtaining human DNA from envelopes, two sample collection methods were tested, involving both non-destructive and destructive methods, differing digestion solution volumes were evaluated, and two DNA extraction methods were compared.

At the Armed Forces DNA Identification Laboratory (AFDIL), envelopes can be used as alternative reference samples to help in the identification process. Currently at AFDIL, envelope DNA extractions are completed by: (1) using a steam bath to open the envelope seal; (2) swabbing the envelope seal to collect epithelial cells deposited when the envelope was licked; and, (3) extracting the swab using an organic extraction and purification method (PCIA, butanol, and ultra-4 column). Mitochondrial DNA (mtDNA) is targeted from envelope DNA extracts because it offers a direct comparison with mtDNA profiles from skeletal remains when nuclear DNA often yields incomplete profiles. Due to the sensitive nature of mtDNA analysis, the steam bath method can yield inconclusive results and introduce contamination from individuals who have previously handled the envelope.

This study developed a new method that maximizes the quantity of authentic DNA recovered from envelopes. To mimic casework conditions, mock envelopes containing a known mtDNA sequence in the seal of an envelope and a different known mtDNA sequence on the outside of the envelope were created for experimentation. These mock envelopes were used to evaluate how the following variables affected nuclear and mitochondrial DNA yields: (1) the amount of digestion buffer and proteinase K volumes; (2) the type of sample collection (steam bath-swabbing vs. cutting); and, (3) the type of extraction — organic vs. QIAamp® DNA Investigator Kit on the QIAcube®. Nuclear DNA yield was assessed by Quantifiler® Duo and mtDNA quality was assessed by mitochondrial DNA sequence data.

By changing the sample collection method to a cutting, the average amount of nuclear DNA recovered from each sample increased by nearly 40pg/μl, with a standard deviation of 9.2pg/μl. Increasing the amount of extraction buffer in the organic extraction process caused a similar increase in the amount of usable DNA. The average DNA recovered from those samples increased by roughly 15pg/μl, with a standard deviation of 8.4pg/μl. Attempts to use the QIAcube® did not prove fruitful, as the increase in recovered DNA was roughly 12pg/μl, while the improved organic extraction was nearly 60pg/μl.

The results indicate that using a cutting of the seal, rather than a swabbing of the seal, yields higher quantities of both nuclear and mtDNA after organic extraction. Secondly, the results show that increasing the volume of digestion buffer from 500μl to 1,200μl increases the quantity of DNA obtained. A comparison with the QIAamp® DNA Investigator extraction protocol performed on an automated QIAcube® instrument revealed that the organic extraction method resulted in higher quantities of DNA. No evidence of mixtures was observed despite the attempts to mimic casework conditions, most likely due to the high quality of the DNA within each mock envelope seal.

Continued evaluation of the method will incorporate heirloom envelopes similar to those encountered by the AFDIL casework sections. Additionally, the effect of Ultraviolet (UV) irradiation will be assessed to minimize the levels of exogenous DNA while not causing significant harm to the DNA contained within the seal.

The opinions and assertions contained herein are solely those of the authors and are not to be construed as official or as views of the United States Department of Defense, its branches, the United States Army Medical Research and Materiel Command, the Armed Forces Medical Examiner System, the Federal Bureau of Investigation, or the United States Government.

DNA Extraction, Envelope, Quantification



B86 Pairwise Comparisons as a Means of Validating Iraqi Muslim and Christian Allele Frequency Databases

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After attending this presentation, attendees will have a better understanding of Iraqi Muslim and Christian allele frequencies as well as an understanding of a novel approach used to examine population substructure using pairwise comparisons.

This presentation will impact the forensic science community by providing a method that can be an additional measure of validity for sample populations used to develop allele frequency databases. Due to the weight placed on DNA evidence in criminal trials as well as civil actions such as establishing paternity or identifying human remains, an additional test of the validity of allele frequency databases can be utilized.

A total of 542 individuals (395 and 147 self-identified Muslims and Christians, respectively) from Baghdad were genotyped at 15 Short Tandem Repeat (STR) DNA markers (D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820, TH01, TPOX, CSF1PO, D19S433, D2S1338, and D16S539) using the AmpFISTR® Identifier™ Polymerase Chain Reaction (PCR) Amplification Kit from Applied Biosystems®. Thirty-two alleles previously unobserved in the Iraqi population were detected in these individuals.

Conventional tests for Hardy Weinberg Equilibrium (HWE) and pairwise Linkage Equilibrium (LE) were performed on these populations and suggested that they were suitable for generating allele frequencies to be used in forensic DNA profiling work within Iraq; however, samples of populations displaying unacceptably high degrees of substructure can pass tests for HWE and LE. As a further test of the possibility of high levels of population substructure, exhaustive pairwise comparisons of the individuals within the Iraqi Muslim and Christian populations (77,815 and 10,731, respectively) were also performed. The distributions of pairwise allele sharing among the populations of Muslims and Christians were roughly Gaussian. The extent of allele sharing between pairs of individuals within Muslim ($\bar{x}=9.30$, $s=2.30$) and Christian ($\bar{x}=9.60$, $s=2.37$) populations was comparable to what was observed in 100 repetitions of these analyses using populations of simulated individuals with the same allele frequencies [Muslim ($\bar{x}=9.35$, $s=2.31$) and Christian ($\bar{x}=9.67$, $s=2.34$)]. The slightly greater degree of average pairwise allele sharing within populations of simulated individuals than what was observed in the actual populations may be an artifact of the slightly greater number of homozygous loci in the actual populations and will be the subject of further analyses with other actual populations. The maximum number of shared alleles observed in pairwise comparisons of actual profiles was 22 of a possible 30, while comparisons of simulated individuals yielded shared alleles of ($\bar{x}=19.65$, $s=0.76$) for Muslims and ($\bar{x}=18.88$, $s=0.70$) for Christians across the 100 simulations of each, indicating that there are no specific pairs of individuals in the Christian or Muslim Baghdad populations that are very likely to be close relatives.

All loci across both populations show high Power of Discrimination ($PD \geq 0.81$). Two-parent expected Power of Exclusion (PE) is greater than 0.50 at all loci except CSF1PO and TPOX in both Baghdad Iraqi Muslims and Christians. Polymorphism Information Content (PIC) was found to be highest for the D2S1338 locus (PIC=0.87 and 0.86 in Muslims and Christians, respectively) and lowest for the TPOX locus (PIC=0.65 and 0.57 in Muslims and Christians, respectively) in both populations. The Combined Matching Probability (CMP) was estimated to be $1.29E^{-18}$ and $9.83E^{-18}$ for the Muslim and Christian populations, respectively.

Iraq, Pairwise Comparisons, Allele Frequencies



B87 Evaluation of a Modified DNA Extraction Approach for Improved Short Tandem Repeat (STR) Recovery From Severely Degraded Skeletal Elements

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After attending this presentation, attendees will learn best practices for processing DNA extracts from degraded skeletal remains where the goal is to increase the purity and concentration of extracts for the purpose of improving the likelihood that more complete STR profiles will be generated.

This presentation will impact the forensic science community, particularly laboratories working on missing persons cases, by presenting a new way to purify and enrich skeletal remains extracts for nuclear DNA (nucDNA) while minimizing the amount of Polymerase Chain Reaction (PCR) inhibitors that co-purify with the DNA.

Degraded skeletal remains generally contain limited quantities of nucDNA and thus, DNA-based identification efforts often target the mitochondrial DNA (mtDNA) control region due to the relative abundance of intact mtDNA as compared to nucDNA; however, in many cases the discriminatory power of mtDNA is inadequate to permit identification and STR analysis becomes essential. Unfortunately, commercial STR kits such as the AmpFISTR® Yfiler® PCR Amplification kit, AmpFISTR® MiniFiler™ PCR Amplification Kit, and PowerPlex® 16 HS PCR Amplification Kit require input DNA quantities greater than what is typically extracted from highly degraded bone samples. As a result, amplification is generally unsuccessful when following the manufacturer's recommendations.

In 2013, a Low Copy Number (LCN) Yfiler® protocol was adopted by the mitochondrial DNA section working on remains from unaccounted-for individuals from past conflicts at the Armed Forces DNA Identification Laboratory (AFDIL). This LCN protocol requires an increased amount of Taq polymerase and more cycles during PCR amplification. To be reported, an allele must be observed above stochastic threshold in at least two out of three or more independent amplifications. This protocol has increased the success rate for generating four or more alleles (the minimum number of alleles needed to report out a sample) to 40%. Unfortunately, in many cases, STR profiles remain incomplete.¹

Currently, 0.2g to 0.25g of bone powder is digested with a demineralization buffer (0.5 M EDTA and 1% N-lauroylsarcosine) and proteinase K; concentrated with an Amicon® Ultra-4 Centrifugal Filter Unit and purified twice with the MinElute® PCR Purification kit. Preliminary optimization experiments started with increasing the amount of bone powder to 0.5g or 1.0g (two or four times in amount) which resulted in a non-linear increase in DNA yield. Therefore, one possible explanation for the low DNA yield observed with the addition of increased amounts of bone powder is the binding competition between DNA and humic acid for the silica membrane of the MinElute® column. The goal of this project was to increase STR typing success rates by increasing the DNA concentration from bone extracts by finding a way to limit the effects of humic acid binding competition.^{2,3}

Presented here is a protocol where multiple 0.2g to 0.25g bone powder samples were extracted, pooled, and concentrated in a way that limited humic acid competition for silica. The extracts were quantified and LCN Yfiler® amplified before and after pooling. Results show that pooling three or four extracts produces the most significant increase in alleles reported. This can also be seen in the table below.

Number of Extracts Pooled	Cases with Equal or Better Results	Total Number of Cases	Success Rate
Two	7	14	50%
Three	3	4	75%



Criminalistics Section - 2015

Four	8	9	88.9%
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LCN STR Amplifications, Skeletal Remains, Pooling



B88 What Do You Get When You Mix a Double Helix With a Becke Line? A Forensic Family

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The goal of this presentation is to present the story of a real forensic family.

This presentation will impact the forensic science community by providing an entertaining yet informative discussion on how two siblings have mentored each other, learned from each other, and impacted the forensic science community together.

The Gross sisters (Ann Marie and Susan) are two forensic scientists who have worked together at the same crime laboratory for 18 years. These sisters can be as different as DNA and trace evidence yet can work together, just as mitochondrial DNA and microscopic hair examinations work hand-in-hand. After working 26 and 20 years, respectively, in forensic science, the sisters not only have family at home but also in their workplace. Ann Marie Gross has spent 26 years working in forensic science at the Minnesota Bureau of Criminal Apprehension (BCA) Forensic Science Services Laboratory. Her career began in 1989 as DNA analysis was being introduced into the forensic arena and she has experience in all DNA technologies — from Restriction Fragment Length Polymorphism (RFLP) to Short Tandem Repeats (STRs) and mitochondrial DNA. Susan Gross has spent 20 years in forensic science, 18 of which have also been at the BCA; her career has involved working in drug chemistry and transitioned into trace evidence. Both have experience in crime scene processing and are currently supervisors in their respective disciplines. How did it happen that two sisters are both working in the exclusive field of forensic science AND in the same laboratory?

This presentation will tell the story of how they entered into the forensic field. “Who followed who?” is a question often posed to the two of them — are their stories the same? Also addressed will be how each has mentored the other and what they have learned from each other. Benefits of having a sibling work in the same field and in the same laboratory will be discussed and the disadvantages of working together will be presented, as well. The challenges of being on crime scenes together and working cases together, which has led to testifying in the same cases, will be described. Finally, research projects the two have collaborated on and future endeavors for the two sisters will be discussed.

Forensic Family, Siblings, Mentoring



B89 A Study of the Formation, Collection, and Microscopic Trace Material and Genetic Makeup of Household Dust Specimens

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After attending this presentation, attendees will recognize how typical household dust specimens (i.e., dust bunnies) are formed and how the fibrous components form a cage-like structure that acts as a snare which in turn entraps an array of human and animal hairs, natural and synthetic fibers, as well as an assortment of particulate materials. Attendees will learn various techniques and strategies for the collection and handling of household dust specimens. The quantity and quality of all the materials collected in household dust bunnies will be presented.

This presentation will impact the forensic science community by demonstrating the probative value of an underutilized yet extremely valuable form of probative physical evidence — household dust.

Over the last century, classic forensic science as developed and practiced by early forensic pioneers (e.g., Dr. Edmond Locard, Dr. Harry Söderman, Dr. Paul L. Kirk, and others) has demonstrated that trace evidence in the form of dust is a valuable source of non-biased probative evidence in criminal investigations. As Dr. Locard asserts in his early writings, trace materials found in dust can be used to identify the individuals involved in a crime, as well as reconstruct the event itself. In the past few years, several papers have been presented which demonstrate that household dust specimens may in fact be unique to a given location. To date, these studies have focused on developing a rapid, accurate, microscopic methodology for tabulating the fibrous materials such as animal and human hair and natural and synthetic fibers, as well as the particulates commonly found in household dust specimens. This study combines the developed microscopic methodology used in these prior studies with new methods used to extract and analyze the human DNA present within each household dust specimen. It is believed that the combination of these different approaches will greatly enhance the discriminating power, as well as the probative value, of household dust by enabling one to not only identify a location but to also identify its habitual occupant(s).

In this part of the combined study, the structure of household dust specimens, the mechanics of how these specimens formed, and simple techniques for their collection were researched. Simple traps were prepared and the dust specimens were observed as they formed over a range of time spans. The simplest traps were prepared by labeling a pre-cleaned microscope slide, photographing it, and placing it on the floor in the subject location. Next, strips of Gel-Film® were cut and affixed to pre-cleaned, labeled microscope slides. Additional dust traps were prepared by attaching one- to two-inch lengths of Scotch® Brand 3M® Double-Sided Tape to pre-cleaned, labeled microscope slides. Finally, dust traps were prepared by sticking 12mm-diameter, double-coated carbon tabs to pre-cleaned, labeled microscope slides.

As in the case of the simplest dust traps, the dust traps containing the adhesive media were photographed and placed on the floor in the subject location. All of the dust traps were allowed to freely collect dust as it settled naturally on their surfaces over a range of time spans. After the collection period, each specimen was recovered and packaged separately. A number of the dust specimens were examined microscopically and their contents tabulated on a dust tabulation sheet while others were analyzed for their human DNA content. Select specimens had both their trace material content tabulated and their human DNA analyzed. For example, trace material content tabulation for specimen No. 4a listed the following: red-, blue-, black-, and pink-colored cotton fibers; blue wool fibers; gray/black nylon fibers; colorless polyester fibers; red-colored acrylic fibers; human skin cells; stellate-shaped plant hairs; manila-colored paper fibers; and one tiny piece of green vinyl tape. Finally, the data from both the microscopic method and the DNA analysis were collected, tabulated, and evaluated.

The results of this combined study demonstrate that human DNA of sufficient quantity and quality can be recovered from household dust. The use of DNA profiles in combination with micro-chemical analysis of the non-biological material in dust bunnies should permit an identification of the room from which the dust originated as well as the occupier(s) of the room.

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Household Dust, Human DNA, Microscopic Method

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B90 Combined Genetic and Micro-Chemical Analysis of Household Dust as a Definitive Trace Identifier of a Room and Its Occupants

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After attending this presentation, attendees will learn how household dust (i.e., dust bunnies) is analyzed for the presence of human DNA. The quantity and quality of human DNA in dust bunnies will be presented. Attendees will also learn enhanced micro-volume strategies for the analysis of human bio-particles identified in dust bunnies.

This presentation will impact the forensic science community by demonstrating the probative value of an underused form of physical evidence — household dust or dust bunnies.

The ultimate goal of trace forensic evidence in a criminal investigation is to identify the people, places, and things involved in the commission of the crime. A commonly found trace material that does not appear to be widely analyzed in operational crime labs at this time is household dust. This is unfortunate since the potential for identification, rather than merely association, with this type of evidence is a realistic possibility. Macroscopic dust bunnies appear to be a unique entangled conglomeration of fibers containing a variety of inorganic and organic particulates from the immediate environment. Dust bunnies are formed over a period of time due to air flow and they accumulate inside rooms (inside homes or the workplace), vehicles (e.g., in the trunk), and even in some outdoor locations. They can be transferred onto, for example, the clothing of a body that has been dragged across the floor prior to the body being taken away and deposited elsewhere. Thus, in principle, if one or more dust bunnies are found associated with a crime it should be possible to positively identify the room from which the dust bunnies originated; however, the probative value of the dust bunny would be enhanced not only if the room could be identified but also the habitual occupier of the room. This might be accomplished by sensitive DNA typing of the cellular material that is trapped inside the dust bunny (likely originating from the habitual occupier of the room).

In this study, dust bunny samples were genetically profiled using two approaches: (1) organic DNA extraction of whole dust bunny samples with standard and increased cycle number Short Tandem Repeat (STR) profiling; and, (2) “smart” analysis with the individual isolation of bio-particles present in the dust bunny samples using micro-manipulation and enhanced micro-volume direct-Polymerase Chain Reaction (PCR) STR profiling. Detectable amounts of human DNA were obtained in 73% (29/40) of the whole dust bunny samples evaluated. DNA profiles (from one to >30 alleles) were obtained in 55% (standard cycle number) to 98% (increased cycle number) of the 40 dust bunnies examined. While admixed DNA profiles were observed in numerous whole dust bunny samples (particularly with increased cycle numbers), highly probative single-source DNA profiles (random match probabilities >10⁶) were recovered in ~25% of the samples.

The use of enhanced micro-manipulation collection techniques and direct micro-volume DNA profiling from individually isolated bio-particles recovered from within the dust bunnies was evaluated (11 samples tested). DNA profiles were successfully recovered from all dust bunnies examined, with profiles ranging in quality and number of alleles. From the more than 600 bio-particle samples evaluated, DNA profiles (from one to >30 alleles) were obtained in ~30% of the bio-particles tested. This enhanced analysis also resulted in the recovery of mainly single-source DNA profiles (97% of the samples tested) with multiple donors identified in some specimens.

The results of these studies demonstrate that human DNA of sufficient quantity and quality can be recovered from household dust (i.e., dust bunnies). The use of DNA profiles in combination with micro-chemical analysis of the non-biological material in dust bunnies should permit an identification of the room from which it originated as well as the occupier(s) of the room.

Household Dust, Dust Bunny, STR Analysis



B91 The Influence of Distance, Depth, and Time on Forensic DNA Profiling of Soil Bacteria

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After attending this presentation, attendees will understand how the bacterial composition of soil changes over space and time and how this affects its traceability back to a particular location. Attendees will also learn that bacterial profiling of soil using next generation sequencing is a viable tool in criminal investigations by linking evidentiary soil to a crime scene.

This presentation will impact the forensic science community by elucidating how spatial and temporal factors affect the bacterial profiles of diverse soils. Next generation sequencing was employed to develop libraries of bacterial sequences which were compared using multiple statistical techniques to determine their effectiveness in assessing how these factors influence profiles.

Microbial profiling has the potential to individualize a soil sample through the identification and comparison of the tremendous variability that exists in soil bacteria. Next generation sequencing can detect sequence differences at the species or strain level, which is much more sensitive than previous profiling techniques, and allows better classification of the bacteria present in a soil sample. This technique is also becoming more timely and cost effective — important considerations for future implementation of soil bacterial profiling in the criminal justice system.

Temporal effects on bacterial profiles were tested by sampling three habitats: a chemically treated yard, an untreated yard, and a deciduous forest. Surface samples were collected once a day for four days, once a week for two weeks, and once a month for one year. Spatial effects on bacterial profiles were tested by collecting at a center sampling site and 5, 10, 50, and 100 feet from the center in the four cardinal directions. Soil depth was also sampled at the same yard and forest, and a different treated yard, at the surface and directly below at 1, 2, 5, 10, 20, and 60 inches. Sample sets were collected twice, six months apart.

DNAs were extracted using a MO BIO PowerSoil® kit, amplified with bar-coded universal 16S rRNA primers, and sequenced on an Illumina® MiSeq®. Sequence libraries were filtered to remove ambiguous bases, aligned to known bacterial sequences, and trimmed to the region of interest. Statistical pairwise comparisons were performed on all libraries in order to examine whether or not the bacterial profiles were significantly different from one another. Further, variation between libraries was calculated using Bray-Curtis dissimilarity index, which was visualized using a multivariate statistical approach to determine relationships among samples based on their placement in multidimensional space.

Surface soil samples collected from the same location over time associated with each other and also separated from other habitats, both pairwise and in multidimensional space. Spatially, pairwise comparisons and the multivariate statistic showed samples collected near the center point of a yard habitat shared similar bacterial profiles, which became more dissimilar as distance increased. In contrast, the forest samples displayed much more heterogeneity even at short distances. The depth samples showed a similar trend: samples collected at or near the surface (zero to five inches) were similar, but beyond that, bacterial profile differences existed.

The statistical measures largely agreed with each other throughout all comparisons. Multivariate evaluations are valuable as they allow a visual assessment of profile similarities and dissimilarities; however, they do not provide a specific statistical significance value. In contrast, pairwise comparisons can be used to determine significance, but the relationship among all samples is difficult to assess, especially when attempting to determine degrees of difference. Forensically, bacterial profiling of soil via next generation sequencing of 16S rRNA variable regions shows strong potential for tracing soil evidence back to a crime scene over time, bearing in mind that spatial considerations are extremely important.

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DNA Profiling of Soil, Soil Bacterial Profiling, Statistical Analysis



B92 Bacterial Profiling of Soil Evidentiary Items Using Next Generation Sequencing

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After attending this presentation, attendees will understand how the bacterial composition of a soil sample can be used to link a piece of evidence to a crime scene, victim, or suspect as well as how the passage of time and storage conditions affect the bacterial community present in a soil sample.

This presentation will impact the forensic science community by elucidating the potential for bacterial profiling in tracing soil evidence back to an individual site. Next generation sequencing was used to produce ribosomal RNA (rRNA) 16S libraries that could be grouped at the taxonomic class level based on sequence differences, building a profile of each soil sample. The sequence data were statistically analyzed via a supervised classification technique, assigning evidence samples to a site of origin both through time and under different storage conditions.

Soil has generally been regarded as class evidence in forensic casework. Bacterial profiling has been proposed to help individualize soil samples and aid in tracing evidentiary items to their source location. This study tested the feasibility of connecting evidentiary bacterial profiles to specific locations over time and examined bacterial profile changes under different evidence storage conditions. The research also established whether an evidence sample originating from a specific location and habitat type can be differentiated from nearby locations having the same habitat type. A preliminary study included examination of varied evidence samples to see if bacterial profiles change over time. A shirt, sock, shoes, shovel, and tire were exposed to soil in a deciduous woodlot, then stored at room temperature for six months. Four soil samples were collected from each evidentiary item. Similar changes in bacterial class abundance and membership occurred across all evidence samples.

In the next set of experiments, eight white cotton T-shirts were rubbed in a two-foot by two-foot area of surface soil in a deciduous woodlot on day zero. Four shirts were stored at room temperature (24°C) and four at 4°C. Small (approximately 1cm²) soil-covered portions of the shirts were collected on day zero and weekly for eight weeks to characterize how soil bacterial profiles changed over time. Surface soil was also collected from the primary deciduous woodlot and eight similar woodlots biweekly to examine whether an evidentiary item can be traced back to a specific location or only classified to a general habitat type, based on statistical comparison of soil bacterial profiles.

DNA extracts were amplified using bar-coded universal 16S rRNA primers and sequenced on an Illumina® MiSeq®. To identify the bacterial classes present, 16S hypervariable regions V3 and V4 were sequenced. Bacterial libraries were filtered to remove ambiguous bases, aligned to a known bacterial sequence, and trimmed to the size necessary for analysis. Sequences were grouped at the taxonomic class level and genetic distances between the samples were calculated using the Bray-Curtis dissimilarity index. A supervised classification technique was used to create a model of the nine deciduous woodlot samples collected over time and to then predict the evidence soil samples' origins.

The soil on the evidence samples was reliably traced back to the primary woodlot immediately following collection and in the weeks following exposure, showing the utility of these methods in individualizing soil evidence. Over time, evidence sample profiles began to exhibit specific class abundance differences from the woodlot where they originated although they never became similar to the other woodlots examined. This measurable change in specific bacterial classes has the potential to act as a biological clock for how long soil has been removed from a habitat. Bacterial class abundance changes on the room-temperature T-shirts mimicked the preliminary evidentiary items study while refrigeration effectively retarded the abundance changes, maintaining similarity to the primary woodlot site. Forensically, this study shows that soil 16S rRNA next generation sequencing shows great potential for tracing soil evidence back to a specific location over time.

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DNA rRNA 16S, Soil Evidence, Bacterial Profiling



B93 Quantitative Analysis of Botanical DNA by Real-Time PCR for Forensic Discrimination and Identification

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After attending this presentation, attendees will better understand a new highly sensitive DNA quantification method for forensically relevant botanical samples.

This presentation will impact the forensic science community by describing a more accurate and effective examination method of DNA quantification for plant specimens. The goal of this study was to develop a Real-Time quantitative Polymerase Chain Reaction (RT-qPCR) analysis for a wide range of botanical species. The amount of genomic DNA required by this method is so small that it is possible to perform more exacting examinations afterward.

Botanical evidence found at a crime scene can provide valuable information in criminal investigations; however, the specimens can be fragmentary, which prevents accurate species identification based on morphological characteristics. Therefore, DNA-based identification has begun to be introduced into botanical forensic analysis.

DNA quantification is required for accurate and effective examination. If the result of the quantitative assay is unreliable, the potential for needing to use more DNA to repeat testing to avoid failure of the analysis is likely. The loss of DNA due to the need for reanalysis affects the ability to perform further important analyses. Furthermore, with short tandem repeat profiling, a common analysis method used in forensic identification, the amount of template DNA needs to be strictly controlled.

Conventional DNA quantification methods, such as Ultraviolet (UV) -visible spectrophotometry and fluorometry, often fail to quantify a dilute DNA solution correctly because of the influence of non-specific background noise. In addition, these methods require a relatively large amount of DNA. One of the significant problems with botanical evidence is the low yield of DNA from damaged samples. In such a case, conventional methods cannot be used to quantify the sample. In contrast, qPCR requires only a small amount of DNA. This method has the ability to estimate dilute DNA solutions accurately and is well-suited for enabling plant-specific quantification.

In this experiment, primers targeting the ribulose 1,5-bisphosphate carboxylase/oxygenase large subunit (*rbcL*) gene were used in a SYBR® green qPCR analysis method. The primers utilized were selected to identify a wide range of plant species previously identified by direct sequencing of specific loci.

To test the newly developed qPCR method, *Arabidopsis thaliana* genomic DNA was extracted from leaf, stem, and root material, then the qPCR was performed on serial dilutions of the extracted DNA. The lower detection limit was 1.6pg and a straight calibration curve was obtained in the range of 1.6pg to 1ng of DNA.

To examine whether quantification by qPCR can be generally used, this method was applied to other plant species. Five families, Asteraceae, Poaceae, Cyperaceae, Rosaceae, and Ranunculaceae, which are popular plants in Japan, were selected. It was determined that all samples could be analyzed successfully using the developed qPCR method.

In conclusion, it is demonstrated that qPCR analysis is a very effective method to quantify DNA from botanical samples. Moreover, this study raised the possibility that qPCR analysis can be applied to a wide range of plant species.

Forensic Botany, Quantitative Real-Time PCR, *rbcL*



B94 Variation in DNA Mixture Interpretation: Observations From NIST Interlaboratory Study Results

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After attending this presentation, attendees will better understand the history and lessons learned from the National Institute of Standards and Technology (NIST) interlaboratory studies on mixture interpretation, including the two most recent studies, in 2005 (MIX05) and in 2013 (MIX13).

This presentation will impact the forensic science community by helping the forensic DNA community to: (1) determine the current “lay of the land” regarding Short Tandem Repeat (STR) mixture interpretation across the United States; and, (2) learn where future training and research could help improve mixture interpretation and reporting.

DNA mixtures of two or more individuals can be challenging to interpret for the forensic DNA scientist. Historically, laboratories have developed protocols to interpret mixtures based upon a combination of multiple areas of investigation including (to name a few): internal validation studies, information present in the scientific literature, training from workshops and scientific meetings, and guidelines as reported by scientific working groups such as the Scientific Working Group on DNA Analysis Methods (SWGAM) or the DNA Commission of the International Society of Forensic Genetics.

Interlaboratory studies enable examination of “big picture” views across many laboratories. In 2005 and again in 2013, interlaboratory challenge exercises were conducted by the Applied Genetics Group at NIST. The 2005 MIX05 study involved data interpretation of DNA mixtures representing four different mock sexual assault case scenarios while the 2013 MIX13 study involved data interpretation for five different case scenarios. Data from these scenarios were generated at NIST with multiple STR kits and provided to laboratories as electrophoretic data. In each MIX05 case, the “evidence” sample result, which was a mixture of genomic DNA from one “perpetrator” and one “victim,” were provided along with the single-source “victim” reference sample. All data were generated on six different STR kits (Profiler Plus[®], COfiler[®], SGM Plus[®], Identifiler[®], PowerPlex[®] 16, and PowerPlex[®] 16 BIO) from the same lot of genomic DNA mixtures. The MIX05 and MIX13 study designs and sample selection processes will be described along with a summary of results obtained from 69 laboratories with MIX05 and 108 laboratories with MIX13.

In MIX05, NIST focused on electronic data for analysis to control for the variation observed in previous studies with extraction and quantification.¹⁻³ One observation in the analysis of the results was the wide range of approaches to interpreting the same data among the different laboratories.⁴ With the publication of the 2010 SWGDAM Autosomal STR Interpretation Guidelines, many laboratories established analytical and stochastic thresholds for mixture interpretation.⁵

The MIX13 interlaboratory study was conducted to determine the current “lay of the land” in regard to STR mixture interpretation across the community. NIST also wanted to gauge the consistency in mixture interpretation across the United States after the publication of the 2010 SWGDAM guidelines. Examples of the mixture interlaboratory study will be shared along with ideas for future training and research to improve mixture interpretation and reporting in the United States.

References:

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4. Butler JM and Kline MC (2005) “NIST Mixture Interpretation Interlaboratory Study 2005 (MIX05)” poster at the 16th International Symposium on Human Identification (Grapevine, TX), September 27-28, 2005.
5. SWGDAM (2010) Autosomal STR Interpretation Guidelines — available at: http://www.swgdam.org/Interpretation_Guidelines_January_2010.pdf

DNA Mixtures, Mixture Interpretation, Forensic DNA

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B95 A Large-Scale DNA Mixture Interpretation Study of DNA Examiners: Inter- and Intra-Laboratory Variability

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After attending this presentation, attendees will understand the inter- and intra-laboratory variation of genotype interpretations that occur during the DNA deconvolution process. This large-scale study attempted to quantify the variability that exists in the DNA interpretation process and was able to gather participation from a large number of DNA examiners from local, state, federal, and international forensic laboratories.

This presentation will impact the forensic science community by serving to explain the current limitations of DNA mixture interpretation, to quantify the genotype variations in the mixtures utilized in the study, and could be used to help train new DNA examiners and guide DNA interpretation protocols.

Forensic laboratories generally deconvolute DNA samples to identify DNA profiles for contributors present in the mixture sample. As the complexity of a sample increases, so does the range of genotype interpretations generated by an examiner. The interpretations may be affected by sample complexity including low template or degraded DNA, increased numbers of contributors in the sample, and the ratios and allelic overlap between each contributor in the mixture. In addition to the genotype interpretation variation generated with a given sample, various forensic laboratories utilize distinct DNA mixture interpretation guidelines and protocols that influence their interpretation of a sample. In some cases, this will determine if a sample is analyzed or deemed inconclusive; however, the degree of variation between examiners within and outside a laboratory has not been quantified.

This study attempts to quantify the variation within and between local, state, federal, and international DNA forensic laboratories using a Genotype Interpretation Metric (GIM) system developed at the Defense Forensic Science Center (DFSC). The GIM establishes a gradient to incorporate the multitude of potential genotype interpretations, converting the genotype interpretation into a quantifiable metric. Six mixtures comprised of two- or three-person contributors with varying contributor ratios were generated at DFSC using Identifiler® Plus and PowerPlex® 16 amplification kits. To establish a variation baseline, a two-person mixture was generated with a clear major and minor contributor ratio that displayed all alleles present at each locus and did not exhibit dropout at any of the loci in the electropherogram. The other five mixtures were more complex and included variations such as number of contributors, contributor ratios, and varying degrees of dropout. The resultant six mixture .fsa files were submitted to more than 275 DNA examiners at more than 50 local, state, federal, and international laboratories and the genotype interpretations were analyzed for variation at the intra- and inter-laboratory levels prior to technical review. Participants varied in their use of Combined Probability Of Inclusion/Exclusion (CPI/E), Random Match Probability (RMP), and Likelihood Ratio (LR) in their mixture interpretations; a smaller subset of examiners utilized probabilistic modeling software systems. GIM scores and an accompanying survey form were generated and analyzed for each mixture, then compared to other examiner GIM scores within and outside their laboratory. The presence or absence of genotype variation among the examiners will contribute to the overall understanding of mixture interpretation, its current limitation, and the variety of interpretational methods. The results of this study can help shed light on sources of variation seen with DNA mixture interpretation. These findings may also inform training programs for DNA examiners with the goal of reducing variation.

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DNA, Mixture, Interpretation



B96 Exploring the Impact of DNA Template Mass on the Ability to Infer the Number of Contributors Using Three Interpretation Methods

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After attending this presentation, attendees will understand the impact DNA template mass has on the ability to infer the true number of contributors. This presentation will assess the accuracy of three methods: (1) Maximum Allele Count (MAC); (2) Maximum Likelihood Estimator (MLE), which is available online; and, (3) via the online tool *NOCIt*.^{1,2}

This presentation will impact the forensic science community by demonstrating that a computational tool that utilizes a continuous probabilistic approach is the preferred method by which to assess the Number Of Contributors (NOC) as it returns higher accuracy rates for low-template samples. Probabilistic approaches also provide the probability distribution over *n* contributors. This provides the user with information regarding not only the most likely number of contributors but the uncertainty associated with the measurement.

The performance of the methods was tested on single-source samples as well as two-, three-, four-, and five-person mixtures, amplified using 29 cycles, and injected for 10 seconds. Samples were amplified using 0.25, 0.125, 0.063, 0.016, and 0.008ng. MAC and MLE rely on setting an Analytical Threshold (AT) to calculate the NOC. In this study, a constant threshold of 50 Relative Fluorescence Units (RFU) was utilized. Application of MAC and MLE also uses a stutter threshold to filter out the peaks in the stutter position of allelic peaks. The stutter filter specified by the manufacturer's manual was used to filter the stutter peaks at each locus. Allele frequencies from the Caucasian population specified in the AppliedBiosystems® AmpFISTR® Identifier® Plus PCR Amplification Kit User's Manual were used to test the *NOCIt* and MLE methods.³ Unlike the MLE and MAC methods, *NOCIt* does not utilize any thresholds. Rather, it relies on a set of calibration standards to train the software and then utilizes this training set to model the baseline noise, stutter ratios, and the non-detection rates of stutter and allele peaks. Thus, 92 single-source samples were amplified utilizing the same amplification, run, and analysis protocols described above. Artifacts such as pull-up, -A, etc., were manually removed. The exported allele table was the calibration file used to train *NOCIt*.

When 75 mock-evidence samples were interpreted, preliminary results suggest that, regardless of the method, DNA template mass had a significant effect on accurately inferring the NOC to a complex stain. Both the MLE and MAC methods resulted in similar accuracy rates, which ranged from 60% to 13% for the 0.25ng to 0.008ng samples, respectively. In contrast, the accuracy rates of *NOCIt* were 87% to 27% for the 0.25ng to 0.008ng samples. MLE and MAC resulted in both overestimates and underestimates. Both methods overestimated 12% of the samples tested. These overestimations were the result of stutter peaks surpassing the stutter ratio threshold. One sample was overestimated when *NOCIt* was used to infer the NOC. Underestimations were typically due to high levels of allele drop-out and/or allele sharing between large numbers of individuals. The percentage of samples resulting in underestimations for MLE, MAC, and *NOCIt* were 43%, 52%, and 43%, respectively.

Unlike MAC, the MLE and *NOCIt* methods provide a probability distribution on the NOC. In all cases, the distribution was unimodal. Further, the uncertainty associated with the result did not change with target but instead increased with the true NOC. For example, a 1:1:2:1:1 mixture amplified using 0.125ng resulted in *NOCIt* returning two significant results: Pr(NOC=4) of 0.872 and Pr(NOC=5) of 0.128, suggesting this sample could have originated from four or five contributors. In summary, when utilizing MLE, 24% of the three-, four-, and five-person samples resulted in at least two NOCs exhibiting significant non-zero probabilities (i.e., Probability \geq 5%). When *NOCIt* was utilized, 9% of the three-, four-, and five-person samples resulted in at least two probable NOCs.

These preliminary results suggest that all methods are limited in their ability to accurately infer the NOC for samples containing low-template quantities. Though *NOCIt* outperformed the other two methods at all templates, these results suggest samples which contain at least one contributor with fewer than ten cells are prone to underestimation. Accuracy rate data from the full study that includes an additional 360 samples will be presented. Data will also be provided regarding the minimum number of calibration samples needed to train *NOCIt*.



Criminalistics Section - 2015

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1. <http://forensim.r-forge.r-project.org/>. Accessed July 24, 2014.
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 3. AppliedBiosystems. *AmpFISTR® Identifiler® Plus PCR Amplification Kit User's Manual*. 1st ed: Applied Biosystems; 2006.
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Number of Contributors, Complex DNA Interpretation, Low-Template DNA



B97 Identification of Contributors in Complex DNA Mixtures Utilizing High-Density SNP Arrays: Influence of Sample Ancestry, Ancestry SNPs, and Reference Population

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After attending this presentation, attendees will better understand the utilization of Single Nucleotide Polymorphism (SNP) genotyping to identify individual contributors to a complex mixture DNA sample and the effect of contributor ancestry, SNPs utilized during analyses, and reference population selection upon results interpretation.

This presentation will impact the forensic science community by demonstrating a practical alternative to Short Tandem Repeat (STR) analysis using capillary electrophoresis for evaluating mixed DNA samples and will also present the factors which could affect evaluation, such as reference population and ancestry of the contributor(s).

An increasing number of samples evaluated by forensic laboratories include DNA from multiple contributors, such as those routinely observed in sexual assaults and touch DNA. Advances in genotyping methodologies, routinely employed by clinical and research laboratories, provide alternatives to the use of capillary electrophoresis and STR testing. In a single assay, high density SNP Arrays can generate data from thousands to millions of genetic markers including ancestry, phenotype, X, Y, mitochondrial, and identity markers. This substantial increase in genetic data may be expected to improve the power with which contributors to a DNA mixture may be identified.

The use of SNPs to determine inclusion or exclusion of a contributor to a mixture involves comparison of the mixture with a reference population. Studies were conducted to evaluate the impact of sample ancestry, ancestry alleles used in the analyses, reference population selection on the determination of inclusion or exclusion, and on the statistics supporting the identification of contributors in sample sets utilizing the QSNP Informatics Software™. This software employs forensically relevant algorithms utilizing genetic markers to estimate the genetic distance between a sample of interest, an evidence sample, and a reference population as proposed in Homer.¹

Two methods were utilized to generate multiple reference populations: Experimental and Bioinformatic. In the Experimental method, reference populations were created by analyzing human Coriell ancestry panel samples with the Illumina® Infinium® Assay and HumanOmniExpressExome-8 BeadChip array. Allele frequencies were developed from the cumulative results of approximately 100 genomes per Coriell ancestry panel samples (AA48 and Cau50). The Bioinformatics Methodology utilized publicly available data from the 1,000 Genomes Project (<http://www.1000genomes.org>). SNPs identified by the 1,000 Genomes Project were filtered to include only those also found on the Exome8 array. Frequencies of SNPs from approximately 1,500 samples from the African and European Ancestries (AFR and EUR) were utilized for comparative purposes.

Four reference populations were compared for their impact on calculated Distance (D) and T-statistic (T) values in matched and mismatched samples of Caucasian and African ancestry. Pairwise comparisons of a matching Caucasian DNA sample (Table 1) show that D and T values are positive, indicating a match. The values for matching Caucasian samples are numerically higher when calculated against reference populations of African ancestry than when calculated against European reference populations. The Bioinformatics-derived reference populations generally provide more positive values than their corresponding experimental reference populations.

Table 1. Summary Statistics for Matched and Mismatched Caucasian Samples

	AA48		Cau50		Pooled AA48 & Cau50		AFR		EUR		Pooled AFR & EUR	
	D	T	D	T	D	T	D	T	D	T	D	T
Mean												
Matched	0.195		0.150	815	0.168	889	0.260	924	0.208	878	0.226	960
St. Dev.	0.002	10	0.002	12	0.002	11	0.002	10	0.002	13	0.003	14



Criminalistics Section - 2015

Mean													
Mismatched	0.01	37		-136		-68	0.021	51					-76
St. Dev.	0.005	16	0.003	17	0.004	20	0.008	19	0.006	20	0.007	22	

The impact of these factors on inclusion and exclusion thresholds will be presented for both major and minor contributors in simulated forensic case scenarios, using both Experimental and Bioinformatics reference populations.

Reference:

1. Homer N, Szeling S, Redman M, Duggan D, Tembe W, et al. (2008) Resolving Individuals Contributing Trace Amounts of DNA to Highly Complex Mixtures Using High-Density SNP Genotyping Microarrays. *PLoS Genet* 4(8): e1000167. doi:10.1371/journal.pgen.1000167

SNPs, DNA Mixtures, Population Genetics



B98 The Validation of a Statistical Tool for the Analysis of DNA Mixtures

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After attending this presentation, attendees will understand a method for the validation of a statistical tool for the probabilistic genotyping of the results of analysis of forensic DNA mixtures.

This presentation will impact the forensic science community by serving as a guideline to describe how laboratories can incorporate probabilistic genotyping into protocols for DNA interpretation of mixtures.

Improvements in time of analysis, sensitivity, and cost result in it not being uncommon to encounter touch DNA samples in the crime lab. The DNA profiles obtained from touch samples are often low-level complex mixtures that are not easily or consistently interpretable using traditional mixture interpretation approaches and statistics. New methods are increasingly available that are better suited for such data.

The Harris County Institute of Forensic Sciences (HCIFS) chose the R-based statistical software package Forensim and validated its use on complex mixtures. Forensim was appealing as it is open source and available at no cost. The Forensim module of interest to HCIFS is LRmix which follows the method developed by Gill.¹ It enables the calculation of likelihood ratios for genotypes derived from complex Short Tandem Repeat (STR) profiles. The system considers allele drop-in, drop-out, and multiple contributors utilizing a semi-continuous approach that does not include peak height.

Initial validation work focused on the calculation of the probability of drop-out and drop-in, values entered manually into the LRmix module before use. The probability of drop-out value of 0.14 was determined using the counting method, the tailed method, and logistic regression for validation samples of varying concentration. The probability of drop-in was determined to be <0.01 by reviewing hundreds of casework negative controls in search of spurious allele peaks below analytical threshold but above instrument baseline. The value was set to 0.01 as that is lowest probability of drop-in LRmix will accept. LRmix module also includes a Monte Carlo probability of drop-out simulator that is run after a profile is evaluated and an assumption of the number of contributors is made. This simulation was run throughout the validation work. The calculated probability of drop-out was found to be within the simulated range reinforcing the appropriateness of the calculated probability. These simulations will also be included in casework done with LRmix.

Forty two-person and three-person complex mixtures were created from known contributors and evaluated using LRmix to demonstrate sensitivity, reproducibility, and precision. All true contributors to the mixtures were correctly associated. The likelihood ratios obtained from these samples ranged from 1×10^5 to 1×10^7 . Over 100 single-source profiles were created from randomly chosen alleles and all were correctly excluded when evaluated against the mixtures. LRmix is also capable of automating this step using the performance check module. The likelihood ratios obtained from these samples ranged from 1×10^{-55} to 1×10^{-7} . Random profiles are created based on the population database used and compared to the mixture profile. The user chooses how many profiles to compare.

Overall, the validation of LRmix demonstrates it is an acceptable method for the calculation of the likelihood ratios for complex two- and three-person mixtures and it is an economical tool that can be incorporated by a laboratory.

Reference:

1. Gill P, Kirkham A, Curran J. LoComatioN: A software tool for the analysis of low copy number DNA profiles. *Forensic Sci Int* 2007;166(2-3):128-138.

Probabilistic Genotyping, LRmix, Validation



B99 Development of a Pre-Screening qPCR Mixture Detection Assay Using High-Resolution Melting Curve Analysis of the Short Tandem Repeat (STR) Loci D5S818 and D18S51

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After attending this presentation, attendees will have a better understanding of how a quantitative Polymerase Chain Reaction (qPCR) mixture detection assay can be used to screen low-level touch DNA samples.

This presentation will impact the forensic science community by demonstrating a way to potentially detect the presence of a mixture earlier in the DNA laboratory workflow process.

With the current forensic DNA laboratory workflow process, it is not possible to determine if a sample is a mixture of multiple contributors until after the capillary electrophoresis step has been performed and the analysis of the DNA profile shows the presence of three or more peaks at two or more loci; however, because of the large number of low-level or “touch” DNA mixture samples that forensic laboratories are receiving as evidence today, mixture detection at an earlier step in the DNA workflow process could be a beneficial tool. Receiving this information earlier would allow the analyst to determine if consumption of the sample is warranted or if protocols should be adjusted to allow for the combining of swabs from different areas of the evidence item prior to STR amplification. The quantitation step is the most logical place to add a mixture screening assay as it is a necessary and required step of the DNA analysis process, uses qPCR which can accommodate multiplexing a mixture detection assay, and because it is early enough in the workflow that changes can be made prior to downstream STR analysis.

STR loci D5S818 and D18S51 were chosen as the targets for a mixture detection assay because they are small in size and they are polymorphic enough to be able to readily determine the presence of a mixture. Fluorescent detection of the STR alleles by qPCR is most easily achieved with the use of an intercalating dye and melt curve analysis; the temperature of the STR melt product will be proportional to the number of polymorphic repeats (length) of the STR allele; however, prior to incorporation of the the STR amplification and detection in the quantitation reaction, it was important to demonstrate that the addition of an intercalating dye does not affect the performance of the quantitation kit. Thus, samples were run with and without the addition of SYTO-64[®] dye on a qPCR platform using a common commercially available human quantitation kit. Data from this study showed that the addition of an intercalating dye does not change the standard curve quality parameters or the quantitation values of unknown samples. Further, all replicates (with and without additional dye) provided values that were well within the normal inter-run reproducibility range (0.0001 to 3.3107). Next, D5S818 STR primers were used to successfully amplify and fluorescently detect standard melt products from both single-source and two-person mixture samples on the same qPCR platform using the same intercalating dye. Three distinct melting curve patterns were identified for the observed genotypes at the D5S818 locus, which was consistent with published studies. A comparison of the melting temperatures for the samples showed that the mixture samples produced melt products from 73.85°C to 74.02°C while the single-source melting curve groups ranged from 73.84°C to 73.95°C. Because there is some overlap between melting temperatures of single-source samples and mixtures, melting temperature alone cannot be used to distinguish between single-source samples and mixtures. Further mixture analysis showed that curve morphologies for the single-source and mixture samples were also very similar; however, it was noted that there was a distinct difference between the peak heights of the shoulder peaks, suggesting that this method could be used to distinguish a mixture from a single-source sample using the D5S818 locus. In order to improve resolution and magnify any melt curve differences between genotypes in future studies, High Resolution Melt (HRM) curve analysis may be more beneficial. Unfortunately, HRM in the available qPCR instrument has a fixed wavelength in the green channel which overlaps with the target fluorophores used in the quantitation kit. Thus, melt curve data from the quantitation experiments with only the intercalating dye added were reexamined. Data from these runs showed there were no melt products detectable after the quantitation amplification reaction. Consequently, it appears that the scorpion molecule used for fluorescent detection of the human quant target prevents the absorption of the intercalating dye. Therefore, high-resolution melt curve analysis, rather than standard melt curve analysis, will be possible. Future experiments will



Criminalistics Section - 2015

include demonstrating the ability to detect all common alleles at the D5S818 and D5S818 loci using high-resolution melt curve analysis on the qPCR platform and EvaGreen® as the intercalating dye for both single-source and mixed samples. To date, data show that HRM analysis of highly polymorphic STR loci during the quantitation step may be a viable method for identification of a mixture prior to STR amplification.

Mixture Detection Assay, qPCR, Quantiplex



B100 Defining the Limits of Forensic DNA Profile Interpretation: An Assessment of the Information Content Inherent in Complex Mixtures

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After attending this presentation, attendees will gain a clearer understanding of the information content in complex forensic DNA profiles. Attendees will learn to support a decision to interpret or not interpret a sample and will be able to base their decision on whether the profile supports a clear separation between a true contributor and a non-contributor.

This presentation will impact the forensic science community by providing more accurate information about forensic DNA profiles that may reliably be interpreted and those that should not be interpreted. This will provide useful information to the legal and judicial system as well as assisting laboratories in efficiently allocating resources and communicating results to their clients.

With the increasing sensitivity of DNA typing methodologies and the increasing awareness by law enforcement of the perceived capabilities of DNA typing, complex mixtures consisting of DNA from two or more contributors are increasingly being encountered; however, it remains to be shown whether it is possible to distinguish a true contributor from a non-contributor in these complex samples. In order to assess this, sets of six Caucasian genotype profiles were simulated and used to create mixtures containing two to five contributors. Each experiment was run 10,000 times. This simulation experiment was intended to illustrate the best-case scenario in which all alleles from the true contributors were detected in the simulated evidence samples. Therefore, the possibility of drop-out was not modeled for this experiment. The computer program DNAMIX was then used to compute Likelihood Ratios (LRs) for both True Contributors (TC) and Known Non-Contributors (KNC), assuming varying numbers of unknown individuals in the mixtures. These complex mixture simulations show that, even when all alleles are detected (no drop-out), true contributors can generate LRs less than one across a 15-locus profile; however, this outcome was rare (seven of 14,000 replicates (0.05%)) and associated only with mixtures comprising five contributors in which the numerator hypothesis includes one or more unknown contributors. For KNCs, LRs were found to be greater than one in a small number of replicates (75 of 14,000 replicates (0.4%)). These replicates were limited to four- and five-person mixtures with one or more unknown in the numerator. Only five of these 75 replicates yielded a LR greater than 1,000.

The informative metric for LRs is not the absolute value, but rather the separation between TCs and KNCs. By plotting the distribution of LRs, it can be seen that the ability to distinguish TCs from KNCs in mixtures containing up to five contributors is remarkably good. This is true even when multiple unknown contributors are required. Overall, these results imply that the strength of evidence that can be derived from complex mixtures containing up to five contributors, under a scenario in which no drop-out is required to explain any of the contributors, is remarkably high. Therefore, such mixtures are worth the time and effort required to perform the statistical analysis required to estimate the weight of evidence.

Mixture Interpretation, Complex Mixture, Likelihood Ratio



Criminalistics Section - 2015

B101 Contributions of Physical Evidence in Human Identifications

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The goal of this presentation will be to focus on the uses of trace evidence, specifically hairs, fibers, and fabric damage, which can aid in the identification of an individual. After attending this presentation, attendees will have a better understanding of the types of evidence criminalists examine and the potential for collaboration between forensic microscopists, forensic biologists, and forensic anthropologists.

This presentation will impact the forensic science community by showing how trace evidence can provide significant contributions in human identification analyses through the examination of physical evidence and collaboration with other specialists.

Microscopical analysis of hairs can provide information on ancestry, cosmetic treatments, and aid in identification through comparison with a known hair sample to potentially include an individual as the possible source of a hair sample. In addition to comparing hairs to a known sample and being a source of mitochondrial DNA, identification of dyes or other artificial treatments found in hair samples can corroborate information about a victim. A microscopic analysis of hair morphology and pigmentation patterns can provide information on ancestry and corroborate skeletal analysis of mixed ancestry. Artifacts of decomposition on hairs, either through postmortem root banding or fungal tunneling along the hair shaft, can provide information which, along with accumulated degree day calculations, may provide probative evidence in a criminal case.

Fibers are another source of physical evidence that can provide significant links in human identification cases. Samples of alternate knowns of the clothing worn by a victim have been used as evidence to prove contact with a suspect or scene when a body has not yet been recovered. Analysis of fabric condition and style may provide information about an individual's size, age, possible ethnicity or group affiliation, and trauma. Fabric damage in conjunction with an anthropological analysis of skeletal remains have been able to indicate a type of weapon, its general dimensions, and the number of injuries in an area covered by clothing. The degree of degradation for clothing depends on the type of fiber(s) that a garment is composed of, the fabric construction, and the environment from which the clothing is recovered.

Including a forensic examiner qualified in hair and fiber examinations to human identification investigations can contribute an area of expertise that can greatly assist in providing information on ancestry, decomposition, trauma analysis, and corroboration of victim identity.

Criminalistics, Trace Evidence, Anthropology



B102 Pollen Evidence of Diet and Environment From a Nebraska Mummy

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After attending this presentation, attendees will understand how pollen and microfossil evidence can be recovered from a mummy. Attendees will learn how control samples can be used to sort out contamination of internal mummy contents through quantification and statistical analysis.

This presentation will impact the forensic science community by showing how the attention to quantification of pollen remains can provide a foundation for understanding the influence of ambient pollen contamination in recovery of pollen during the autopsy process. In this way, victim diet and activities within a region is able to be discerned.

Palynological investigation of archaeological mummies established methods of environmental and dietary study.^{1,2} These methods have been applied to mummies in criminal and Missing in Action (MIA) situations.

The case of a mummified homicide victim in Nebraska provided an opportunity to apply these methods to actual forensic material. The individual was thoroughly desiccated in such a manner that the thorax was essentially hollow. Control samples from the area in which the corpse was located provided an idea of the normal ambient pollen of the crime scene. Samples from the victim's sacrum as well as a section of intestine were analyzed. Another internal control sample of powder was recovered from the area inferior to the diaphragm. Finally, pollen was washed from the victim's hair. A section of intestine was rehydrated and microfossils were recovered from the inside of the section.

Quantification was based on the Lycopodium method based on adding a known number of spores to a known amount of sample.¹ The number of pollen grains per gram ranged from 980 to 1,680. This provided a quantitative basis for analysis.

The control samples were dominated by wind-pollinated, environmental types. The internal intestine sample was dominated by dietary pollen. The sacrum sample was also dominated by dietary types, with some environmental types. The internal powder of the corpse exhibited a dominance of environmental types which suggests that contaminant pollen entered the corpse at the time of autopsy. It is suspected that the vibration of the Stryker autopsy saw caused some external pollen grains to fall into the corpse. The intestinal section method, proven important in archaeological investigations of mummies, can be directly transferred to forensic investigations.

Pollen consistent with the genus Brassica (broccoli type) was most common with 980 grains per milliliter of the final material recovered from the sample. Traces of oak, hackberry, and clover were present. Pollen was more abundant in the sacrum sample. Brassica-type was most common and approximately 1,680 pollen grains were present per gram. Other dietary types included maize (200 grains per gram) and prickly pear cactus (80 grains per gram). A variety of environmental types including goosefoot, ragweed, juniper, dandelion, grass, pine, cottonwood, oak, and cattail were also encountered. All of these types grew in the vicinity of the victim's house.

The powder from the diaphragm area contained only air-borne ambient pollen consistent with the pollen from control samples. The victim lay on a rug which was heavily contaminated by air-borne pollen leading to the transfer of pollen from the rug to the victim's chest and abdomen. This transfer provided a source of contaminant pollen which apparently entered the hollow thorax during the autopsy, probably related to the vibration of a Stryker autopsy saw.

A second case involved an analysis to help determine the identity of an MIA case from the Korean War. The goal of this analysis was to determine the decedent's diet to assess his military identity. The analysis of a coprolite recovered from the individual revealed a diet completely composed of indigenous Korean plant foods. Comparison with Korean mummies that are currently under analysis in the laboratory confirmed that the MIA diet and medicine pollen is completely consistent with Korean culinary traditions that persisted for hundreds of years. The individual components of the diet are consistent with Korean food and medicinal teas. It appeared that this was the body of an indigenous individual; however, the remains were ultimately identified as those of a United States serviceman. Thus, the decedent was subsisting on indigenous foods in the days immediately before he was killed. In such cases, adaptation of military personnel to indigenous foods obscures cultural identity.



Criminalistics Section - 2015

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Palynology, Pollen, Mummy



B103 Recent Applications of Stable Isotope Forensics for Tracking Region-of-Origin and Residence Patterns of Unidentified Individuals

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The goal of this presentation is to highlight recent applications of stable isotope forensics for predicting region-of-origin and residence patterns in unidentified human remains. After attending this presentation, attendees will better understand the applications and limitations of stable isotope forensics as an investigative tool in human identification.

This presentation will impact the forensic science community by demonstrating how new approaches in stable isotope forensics can aid in the resolution of unidentified remains cases.

Over the past decade, the use of stable isotope analysis has become an increasingly important part of forensic casework. More recently, stable isotope analyses have been applied to unidentified human remains cases from local jurisdictions, United States service personnel from past wars and conflicts, undocumented border crossers, and victims of genocide. These applications of stable isotope forensics have been successful due to the recent development of baseline water and geological “isoscape” maps for various regions. In addition, recent collaborations between forensic anthropologists and analytical chemists have provided opportunities to develop and test new methods by evaluating samples of known origin.

Stable isotope ratios measured in human tissues, such as bones, teeth, and hair, provide a history of a person’s diet and residence patterns. Bulk stable carbon and nitrogen isotope values of bone collagen or keratin (e.g., hair, nails) provide information on the types of foods consumed during life, which in turn may reflect regional or cultural dietary patterns. Stable oxygen isotopes of tap water and precipitation water vary between regions due to environmental factors (e.g., distance from the coast, elevation, aridity) and are incorporated into teeth and bone bioapatite at the time of tissue formation. Strontium isotopes are a product of the geological age of the underlying bedrock in a region and are incorporated into local plants and the animals that consume them, including humans. Thus, stable oxygen and strontium isotopes can be used together as an effective “geolocation” tool for predicting region-of-origin or residence patterns of unidentified individuals. This multi-isotope approach provides independent lines of evidence that narrow down residence patterns and/or region-of-origin of unidentified remains from tissues that form at different intervals (e.g., tooth enamel, bone, hair), which can generate new investigative leads. The approach is most effective in cases where the decedent was born outside of the area or was a recent traveler to the area in which he/she died.

A case study from the western United States demonstrates this potential for narrowing down region-of-origin through comparison of oxygen and strontium isotopes of tooth-versus-bone bioapatite, which represent childhood and adult residence patterns, respectively. Isotopic data indicate that the decedent was not local to the area based on oxygen and strontium isotope values of tooth enamel bioapatite; however, bone bioapatite oxygen isotope values were not inconsistent with the region-of-origin and include other possible regions such as western North America.

A second case study discusses the application of stable isotope forensics for predicting region-of-origin of human bone obtained by the Joint POW/MIA Accounting Command, Central Identification Laboratory. Previous research using stable carbon isotopes of bone collagen and bioapatite found that Southeast Asians could be discriminated from United States Americans with 96.3% accuracy (cross-validated) based on cultural differences in diet (mixed C₃/C₄ diet among United States Americans versus predominately C₃ diet among Southeast Asians).¹ These samples were recovered from various sites in Southeast Asia and were selected from known incidents and were of known origin. Analysis of stable oxygen isotopes of bone bioapatite from three samples allowed for more specific region-of-origin prediction, which could be further narrowed down based on contextual information on the incidents.

The use of stable isotope analysis has enormous potential in forensic science and recent approaches have been successful in predicting probable region-of-origin and residence patterns in undocumented border crosser cases and in victims of genocide.



Criminalistics Section - 2015

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Forensic Anthropology, Stable Isotope Analysis, Human Identification



B104 Lessons Learned From the DNA Analysis of More Than 11,000 Skeletal Samples: More Than 20 Years of Process Improvements

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After attending this presentation, attendees will better understand how rapid adoption and implementation of new procedures within their laboratories will increase DNA reporting success rates for skeletonized remains that are more than 40 years of age.

This presentation will impact the forensic science community by providing a framework of adaptations implemented by the Armed Forces DNA Identification Laboratory (AFDIL) and the Joint POW/MIA Accounting Command Central Identification Laboratory (JPAC-CIL) that can serve as a model for any laboratory seeking to improve DNA recovery from skeletonized human remains.

Since the creation of the laboratory in 1992, the AFDIL has striven to provide world-class DNA analysis to aid the JPAC-CIL in the identification of missing United States service members. Over the past 22 years, procedures have been constantly adapted and improved in order to stay at the cutting edge of DNA technology. This presentation will show how changes to mitochondrial DNA (mtDNA) and autosomal Short Tandem Repeat (auSTR) amplification strategies, as well as DNA extraction and general laboratory protocols, have significantly increased results over time. The increase in DNA reporting success rates will be of particular use to any laboratory performing work on missing persons or cold cases due to the similarity in degradation patterns over time.

Thoughtful application of modifications to standard operating procedures can reap enormous benefits when applied within practicing laboratories. In 1999, the introduction of mini-primer sets allowed for mtDNA sequence data to be generated from previously unreportable samples due to amplicon size. In 2001, a customized LIMS was introduced, which allows for the tracking of samples from cradle (arrival) to grave (final report). Specially designed modules provide for automated comparisons of casework mtDNA profiles to thousands of family references samples in a matter of seconds, allowing analysts to spend more time in the laboratory rather than manually calculating comparisons. Introduction of capillary electrophoresis in 2002 further improved the speed by which samples could be processed within the laboratory.

While all of these previous modifications showed a positive impact on sample processing, the sampling procedure at JPAC-CIL remained essentially the same: remove a 5.0g fragment of compact bone to send to AFDIL for DNA processing of which 2.0g was needed for extraction. This technique generated successful results nearly 85% of the time, but cases that had much smaller bone fragments or fragments of poor quality were unable to be sampled. In 2007, the demineralization extraction protocol was implemented. The success rate for mtDNA processing jumped to more than 92% and the sample size for extraction was reduced ten-fold. In the years following implementation of this extraction technique, JPAC-CIL has been able to slide their sampling strategy to increasingly smaller samples. Over 24% of samples submitted currently weigh less than 2.0g. Cases that would have previously been shelved due to size of available bone fragments can now be processed with positive results. A simple improvement in the extraction procedure removed the restrictions of both sample size and quality, allowing almost any skeletal element to be sampled successfully.

In the summer of 2013, AFDIL incorporated an inorganic purification step into the extraction procedure, completely eliminating phenol, chloroform, and butanol from use. While this modification has had only a moderate impact on mtDNA reportability, autosomal success has more than doubled. AFDIL has used commercially available auSTR and Y-chromosomal Short Tandem Repeat (Y-STR) kits since 2007 to support mtDNA matches or separate individuals with common mitotypes. Both auSTR and Y-STR success rates have jumped from 25% to more than 45%, thus making the use of nuclear DNA testing on aged skeletal samples a more consistently viable option.

Progressive improvements will only provide positive results to any forensics laboratory working on missing persons or cold cases, as examination of changing mtDNA, auSTR, and YSTR success rates from more than 11,000 osseous samples demonstrates. The adaptations described above and implemented by AFDIL and JPAC-CIL serve as a model for any laboratory seeking to improve DNA recovery from skeletonized human remains, to include laboratories performing identification of Unknown Human Remains (UHR).

The opinions or assertions presented are the private views of the authors and should not be construed as official or as reflecting the views of the Department of Defense, its branches, the United States Army Medical Research and Materiel Command, the Armed Forces Medical Examiner System, or the Joint POW/MIA Accounting Command-Central Identification Laboratory.

DNA, Skeletonized Remains, Process Improvement

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B105 Modeling 3D Facial Appearance in Relation to Sex, Genetic Ancestry, and Individual Genes Enables Facial Prediction From DNA

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After attending this presentation, attendees will better understand the variation in 3D facial shape across the globe and how it can vary with respect to sex, genetic ancestry, and individual genes. Attendees will also have a better idea of the methodological challenges involved in predicting complex physical traits using these three variables.

This presentation will impact the forensic scientific community by introducing an approach that represents an important advance in predicting physical appearance from genetic data.

Genomic knowledge, analytical methods, and technology are approaching a stage where questions regarding the evolutionary-genetic architectures underlying variation in human physical traits can be formulated and directly addressed. The recent successes in discovering the genes and histories of skin pigmentation provide compelling examples of this approach. This presentation will review these results and discuss the fundamental considerations in Genotype-to-Phenotype (G2P) mapping especially with regard to the definition and measurement of traits and the use of admixed populations to study recently evolved traits. Using facial features an example trait, both the benefits and challenges inherent in studying phenotypes that are not only multivariate, but are owing to complex patterns of modularity are also multipartite. An approach that has been recently developed to investigate facial variation, called Bootstrapped Response-based Imputation Modeling (BRIM) will also be discussed. This presentation will demonstrate how this technique was used to model the effects of sex, ancestry, and individual genes on the face.

BRIM is unique in that it allows for the compounding of multiple independent response variables into a single scalar customized to the predictor variable of interest. By combining subjective observer assessments of the face with these methods, the capacity to both discover and model the effects of genes on facial features has been demonstrated and provides support for the hypothesis that the pressures of sexual selection shaping the human face are both substantial and dynamic and have also affected the human facial perceptual facilities. These findings will be discussed in light of forensic predictions.

Criminalistics, Molecular Photofitting, Genetics



B106 The Identification Process Involving American Citizen (AMCIT) and Undocumented Border Crossers (UBCs): The Pima County Office of the Medical Examiner (PCOME) Experience

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After attending this presentation, attendees will better understand the complexities encountered during the identification process. Additional postmortem descriptors may be necessary to complete some identifications.

This presentation will impact the forensic science community by describing how personal identification may require more than the match between a databased DNA profile or fingerprint record. Unless the true name and nationality can be deciphered, Next Of Kin (NOK) notification is usually not possible. Emerging technologies that can be utilized to extend the postmortem description beyond DNA profiles and fingerprint records can address this issue.

The PCOME is actively monitoring the case files of more than 1,000 unidentified persons dating back 50 years. The great majority of these cases have been sampled for DNA analyses, with more than 600 of these cases having already yielded complete or nearly-complete DNA profiles. Many of these profiles have been entered, or are in the process of being evaluated for entrance, into the Federal Bureau of Investigation's (FBI's) Combined DNA Index System (CODIS). Although the vast majority of these unidentified individuals are currently believed to be foreign national migrants who died while in transit from Mexico to Arizona as UBCs, their unidentified status requires the PCOME to consider each case as a potential AMCIT until identified as a foreign national. Thus, all technological tools available within the United States medicolegal sphere to assist with the identification process are utilized. In addition to CODIS, the FBI's Automated Fingerprint Identification System and National Dental Image Repository, the Department of Homeland Security's Biometric Support Center's US-VISIT and IDENT databases, the Open-GIS Mapping System, the National Missing and Unidentified Persons System, and the National Center for Missing and Exploited Children are all employed toward this end. While the PCOME actively engages in the identification process armed with DNA profiles, fingerprint records, dental records, and missing person reports, the suspected nationality status of the unidentified decedents is regularly being reviewed. Thus, any identification technology, emerging or long-established, that will assist with global region of birth or a migratory pattern is of great interest. Recent advances in technologies such as isotope ratio analysis may allow the PCOME to be more confident in the categorization of unidentified individuals as either likely UBCs or likely AMCITs. The importance of this categorization lies in the fact that a missing person's antemortem records, essential in the identification process, may be more readily located if a specific nationality is suspected or has been excluded from consideration. Examples of "identified" foreign nationals, who have matched to their own DNA profiles in CODIS but remain unidentified according to PCOME protocols because an alias name was provided to United States law enforcement, will be discussed. In these cases, the true name, nationality, and thus NOK remain unknown. This discussion should highlight the need for additional postmortem descriptors in order to achieve a more complete resolution of some unidentified cases. Innovative and emerging technologies that provide these additional descriptors hold the promise to improve the identification process by allowing for a more thorough comparison of antemortem and postmortem records.

Identification Process, Postmortem Descriptors, Emerging Technologies



B107 A Novel Software-Based Toolset for Latent Print Examination

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After attending this presentation, participants will better understand the features and benefits of using a novel automated system to augment their existing tools for latent print searching and matching.

This presentation will impact the forensic science community by reporting on the development and testing of a novel toolset for latent print examination. The toolset provides a larger candidate list with latent features mapped onto the reference print, thereby potentially increasing the likelihood of identification if the database contains the reference print. The use of this toolset has potential cost-saving benefits in reducing examiner time and increasing the efficiency of expert examiners.

This presentation will introduce innovative toolsets for latent fingerprint examination that significantly reduce the labor intensity of examination and make possible the detailed comparison of a latent print to large databases of reference prints. The toolsets result from an innovative technology that captures ridge and furrow information in a fingerprint via a concept of Ridge-Specific Markers (RSMs).¹ RSMs are exploited to produce invertible overlays between a latent and a reference print that removes the local non-linear distortion between the prints. Each overlay is scored at the pixel level by an algorithm that evaluates the accuracy of the overlay at the pixel level within ridges and furrows. The overlay technology and scoring algorithms were previously presented and the performance of the RSM technology using the National Institute of Standards and Technology (NIST) Special Database 27 latent print data set was demonstrated; this presentation reports on new results of testing the current toolset by latent print examiners within various organizations.¹⁻³

Latent prints often offer the best possibility for identification of suspects in criminal and terrorist cases; however, latent prints are also the most difficult to analyze by conventional automated methods due to poor image quality and limitations of the number of minutiae available for identification. The challenge in matching latent prints becomes one of establishing identity within the constraints of the limited information provided.

The presented toolset incorporates a unique method that establishes how well one fingerprint will overlay onto another. The overlay leads to a score that provides a quantitative assessment of the fit between prints with the objective of determining whether two fingerprints potentially came from the same finger. This method permits fingerprint matching using the rich feature set provided by the ridge structure enabling matching to take place without reliance on traditional minutiae (i.e., ridge edgings and bifurcations).

The toolset runs a latent against a reference print database, such as the prints returned from an Automated Fingerprint Identification System (AFIS) search or a set of prints of interest in a specific case. The toolset performs totally automated processing of the latent and reference images to include: (1) quality masking of the images; (2) binarization of ridges and furrows; (3) invertible non-linear overlays of the latent on each reference image; (4) pixel-based scoring of overlays to reference images based on the accuracy of the overlays; (5) automated locating and minutiae markup in the latent and all reference images; (6) visual assessment of level-3 features (when level-3 feature information is available in the images); and, (7) interactive image overlays supporting comparisons of latent and reference images that allow real-time visual assessment of corresponding locations in the latent and each reference print.

The magnitude of the score indicates the degree of similarity between the latent and the reference image. Scores are pixel-based for pixels along the one-pixel-wide centerline of both ridges and furrows; that is, scoring is an aggregate of the similarity of a latent and a reference print on the totality of ridges and furrows within an examiner's region of interest as marked on the latent print.

This presentation will also showcase the listed functionalities through a live demonstration of the toolset, specific to latents and reference print databases. Through this presentation, latent print examiners will become aware of enhancements to latent print examination through the RSM technology that potentially reduce examiners' labor and permit a broader quality range of latent prints to be exploited.



Criminalistics Section - 2015

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Fingerprints, Minutiae, Ridge Flow



B108 Fingerprint Identification and Error-Rate Estimation Based on the Congruent Matching Cell (CMC) Method

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After attending this presentation, attendees will learn how to discriminate between matching and non-matching fingerprint image pairs by determining congruency of cell pairs divided from the original fingerprint image pair, as well as the error-rate estimation procedure.

This presentation will impact the forensic science community by providing fundamental scientific and statistical support to fingerprint identification.

The CMC method is an approach originally developed at the National Institute of Standards and Technology (NIST) for the accurate forensic identification of firearm tool marks. The CMC method is applied in fingerprint identification, aiding in the evaluation of the strength of fingerprint evidence by providing a technique to determine the random match probability of fingerprint evidence as well as the probability of false positives and negatives. In this presentation, promising initial results on the application of the CMC method to fingerprint identification are described.

The CMC method for comparing two samples is based on the correlation of pairs of small cells instead of the entire sample. Two sets of cells are a congruent matching pair if they have a high degree of similarity, typically expressed by the maximum value of the area Cross-Correlation Function (CCF_{\max}) and if their registration position and orientation are consistent with those of other congruent cells. Thus there are three sets of parameters identifying cell pairs originating from the same source: (1) the CCF_{\max} value; (2) registration position x and y ; and, (3) registration angle θ , with associated thresholds T_{CCF} , T_x , T_y , and T_θ . An identification requires a certain minimum number of CMCs. It was observed that the correlation of cells instead of the entire surface yields a good statistical separation between the number of CMCs for matching and non-matching samples, even if major sample areas have missing features. The CMC method also enables an approach to estimating error rates. The combined false positive and false negative identification probability for each correlated cell pair, P_1 and P_2 , can be estimated from the statistical distributions of the three sets of identification parameters and their thresholds. These probabilities are then used to estimate the probability of a false exclusion or false identification for a given number of compared cells and observed CMCs.

After modifications, the CMC method was applied to fingerprint identification. Forty-four fingerprint images randomly selected from the NIST fingerprint (10-print) database were compared. Each fingerprint image was divided into a cell array with an average of 225 cells. The number of CMCs for the 924 Known Non-Matching (KNM) fingerprint pairs were distributed in a range from 0 to 5. The number of CMCs for the 22 Known Matching (KM) fingerprint pairs were distributed in a range from 8 to 60. The initial results show significant separation between the KM and KNM CMC distributions and no false identifications or false exclusions were made. This study hopes to obtain a wider separation after tailoring the registration algorithms and CMC criteria to fingerprint patterns. Error-rate estimation will require tests on larger and more varied fingerprint sets to estimate typical "local" cell-matching probabilities in the patterns for images of various quality levels. It avoids errors introduced by incorrect feature identification, which lowers identification accuracy when comparing imperfect fingerprint images.

Fingerprint Identification, Error Rate, Congruent Matching Cells



Criminalistics Section - 2015

B109 Development of Fingerprints From Items Submerged in Fresh Water Over Time

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After attending this presentation, attendees will better understand which fingerprint development and analysis techniques to use in cases involving items submerged in different bodies of fresh water.

This presentation will impact the forensic science community by raising awareness of the fact that little research has been conducted on developing fingerprints from items that have been submerged in water environments. Previous research on obtaining fingerprints from submerged items has been performed in a laboratory setting using deionized water. Since deionized water is not commonly found outside a laboratory setting, the results from the previous study are not beneficial for possible crime scene settings. It is expected that the results from previous studies will vary from the results of this study. Unpublished research has been performed on developing fingerprints from items submerged in saltwater environments.

In this study, a variety of types of items were placed in three different fresh water environments: a tub filled with tap water, a chlorinated pool, and a lake. Items that were placed in the pool and lake setting were placed in a mesh laundry bag for ease of recovery. The items that were examined included glass bottles, plastic bottles, copper piping, stainless steel piping, PVC piping, duct tape, and plastic bags. These items were selected because they are non-porous and are frequently found as items of evidence. Two different types of metal piping were selected to show that different metals produce different results in the same environments. Non-porous items were selected because the methods used for development work best on these types of items. Two sets of items were set up for each day. A depletion series of five thumbprints from the primary investigator's right hand was placed on each item. The items were then submerged in one of the three different environments, and removed in 24-hour increments. The first set was developed using Small Particle Reagent (SPR) and the second set was allowed to dry. After the items were dried, they were developed using cyanoacrylate fuming. The cyanoacrylate fuming was performed at the Montgomery County District Attorney's office in Norristown, PA. Based on this research, fingerprints containing level 2 detail can be developed from items submerged in fresh water for multiple days using SPR and cyanoacrylate fuming, meaning that investigators should attempt recovery of fingerprints from items even though the item has been submerged in a fresh water environment.

Fingerprints, Submerged Items, Small Particle Reagent (SPR)



B110 DNA Profile From a Fingerprint Developed With a Columnar Thin Film

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After attending this presentation, attendees will understand that an emerging fingerprint-development technique preserves DNA from body fluids on a fingerprint and that good quality DNA profiles can be obtained from the fingerprint. It is hypothesized that the developed body-fluid fingerprints will be of better quality than those that have not been developed and that the fingerprints will give the same quality DNA profiles.

This presentation will impact the forensic science community by promoting knowledge of the options available to forensic scientists in developing body-fluid fingerprints. Moreover, this presentation describes how to obtain DNA from fingerprints and subjectively and objectively grade the quality of those fingerprints. The Columnar Thin Film (CTF) method is also analyzed in regard to fingerprint quality and the potential for DNA preservation.

DNA and fingerprint analyses are the two major methods of identification used in forensic science. If these two methods can be implemented on the same sample, identification of a suspect or a victim can be greatly improved. In this research, blood was collected by pricking the finger and smearing it on the fingertip. Saliva was collected by dipping fingers into saliva. Sixty fingerprints produced with blood and 40 fingerprints made with saliva (100 total fingerprints) were placed on brass. Fingerprints were allowed to dry at room temperature and CTF deposition occurred the following day. Half the fingerprints were developed with CTF technique and the other 50 remained undeveloped.

The CTF technique involves the resistive heating of a material, which sublimates and condenses conformally as a tight stack of upright nanoscale columns atop a fingerprint. The CTF entombs the entirety of the residue, serving as a barrier between the residue and the environment, and potentially preserving DNA in the residue.

After the fingerprints were developed, the substrate was swabbed to collect the residue comprising the fingerprint emulsion, the body fluid, if necessary, and the CTF. All samples underwent DNA analysis. In the case of the fingerprints with body fluids, the DNA was extracted from the collected residue and quantitated using Real-Time Polymerase Chain Reaction (PCR) technology. Concentration of DNA ranged from 0.226ng/ μ L to 7.05ng/ μ L. Approximately 1.0ng of DNA was amplified with the Identifiler® Plus amplification kit for all fingerprint samples. DNA from the amplified product was detected by capillary electrophoresis injection on the Applied Biosystems® 3130xl Genetic Analyzer. The generated data was analyzed using GeneMarker® HID Software from SoftGenetics®.

Latent fingerprints without body fluids were also harvested and placed on glass. With these latent fingerprints, the extraction procedure involved low-template DNA-analysis methods previously used in this study's laboratory. DNA was quantified using the Trio kit from Applied Biosystems® and the InnoQuant™ kit from InnoGenomics. The yield of DNA from fingerprints, which had been aged for defined periods of time, allowed the researchers to compare the degree of degradation which took place between the enhancement techniques.

This research indicates that fingerprints wetted with body fluids and developed with the CTF technique are better quality than latent fingerprints. It was possible to generate complete DNA profiles from 100% of the fingerprints wetted with body fluids and developed by the CTF technique. The profiles were concordant with the reference samples of the donors. Of the fingerprints wetted with body fluids and not developed by the CTF technique, complete profiles were obtained from 83.33% of the samples, partial profiles were generated from 8.33% of the samples, and no profile was obtained from 8.33% of the samples. Since a measurable amount of DNA and complete DNA profiles were obtained from these fingerprints after CTF exposure, it was determined that the CTF had no negative effect on the methods of DNA analysis, and therefore it is believed that the DNA profiles obtained from CTF-developed fingerprints are of the same quality as non-developed fingerprints. This research also indicates that there is a sufficient quantity of DNA in multiple latent fingerprints to analyze DNA amplicon length ratios. Thus, it is possible to determine the amount of degradation in the sample. Overall, the combination of the CTF-development technique and DNA analysis could prove useful for identification purposes in a crime laboratory.

Fingerprints, DNA, Columnar Thin Film



B111 Mobility Empowered and Sustainable AFIS (MESA)

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After attending this presentation, participants will better understand the capabilities as well as the possibilities of off-the-shelf smartphone technology as a fingerprint-capture device in support of identity matching.

This presentation will impact the forensic science community by reporting on the development and testing of a novel approach called MESA (Mobility Empowered and Sustainable AFIS (Automated Fingerprint Identification System)) technology for using the forward camera on a conventional smartphone as a fingerprint-capture device. No additional sensors are used on the device. MESA represents a software-only solution and the use of this technology will greatly broaden the availability of fingerprint data for law enforcement, intelligence, and defense purposes.

This presentation will introduce MESA as a means of capturing useful fingerprint information through conventional smartphones. Smartphones are ubiquitous devices with very powerful sensor capabilities. The typical smartphone has an eight-megapixel camera which offers the opportunity for high-resolution photography of fingers and hands suitable for biometric identity matching. Fingerprints are truly the “human barcode” and among the best measures of human identity available. Fingerprints are similar to DNA as biometric identifiers because they can be obtained either directly from individuals or from items that individuals have touched or places they have been. An additional advantage of fingerprints is they are readily captured through well-proven techniques; however, “traditional” fingerprints represent only a portion of what the hand offers in terms of identity. Other data available takes the form of “palmprints” which is a class that includes not only the palm but also includes the second and third joints of the fingers and the finger sides and tips. Because the focus of current fingerprint-capture technology is on fingers, the palm class of prints is often ignored. In fact, most portable scanners have no palm capability — but smartphones, through their high-resolution cameras, do have the capability of capturing images from the palms as well as the fingers.

Underlying MESA is an innovative method for matching latent fingerprint fragments only a few millimeters in diameter. The method is based on the ridge-specific marker technology that employs a unique algorithm that establishes how well one fingerprint will overlay another and can be used for matching as well as comparing multiple images from the same finger to obtain the best composite result. This method is important when dealing with fingerprint photographs from smartphones since there are many factors — focus, movement, and image occlusion due to lighting — that can cause difficulty during the matching process.

The discussion will address the issue of standards as they relate to fingerprints generated from photographic images and touch on the issue of fingerprints as “big data” given the opportunity to expand collection. Finally, the presentation will showcase the power of smartphone fingerprint capture through a live demonstration of a fingerprint captured in real time and matched against a laptop-based AFIS. Through this presentation, all practitioners who work with fingerprints as an identification tool will become aware of how a widely used tool can become a critical asset in the identification process.

Fingerprint, Mobile, AFIS



B112 Bloodstain Patterns Associated With Gunshot Wounds — Misconceptions

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The goal of this presentation is to provide attendees with a better understanding of the conditions under which gunshot wounds will produce blood droplet spatter.

This presentation will impact the forensic science community by having a positive impact on bloodstain pattern interpretation in shooting cases by clearing up misconceptions.

Much confusion appears to exist concerning bloodstain patterns produced by gunshots. This confusion can lead to misinterpretations in attempts to reconstruct past shooting events transpiring at crime scenes. Confusion on the part of laypersons such as lawyers, judges, or the members of a jury may be at least partially attributable to graphic media portrayals of shootings in film and television where volumes of blood spurt out of the wounds. If transcripts of testimony and textbooks are a good indication, confusion also exists among some experts charged with the responsibility of reconstructing shootings. The latter may be attributable to poor training, lack of a suitable scientific education, poorly designed experiments, and inappropriate published photographs in training materials.

Projectiles (e.g., bullets) impacting or traversing materials often can be expected to produce secondary projectiles. These secondary projectiles can consist of fragments of the materials struck or fragments of the projectile itself in the case of a hard target surface such as concrete or steel. The type of secondary projectile which predominates depends on the hardness of the initial projectile relative to the hardness of the material with which it interacts. In controlled experimental work, the secondary projectiles can be studied using high-speed photography and/or by placing “witness papers” near the point of impact or projectile exit. Witness papers are specially prepared surfaces, commonly white paper supported by a frame, used to reveal the path of a projectile or secondary projectiles. Of course, in “real world” case investigations, neither of these recording mediums is typically available. Any record produced of such secondary projectiles will depend upon the fortuitous proximity of surfaces capable of retaining evidence of such secondary projectiles.

Published photographs of a bullet traversing a blood-saturated sponge are very misleading. Something in the vicinity of 90% to 95% of the weight of a blood-soaked sponge or section of open cellular foam is due to the blood contained therein. This is not a good experimental model for shots traversing many regions of the human body. Only about 5% to 7% of the weight of a human body is blood. In addition, the blood is not evenly distributed throughout the body. In most regions and tissues in the body, the blood is confined to capillary beds. The function of the fine-diameter capillaries is to provide more intimate contact between the oxygen carrying red cells and somatic tissues dependent upon oxygen. Life-sustaining oxygen transport would be inefficient without hemoglobin and small-diameter capillaries. The capillaries have inside diameters of approximately 5 μ m. Some publications discussing blood spatter produced by gunshot wounds refer to drops with stain diameters of 1mm as being diagnostic for gunshot-derived blood spatter. This dimension is 200 times that of the capillary diameter. Thus, for a bullet traversing capillary-supplied tissue, no coalesced volumes of liquid blood are encountered. The bullet may carry tissue and fragments of capillaries with it and deposit them on a surface near the exit wound (or even on a surface near the entrance wound). In this circumstance, there will be no droplets of blood deposited. In actual cases, histological examination of the deposited tissue fragments has revealed fragments of capillaries with red cells lined up single-file within them. Again, there are no blood droplet stains. On the other hand, if the bullet encounters a volume of coalesced blood along its path, such as that contained in larger blood vessels or even the heart, fine droplets of blood can contribute to the spatter pattern. After a bullet has traversed capillary-supplied tissue, blood will flow into the wound channel, coalesce to form larger volumes, and may be available for spattering during a follow-on shot to the same area. Clothing which becomes saturated with blood flowing from a wound may behave like the blood-soaked sponge discussed above, if it is struck by another bullet. Additional ways in which gunshots can produce fine droplet spatter will be discussed.

The point needs to be made that special conditions are required in order for a gunshot to produce airborne droplet spatter patterns. Principally, there must be a volume of coalesced blood along the bullet path along with a suitable nearby target to collect and record the pattern of any blood droplets that are projected from the wound. These points will be illustrated using experimental models and actual case examples.

Blood Spatter, Gunshot, Shooting Reconstruction



Criminalistics Section - 2015

B113 The Hunt for Aaron Bassler: A Multidisciplinary Criminalistics Case Study Incorporating Proper Crime Scene Evidence Collection, DNA, Officer-Involved Shooting, and Firearms Comparison

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After attending this presentation, attendees will better understand a criminalistic's case study that encompasses crime scene response, proper crime scene evidence collection, DNA, and firearms comparison.

This presentation will impact the forensic science community by reviewing a comprehensive criminalistics case that explores the inter-relationship between client agency investigations and the criminalistics laboratory.

On August 11, 2011, a gunshot homicide victim was found in a remote forest area of Mendocino County, CA, by the Mendocino County Sheriff's Office. The unknown suspect had defecated at the scene and the Mendocino County Sheriff's Office believed this to have good evidentiary value for a DNA and Combined DNA Index System (CODIS) search. Detectives from Mendocino County Sheriff's Office contacted a senior criminalist at the California Department of Justice Eureka Laboratory regarding submission of feces evidence left at a crime scene. The detectives were advised that the sample was inappropriate for DNA analysis. After determining there was no firearm evidence left at the scene, they were advised to return to the scene to look for a more appropriate sample such as a bottle, can, partially eaten food, or cigarette butt. Mendocino County Sheriff's Office personnel returned to the scene and collected a tinfoil marijuana joint.

On August 27, 2011, a second gunshot homicide victim was located in a remote forest area near the town of Fort Bragg, CA, also in Mendocino County. A witness to this shooting helped Mendocino County detectives identify Aaron Bassler as a possible suspect. Due to the apparent type of weapon used, the wilderness location of the homicide, and familiarity with the suspect, the Mendocino County Sheriff's Office detectives believed that Aaron Bassler was the suspect for both homicides. Another senior criminalist at the California Department of Justice Eureka Laboratory was called in to assist in processing the scene of the second gunshot victim. Among the items collected at the scene was a tinfoil marijuana joint. The first senior criminalist collected swabs from the marijuana joints at each scene and submitted the swabs to the California Department of Justice Redding Laboratory for DNA analysis. Due to the fact that the suspect had not been apprehended, a reference bloodstain was made from a blood sample from Aaron Bassler's previous Driving Under the Influence (DUI) submission. The DNA profiles from the evidence from both scenes were the same as Aaron Bassler's.

Two homicide victims in a small, rural county caused great concern for the citizens of Mendocino County. The Mendocino County Sheriff issued multiple press release statements declaring that apprehending the suspect was a top priority. A five-week manhunt ensued that involved Special Weapons And Tactics (SWAT) teams from Mendocino County, Humboldt County, Napa County, Sacramento County, and Alameda County as well as United States Federal Marshals, numerous search and rescue teams, and K-9 units. The suspect was able to elude the agents at length due to his familiarity with the remote surroundings, the very difficult terrain, and his survivalist mentality. At one point during the manhunt, Bassler was observed by an Alameda County SWAT officer and gunfire was exchanged. After he had fled the area, fired cartridge cases were collected from the rifle used by Bassler during this incident.

The manhunt finally came to a conclusion when Sacramento County SWAT officers shot and killed Bassler. The weapons from the officers involved in the fatal shooting of Bassler as well as Bassler's rifle were submitted to the first senior criminalist for comparison to the fired cartridge cases from the second homicide scene and both officer-involved shootings. The cartridge cases were most likely fired from Bassler's rifle.

Crime Scene, DNA, Firearms Comparison



B114 Polymer Replication of Reference Bullets Using Advanced Casting and Metal-Coating Techniques

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After attending this presentation, attendees will understand the concepts of creating polymer replicas from reference bullets. Vacuum-degassing and pressurization techniques are discussed for removing micro-bubbles. Sputter-coating techniques are also discussed which give hard-coating surfaces to the replicas, thereby increasing the durability.

This presentation will impact the forensic science community by providing a high-quality method for the transfer of evidence between jurisdictions. This normally results in a chain-of-custody issue; however, the transfer of replicas instead of original evidence allows agencies to work together in solving violent crimes. The sharing of evidence could lead to breakthroughs in criminal cases where it was not previously possible.

This presentation describes the polymer casting/replication and metal-coating techniques that are being used at the National Institute of Standards and Technology (NIST) to improve the quality and durability of replicated reference bullets using polymer casting methods.

Tool marks created by a firearm on a bullet or cartridge case are very important in the forensic examination of violent crimes. In many cases where violent crimes are committed in multiple jurisdictions, the transfer of ballistic evidence from one jurisdiction to another would be beneficial in helping to solve these cases; however, the transfer of ballistic evidence from one laboratory to another is typically not possible due to chain-of-custody requirements. The replication of ballistic evidence using polymer casting techniques is a perfect solution to this problem. High-quality replicas of the bullets can be distributed and analyzed, preserving chain-of-custody requirements of the original evidence.

NIST has been developing a polymer replication technique based on earlier work at the Bundeskriminalamt in Germany.¹ A vacuum-degassing process has been developed that removes the majority of micro bubbles from the replication materials during mixing. Pressurized chambers are also used during the curing phase to eliminate any remaining bubbles from the material so that near-perfect replications of the original bullets are created.

The process of making a replica bullet is explained with emphasis on the vacuum-degassing and pressurization steps that are necessary in creating clean replicas free of micro-bubbles. Comparisons of the replicas to the original master bullets are performed by measuring or imaging the striated features. Optical bright field microscopy is used for qualitative comparisons and high-resolution measurements of the surface topography are performed using disk-scanning confocal microscopy and surface profilometry. Analysis of the surfaces using mathematical correlation algorithms is then performed to make objective comparisons of the replicas and quantify their similarity to the master bullets.

The NIST Standard Reference Material (SRM) 2460 Bullets are used as a basis for the replications. The SRM bullets were originally manufactured using a computer numerical control-based diamond turning process. This is a highly repeatable process that precisely machines the striated markings onto the bullet surface. The surface topography of these bullets is well defined and each one is virtually identical to the other. Validation of the replication process is possible through the quantitative analysis of the SRM bullets and their replicas.

Durability of the replicas has also been investigated. Due to the relatively soft nature of polyurethane, additional hardening of the surfaces is required for the replicas to be durable enough for forensic work. A metal sputter-coating process is used that coats the replicas with a very thin layer of metal. The specific type of metal coating can be varied depending on the application. A thin layer of gold coating is an excellent barrier to tarnish/aging of the base material. It also reduces static charging and can be useful if scanning electron microscopy analysis is utilized in the examination. If additional hardness is required to reduce the risk from handling or abrasives, the gold coating can also be combined with other elements such as chromium or platinum. These coatings leave an excellent finish that is both durable and good for use with optical inspection methods. The sputter-coating process will be discussed, as well as additional correlation analysis showing comparisons from before and after the coatings.



Criminalistics Section - 2015

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Bullet Replication, Polyurethane Casting, Sputter Coating



B115 Reporting Error Rate for Firearm and Tool Mark Identifications in Forensic Science

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After attending this presentation, attendees will appreciate the challenges of conducting evidence-based research to establish a scientific procedure for quantitative error rate reporting to support ballistics and tool mark identifications in the United States.

This presentation will impact the forensic science community by explaining a new method, Congruent Matching Cells (CMC), developed for accurate ballistics identification and error rate calculation.

Side-by-side image comparisons for ballistic identification have more than a 100-year history; however, as stated in a 2008 National Research Council Report: (1) “The validity of the fundamental assumptions of uniqueness and reproducibility of firearms-related toolmarks [sic] has not yet been fully demonstrated.”; (2) “Recent court decisions in which admissibility of firearms toolmark [sic] evidence was in question have generally accepted that the field has validity but have refused to accept ‘exclusion of all other firearms’ arguments.”; and, (3) “Since the basis of all forensic identification is probability theory, examiners can never really assert a conclusion of ‘identification to exclusion of all others in the world,’ but at best can only assert a very small probability of a coincidental match”.¹⁻²

However, for DNA-based identifications, the 2009 National Academy of Sciences Report, *Strengthening Forensic Science in the United States: A Path Forward*, stated: “The courts already have proven their ability to deal with some degree of uncertainty in individualizations (with its small, but nonzero, error rate)”.³

It has been a fundamental challenge in forensic science to conduct evidence-based research for establishing a scientific procedure for quantitative error-rate reporting to support ballistics and tool mark identifications in the United States.

The National Institute of Standards and Technology’s (NIST’s) Forensic Topography and Surface Metrology Project seeks to support United States forensic identifications by developing novel physical standards and measurement systems. The research team has developed NIST Standard Reference Material (SRM) bullets and cartridge cases which are being used in the United States and foreign crime laboratories as reference standards.^{4,5} The team also developed topography measurement systems and correlation software for bullets and cartridge cases, which have been used in support of the 2008 National Academies Study and in a laboratory comparison of ballistic imaging systems in crime labs.^{4,6,7} In addition, results of two studies have shown a high-correlation accuracy using the NIST topography correlation system relative to a commercial system.^{6,8}

NIST researchers have recently developed the new CMC method for accurate ballistics identification and error-rate calculation. The CMC method is based on correlations of pairs of small correlation cells instead of correlations on the entire images. Three types of identification parameters are created for uniquely identifying correlated cell pairs originating from the same firearm. This enables an approach to calculating error rates based on the total number of correlation cells, the number of qualified CMC cell pairs, and the false positive and false negative identification probability for an individual cell pair. Validation tests of the CMC method have recently been completed using 40 cartridge cases fired with consecutively manufactured pistol slides. These tests include 717 Known Non-Matching and 63 Known Matching image correlations. The results do not produce any false positive or false negative identifications and so provide strong initial support for the effectiveness of the CMC method. A method for calculating error rates has also been developed using the CMC approach. It is believed that this method can be adapted to large databases and used for supporting ballistics identifications in court proceedings in a manner similar to methods used for DNA identifications.



Criminalistics Section - 2015

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Forensic Science, Firearm Identification, Error Rate



B116 NIST Reference Ballistic Tool Mark Database for Research and Development of Identification Systems and Confidence Limits

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After attending this presentation, attendees will understand how the National Institute of Standards and Technology (NIST) established its reference ballistic tool mark database. This includes meta data categorization, standard file exchange format, and test fire selection. Attendees will also learn why this reference database is important to the evolving field of objective firearm and tool mark analysis.

This presentation will impact the forensic science community by sharing how the research database and the infrastructure developed in this project provide a growing, shared, scientific knowledge base on the degree of similarity that can be found between marks made by different firearms and the variability in marks made by an individual firearm. This is achieved through a large variety of challenging datasets representing: (1) test fires conducted using consecutively manufactured barrels, slides, firing pins, and other firearm parts; (2) test fires conducted using the same firearm, with large numbers of intermediate firings to represent varying degrees of firearm wear; and, (3) test fires conducted using different brands of ammunitions.

The database contains test fires characterized by using state-of-the-art measurement equipment and measurement protocols at NIST. The database will drive the development and validation of mathematical criteria, algorithms, and systems for objective firearms identification. This is achieved through a unique focus on challenging scenarios, such as consecutively manufactured firearm components, persistence firings, and different ammunition types. These research datasets cannot be obtained from existing forensic databases such as the National Integrated Ballistic Information Network (NIBIN). It is not economically feasible for a single entity such as a university or system developer to generate the variety of data sets required for broadly applicable results. The database will contain both reflectance microscopy images and 3D surface topography data to ease the transition to 3D surface topography metrology.

The challenging identification scenarios provide researchers, for the first time, with the large variety of data needed to assess worst-case variability and repeatability, providing a path to the development of scientifically justified methods that yield identification results with well-characterized, quantitative confidence limits.

The project objective is an open-access research database of bullet and cartridge reference data, consisting of traditional reflectance microscopy images and 3D surface topography. The database will foster the development and validation of advanced algorithms, mathematical similarity criteria, and quantitative confidence limits for objective ballistics identification.

The 2009 National Academy of Sciences Report, *Strengthening Forensic Science in the United States: A Path Forward*, called into question, among other issues, the objectivity of visual tool mark identification by firearms examiners.¹ The National Academy of Sciences recommended development of objective tool mark identification criteria and error-rate estimates. Industry, academia, and government laboratories are pursuing two promising approaches toward this goal: (1) development of mathematical criteria and advanced algorithms for the objective and automated identification and scoring of potential matches; and, (2) supplementing traditional reflectance microscopy images with 3D surface topography measurement data.

Development and validation of both of these approaches to objective tool mark identification are hindered by a lack of access to tool mark data sets that: (1) represent the large variety of ballistic tool marks encountered by tool mark examiners; and, (2) represent challenging identification scenarios, such as those posed by consecutively manufactured firearms components. It is not economically feasible for individual companies or institutions to generate their own data sets. This makes it difficult for these entities to objectively evaluate their solutions. During the symposium held at NIST entitled, "Measurement Science and Standards in Forensic Firearms Analysis 2012," one of the priority requests from the attendees was the construction of a database where bullet, cartridge case, and tool mark surface data can be shared between researchers to facilitate testing, refinement, and comparison of new systems, methods, and algorithms.



Criminalistics Section - 2015

The database will provide the representative variety of tool mark data required, ranging from crime laboratory test fires to test fires conducted using consecutively manufactured barrels, firing pins, slides, and other firearm surfaces. The database will contain both reflectance microscopy images and 3D surface topography data. The database will consist of indexed surface data acquired at NIST using state-of-the-art instruments and measurement procedures. Some of the data collected will be stored in a closed database for possible future application to the validation and comparison of correlation software.

The database will enable researchers to test and validate new approaches to objective, mathematics-based tool mark identification while easing the transition to 3D surface topography data. The database will provide a foundation for a scientific knowledge base on the degree of similarity that can be found between marks made by different firearms and the variability in marks made by an individual firearm. The current “fairly limited” knowledge base is a fundamental barrier to the development and validation of objective mathematical similarity criteria and associated confidence limits applicable to a broad range of firearms and ammunition brands.¹

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Reference Database, Ballistic Tool Marks, Confidence Limits



B117 Improved Congruent Matching Cells (CMC) Method for Optical Images Identification of Cartridge Cases

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After attending this presentation, attendees will understand a new theory of ballistics identification, the implementation of this method, and the applications on cartridge case identifications.

This presentation will impact the forensic science community by applying the proposed CMC method to cartridge case identification. The method can decrease the effect from “invalid areas” of images and improve the accuracy of identification.

The CMC method was invented by the National Institute of Standards and Technology (NIST) with the ultimate goal of providing objective and high-accuracy ballistics identifications and evidence searches. The CMC method is based on the correlation of pairs of small correlation cells instead of correlation on entire images to identify the “valid correlation areas” and eliminate the “invalid correlation areas.” Four identification parameters are proposed for uniquely identifying correlated cell pairs originating from the same firearm. The correlation conclusion (matching or non-matching) is determined by the qualified CMC numbers $C \geq 6$.¹ This method has already been validated by a set of 780 pair-wise 3D topography images captured on 40 cartridge cases fired from handguns with ten consecutively manufactured pistol slides; however, most ballistics images stored in current national databases are optical intensity format.² As a result, the reliability of applying the CMC method on optical intensity images is an important issue.

To verify the effectiveness of the CMC method on the optical images, this presentation initially introduces the implementation of the CMC method and provides a fast correlation method and the strategy for threshold determination. Then the optical intensity images captured on the same set of 40 cartridge cases under the top-ring lighting source (which provides uniform light conditions) are correlated and analyzed. This includes 780 correlations of 63 pairs of matching images and 717 pairs of non-matching images. The tests of the method do not produce any false identification (false positive) or false exclusion (false negative) results, which supports the CMC method and the identification criterion $C=6$ for firearm identifications using optical intensity images. To improve the identification accuracy of the CMC method, a new method has been developed to process cell correlations at each rotation angle and combine the forward and backward comparisons to improve the identification accuracy. In the initial test, the CMC method identifies all of 780 correlations correctly. Compared with the result of the original method, the improved result shows a larger gap between the matching and non-matching correlations, which proved that the improved CMC method is effective in improving the accuracy of identification. Based on results of the study, it is considered that the principle of the CMC method is effective in firearms identifications in both 3D topography images and optical images of cartridge cases. The proposed identification criterion $C=6$ is also working well in current experiments.

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2. W. Chu, M. Tong and J. Song, Validation Tests for the Congruent Matching Cells (CMC) Method Using Cartridge Cases Fired with Consecutively Manufactured Pistol Slides, *AFTE Journal*, 45, 4, 2013, p361-366.

Ballistics Identification, Cartridge Case, Congruent Matching Cells (CMC)



B118 Toward a Novel, Fast, and Accurate 3D-Topography Imaging and Analysis System for Firearm Forensics

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After attending this presentation, attendees will be familiar with a novel 3D imaging and analysis system, the image acquisition technology, and the results of several cartridge casing matching experiments. This presentation will describe TopMatch-GS 3D, an accurate, fast, and low-cost 3D imaging and analysis system for cartridge casings.^{1,2}

This presentation will impact the forensic science community by educating attendees on the latest generation of technology for 3D tool mark imaging and analysis. The use of a confidence score and interpretable match results will provide practitioners with a firm foundation on which to place identification results.

The work proposes that the 3D scanning system, TopMatch-GS 3D, is capable of acquiring high-resolution surface scans, visualizing these scans, and accurately comparing/matching pairs of casing scans. The study investigated if the matching algorithm is capable of recognizing known matches with high accuracy while keeping the rate of false positives to near zero.

The prototype TopMatch-GS 3D scanner incorporates the GelSight[®] retrographic sensor to measure 3D surface topography at a resolution of 1.4 microns per pixel.^{3,4} The system was evaluated on a large data set containing casings from several hundred 9mm Luger[®] firearms, representing more than 20 firearm manufacturers and seven ammunition types. The firearms come from several crime laboratory reference collections and were not selected on their ability to produce strong tool marks. The casings represent the types of evidence and test-fires seen in a real-world setting. They contain milled, filed, granular, and striated marks as well as poorly marked casings. Data collection took place both in-house and at several collaborating California crime laboratories. Participants across these locations include trained firearms examiners, technicians, forensic science students, and university faculty.

The algorithm's match score is a function of the similarity between the casings' true breech-face impression and their aperture shear. For breech-face impression comparison, automatically identified distinctive features (corresponding to informative microscopic tool marks) are used to match and align two casings. By requiring spatial coherence of matched features, the methodology is able to strongly indicate when two casings were fired through the same firearm. In contrast to cross correlation-based methods, feature-based techniques compute the match score using only the portions of the surface identified as informative (i.e., the matching microscopic tool marks). The algorithm compares aperture shears by first extracting the linear shear profile and then aligning two profiles while accounting for baseline correction and warping.

The scoring function is a confidence score where each candidate match (pair of casings) is scored based on the likelihood that the two casings were fired through the same firearm. Unlike other systems, the TopMatch score reflects the true confidence of the match. The confidence score allows examiners to efficiently limit their search. In other words, examiners only need to consider candidate matches that score above a threshold.

The TopMatch system is able to match casings with high accuracy. True positives (known matches) have extremely high scores while true negatives (known non-matches) have low match scores. There are virtually no false positives (i.e., known non-matches mistakenly identified as a match).

The TopMatch-GS 3D imaging and analysis technology represents a promising new method for measuring and correlating the true 3D surface topography of cartridge casings. The results from several deployment studies and the large set of real-world casings demonstrate accurate matching results with an extremely low false positive rate (near zero as of the time of this writing).



Criminalistics Section - 2015

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 2. Lilien, Brubaker, Duez, and Weller. "Progress Towards a Novel 3D-Topography Imaging and Analysis System for Firearm Identification, TopMatch-GS 3D" The Association of Firearm and Tool Mark Examiners (AFTE) Annual Training Seminar (Seattle), 2014.
 3. Johnson and Adelson. "Retrographic Sensing for the Measurement of Surface Texture and Shape." Proc. of the IEEE Conf. on Computer Vision and Pattern Recognition (CVPR), 1070-1077, 2009.
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Firearm Forensics, Tool Mark Analysis, 3D Surface Topography



B119 Evaluating the Gray Scale Response Difference Associated With Bullet Comparisons Using Optical Microscopy

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After attending this presentation, attendees will gain a greater appreciation for how tedious and delicate the task of bullet comparisons is and understand that the inherent error associated with these types of comparisons can be overcome with the proper approach.

This presentation will impact the forensic science community, specifically the firearms examiner faction, by providing results from a controlled experiment with a known bullet profile. The reproducibility of this research will provide an opportunity for a starting point for future methodology that produces bullet comparisons with greater reliability.

Firearms examiners have long been interested in a method that would image the striae of a bullet so that it could be entered into a database for subsequent comparison to other bullets. As the development, use, and application of these databases grew, so did the problems associated with them. The algorithms were breaking down and the software was not functioning at the level of precision expected by firearms examiners, thus analysts stopped using the databases and returned to the traditional laborious methods of bullet analysis using the comparison microscope; however, the use of the comparison microscope is not without its own set of limitations. When using a comparison microscope, two separate stages with two separate light sources are necessary because the determination of the distribution of striae on a bullet is sensitive to bullet position and orientation. It is suggested that many of the problems associated with bullet-based database systems are directly related to methods in which images are captured, along with the number and type of images.

The goal of this research was to ultimately determine if bullet comparisons using optical microscopic imaging were possible, rather than live bullet-to-bullet comparisons. Once that was determined, which microscope would produce the best results for bullet comparison utilizing captured images and the minimum number of images that should be taken of a single land impression to account for all potential variations in bullet profiles was identified. To identify profile measurement error inherent in orientation and rotational and translational mis-orientation, the profile of a National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 2460 standard bullet was measured while misoriented in systematic ways using a light microscope. These measurements were then repeated with a Zeta 3D-20 optical microscope and a Leica® FSC comparison microscope to determine which microscope produced the greatest correspondence for misoriented NIST bullet profiles. The method applied utilized captured images that were converted into light contrast profiles, which were a representation of the bullet surface based on light intensities. From these profiles, identified peaks and valleys were grouped into resolution bins or segments of a particular length which were used to subdivide the land impression of the bullet and discriminate peaks and valleys. The results showed that the light microscope produced the highest level of correspondence with 100% total peak correspondence for both laterally and rotationally shifted profiles, 330µm and 6° respectively, with a 20µm bin resolution.

Based on this study it was concluded that bullet comparison through the use of images captured by optical microscopy is possible. It is thus suggested that something similar to the Zeiss Universal light microscope with reflected through-the-lens illumination be used for this methodology for bullet analysis and comparison using a bin resolution no less than 15 microns. With this microscope, the minimum number of images required to account for potential misorientation, or bullet profile variations, are 11 images per land impression.

NIST Bullet Comparisons, Bullet Comparisons, Bullet Images



B120 Critical Angles for 9mm Parabellum Bullets

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The goal of this presentation is to introduce attendees to the angle at which a ricochet will no longer occur when a bullet strikes some common substrates.

This presentation will affect the forensic science community by providing data that can be used as background when assessing a ricochet at a shooting scene.

Determining the angle at which a bullet will successfully ricochet is essential information when a shooting investigation involves indirect fire. This information provides the forensic scientist with fundamental data required for the scientific reconstruction and assessment of a shooting scene. Depending upon the substrate, the bullet's design, velocity, construction, and its angle of impact, a bullet may fail to ricochet upon impact or the bullet will successfully ricochet. Knowledge of bullet behavior with common substrates provides valuable information for scientific investigations of shooting scenes where bullets have impacted intermediate surfaces. A timely and accurate scene reconstruction is imperative in both the investigative and the adjudicative stages of a shooting incident.

Bullets discharged from firearms have a considerable amount of kinetic energy and achieve high velocity during the very short time they are accelerating down the bore of the firearm. Even accounting for some frictional losses as it is forced through the bore, the once-stationary bullet emerges as a fast-moving energetic projectile.

A bullet that is discharged from a firearm can ricochet off of the many different surfaces present at a typical shooting scene. Although bullets will behave differently depending upon the material they encounter, they are nevertheless bound by the laws of physics.

Potential ricochet surfaces may be categorized based on how the bullet interacts with the substance. Hard surfaces traditionally remain intact after low-angle bullet ricochet because they will not yield to the bullet's impact energy. Materials such as concrete, marble, granite, and steel are just a few examples that fall into this category. Bullet ricochets off of hard unyielding surfaces consistently have ricochet angles (the angle created between a line designating the bullet path and a line designated by the substrate surface) that are less than the bullet's angle of impact (the acute angle at which the bullet approaches the substrate surface).¹ Soft surfaces will not remain intact but instead will deform, deflect, or fail entirely, yielding to the bullet's impact energy. Materials such as wood, turf, sand, paneling, wallboard, thin automobile sheet metal, and water are just some examples of surfaces that fall into this category. Bullet ricochets off of relatively soft yielding surfaces routinely have ricochet angles that are greater than the impact angle of the bullet.² When a bullet is fired at either of these types of materials, whether hard or soft (i.e., unyielding or yielding, respectively), there is an angle at which a ricochet will no longer occur. At (and above) this "critical angle" the bullet may break apart into fragments after hitting the surface in the case of a hard unyielding substrate or it may remain intact, to either penetrate (embed but not exit) or perforate (enter and exit) the material in the case of a soft yielding substrate.

The critical angle for the 9mm full metal jacket bullet used in this study was determined to be between 7° and 8° for gypsum drywall, between 5° and 6° for automotive sheet metal, between 11° to 15° for sand, and approximately 15° for heavy steel plate, marble, and modular concrete block. For the purposes of determining the critical angle for the unyielding substrates, the retained weight of the bullet was used as the criterion to decide whether the angle had been reached. Once the recovered bullet lost more than 5% of its original mass, the critical angle was considered achieved; however, it must be appreciated that a fast-moving bullet with 95% of its original mass must still be treated with respect.

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1. Haag, L. Shooting incident reconstruction. *New York: Academic Press*, 2006: 123
2. *Ibid.* 131

Ricochet, Critical Angle, Shooting Reconstruction



B121 A Study of the Presence of Gunshot Residue in Pittsburgh Police Stations Using SEM/EDX and LC-MS/MS

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After attending this presentation, attendees will be informed about a novel approach that was used in the investigation of the likelihood of Pittsburgh police vehicles and stations being sources of both inorganic and organic secondary Gunshot Residue (GSR) contamination. Attendees will also better understand how to analyze both inorganic and organic GSR from a carbon-coated adhesive aluminum stub.

This presentation will impact the forensic science community by investigating the presence of both inorganic and organic GSR contamination in police stations and vehicles — something that has yet to be investigated. Several studies have shown that environmental sources such as cars, fireworks, or paints can produce particles that may be misinterpreted as characteristic inorganic GSR. Yet, by testing for both organic and inorganic GSR, this possibility of misinterpretation by environmental sources could potentially be eliminated, which in turn would greatly enhance the significance of the evidence.

A police station or vehicle contaminated with GSR could lead to arrested individuals testing positive for GSR even though they have neither recently fired a gun nor been in the vicinity of a fired gun. This in turn could result in the incrimination of the innocent as well as lessen the legitimacy of a positive GSR result. Due to the risk of secondary transfer of GSR by a police vehicle or station onto the suspect prior to GSR sampling, it is necessary to create a baseline for the amount of GSR present in a police station or police vehicle. The potential of police stations and vehicles being sources of secondary inorganic GSR contamination has been investigated using Scanning Electron Microscopy with Energy-Dispersive X-ray Analysis (SEM/EDX); however, the presence of organic GSR contamination has not been examined.

This research takes a novel approach by investigating the likelihood of Pittsburgh police vehicles and stations being sources of both inorganic and organic secondary GSR contamination. Seventy-three samples were collected from four Pittsburgh police stations and vehicles using carbon-coated adhesive aluminum stubs. The samples were automatically analyzed using the SEM/EDX for the presence of non-crystalline particles containing approximately 10% by weight antimony, barium, and lead. These particles were classified as characteristic inorganic GSR, while non-crystalline particles containing various combinations of lead, antimony, and barium with aluminum, titanium, and zinc were classified as consistent with inorganic GSR. For a sample to be considered positive for GSR, there must have been at least three characteristic inorganic GSR particles present. Only one characteristic inorganic GSR particle was found; no sample was classified as positive for GSR. These results suggest there is a small potential of secondary transfer of inorganic GSR by a police vehicle or station onto a suspect. To test for the presence of organic GSR, these same 73 samples were then extracted with methanol and analyzed using Liquid Chromatography with Tandem Mass Spectrometry (LC-MS/MS). Optimized liquid chromatography parameters and an optimized multiple reactions monitoring scanning method were developed to obtain the greatest sensitivity for the following investigated compounds: akardite II, ethyl entralite, diphenylamine, N-nitrosodiphenylamine, 4-nitrodiphenylamine, and 2-nitrodiphenylamine. The limit of detection for each of the above compounds was found to be 0.53, 0.40, 1.18, 3.78, 2.33, and 2.33 nmol L⁻¹, respectively. Recovery percent from spiked SEM stubs was also investigated.

Gunshot Residue, SEM/EDX, LC-MS/MS



B122 Detection of Organic Components of Gunshot Residue (GSR) of Carbon SEM Stubs by Raman Spectroscopy

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After attending this presentation, attendees will better understand the analysis of organic components of GSR by Raman spectroscopy and the increased probative value of the data produced. Attendees will further understand initial efforts at development of an analytical protocol which can identify both organic and inorganic components of GSR using a standard 12.7mm Scanning Electron Microscopy (SEM) stub with carbon adhesive tab.

This presentation will impact the forensic science community by demonstrating the applicability of Raman spectroscopy to an analysis scheme which currently relies on Scanning Electron Microscopy with Energy-Dispersive X-ray Spectrometry (SEM/EDS) to identify individual particles having a specific morphology and elemental composition.

Current methods of GSR analysis concentrate on identification of the inorganic components of ammunition primers. Single particles with morphology indicative of formation in a high heat environment and containing the elements lead, barium, and antimony are considered characteristic of GSR. SEM/EDS is the widely accepted method to determine both elemental composition and morphology. Although this is a very effective method, the probative value of inorganic GSR analysis is limited. Not only are there limitations on the sample containing three component particles, but current and future formulations of “green” lead-free ammunition will lead to an increasing probability of false negatives.

The organic component of the GSR has not been utilized during analysis thus far. Chromatography with mass spectrometry has been proposed as a way to identify the components of these organic compounds, but at the expense of the sample. There would be no way to identify if the inorganic components are present after this examination. With recent advancements in Raman spectroscopy technology, new techniques are able to be developed.

This study introduces early research into the ability to detect and characterize organic residues deposited on the standard 12.7mm SEM stubs, while still allowing for subsequent traditional SEM/EDS analysis.

Previously published results of the viability of organic GSR analysis by Raman spectroscopy were reproduced by firing several types of ammunition at short range into cloth targets and confirming the presence of particles of partially combusted propellant. The spectra of the unfired propellant were compared to the partially burnt propellant picked off of the fabric and were compared and observed to be consistent. To expand the scope of the initial analysis, 12.7mm SEM stubs with adhesive carbon tabs were mounted three inches on either side of the cloth target. Spectra consistent with results from unburnt propellant were able to be obtained by targeting individual particles on the surface of the carbon tab. Positive results show that it is possible to identify organic GSR components even in the presence of broad, dominant carbon D band at $1,350\text{cm}^{-1}$ and G band at $1,582\text{cm}^{-1}$. By using this non-contact and non-destructive approach, the GSR stub is available to be used for subsequent analysis on the SEM/EDS.

The next phase in the research involved a more realistic collection scenario. After each test firing, the shooter’s hands were sampled with individual GSR stubs in accordance with normal collection of inorganic GSR. Initial manual scans detected a small number of particles consistent with organic components of the propellant of the discharged ammunition. Although the initial number of particles detected was small, the findings are considered an important proof of concept that organic portions of GSR can be detected on samples using existing collection protocols.

Future areas to be researched are the optimization of instrument parameters to be able to accurately detect organic GSR particles, correctly characterize and classify particles by propellant type, and implement the use of software mapping features to set up an automated run similar to the inorganic GSR analysis by SEM.

This study indicates that it is possible to detect organic components of GSR on a standard 12.7mm SEM stub with adhesive carbon tab using already existing collection techniques. Since organic GSR shows far more variation both by manufacturer and even by individual types of ammunition by the same manufacturer, it may be possible to identify differing types of propellants, greatly increasing the probative power of GSR analysis.

Gunshot Residue, Raman, Organic



B123 Development of a Spectral Camera for Estimating the Age of Bloodstains in Casework

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After attending this presentation, attendees will understand the principles of hyperspectral imaging and the chemical reactions taking place within a bloodstain.

This presentation will impact the forensic science community by showing the possibilities and limitations of a technique for age estimation of bloodstains at the crime scene.

If the age of bloodstains could be determined, this would provide investigators with valuable information regarding the timeline of events. Although several techniques for the age estimation of bloodstains have been explored in forensic laboratories, no method has yet been applied in forensic investigations. The transition from laboratory measurements to crime scene analysis brings some typical challenges. While ideal substrates are selected in a laboratory setup, many different and far-from-ideal substrates can be encountered at a crime scene. Likewise, the measurement setup is less controlled.

Recently, it was demonstrated how diffuse reflectance spectroscopy could be used to measure the concentration change of oxyhemoglobin, methemoglobin, and hemichrome, all reaction products of hemoglobin, in order to estimate the age of bloodstains in a laboratory setup without destroying or even touching the sample. Measurements were demonstrated on 40 bloodstains performed at ages ranging from fresh to 200 days old and estimated the age with an error margin of approximately 20%. This method has now been adapted to the specific requirements of forensic practice. A wireless spectral camera was introduced to replace the optical fibers used for point measurements in the laboratory. By combining spectroscopy and digital imaging, spectral imaging enables investigators to record the reflectance spectra and the distribution of many bloodstains simultaneously. To be able to perform measurements on (non-ideal) substrates possibly found at the scene, the algorithm used for the age estimation was adapted to correct for background absorptions.

The developed technique was successfully tested at a simulated crime scene in which bloodstains of five different ages were deposited. The absolute error of the age estimation task increased with age, with a median relative error of 13.4% of the actual age. Finally, the technique was applied in several real murder cases. In these cases, the research questions were twofold: (1) what is the age of these bloodstains; and, (2) were these bloodstains deposited during the same event?

Regarding the first question, when an absolute age estimation is asked, the influence of humidity and environmental temperature on the speed of the chemical reactions has to be taken into account. When this information is not available, it may be possible to perform a relative age estimation, which gives investigators information about the sequence of events.

In this presentation, the theory of bloodstain age estimation and spectral imaging is explained, followed by a description of the experimental verification and several case examples. Insight is given not only in the possibilities of this technique but the limits and challenges of this technique in forensic practice are also described. Applied in casework, this technique provides new objective information which can be used by investigators to create a timeline of events.

Spectral Imaging, Bloodstain Age, Timeline



B124 DNA Methylation Patterns as Markers in Forensic Investigation

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After attending this presentation, attendees will understand the application of epigenetics markers in forensic casework and the importance of this new tool of identification in the resolution of a crime scene.

This presentation will impact the forensic science community by providing crucial information on the development/validation of DNA methylation markers for forensic use. The new and improved technique for the identification of cell type and age will provide the community with new and improved methods to interpret the crime scene.

Body fluids recovered from crime scenes are considered one of the most important types of evidence in forensic cases. DNA obtained from body fluids can be used to identify the donor of the biological material but as presently used it cannot reveal the tissue source or the possible age of the donor. Determining the type and origin of the fluid can provide important assistance in reconstructing crime scenes. DNA presents the ideal source for identification of tissue type since it provides quantitative results and is more stable than RNA. In addition, the extracted DNA target is already present in the laboratory.

DNA methylation is an epigenetic modification involved in transcriptional regulation.¹ It is known that methylation is important in cell differentiation and genomic loci are differentially methylated between tissues. Because of this, different methylation patterns between tissues and cells can provide the basis of an assay for body fluid identification. The ability to determine the age of the sample donor based on DNA would also be a powerful tool for forensic investigation. Human ageing is associated with epigenetic modifications such as DNA methylation. Several studies have investigated biomarkers for ageing which can be used to track donor age, presenting practical implications in forensic analysis.^{2,3}

The most common body fluids found at crime scenes are blood, semen, and saliva. A set of epigenetic markers, C20orf117, ZC3H12D, BCAS4, and FGF7, has been developed which produce unique and specific patterns of DNA methylation that can be used to identify these body fluid types; however, to ensure the efficiency of these epigenetic markers, developmental validation studies needed to be performed to determine the conditions and limitations of this new tool for forensic analysis.⁴ For this research, all validation studies were performed according to Scientific Working Group on DNA Analysis Methods (SWGDM) guidelines. Also, five genes previously found to be DNA methylation age-associated, NPTX2, TRIM58, GRIA2, KCNQ1DN, and BIRC4BP, were tested for prediction of age using a variety of body fluids and a pyrosequencing system.³ DNA was extracted and bisulfite conversion was performed using the Epitect® Bisulfite Kit. Bisulfite modified DNA was then amplified using specific primers for methylated target regions. The last step was analysis by pyrosequencing. All pyrosequencing reactions were performed using the PyroMark® Q24 Pyrosequencer. Data analysis was performed using the PyroMark® Q24 assay software for CpG methylation quantitation and the corresponding percent methylation values for each site and the data were displayed as a pyrogram.

The versatility of these new markers will be presented by showing the results of validation studies on sensitivity, human specificity, age, and mixture resolution. When testing the markers using different species samples, amplification occurs not only for human DNA but for some animal samples; however, when the amplified DNA is pyrosequenced, the sequencing primer increases the specificity and eliminates results from any non-human samples. All the other samples show a pyrogram equivalent to the one obtained for the negative control. In another test, samples were degraded by being heated at different temperatures. All degraded samples showed good pyrosequencing results. In a study examining the effect of Polymerase Chain Reaction (PCR) inhibition, all samples were amplified and showed good pyrograms when hematin (0.08mM) and humic acid (0.24mg/mL) were added before bisulfite modification. When these same inhibitors were added after bisulfite modification and before PyroMark® PCR, there were no satisfactory results. This data indicates that the purification process at bisulfite conversion removes inhibitors during the washing steps. Sensitivity and mixture studies were also performed. In general, the results generated indicate a good perspective for the overall validation of the tested markers.

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Criminalistics Section - 2015

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DNA Methylation, Body Fluid, Pyrosequencing



B125 Effects of Decomposition on the Recoverability of Biological Fluid Evidence

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After attending this presentation, attendees will understand how decomposition and environmental factors can affect traditional methods for detecting and identifying blood and semen evidence as well as the ability to recover human DNA from such samples.

This presentation will impact the forensic science community by demonstrating that traditional methods of detection and testing may not readily identify blood and semen evidence present on the clothing of a decomposing body, indicating that alternate methods of testing and sampling should be considered.

Several factors that influence the rate of human decomposition have been described, including temperature, access by insects, humidity, and rainfall.¹ These environmental factors, as well as purge fluid released during decomposition, can also interact with any evidence deposited on the clothing of a deceased individual.² This present research assessed how these combined factors affected the detection and identification of blood and semen evidence.

A 35- to 45-pound feeder pig (Postmortem Interval (PMI) <3 hours) was placed on a grassy area within the Boston University Outdoor Research Facility for a period of 22 days during late spring, the temperature averaging 61.8oF. Aliquots of 30µl of either human blood or semen were pipetted onto 1"x1" sections of a 95% cotton T-shirt. Twenty-two samples of each type were placed on top of and underneath the pig, as well as a similarly weighted bag of sand (control). One bloodstain and one semen stain were collected each day for a period of 22 days from each location, yielding eight samples per day. Each sample was analyzed within 30 hours of collection.

The blood samples beneath the control showed that environmental factors influenced the results of testing. Rain caused dilution and diffusion of the bloodstains and the color of the stains changed from red-brown to green-yellow. Kastle-Meyer (KM) testing was positive for all samples and ABACard® HemaTrace® testing was positive for 14 of 22 samples, with the negative results occurring during days 12 to 22. Two stains that were negative at ten minutes turned positive shortly thereafter, suggesting that perhaps a longer development time may be required for compromised samples. The blood samples placed beneath the pig yielded positive KM results on all 22 days and positive HemaTrace® results through day ten. All bloodstains placed on top of the pig and control yielded positive KM and HemaTrace® results.

Semen samples from beneath the control began to show a decrease in fluorescence using an Alternate Light Source (ALS) by day three, and some areas of fluorescence occurred in a different location, indicating that the soluble components had diffused outward from the Original Region Of Deposition (ORD). Results for Acid Phosphatase (AP) and ABACard® p30 were mostly positive through day 16. By day 17, the ORD no longer fluoresced or yielded positive AP or p30 results. With the exception of day ten, sperm were identified on all samples. Semen results from beneath the pig showed that even on day one, the ORD were only weakly fluorescent and by day four, fluorescent regions began appearing outside of the ORD. These migrated regions of fluorescence yielded positive results with AP spot and p30 testing but showed few or no spermatozoa when examined microscopically. As the days passed, the ORD were no longer fluorescent and AP mapping and p30 testing yielded negative results; however, spermatozoa could still be identified in almost all of the ORD through day 22.

Semen samples collected from on top of the control showed that semen stains retained fluorescence and tested positive for AP, spermatozoa, and p30 through 22 days of testing. Semen samples collected from on top of the pig yielded similar results until day 16, when the fluorescence began to fade and AP testing did not yield traditional color changes associated with a positive result. By day 18, fluorescence was no longer visible with an ALS at 450nm or 495nm; however, Ultraviolet (UV) light yielded positive fluorescence when used during days 19 to 21. Spermatozoa and p30 were identified on samples saturated with products of decomposition, even when presumptive screening techniques were negative (450nm-495nm) or showed an altered appearance (AP).

DNA analysis results of select stains will be presented.



Criminalistics Section - 2015

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Blood, Semen, Decomposition



B126 Transfer of Sperm Cells During the Laundering Process

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After attending this presentation, attendees will understand the possible transfer of spermatozoa from one fabric item containing an original semen stain to another previously unstained fabric item during the laundering process.

This presentation will impact the forensic science community by potentially influencing how examiners in a forensics laboratory might treat future casework and what items of evidence criminal investigators might collect if the cases they encounter involve clothing items which have been laundered together.

During a sexual assault investigation, clothing and bed sheets are often collected due to their potential evidentiary value. Sperm cells recovered on such items may provide insight into the allegation but may also produce challenges when interpreting such results. The question then becomes whether the findings were from a direct deposition (potentially establishing sexual contact between suspect/victim) or from an unrelated event (transfer in the washing machine).

There have been several studies which have addressed the persistence of seminal fluid during the laundering process; however, the literature is scarce regarding the transfer of spermatozoa between items during the laundering process. Between the published papers specifically addressing transfer, there are conflicting reports on whether or not transfer occurs.

This project was designed as an initial study to assess the transfer of sperm during the laundering process. Seminal fluid was obtained from a fertility clinic and anonymous donors. A total of 24 cold water (18°C) washes were conducted using the same top-loading Maytag® Performa washer. Each wash cycle was completed using a “mini” water load and set to the “normal” wash cycle. Two types of detergents were tested, Liquid Tide® (phosphate) and Seventh Generation™ Detergent Pods (phosphate-free). Then 2mL of semen was deposited onto three types of “source” (stained) fabrics: 100% cotton panties, 100% cotton towels, and 60% cotton/40% polyester bed sheets. Each of the 24 wash cycles contained one “source” item and three equal size “receiving” (unstained) fabrics. Half of the stained “source” fabrics were air dried for 24 hours prior to washing and half were allowed to air dry for seven days. After washing, all fabrics were air dried.

Each “source” and “receiving” item was examined visually with an Alternate Light Source (ALS). Each item was then tested for acid phosphatase using AP Reagent solution and for the presence of the p30 protein using Seratec® PSA cards. Cuttings from each item were treated and extracted onto a microscope slide, dried, and stained using the Christmas Tree dyes, and examined microscopically.

Twenty-one out of 24 “source” fabrics tested positive for acid phosphatase and 20 out of 24 “source” fabrics tested positive for p30 protein. Fifteen out of 24 “source” items had visual fluorescent stains under an ALS. None of the “receiving” fabric items fluoresced under the ALS and none tested positive for AP or p30 protein. For the “receiving” items air dried for 24 hours prior to washing with a “source” item, 29 were negative for sperm while the remaining 19 had sperm counts ranging from 1 to 50+; with an average of 6.5 sperm/item. For the “receiving” items air dried for seven days prior to washing with a “source” item, 23 were negative for sperm while the remaining 25 had sperm counts ranging from 1 to 31, with an average of 5.6 sperm/item.

Cuttings from each “source” and “receiving” item were tested for DNA using polymerase chain reaction techniques. Twenty-two out of 24 “source” items provided full profiles consistent with the donors. One “receiving” sheet and two “receiving” towels provided full profiles consistent with the donors. Three “receiving” sheets, eight “receiving” panties, and three “receiving” towels provided information at a minimum of six loci, providing partial profiles in which the donors could not be excluded. All samples were extracted using a QIAGEN® EZ1® and quantified using Plexor® HY on an AB 7500®. Following the quantitation results, all of the samples were amplified using Identifiler® on an AB 9700®, and capillary electrophoresis was performed using an AB 3130xl.

In this study, sperm heads were visualized on the “receiving” items, demonstrating that transfer of spermatozoa during the laundering process can occur. This is important information to be aware of for investigators and laboratory scientists. Additional work is needed to develop enhanced evidence-collection guidelines. The results of future testing will help define laboratory sampling protocols and data interpretation procedures.

Sperm, Transfer, Washing Machine



B127 High-Resolution Melt Analysis of DNA Methylation Status as a Novel Method for Human Semen Identification

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After attending this presentation, attendees will understand how DNA methylation patterns differ between cell types and how high-resolution melt curve analysis can be used in conjunction with bisulfite conversion to identify fluids based on their methylation status.

This presentation will impact the forensic science community by introducing a new method for semen identification that is based on DNA methylation and relies on instrumentation that is affordable and currently found in forensic laboratories. The method also offers the potential of being extended to multiple body fluid types.

Identification of the body fluid origin of a biological stain found at a crime scene can shed light on events that may have occurred. Unfortunately, current methods of body fluid identification rely on techniques that are subject to both false positives, through cross-reactivity between multiple fluids, or false negatives, due to protein degradation in the fluid. A recent study shows that it is possible to distinguish body fluids by examining the differentially methylated DNA in the fluid by pyrosequencing.¹ The location of methyl groups on DNA is known to regulate gene expression, and is thus tissue specific. It is also thought to be stable over long periods of time in deposited stains; however, pyrosequencing is a fairly new technique that is time-consuming and uses expensive equipment that is not currently available in most forensic laboratories.

The goal of this study was to develop a cost- and time-effective technique for the identification of body fluids by DNA methylation, using instrumentation typically available in crime laboratories.

Six blood, five saliva, four urine, and four vaginal fluid samples were obtained from 11 volunteers and five semen samples were obtained from different donors in a conical vial by self-collection. All semen samples were then deposited (5 μ L) on sterile cotton-tipped swabs. Bloodstains from 1998, 2003, and 2010 were also obtained and used to assess the stability of methylation over longer periods of time.

DNA was isolated from each swab using either organic extraction (saliva, urine, and vaginal fluid) or Chelex[®] 100 extraction (blood and semen) and subjected to bisulfite modification using a Zymo Research EZ DNA Methylation-Gold[™] kit, to convert unmethylated cytosines to uracil. The Qiagen[®] Epiect[®] High Resolution Melt (HRM) PCR Kit was used for melt curve analysis of amplicons containing known sites of differential methylation.^{2,3} Primers used in this kit are proprietary.

All body fluid stains were analyzed in triplicate. Results indicate that semen can be identified using post-amplification melting temperature differences compared to the other fluids used. This shift in melt temperature was found to be statistically significant for all five body fluids using a one-way Analysis of Variance (ANOVA) with a 95% confidence interval ($p=6.34 \times 10^{-5}$), as was expected for genes actively transcribed in tissue samples due to the lack of methyl groups, and thus bisulfite conversion of cytosine residues; however, when semen was removed from the statistical analysis, the remaining body fluids were found not to be statistically different from each other (one-way ANOVA at 95% confidence interval, $p=0.141$). Extraction and analysis of DNA from the 1998 blood case sample resulted in a melting temperature consistent with blood samples from volunteers indicating that the age of the stain does not affect this analysis. The results from case samples 2003 and 2010 yielded insufficient results. This method is also short tandem repeat-typing compatible due to DNA extraction prior to methylation analysis. Although only semen could be identified with this study, there are other potential primer sites that could help identify the other body fluids using the same method. This study does show that body fluid identification is possible for both old and fresh samples using DNA methylation and HRM analysis.

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DNA Methylation, Melt Curve Analysis, Semen Identification



B128 Persistence of Sperm: What the Literature Really Says

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The goal of this presentation is to shed light on the inconsistencies in the forensic literature regarding the persistence of spermatozoa in the vaginal cavity and cervix. Specifically, this presentation will show that the literature citations of spermatozoa persisting for 17 and 19 days are not reliable. This presentation will leave attendees more aware of how to critically read and process scientific literature.

This presentation will impact the forensic science community by increasing awareness of how journal articles are cited in the literature and the potential pitfalls in relying on secondary sources for setting laboratory and investigative policies and guidelines.

During the investigation of an alleged sexual offense, the timely recovery of physical evidence is crucial; however, for a variety of reasons, a victim of sexual assault may not report the alleged incident immediately. It is in these extended intervals between the alleged offense and reporting (post-assault interval), that key decisions must be made by investigators and laboratory examiners regarding if any physical evidence may still exist and how that evidence can best be recovered. The identification of semen can be of great importance in establishing sexual contact between an alleged victim and subject. It is the recovery of semen from the female victim that is frequently the focus of the investigative decision making efforts. The literature can give a misleading picture as to how long semen can be recovered from body cavity swabs taken during a sexual assault exam. Based on the scientific literature review, most of the citations state that spermatozoa can be detected approximately three days in the vaginal cavity and five to seven days in the cervix; however, there are a small number of published accounts relating that spermatozoa can be detected much later than a week in the vaginal cavity. These outliers in the literature can cause confusion as to when reliable semen results can be obtained from swabs collected during a sexual assault exam and identified during laboratory testing.

A literature review was performed to determine the veracity of two separate claims that spermatozoa can be recovered from the vaginal cavity up to 17 and 19 days post coitus. The specific source that cites a 17-day claim was traced to an article written in Italy in 1891. In that reference, the author was focused on artificial impregnation and fertilization. Based on the success of fertilization, the author determined spermatozoa may remain alive for as long as 17 days. The sources that reference this paper illustrate this claim is dubious at best. The claim of finding spermatozoa 19 days post coitus was traced to a conference abstract from 1977 that referred to data which was eventually published; however, the published paper did not address the 19-day findings stated in the conference proceedings. The data referenced in the conference proceedings was based upon self-reporting by female volunteers. In this case, the 19-day findings were regarded as “possibly correct” because very few spermatozoa were routinely observed after day ten. In the same reference, the authors noted that the reported post-coitus times of one month to many months where spermatozoa were found were incorrect because there were a high number of spermatozoa detected that did not show degenerative changes that would be expected. Even though these outliers are mentioned in these two papers, the preponderance of the data in these citations and others support the trend that spermatozoa can be detected three days in the vagina and up to seven days in the cervix post coitus.

During the laboratory’s search of the literature, these two outliers were found to be cited several times in an effort to illustrate that semen can persist outside of the traditionally expected time frame of three to seven days post coitus. The problem is the original sources that mention these figures are typically not cited directly in the literature. Instead, the literature articles cite various secondary sources to illustrate these claims. This made it very difficult to determine the origin of the data. Having knowledge of what the original sources of these claims say about the persistence of sperm has made it possible to educate submitters about the likelihood of obtaining usable data from samples collected 17 and 19 days post coitus.

The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

Sperm, Persistence, Semen



B129 Persistence of DNA: A Case File Review

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The goal of this presentation is to educate attendees on how long semen and DNA persist on casework samples collected during a sexual assault exam from or using actual casework data.

This presentation will impact the forensic science community by increasing awareness of how long semen can persist on samples collected during a Sexual Assault Nurse Exam (SANE) and the impact of time on the ability to recover DNA using standard DNA techniques that are currently utilized at the United States Army Criminal Investigation Laboratory (USACIL).

During the investigation of an alleged sexual offense, the timely recovery of physical evidence is crucial; however, for a variety of reasons, a victim of sexual assault may not report the alleged incident immediately. It is in these extended intervals between the alleged offense and reporting (i.e., the post-assault interval) that key decisions must be made by investigators and laboratory examiners regarding if any physical evidence may still exist and how that evidence can best be recovered and subsequently tested. Identifying semen and obtaining a DNA profile are not synonymous. The identification of semen can be of great importance in establishing sexual contact between an alleged victim and subject. It is the recovery of semen from the female victim that is frequently the focus of the investigative decision-making efforts. Though there is literature on the subject of semen and DNA persistence in the vagina and cervix, the sensitivity and availability of the techniques used to detect semen and DNA vary from laboratory to laboratory. In an effort to give more accurate guidelines to SANEs who collect the evidence and to federal agents who submit evidence to the USACIL, past case files were reviewed in order to determine a realistic time frame in which semen and DNA can be recovered.

A search of the USACIL DNA casework files from 2012 to 2014 from all branches of the military was performed in order to determine the time frame of obtaining serology results and DNA profiles from post-coital swabs taken from casework samples. In addition, this review also included the amount of DNA obtained from different types of body swabs in relation to the post-assault activity and interval, a tally of the number of sexual assault submissions without a Sexual Assault Evidence Collection Kit (SAECK), and the reason such a kit was not obtained.

In conjunction with the review of the USACIL cases, a review of United States Air Force (USAF) cases was also performed. This review focused on sexual assault cases reported to and investigated by the Air Force Office of Special Investigations (AFOSI) during calendar year 2013. The AFOSI review focused on the following contextual data: (1) the average time between the alleged assault and obtaining a SAECK; (2) the number of cases where no SAECK was obtained and why; (3) the number of cases where a SAECK was collected and submitted to the USACIL for analysis; (4) the number of cases where a SAECK was collected but not submitted to the USACIL for analysis and why; (5) the number cases where a SAECK was submitted to the USACIL but not processed and why; and, (6) the outcome of the cases. The time between alleged incident and collection, serology and DNA results for body cavity swabs, the number of sexual assault cases submitted without a kit, and the reason that a kit wasn't collected was recorded and tallied.

The data collected is important for providing a sound basis for guidelines used by the laboratory and the USAF regarding the submission and testing of SAECKs that are collected in the field.

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Semen, Persistence, DNA



B130 Intra-Bone Variation of Recoverable Nuclear and Mitochondrial DNA in Femora

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After attending this presentation, attendees will learn: (1) that DNA content varies significantly along the length of the femur; (2) the implications this has on recovering nuclear and mitochondrial DNA from skeletal remains; and, (3) practical recommendations for improving DNA recovery from femoral tissue.

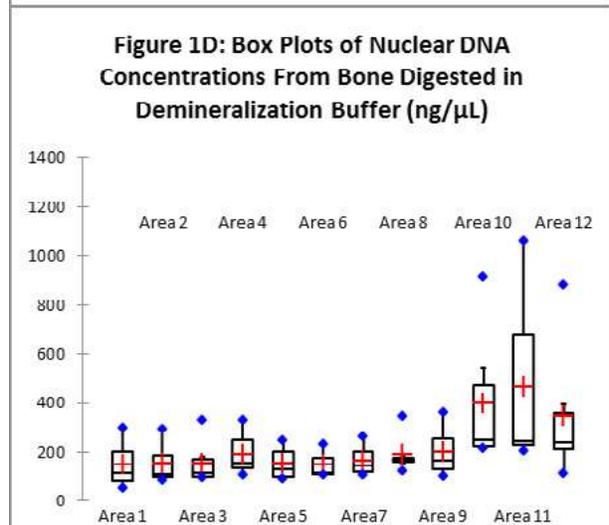
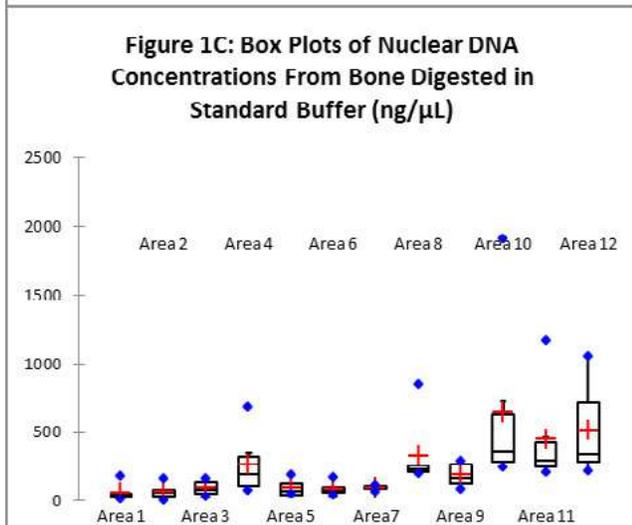
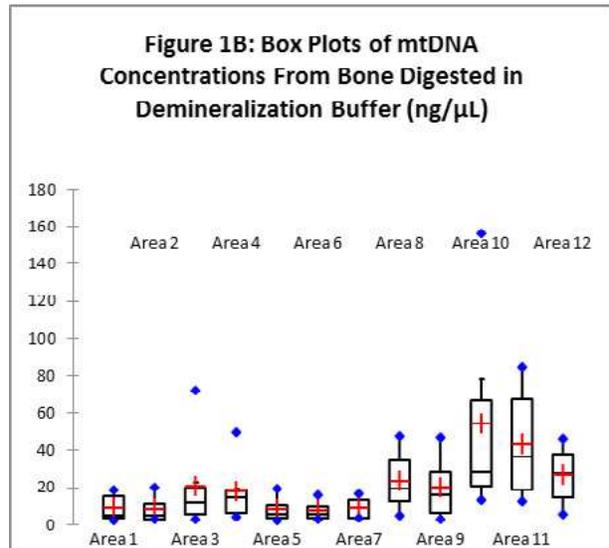
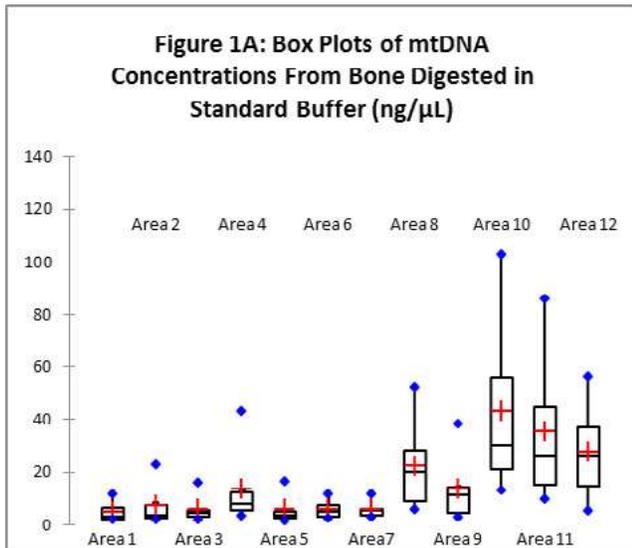
This presentation will impact the forensic science community by informing practitioners about intra-bone variation of recoverable DNA from femora. Further, these findings could have immediate impact for practitioners by improving sampling strategies, increasing first-pass success rates of generating a DNA profile, and saving forensic laboratories time, money, and resources.

Positive identification of skeletal remains is not always possible owing to a lack of antemortem records, their level of preservation, or the remains being substantially incomplete or fragmented. Identification in these instances frequently relies on obtaining DNA from skeletal material. Research and practice have shown that successfully recovering DNA from bone is based upon several factors, including environmental conditions, extraction methodology, and different bones of the body.¹⁻³ Further, cortical osseous tissue from weight-bearing long bones (e.g., the femur and tibia) tends to be a better target for obtaining amplifiable DNA than trabecular bone and because midshaft diaphysis of the femur and tibia contain an abundance of cortical bone, they have become a preferential target for DNA extraction; however, there is currently a lack of research on intra-bone DNA variation.^{1,4} As an example, the femur, with its large size, surface area, and varied distribution of osseous tissue types, has the potential to contain a wide array of DNA variability.

In this research, fresh bovine femora were first macerated in a boiling 1% Terg-a-zyme[®] solution to remove soft tissues. Twelve regions of the femur, including eight equidistant sections of diaphysis extending proximally and distally from the midshaft as well as the proximal and distal epiphyses were drilled using a Dremel[®] tool with a 7/64-inch cobalt drill bit. Bone powder from each region was divided between two digestion methods: standard laboratory digestion buffer (20mM Tris, — pH 7.5; 50mM EDTA; 0.1% SDS) or demineralization buffer (0.5 M EDTA — pH 8.0; 1% Laural-Sarcosinate), extracted organically, and concentrated using Amicon[®] filtration columns. DNA quantification of extracts utilized a TaqMan[®] real-time polymerase chain reaction assay targeting the nuclear MC1R and mitochondrial ATPase8 loci. To determine quality, DNAs were amplified utilizing a series of primer sets that generated ~200, 400, 600, and 1,000 base pair amplicons. All results were examined statistically at an α of 0.05 utilizing analysis of variance in conjunction with post-hoc pairwise comparisons to determine areas of significant difference.

Resulting data (Figures 1A–D) showed substantial variation in DNA yields across femoral regions. Significantly more nuclear and mitochondrial DNA was obtained from the distal and proximal femoral epiphyses than the diaphysis. Areas of diaphysis close to the epiphyses, as well as auricular surfaces, had more DNA than midshaft diaphysis, which consistently had the lowest amount of recoverable DNA. The mitochondrial (1,000 base pairs) and nuclear (400 base pairs) DNA amplicons were consistently generated from all regions of the femur; however, larger nuclear DNA amplicons (600 and 1,000 base pairs) were inconsistently obtained across the femur.

These findings indicate that substantial variation in DNA levels exists along the femur. Furthermore, midshaft femur, commonly sampled by forensic practitioners, appears to be suboptimal for obtaining recoverable nuclear and mitochondrial DNA. Given the value of DNA in establishing the identity of skeletal remains and the common use of weight-bearing long bones such as the femur for obtaining that DNA, this research has the potential to be of considerable utility to forensic laboratories, and thus, to the criminal justice system.



Legend:

Area 1 (Midshaft Diaphysis)

Area 2 (Diaphysis Proximal to Area 1)

Area 3 (Diaphysis Proximal to Area 2)

Area 4 (Proximal Metaphysis)

Area 5 (Midshaft Diaphysis Distal to Area 1)

Area 6 (Diaphysis Distal to Area 5)

Area 7 (Diaphysis Distal to Area 6)

Area 8 (Distal Posterior Auricular Surface)

Area 9 (Distal Metaphysis)

Area 10 (Articular Surface of Distal Epiphysis)

Area 11 (Femoral Head)

Area 12 (Greater Trochanter)

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DNA Quality and Quantity, Skeletal Variation, DNA Recovery



B131 Effect of Skeletal Sampling Technique on DNA and Elemental Analysis Results

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After attending this presentation, attendees will understand the relationship between skeletal sampling technique and recovery of nucleic acids and elemental materials and the reduction in the quality of Short Tandem Repeat (STR) results due to DNA damage from laboratory processing and Polymerase Chain Reaction (PCR) inhibition from co-isolated elements.

This presentation will impact the forensic science community by demonstrating the effect of skeletal sampling technique on the DNA recovered and mineral constituents co-isolated from DNA extraction of human skeletal remains. Presented data include measurement of DNA quality and quantity, genetic analyses, and determination of elemental contents.

The goal supported by this work is to identify more people from skeletal remains by developing improved procedures for extracting DNA from bone samples. Bones are considered one of the most challenging sample types due to laborious laboratory processing that may result in incomplete genetic results or amplification failure. Causes of poor results may include DNA damage, inhibition of PCR, and/or inefficient extraction. These experiments will determine whether skeletal sampling technique contributes to poor quality DNA analysis results and resulting data will provide a foundation for the development of improved procedures to extract DNA from skeletal remains.

Cadaver long bones were obtained through the Willed Body Program at the University of North Texas (UNT) Health Science Center and samples were cut from the diaphysis of a left tibia. Three extraction sets, each comprised of five samples and a reagent blank, were tested for DNA and elemental contents. The three sets included the following: whole bone pieces weighing between 0.837 and 0.935 gram, numerous bone cuttings with a total sample weight between 0.887 and 0.915 gram, and pulverized samples weighing between 0.905 and 0.912 gram. DNA extraction was performed using the UNT Center for Human Identification "Demineralization Extraction of Skeletal Remains" protocol, a modified version of the Loreille et al. method.¹ Samples were weighed and placed into tubes. Demineralization buffer and proteinase K were added to each sample and incubated overnight. An equal volume of Phenol-Chloroform-Isoamyl Alcohol (PCIA) was added and samples were vortexed and centrifuged. A 1mL aliquot was removed from the aqueous phase for ethanol purification and subsequent testing and the remaining aqueous phase was transferred to a centrifugal filtration device. Samples were centrifuged through the device and the retentate was purified using a chaotropic salt solution and a centrifugation column.

Two fractions were examined: the aqueous phase following PCIA purification, subsequently purified with ethanol; and the fully purified samples, consisting of the retentate from the centrifugal filtration and purified using a second column. Contents of the aqueous phase formed crystals as a result of ethanol purification. These samples were dissolved in TE⁻⁴ buffer and the DNA content evaluated using a microfluidic platform. The crystals were again formed with ethanol and examined by X-ray diffraction. Fully-purified samples were tested for DNA content using the following methods: sizing on a microfluidic platform in duplicate; two qPCR DNA quantification kits in triplicate; STR amplification in triplicate; and, genetic analysis on two models of capillary electrophoresis instruments. Elemental analysis of both fractions was performed using Inductively Coupled Plasma/Mass Spectrometry (ICP/MS). Baseline and subsequent analyses were performed in solution mode ICP/MS. Acid digestion was used for baseline sample preparation; approximately 100mg of sample was dissolved in concentrated nitric acid (HNO₃), and then diluted with 1% HNO₃. Dilution factors varied between 20 and 100 for aluminum, calcium, copper, iron, nickel, and lead. All samples were measured in triplicate.

Results were successfully obtained for each assay attempted. DNA quantification yields for pulverized bone were 16.7 to 93.3 times higher than whole or cut bone pieces; however, STR profiles obtained for the cut bone pieces were superior to whole or pulverized bone samples. STR profile quality was assessed by determining the percent of fluorescence exhibited by the highest molecular weight allele versus the lowest molecular weight allele for a dye channel. The range of percent recovery of the highest molecular weight allele for whole bone was 1.72% to 35.66%, cut bone was 4.82% to 49.79%, and pulverized bone was 0.96% to 18.05%.



Criminalistics Section - 2015

Reference:

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Skeletal Remains, DNA Extraction, Elemental Analysis



B132 Development of a Multiplex Quantitative PCR (qPCR) Assay for Simultaneous Quantification of Human Nuclear and Mitochondrial DNA From Forensically Relevant Samples

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After attending this presentation, attendees will have a better understanding of ongoing efforts to develop a multiplex quantitative Polymerase Chain Reaction (qPCR) assay that enables simultaneous quantification of human nuclear and mitochondrial DNA (mtDNA). Additionally, principles of droplet digital PCR (ddPCR), a newly emerging quantitative technique, will be presented.

This presentation will impact the forensic science community by providing a method for comprehensive quantitative analysis of DNA in forensically relevant samples.

In forensic casework, multilocus Short Tandem Repeat (STR) typing is often the preferred method of analysis due to its high power of discrimination however, many evidentiary samples contain low amounts of DNA or degraded DNA that is not suitable for STR typing. In these cases, mtDNA sequence analysis is typically performed. Determining which investigative approach is most suitable can be challenging, especially in cases where the sample or extract is limited. Here, a powerful multiplex 5' nuclease real-time PCR assay is described that enables simultaneous quantification of both human nuclear and mitochondrial DNA from a sample extract. This tool provides specific quantitative data that can be used to determine the most appropriate analytical workflow without consumption of additional sample or increase in labor compared to methods currently used in crime laboratories.

The nuclear target for this custom qPCR assay is the 275bp Alu Yd6 mobile element originally described by Xing et al.¹ High sensitivity of nuclear DNA quantitation using the multicopy Alu Yd6 marker has previously been reported and a qPCR assay designed for this target has been used successfully.^{2,3} The mtDNA target sequence corresponds to a 105bp segment of the NADH dehydrogenase subunit 5 gene and is described by Kavlick et. al. for use in an assay that is utilized routinely in several forensic laboratories.⁴ Both targets have been shown to exhibit little to no cross-reactivity with non-human sources.^{2,4} In addition to these primary targets, an Internal Positive Control (IPC) has also been included for assessment of possible PCR inhibition.

Initially, 2800M human control DNA was characterized using ddPCR for use as a qPCR standard. This absolute quantitative technique enables accurate and precise quantitation of nucleic acids. Briefly, a 20 μ L sample containing TaqMan[®] PCR reagents and sample DNA extract is partitioned into approximately 20,000nL-sized droplets. The droplets then act as independent microreactors where fluorescent reporter dyes are liberated from target specific probes during PCR. Following PCR, the droplets are counted as target positive or negative depending on the presence or absence of fluorescence detected by a droplet reader. Target concentrations are estimated by software using a Poisson model. Human nuclear and mtDNA in control sample 2800M were separately quantified in singleplex ddPCR reactions. This data was then used to prepare a single standard dilution series of 2800M DNA with quantitative ranges of 10ng/ μ L to 1fg/ μ L of nuclear DNA and 400,000 copies/ μ L to 0.04 copies/ μ L of mtDNA. Each qPCR quantitation was performed both as a duplex assay (primary target and IPC together) and as a multiplex assay (both primary targets and IPC together). Resulting PCR efficiencies and sample quantitations were compared. Preliminary data suggests that multiplexing has a minimal derogatory effect on the efficiency of each assay and, as a result, sample quantitations are similar when using singleplex or multiplex qPCR.

The assay described herein offers a high degree of specificity and sensitivity and facilitates preservation of limited samples while allowing the analyst to determine the optimum analytical approach for each sample. In the future, a larger target of the mtGenome will also be included in the multiplex assay to enable assessment of DNA degradation.



Criminalistics Section - 2015

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Quantitative PCR, Droplet Digital PCR, Multiplex



B133 Utility of a Novel and Sensitive DNA Multiplex for Highly Degraded Missing Persons Samples

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After attending this presentation, attendees will understand the application of a novel DNA marker system for use with missing persons samples.

This presentation will impact the forensic science community by serving as a key aspect for the analysis of degraded forensic DNA samples, as it can augment or replace traditional DNA testing systems in cases where those systems do not produce results due to the presence of highly degraded DNA.

The Missing Persons unit of the UNT Center for Human ID (UNTCHI) annually processes more than 2,600 specimens including human remains, family reference samples, and direct reference samples. The ability to successfully type these human remains samples using DNA analysis is of utmost importance for their identification. In these cases, identification is achieved primarily through Short Tandem Repeat (STR), Y-chromosomal Short Tandem Repeat (Y-STR), and mitochondrial DNA analyses; however, this approach is often restricted by common haplotypes and the excessively degraded condition of samples, thereby limiting the discrimination power of DNA using these technologies. To address these limitations, the UNTCHI strives to optimize and validate new techniques to assist with the identifications in missing persons cases. This presentation focuses on the utilization of a novel DNA marker system, for use with the types of samples commonly encountered in the Missing Persons unit at UNTCHI.

InnoTyper™ is a marker system that utilizes Retrotransposon Insertion Polymorphisms (RIPs). Retrotransposable Elements (REs) consist of Long Interspersed Nuclear Elements (LINEs) and Short Interspersed Nuclear Elements (SINEs). This group of bi-allelic markers can be useful for human identity testing. Among the advantages of using RIPs are that they do not yield stutter artifacts due to slippage during the PCR, there are no known genetic mutations since they are identical by descent only, they are present in very high copy numbers, and they have a well-defined genetic lineage, which makes RIPs useful for relationship determinations; however, until recently due to the inherent size difference (>300bp) associated with insertion and null alleles, the use of RIPs has not been practical for forensic applications. To circumvent the allele size disparity, a novel primer design methodology was used to remove the intra-specific locus competition that occurs in heterozygotes. This innovative primer design allows for the amplicon size to be reduced to a size smaller than currently used STR markers, such that substantially degraded DNA samples can be analyzed. Utilizing this primer design, a more simplified, rapid, and automated typing technology can be applied to RIP typing.

The multiplex utilized in this study consisted of 13 RE markers plus the gender identifying marker, amelogenin. All amplicons in this PCR multiplex range in size between 50bp to 125bp. To test the utility of this system in a forensic casework missing persons laboratory, several studies were performed, including sensitivity and testing on non-probative human remains. Results show the system to be highly robust and sensitive. Complete 13 RE marker profiles were obtained with as little as 16pg. These results were compared to the low copy procedure used by UNTCHI. The small amplicon sizes result in an extremely sensitive, rapid, and useful multiplex for typing highly degraded forensic samples as well as for high-quality DNA samples.

Data to be presented supports the usefulness of this system for analyzing missing persons samples which did not produce usable STR results but did provide InnoTyper™ results with high discrimination power. This system will prove very useful for analyzing single-source degraded DNA samples such as those found in mass disasters and other human identification efforts.

DNA, Degradation, Retrotransposon



B134 Hair and Calcified Tissue DNA Extracts: qPCR-Based Guidelines/Strategies for Streamlined Mitochondrial DNA (mtDNA) Amplification and Improved mtDNA Sequence Recovery

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After attending this presentation, attendees will better understand how mtDNA quantitative Polymerase Chain Reaction (qPCR) values can be used to increase sequence data recovery and minimize sample consumption.

This presentation will impact the forensic science community by providing practical, data-driven mtDNA-processing guidelines for streamlined mtDNA workflows.

Mitochondrial DNA (mtDNA) is a valuable forensic marker for samples such as hair and calcified tissue, as they routinely fail upon nuclear DNA (nDNA) analysis due to DNA degradation or insufficient template. Though mtDNA can generally be recovered from these sample types, the mtDNA content is often still quite limited and the source material is almost always limited. Under these circumstances, a mechanism for determining the most efficient and successful amplification strategy would lead to reduced sample consumption and an increase in the quantity of sequence information obtained.

The mtDNA Control Region (CR, rCRS 15998-616) is routinely targeted for analysis due to its high inter-individual variation. Historically, CR data have been recovered at the Federal Bureau of Investigation (FBI) laboratory with four amplicons (designated HV1A, HV1B, HV2A, HV2B, each ~230bp to 290bp) that sit within mtDNA Hypervariable Region 1 (HV1, rCRS 15998-16390) or Hypervariable Region 2 (HV2, rCRS 49-408). Though amplification of smaller fragments is also often performed, previous studies and two decades of casework experience have shown that larger fragments are seldom recoverable. As a result, they are rarely targeted for amplification.

With the recent implementation of new extraction procedures for calcified tissues and hairs, a dramatic improvement in the quantity/quality of mtDNA recovered from these specimen types has been observed. Studies have shown a 2- to 30-fold increase in mtDNA copy number (as determined by a custom real-time quantitative PCR (qPCR)) and established that fragments >350bp are now recoverable.¹ In addition, results show that the qPCR values, though based on a 105bp amplicon, provide an indication of CR amplification success with amplicons of varying size. Not surprisingly, extracts with greater qPCR values tend to produce amplicons of greater size.

In order to further define the mtDNA quantities required for successful amplification and develop practical guidelines for casework implementation, representative extracts were quantified by qPCR and then amplified with primer sets targeting CR fragments of various size. Amplicons ranging from ~230bp to 1,100bp (the entire CR) were tested, then extracts producing successful amplification were evaluated for their corresponding mtDNA quantities. Minimum mtDNA quantities required to produce successful amplification at a given fragment size were conservatively established based on the highest quanting extracts (75%) that led to amplification product. Based on this criterion, guidelines were established to amplify entire HV1 and HV2 region amplicons (~360bp to 390bp) from hair extracts when $\geq 1,500$ copies/ μ L are detected (100% successful in studies) and entire CR with values $\geq 8,000$ copies/ μ L (95% successful in studies). Similarly, for calcified tissue, the entire CR can be amplified with values $\geq 30,000$ copies/ μ L (85% successful in casework and studies) and HV1 and HV2 can be targeted with as little as 400 copies/ μ L (100% successful in casework and studies). As a result of these guidelines, approximately 60% and 85% of calcified tissue and hair cases, respectively, have successfully yielded HVI and HVII amplicons. Moreover, the entire control region (~1100bp) has been successfully amplified from casework hair and calcified tissue specimens, whereas previously this would have not even been attempted.

The guidelines are continually being updated with casework data to maintain the most efficient and successful use of DNA extracts for current mtDNA analyses. In addition, it is expected that qPCR-based guidelines may be similarly useful with forthcoming Next Generation Sequencing (NGS) assays and applications. In fact, recent experiments have shown that 2kb to 3kb fragments spanning the entire mtGenome can be recovered from some hair samples with values of $\geq 30,000$ copies/ μ L and that these amplicons can be successfully sequenced using the Nextera[®] XT DNA Sample Preparation Kit and the Illumina MiSeq[™].

For poor quality evidentiary material containing damaged and/or low quantities of DNA, knowledge of the amplifiable mtDNA content has led to more informed casework decisions on downstream PCR. This in turn has led to decreased sample processing time and cost, more judicious use of limited sample material, and, most importantly, more successful mtDNA testing outcomes.



Criminalistics Section - 2015

Reference:

1. Kavlick, M.F., Lawrence, H.S., Merritt, R.T., Fisher, C., Isenberg, A., Robertson, J.M., and Budowle, B. 2011. Quantification of human mitochondrial DNA using synthesized DNA standards. *J Forensic Sci.* 56:1457-1463.
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mtDNA, NGS, qPCR



B135 Recovering Touch DNA From Cartridge Casings Using a Method of Tape Lifting

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After attending this presentation, attendees will better understand a more effective method to recover DNA from unfired cartridge casings compared to conventional wet-swabbing techniques.

This presentation will impact the forensic science community by providing a more effective process for recovering DNA from copper and copper-alloy surfaces.

Previous studies suggested that copper-induced degradation significantly lowered the amount of DNA recovered when copper or copper alloys were wet-swabbed. This phenomenon will reduce the recovery of epithelial cells and Cell-free Nucleic Acids (CNAs) deposited on the surface of a casing when loading a gun. To overcome this issue, tape-lifting was evaluated as a method to remove cells from the surface of cartridge casings.

In this experiment, the method of tape-lifting was used to recover touch DNA from unfired brass and nickel casings. Tape-lifting prevents any interaction between the copper component of brass and the aqueous solutions. Nickel casings were used as an experimental control. To evaluate the efficiency of the tape-lifting method, multiple individuals were asked to deposit touch DNA on cleaned brass and nickel cartridge casings. Four experiments were performed. Three donors participated in all four experiments, while three other donors participated in one experiment each. The donors were asked to roll a single casing in each hand for one minute, switching the casings from hand to hand every 15 seconds in order to try to equalize the amount of touch DNA on each casing. The DNA was allowed to stand on the casings for 48 hours before collection and extraction. In cases where donors needed to provide touch DNA on more than two casings for the experiment, the second set of casings were made available to the donors one hour after their first donation in order to maximize cell shedding between donations. The DNA was collected from the casings using both wet-swabbing and tape-lifting methods for comparison. DNA extraction was performed using Qiagen® QiaAMP® DNA Investigator® Kit. Quantitation was performed using an in house TaqMan® assay targeting autosomal DNA plus an internal control for inhibition developed for the Cepheid® SmartCycler® automated real-time PCR system.

Experimental results showed that the tape-lifting method recovered significantly more DNA than the wet-swabbing method from the brass cartridge casings, while no significant difference was observed between the two methods on nickel casings. Even though the amount of DNA recovered from brass cartridge casings was improved with the tape-lifting method, it was still significantly lower than the amount of DNA extracted using tape from nickel cartridge casings. Therefore, with the overall amount of DNA recovered from nickel being higher than that recovered from brass, the results suggest that degradation still occurs even without an aqueous medium. The average amounts of total DNA collected and extracted from brass using the swabbing and tape-lifting methods were 0.03181ng±0.03905ng, and 0.09965ng±0.06456ng, respectively. The average amounts of total DNA collected and extracted from nickel using the swabbing and tape-lifting methods were 0.16060ng±0.15795ng and 0.19068ng±0.15276ng, respectively. The above calculations were based on interim results of nine replicates of brass casings and seven replicates of nickel casings for both swabbing and tape-lifting methods. To confirm sample source, STR amplification was performed using AmpFISTR® Identifiler® Plus and analyzed on an ABI® Prism 3130® Genetic Analyzer. Although STR analysis yielded partial profiles in most cases, the lowest being 2/16 loci, the highest being 16/16 loci, and the average being 9.5/16 loci, the DNA recovered from the sampled surface matched the appropriate donor.

Touch DNA, Tape Lifting, Cartridge Casings



B136 Evaluation and Optimization of DNA Recovery From Bullet Cartridge Cases

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After attending this presentation, attendees will be aware of optimized methods for the recovery of nuclear DNA from spent cartridge cases.

This presentation will impact the forensic science community by examining the ability forensic biologists have to obtain useful DNA profiles from cartridge cases through the evaluation and optimization of collection and extraction techniques.

Firearms were used in 69.3% of homicides, 41% of robberies, and 21.8% of aggravated assaults in the United States in 2012. Accordingly, both fired and unfired cartridge cases are commonly encountered at crime scenes; however, despite the prevalence of this sample type, DNA testing is not often sought as it frequently fails to produce an interpretable profile. A common explanation for this poor/minimal “touch type” DNA transfer to non-porous surfaces. Another explanation is possible damage to DNA by the heat generated during the firing process. This degradation would lead to preferential amplification of those loci with shorter base pair lengths and the loss of amplifiable alleles at larger loci. It has also been hypothesized that co-eluting reactive metal ion species from Gun Shot Residue (GSR) or from the cartridge case itself can result in either inhibition and/or the accelerated degradation of DNA.

In order to optimize DNA recovery, different collection and extraction techniques were evaluated with cartridge cases of various metal compositions. Two collection techniques, double-swab and sonication, were evaluated by sampling sixty .22LR caliber cartridge cases. Additionally, two extraction techniques, a robotic extraction technique utilizing Qiagen®’s EZ1® Advanced XL system and a manual phenol:chloroform extraction protocol, were assessed by sampling 200 .45 caliber rounds. All samples were quantified using real-time quantitative PCR with Life Technologies® Quantifiler® Human DNA Quantification kit, amplified using Promega’s® PowerPlex® 16HS amplification kit, and analyzed using an Applied Biosystems® 3130 Genetic Analyzer with ABI’s® GeneMapper® software.

It was determined that the use of sonication for collection along with a phenol:chloroform extraction method results in significantly greater DNA recovery than using a double-swab collection technique or a robotic extraction technique. Sonication recovered an average of 50% of DNA compared to an average 11% recovered with a double-swab technique. Although the robotic extraction technique produced more consistent results, the average DNA recovery was significantly lower as compared to the phenol:chloroform extraction method. The robotic extraction technique resulted in an average DNA recovery of 3.65pg/μL with a standard deviation of 1.9, while the phenol:chloroform extraction technique allowed an average DNA recovery of 7.65pg/μL with a standard deviation of 10.6.

In order to assess the role GSR has on observed differences in the recovery of DNA from the various extraction methods used, a suspension of GSR was created using internal barrel swabs of recently fired guns. Increasing volumes of this suspension (1μL, 5μL, 7.5μL, and 10μL) were added to eight samples of 2800M positive-control DNA. These samples were then extracted using either the EZ1® protocol or the phenol:chloroform protocol. The EZ1® extraction protocol resulted in DNA profiles with significant variability and poor inter-locus signal balance. Several of the samples extracted using the EZ1® protocol demonstrated preferential amplification or peak height suppression. Samples which were extracted using the phenol:chloroform protocol produced DNA profiles with consistently high and well-balanced allelic peaks across all loci.

In order to assess the role which the extraction method plays in samples from handguns, 18 swabs were prepared under controlled laboratory conditions from the slide serrations of handguns known to contain multiple contributors. Half of each sample was extracted using the robotic protocol and the remaining half was extracted with a phenol:chloroform protocol. The EZ1® extraction protocol resulted in DNA profiles with an average RFU value of 60. Samples which were extracted using the phenol:chloroform protocol produced full DNA profiles with an average RFU value of 2,000.

This research offers an optimized method for DNA recovery from cartridge cases and demonstrates that the adverse impacts on DNA profiles associated with samples originating from handguns could be due to the inability of commonly employed robotic extraction methods to remove co-eluting species which have been demonstrated to affect downstream analysis.

Cartridge Cases, STR Typing, Touch DNA



B137 Evaluation of a Vacuum-Swab Protocol for Recovering Epithelial Cells From Handled Evidentiary Samples

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After attending this presentation, attendees will be able to implement a cost-effective and efficient collection technique for the recovery of epithelial cells from handled forensic samples.

This presentation will impact the forensic science community by introducing an efficient collection technique for the recovery of touch DNA from forensic samples. This vacuum-swab technique can easily be implemented into current forensic laboratories without the need of purchasing expensive equipment.

Handled evidence, such as clothing, firearms, and tools, are frequently submitted to forensic biology laboratories for Short Tandem Repeat (STR) typing. This has resulted in a growing need for a cost-effective and efficient collection technique for recovering biological material from handled items of evidence. Due to the limited quantities of DNA that can be left behind on handled objects, it is essential that the recovery method employed provides the maximum possible percent recovery of epithelial cells and therefore the greatest chance of generating a quality genetic profile. Current recovery techniques used to collect epithelial cells include the double-swab method, the tape-lift method, and the scraping method. On hard, non-porous surfaces, the double-swab technique has been shown to be the most effective. In a comparison using filter paper, single-swab, and double-swab techniques, the double-swab technique yielded the greatest quantity of DNA. The use of adhesive tape has been shown to be the most effective on porous samples, yet recovered only 55% or less of the donor's genetic profile. An additional collection method using a vacuum apparatus has been shown to produce promising results from porous items of evidence. This system sprays a buffer onto the surface of an item of evidence while simultaneously suctioning from the surface, thus collecting cellular material in a large volume of buffer; however, this necessitates the use of an additional filtration step to collect cellular material for DNA extraction. The current study evaluated these DNA recovery techniques in comparison to an alternative vacuum-swab technique to determine the optimum method of collecting epithelial cells from porous substrates.

Buccal cells were collected from a single donor and deposited on fabric at varying concentrations and allowed to dry. For the double-swab method, a swab moistened with 2% Sodium Dodecyl Sulfate (SDS) was first passed over the surface of the fabric ensuring maximum contact between the swab and surface. A second dry swab was then passed over the fabric and combined with the initial swab for extraction. For the tape-lift method, water-soluble tape was firmly pressed against the surface of the fabric and directly added to tubes for extraction. For the scraping method, a scalpel blade was passed over the fabric held at an angle to recover cellular material on the surface of the fabric. The vacuum-swab technique used a sterile glass tube with a cotton plug attached to a rough pump vacuum apparatus. All samples were quantified and then amplified at 16 polymorphic loci and analyzed using capillary electrophoresis. Quantitative values were compared for each method and resulting genetic profiles were assessed to ensure no allelic drop-in or sample contamination.

Equal volumes of liquid buccal cell slurry were extracted with samples that had the same volume deposited on fabric prior to collection. This was used to calculate a theoretical DNA quantity deposited on the fabric swatches and, therefore, the percent recovery of each collection protocol. Significant differences were found among the four methods of recovery ($p \leq 0.001$). The double-swab and scraping methods showed the poorest percent recovery, while the tape-lift and vacuum-swab methods showed the best percent recovery. At each concentration assessed, the tape-lift and vacuum-swab methods collected on average two to five times greater DNA concentrations, respectively. Amplification results demonstrated the absence of contamination in all cases. This data illustrate that the vacuum-swab and tape-lift collection protocols employed can enhance the quantity of recovered DNA from handled items and, therefore, are best suited for use in forensic biology laboratories for submitted porous evidence types.

STR Typing, Vacuum Swabbing, Touch DNA



B138 Evaluation of a Novel Approach to Low-Copy Number (LCN) DNA Methodologies for Generation of Short Tandem Repeat (STR) Profiles

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After attending this presentation, attendees will be familiar with the relative impact of different approaches to low-copy DNA analysis on stochastic effects as compared to increased Polymerase Chain Reaction (PCR) cycle number protocols.

This presentation will impact the forensic science community by assessing alternative methods for analyzing low template DNA from evidentiary material that will allow for the generation of more complete STR profiles. With increased allelic recovery in addition to decreased stochastic events as compared to other LCN methods, an improved protocol for LCN samples will increase the ability of forensic laboratories to generate useful profiles.

Producing genetic profiles from samples with 100pg or less of template DNA, also known as LCN samples, is difficult using standard PCR methods. Modified sample preparation protocols which increase the cycle number used for amplification up to 34 cycles have been developed to improve profile recovery from these sample types; however, increasing the PCR cycle number produces allelic imbalance and drop out as well as other stochastically induced events, such as increased stutter ratios. Increasing the number of PCR cycles can result in either more complex mixtures due to the amplification of trace DNA that would not otherwise be detected or in the amplification of trace DNA contaminants introduced during sample processing that would not otherwise have been detected. These effects can complicate the interpretation of resulting STR profiles. The method assessed in this study seeks to increase the percent recovery of a genetic profile as compared to standard PCR methods without producing the increased stochastic effects observed with increased cycle number methods.

Dilutions of extracted reference quality DNA were prepared so that the maximum input volume for amplification (in this case 17.5µl) would result in the addition of the following amounts of DNA: 245pg, 122.5pg, 85.8pg, 42.9pg, 30.0pg, 15.0pg, 10.5pg, 5.3pg, 3.7pg, and 1.8pg. Autosomal STR profiles were developed from each dilution in the series by four different assay methods (five replicates each). Samples in treatment group one were amplified following the manufacturer's recommended protocol with 30 amplification cycles. Samples in treatment group two were amplified following the manufacturer's recommended protocol with the addition of four extra amplification cycles (34 amplification cycles total). Samples in treatment group three followed the manufacturer's recommended protocol with 30 amplification cycles except that 50µl of DNA extract was dried down and reconstituted in 17.5µl TE⁻⁴, thus resulting in a greater overall input of DNA. Samples in treatment group four followed the manufacturer's recommended protocol with 30 amplification cycles except that 50µl of DNA extract was added to a centrifugal filter and concentrated in 17.5µl TE⁻⁴, thus resulting in a greater overall input of DNA. All samples were amplified using an Applied Biosystems® GeneAmp® PCR System 9700 using a Promega® PowerPlex® 16 HS System and analyzed using capillary electrophoresis on an Applied Biosystems® 3130 Genetic Analyzer. Resulting genetic profiles were compared based on percent recovery of donor alleles, stutter ratios, and allelic balance, as well as for allelic drop in.

Full DNA profiles were consistently obtained with as little as 15pg template DNA for dried down samples. Samples at the same initial concentration, 0.30pg/µl, only yielded 35.5%, 45.8%, and 29% of donor alleles for standard samples, increased cycle number samples, and samples concentrated with filters prior to amplification, respectively. When compared to samples with the same input of template DNA prepared following the other methods, dried down samples did not show significant increased stutter nor was any peak height imbalance or allelic drop-in observed; however, increased stutter, peak height imbalance, and drop-in was observed in those samples that underwent increased PCR cycle number. Several replicates of dried down samples even produced full profiles with only 10pg template DNA. Resulting data illustrates that concentrating extracts prior to amplification by drying them down is a method well suited for use in forensic biology laboratories when attempting to develop genetic profiles from low copy number samples.

STR Typing, LCN DNA, PowerPlex® 16HS



B139 Use of the Ion PGM™ System for Typing Human Identity Marker Systems

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After attending this presentation, attendees will understand the methodology and sequencing chemistry used in massively parallel sequencing on the Ion Personal Genome Machine® (PGM™) platform and the capability of this system to sequence and interpret large genetic marker panels.

This presentation will impact the forensic science community by providing current progress on the use of massively parallel sequencing as it pertains to forensic DNA analysis. In addition, attendees will learn about the quantity and quality of the data generated by Ion PGM™ system.

Massively Parallel Sequencing (MPS), also known as next generation sequencing, technologies provide the scientific community with novel and enhanced approaches to DNA typing. While capillary electrophoresis-based technologies have been considered the gold standard for human identity typing applications, the current technology has notable limitations in intra-repeat variation resolution, sample scalability, and throughput of markers that can be typed. MPS has the potential to overcome these limitations with its ability to multiplex different types of forensically relevant genetic markers, analyze a large number of markers simultaneously, and sequence multiple samples per run. Additionally, MPS data may offer a new avenue for interpretation of mixtures. The Ion Torrent™ PGM™ is a benchtop sequencer that uses semiconductor sequencing chemistries and laboratory workflows that enable high-throughput and quick run times at a reasonable cost.

Twelve genomic samples, containing total DNA ranging from ~42ng to 280ng, were provided by a third party (the Green Mountain Conference) for a blinded genetic study. The mitochondrial genome and three Ion PGM™ panels containing human identity Single Nucleotide Polymorphisms (SNPs), ancestry informative SNPs, and Short Tandem Repeats (STRs) were sequenced on the Ion PGM™ system and analyzed for these 12 samples. Sequencing and genetic analysis for all four genetic systems were completed in a reasonably quick time frame by one individual. Completeness of genetic profiles, depth of coverage, strand balance, and allele balance were evaluated as informative metrics for the quality and reliability of the data produced. The autosomal SNPs from the human identity SNP panel reached an average read depth of 2,233. For this study, 99% of these SNPs had an average strand balance of 60% to 100%, and 100% of these SNPs had an average allele coverage ratio of 30% to 50%. The Y-chromosome Single Nucleotide Polymorphisms (Y-SNPs) from the human identity panel reached an average read depth of 975 while 97% of these SNPs had an average strand balance of 60 to 100 percent. The SNPs from the ancestry SNP panel reached an average read depth of 1,511 and 98% of these SNPs had an average strand balance ratio of 60% to 100% and 94% of these SNPs had an average allele coverage ratio of 30% to 50%. The large number of SNPs in these panels provides information on individual identification, familial relationships, and population background for the samples analyzed. The average allele coverage ratios for the STR markers ranged from 70% to 100% and the STR genotypes generated by MPS were in complete concordance with genotypes generated by standard capillary electrophoresis-based technologies. Additionally, intra-STR SNPs identified by MPS offer the potential for increased discriminatory power and improvement in mixture analysis. MPS coverage across the entire mitochondrial genome ranged from 489 to 7,029 in read depth. The average percent positive strand coverage for the entire mitochondrial genome ranged from 30% to 86% and indicated that sequence data from both strands of the mitochondrial DNA were captured. Mitochondrial variants identified among this sequence data were used to generate haplogroups for each sample and ultimately provide information on population background and maternal relationships. Sample information provided by Green Mountain subsequent to the blinded study supported that reliable results had been produced for all 12 genomic samples. The strength and depth of these results warrant expanded validation studies of current MPS technologies (which are ongoing) and continued development of tools for data analysis.

Massively Parallel Sequencing, Forensic DNA Typing, Human Identity



B140 Increased Ancestry Prediction of the United States Population Using Short Tandem Repeat (STR) Data in Addition to 32 Ancestry-Informative SNPs

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After attending this presentation, attendees will learn that the STRs used for Human Identification (HID) can support Single Nucleotide Polymorphisms (SNPs)-based ancestry prediction on United States-population individuals.

This presentation will impact the forensic science community by demonstrating that STRs can aid in increasing the accuracy of ancestry inference.

Often a very limited amount of information is available on the perpetrator of a crime, especially when there are no eyewitnesses, when the DNA left behind does not match any suspect, or the DNA isn't present in the databases; however, it is still possible to garner investigational information by analyzing Ancestry-Informative SNPs (AISNPs). An SNP assay was developed at George Washington University that predicts the most likely ancestry of an individual between the primary United States populations (grouped as African American, East Asian, European American, and Hispanic/Native American). The prediction is made by first determining the probability of the SNP profile within each population (i.e., the Random Match Probability (RMP)). The RMP is the probability of randomly finding an unknown individual with that specific profile in the given population. Then a Likelihood Ratio (LR) is calculated for each of the four populations. In this LR, the numerator is the RMP of the SNP profile from the population in which it is highest and the denominator is the RMP of the profile in the specified population. The LR expresses how much more likely it is to observe the profile if it originated from the population in the numerator versus if it originated from the population in the denominator. An empirical threshold of 1,000 was chosen, above which it is considered significant for a sample to be classified as belonging to one of the four populations. A sample with an LR lower than 1,000 was classified as inconclusive between the two populations with the highest RMPs, inferring that the sample most likely belongs to one or both of those populations. The samples are divided into four categories based on the results obtained: correct, incorrect, inconclusive correct, and inconclusive incorrect. Correct corresponds to the situation where the sample is classified as belonging to a single population, which is the same one as reported by the donor. Incorrect corresponds to the situation where the sample is classified as belonging to the wrong population. Inconclusive correct corresponds to the situation where the sample is classified as belonging to two populations and the donor reported one or both of these populations. Inconclusive incorrect corresponds to the situation where the sample is classified as belonging to two populations but these populations were not reported by the donor.

Thirty-two AISNPs were used to predict the ancestry of 134 samples (with self-reported ancestry information) using the method described above. Of these samples, 72% were correctly classified as belonging to one population. One sample was classified as inconclusive incorrect. The rest of the samples (27%) were classified as inconclusive correct. No samples were classified as incorrect.

To assess the impact of including HID STR allele frequencies into the ancestry prediction, the 134 samples were also genotyped with AmpflSTR® Identifier® Plus. The random match probability was calculated with the STR data obtained and factored into the SNP RMPs. The LRs were then recalculated and the accuracy of the prediction was reevaluated. With the inclusion of the STR data in the analysis, the accuracy of the prediction increased to 80% correctly classified samples. This 8% increase is statistically significant at a 95% confidence (Z -score=-1.7252154; p =0.0422). This indicates that although STRs were not selected to provide ancestry information, they can improve the prediction of an SNP-based assay. Thus, available STR data should be included in ancestry prediction.

SNPs, Ancestry Prediction, STRs



B141 Simulating the United Arab Emirates Crime Scene Samples and Generating DNA Profiles From Them Using the RapidHIT™ System

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After attending this presentation, attendees will understand how quickly DNA profiles can be obtained from environmentally challenged samples even by non-scientists.

This presentation will impact the forensic science community by demonstrating that a robust automated system can be used to obtain DNA profiles from samples exposed to detrimental environmental conditions.

The average time needed to obtain a DNA profile from an evidence sample in crime laboratories is approximately 12 hours, which is time-consuming and labor intensive. In contrast, the RapidHIT™ Human DNA Identification System provides a faster method to generate DNA profiles in approximately 90 minutes. The operator only needs to insert the samples in the instrument cartridge. The system then extracts, amplifies, and generates DNA profiles through capillary electrophoresis.

The goal of this research was to test the sensitivity and robustness of the newly introduced “Run Other Samples” instrument protocol of the RapidHIT™ System in generating DNA profiles from simulated crime scene samples. The samples used in this study were blood and saliva, with varying amounts of each deposited on different types of substrates. The blood samples were obtained from one deceased male and one deceased female. The saliva sample was obtained from a living female donor.

The substrates chosen for this study are commonly found in crime scenes in the United Arab Emirates. The substrates used for deposition of saliva samples included cotton swabs, cotton-tipped applicators, stainless steel spoons, plastic spoons and forks, straw, mint-flavored chewing gum, stones covered with soil, the mouth area of water bottles, stamps, envelopes, and oil-based paint. Blood was deposited on substrates that included cotton swabs, cotton-tipped applicators, paper coated with latex-based paint, paper coated with water-color paint, tile, finished wood flooring, unfinished wood blocks, laminated flooring, broken tempered glass, stones covered with soil, pieces of wood covered with soil, denim jeans, synthetic leather, carpet fibers, delicate task wipes, clear plastic bags, and white scarves typically worn by men in the United Arab Emirates. Similar amounts of blood from deceased donors and an approximately similar amount of saliva from a living donor were deposited on each of these items. The substrates containing body fluids were then kept at room temperature for approximately 24 hours. Furthermore, the substrates were environmentally challenged by heat and humidity, factors similar to the weather conditions encountered in the United Arab Emirates.

Each substrate containing one body fluid was either swabbed or cut and deposited in cartridges using the RapidHIT™ System. Autosomal Short Tandem Repeat (STR) loci and the amelogenin gender locus were amplified using the PowerPlex®16 HS multiplex amplification kit chemistry. The generated data were analyzed using GeneMarker® HID Software Version 2.4.0. The DNA profiles were compared for concordance within and between the substrates used in this study.

This study shows that the RapidHIT™ System is capable of producing complete and concordant profiles from blood and saliva samples deposited on simulated crime scene substrates commonly encountered in the United Arab Emirates. The “Run Other Samples” instrument protocol worked effectively to generate DNA profiles from pristine and challenged samples.

Complete profiles were obtained from all of the 87 samples tested. Replicates were not run since full profiles were generated from all 87 samples. The Peak Height Ratios (PHR) and the Relative Fluorescence Units (RFU) values decreased with decreased volumes of blood. The PHR values were above 70% with 1.0µl, 0.5µl, and 0.25µl of blood while PHR values below 70% were observed when 0.125µl and 0.0625µl of blood were amplified.

DNA profiles with all 16 loci were obtained from 1.0µl, 0.5µl, 0.25µl, and 0.125µl of the neat male blood sample. Additionally, 50.0µl, 40.0µl, 30.0µl, 20.0µl, and 10.0µl of saliva directly deposited on the swabs yielded complete DNA profiles. As expected, PHR values decreased when the amount of saliva was reduced.

Various volumes of the blood and saliva samples deposited on different, potentially inhibiting substrates generated complete profiles with excellent peak signals and balanced peak height ratios. Additionally, high heat and humidity similar to that experienced in the United Arab Emirates did not adversely affect the RapidHIT™ System’s ability to yield complete profiles on simulated crime scene samples.

United Arab Emirates, RapidHIT™ System, DNA Profiles

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B142 Development of a Portable, Laminated Dynamic Solid-Phase DNA Extraction Method on a Rotationally-Driven Platform

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After attending this presentation, attendees will better understand the growing field of microfluidics and the design and development toward a novel, inexpensive, and portable alternative for extracting DNA from forensically relevant samples.

This presentation will impact the forensic science community by introducing a technique that adapts DNA extraction to a microfluidic platform for the potential of an inexpensive, on-site DNA extraction system in resource-limited areas with increased sample efficiency compared to current benchtop methods.

Solid-Phase Extraction (SPE), a critical step in genetic analysis, relies on the binding of DNA to silica-coated particles.¹ Dynamic SPE (dSPE) manipulates silica-coated magnetic particles during the extraction process to ensure optimized trapping and elution of DNA. Adapting dSPE to a microdevice enables advantages over benchtop methods including: increased sample efficiency, decreased reagent volumes, decreased assay cost, a closed system to prevent contamination, and the ability for integration with downstream processing via STR or next generation sequencing.

Polyester (Pe) can be patterned and bonded with Toner (T) to create multilayer microfluidic PeT devices for <\$2 in <15 minutes.^{2,3} Successful demonstration of dSPE via pressure-driven flow in rapidly fabricated PeT devices has been shown via manual pipetting.² Recent developments with open architecture PeT devices, hydrophobic valving, and centrifugally-driven fluid flow provide an attractive new platform for automated dSPE. The proposed dSPE system functions at low spin speeds (<1,500rpm), incorporates valving for non-aqueous solutions, is low cost (\$0.25 per assay), and is amenable to rapid, simple fabrication. For the first time, DNA extraction from whole blood on a disposable plastic PeT microdevice using an automated, rotationally-driven platform is described.

The proposed PeT microdevice is composed of four layers and is fabricated using laser printer lithography. The device, when loaded with dSPE reagents and sample, can be run through a five-speed bidirectional spin program (0 to ~1,276rpm) on the home-built system. Binding of sample DNA to the particles is driven by an Alternating Magnetic Field (AMF). A novel combination of a hydrophobic valve and backpressure prevents the wash and elution buffers from entering the bound DNA chamber. After binding, IPA is released by centrifugal force (overcoming backpressure) at ~293rpm, followed by a TE wash through hydrophobic valve bursts at ~340rpm. After washing, the bound DNA is eluted and mobilized to a separate elution chamber at ~1,276rpm using “stop” valves positioned below the main DNA chamber. Approximately 10 μ l of purified DNA results from this process and is accessible by puncturing the Poly(DiMethyl) Siloxane (PDMS) covering the elution chamber. To demonstrate that the spin system effectively purifies PCR-ready DNA from 2 μ L of whole blood, the target β -globin was successfully amplified via microchip electrophoresis. In addition, samples extracted from FTA[®] cards were successfully amplified for STR analysis and were shown to display full profiles.

The proposed PeT microdevice has the potential to extract forensically-relevant samples when compared to the Qiagen[®] EZ1[®] instrument. Preliminary results suggest that DNA extracted from the microdevice and the EZ1[®] from buccal swabs were nearly equivalent in concentration, 0.28 \pm 0.1ng/ μ L and 0.27 \pm 0.7ng/ μ L, respectively. Furthermore, full STR profiles have been obtained with both methods. PeT-based peak heights were 1,619 \pm 1,054 Relative Fluorescence Units (RFUs) compared to the EZ1[®] peak heights at 1,774 \pm 705 RFU. Current efforts are underway to increase extraction efficiency and reproducibility. Overall, this is the first demonstration of a PeT microdevice used for chaotrope-driven dSPE on a rotationally-driven platform. The portable system has the potential to provide a cost-effective alternative to current SPE kits and once fully optimized, can be coupled to other pre- and post-processing DNA steps including preliminary DNA detection and amplification.

The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.



Criminalistics Section - 2015

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Microfluidics, Extraction, DNA



B143 Use of Rapid DNA Systems in Disaster Victim Identification

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After attending this presentation, attendees will understand how Rapid DNA systems can be implemented for use in disaster victim identification.

This presentation will impact the forensic science community by informing attendees about how Rapid DNA systems can shorten the time it takes to process, compare, and identify victims of a mass fatality incident.

Disaster Victim Identification (DVI) can be a very meticulous and lengthy process despite the number of casualties. This is especially true if DNA testing is part of the DVI process and is used to assist in the identification of victims. Processing and analyzing DNA samples from decedents or relatives can take several days. Rapid DNA typing systems eliminate this wait by utilizing a tabletop, self-contained, and automated instrument. Within the past few years, several companies have developed and improved such systems. Rapid DNA systems reduce the time needed to process a DNA sample from days to less than two hours. In contrast, traditional methods of DNA profiling from cheek swabs require upwards of ten hours and involve multiple laboratory personnel. The currently available rapid DNA systems allow for DNA extraction, amplification, separation, and genotyping analysis to be completed in less than 90 minutes, without analyst intervention. This represents a significant savings of time and personnel over traditional STR typing methods. Among the rapid DNA instruments that are available, each generates a DNA profile in a similar manner but with varying sample capacity, run times, and cost.

Rapid DNA systems are particularly practical for disaster recovery and identification. Besides being fast, the systems are mobile, rugged, and simple to use, with all consumables provided in a disposable, ready-for-use format. With rapid DNA systems on-site at a Family Assistance Center (FAC), DNA analysis of samples collected from family members can be processed and the resulting DNA profiles analyzed in a family pedigree chart.

Concurrently, if disaster recovery is occurring and a mobile morgue is set up, postmortem samples can be collected on-site and processed immediately. Transport, sample tracking at the laboratory, and the requirements of multiple laboratory personnel are eliminated. The DNA team at the mobile morgue can collect and process samples immediately. The profiles generated from decedents at the mobile morgue and relatives at the FAC can be readily input into a current victim identification program and kinship analysis performed.

The New York City Office of Chief Medical Examiner (NYC OCME) Department of Forensic Biology has evaluated two rapid DNA systems in order to evaluate their application for DVI: the IntegenX RapidHIT® Human Identification System and GE Healthcare Life Sciences (GE) and NetBio DNAScan™ Rapid DNA Analysis™ System. Initial evaluations were conducted at the NYC OCME and the performance of each instrument was further evaluated at a full-scale mass disaster exercise. Specifically, the RapidHIT® and DNAScan™ Rapid DNA systems were evaluated on-site at the FAC and DNA unit of the mobile morgue during the Fifth Annual Regional Mass Fatality Management (MFM) Training, which occurred from June 2 to 6, 2014, at Fort Hamilton Army Base in Brooklyn, NY. During the FAC portion of the exercise, reference samples were collected from volunteers simulating family members and processed on both instruments. During the mobile morgue portion of the exercise, eight postmortem muscle and two postmortem blood samples, simulating recovered remains, were processed and typed in the DNA unit of the mobile morgue on both instruments. Samples were loaded into either the RapidHIT® system sample cartridges or the DNAScan™ BioChipSet™ cassette, both using Promega's® PowerPlex® 16 HS kit optimized for rapid DNA analysis. The DNAScan™ data were automatically analyzed using NetBio's integrated software with fixed analysis parameters. The RapidHIT® data were automatically analyzed with the on-board SoftGenetics® GeneMarker® HID STR Human Identity Software.

The output data for both rapid DNA systems were compared to a reference database of traditionally-typed STR profiles. Output data were also manually confirmed by a DNA analyst and evaluated based on alleles called, profile completeness, and peak height balance. See data in Tables 1 and 2 below.

The MFM exercise has demonstrated that rapid DNA systems can be utilized in DVI. Sample preparation for input into cassettes, respective software analysis parameters, and logistics, as far as instrument transport and chip storage, need to be considered and incorporated into a standard operating procedure for rapid DNA system operations in DVI.

Table 1 — GE/NetBio DNAScan™ DNA Morgue and FAC Results with Promega® PowerPlex® 16 HS Chemistry

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Criminalistics Section - 2015

Sample	Ref. Profile Alleles	#Alleles		% Alleles		% Alleles Recovered by Analyst Review	
		Auto	Confirmed**	Auto	Confirmed**		
Degraded Muscle Tissue	D1	29	0*	14	0%	48%	48%
	D3	26	0*	5	0%	19%	19%
	D4	29	0*	0	0%	0%	0%
	D5	29	0*	7	0%	24%	24%
Fresh Muscle Tissue	F1	28	0*	11	0%	39%	39%
	F2	27	25	26	93%	96%	4%
	F4	26	24	24	92%	92%	0%
	F5	28	28	28	100%	100%	0%
Bloodstained FTA® Card	363	29	0*	0	0%	0%	0%
	359	29	0*	26	0%	90%	90%
Buccal Swabs	1	29	29	29	100%	100%	0%
	2	26	0*	22	0%	85%	85%

Average % Alleles Recovered = 26%
 * = DNAScan™ Software flagged alleles as “NR” or not reliable, based on fixed analysis parameters.

** = DNA analyst visually reviewed electropherograms and confirmed peaks labeled as “NR”

Table 2 — IntegenX RapidHIT® Human Identification System DNA Morgue and FAC Results with Promega® PowerPlex® 16 HS Chemistry

Sample	Ref. Profile Alleles	#Alleles		% Alleles		% Alleles Recovered by Analyst Review	
		Auto	Manual**	Auto	Manual**		
Degraded Muscle Tissue	D1	29	11	23	38%	79%	41%
	D3	26	6	15	23%	58%	35%
	D4	29	22	29	76%	100%	24%
	D5	29	7	14	24%	48%	24%
Fresh Muscle Tissue	F1	28	19	29	68%	104%	36%
	F2	27	0*	17	0%	63%	63%
	F4	26	21*	23	81%	88%	8%
	F5	28	0*	20	0%	71%	71%
Bloodstained FTA® Card	363	29	29	29	100%	100%	0%
	359	29	0*	20	0%	69%	69%
Buccal Swabs	1	29	29	29	100%	100%	0%
	2	26	26	26	100%	100%	0%

Average % Alleles Recovered = 31%
 * a very high signal peak (saturated) resulted in the primer trim step cutting into read region during auto analysis

** Manual = Raw data re-analyzed in SoftGenetics® GeneMarker® HID STR Human Identity Software version 2.6.0 by a DNA analyst.

Mass Disaster, Rapid DNA, Disaster Victim Identification

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B144 Human Identification in Less Than 45 Minutes: A Rapid and Fully Portable DNA Solution

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After attending this presentation, attendees will be aware of an ongoing effort to develop a human DNA identification system that provides a discriminatory power of more than 98% and is performed on a commercially available, rapid, portable, and ruggedized instrument. Attendees will gain insight into the research and development of the system and the flexibility of using Single Nucleotide Polymorphisms (SNPs) for DNA identification.

This presentation will impact the forensic science community by introducing a system that can be used to develop investigative leads through the analysis of biological evidence on-site at crime scenes or areas of interests. The presented system will also support the applicability of SNPs to forensic DNA technologies and human identification.

In recent years, major progress has been made toward the development of fully integrated rapid DNA analysis devices; however, most of these instruments are not amenable to on-site DNA processing at the crime scene due to their size. Overall, the existing rapid DNA devices are large and mobile laboratories would be necessary to transport the systems to a crime scene. To address the need for a portable rapid DNA analysis system, The Bode Technology™ Group, Inc. has developed a human identification SNP assay for use with the RAZOR® EX instrument. The RAZOR® EX is a lightweight real-time thermal cycler typically used for the detection and identification of pathogens and biothreat agents. Commercially available RAZOR® EX assays are performed in under an hour using a rapid extraction method and a specialized pouch containing lyophilized amplification reagents. Additionally, the device has an on-board Liquid Crystal Display (LCD) screen that displays the assay results in real time.

To adapt the RAZOR® EX instrument for human DNA analysis, a multiphased approach was taken for development of a human identification system: (1) a simple, rapid, and efficient extraction method was created that is capable of lysing blood, semen, and epithelial cells in less than 15 seconds; (2) a customized TaqMan® allelic discrimination assay capable of amplifying forensic samples was developed using SNPs that were previously characterized for forensic use; (3) two amplification controls were designed to estimate the quantity and quality of the forensic sample; and, (4) the amplification reagents were lyophilized to ensure the stability of the assay at room temperature. While the assay results can be easily interpreted from the RAZOR® EX's LCD screen, a software application was also developed to provide more accurate data analysis and to allow population statistics to be applied to the sample. The entire process, from collection to result, has been performed on mock evidentiary samples in less than 45 minutes.

The current version of the rapid portable human identification system has generated robust results both in the laboratory and in mock field testing. The discriminatory SNP assays have been tested on over 400 forensically relevant samples. Prior to lyophilization, initial wet assay testing examined 25µL of blood (neat and diluted 1:50), semen (neat and diluted 1:50), and saliva (neat and diluted 1:10) deposited on glass, plastic, rusted metal, adobe brick, red brick, concrete, ceiling tile, MDF, duct tape, plywood, cotton fabric, denim, and paper. Full SNP profiles were generated from 100%, 86.5%, and 43.6% of the neat blood, semen, and saliva samples, respectively. As expected, fewer full profiles were generated from the diluted fluid samples, with a 30.8% success rate for diluted blood, a 23.1% success rate for diluted semen, and a 28.2% success rate for diluted saliva. Across all sample types, assay success rates were higher for samples deposited on non-porous substrates. Following lyophilization of the assay, 27 mock forensic samples were amplified with the lyophilized pouches on-site at mock crime scenes. The mock forensic samples consisted of blood, semen, and saliva (100µL) deposited onto metal, concrete, plastic, and cotton substrates that were exposed to uncontrolled indoor or outdoor conditions for a minimum of 12 months. Of these samples, 67% generated full SNP profiles. Additional controlled laboratory testing has been performed to examine the reproducibility and sensitivity of the lyophilized assay. A full developmental validation is anticipated to determine the system limitations.

Rapid, Portable, SNPs



B145 Validated and Straightforward Multiplex PCR Method for High-Quality Analysis of the Expanded CODIS STR Loci Set

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After attending this presentation, attendees will know about the benefits of QIAGEN's® Combined DNA Index System (CODIS) expansion Polymerase Chain Reaction (PCR) kit, how to evaluate the amplification efficiency of the PCR in a very convenient way, and how to choose the most appropriate rework strategy.

This presentation will impact the forensic science community by showing new technical features for a Short Tandem Repeat (STR) analysis with the benefits of reducing costs and increasing efficiency, productivity, and quality for laboratory operations by using the unique and novel quality sensor system.

QIAGEN® has developed and validated two multiplex PCR kits for reliable genotyping of the expanded CODIS STR loci set: the Investigator® 24plex QS Kit and the Investigator® GO! Kit. The former kit is designed for purified DNA from casework and reference samples, the latter kit is optimized for direct amplification of reference samples, like blood or buccal cells on FTA® paper or swabs. For buccal swabs, a five-minute lysis protocol is provided to prepare samples for direct amplification.

To verify the quality of the DNA sample and the performance of the PCR, both kits contain a novel quality sensor as an internal performance control. Forensic analysts are often faced with challenges when it comes to interpreting STR results. What is the reason that no peaks are visible in the electropherogram? Did the PCR fail? Was the DNA concentration too low? Was the DNA degraded? The kits of the Investigator® 24plex family contain a unique and novel Quality Sensor that is useful for evaluating the amplification of the PCR reaction. The system consists of two internal PCR controls (Quality Sensor QS1 and Quality Sensor QS2) located at the borders of the purple dye channel at 74bp and 435bp, respectively. To ensure that the quality controls do not cause any unspecific amplification during PCR, the internal control template was designed using a random algorithm and the obtained sequences were checked for absence of significant similarity to any known sequences. The 74bp QS1 shows very stable amplification, even in the presence of extremely high inhibitor concentrations (e.g., 1,000µM hematin). In contrast, the 435bp QS2 is more prone to inhibition and typically drops out before the first STR marker drop out is observed. Relative signal heights of QS1 and QS2 can thus be used to indicate inhibition. In the case where both sensors are unaffected but the sample shows a ski-slope effect with poor amplification of high molecular weight markers, degradation of the DNA sample is most likely. This information can be used to choose the most appropriate rework strategy.

Both assays use a new 6-dye technology to shorten the overall amplicon length and minimize overlap of the 23 markers which might lead to errors in data interpretation. The kits feature a very robust gender typing by offering small amplification fragments both for amelogenin and DYS319, leading to correct gender typing even for difficult samples (degraded or inhibited DNA) of amelogenin null mutant individuals. The developmental validation of the Investigator® 24plex QS kit based on the revised guidelines of the Scientific Working Group on DNA Analysis Methods (SWGDM) and the recommendations of the European Network of Forensic Science Institutes (ENFSI) has recently been completed. In a sensitivity study, full profiles were consistently obtained with 125pg template DNA. First stochastic allele drop outs were observed at 63pg template. Inhibitor studies revealed full DNA profiles in the presence of up to 200ng/µl humic acid, 750µM hematin, 3mM calcium, 12mM indigo carmine, 4000ng/µl tannic acid, and 200ng/µl collagen. These kits are in the process of being evaluated for approval for use at the National DNA Index System (NDIS).

CODIS, PCR, Quality



B146 Improving Processing Efficiency for Forensic DNA Samples

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After attending this presentation, attendees will be familiar with several approaches used to reduce sample processing time by ~33% for databasing reference samples, including extraction normalization, fast Polymerase Chain Reaction (PCR), and quicker genetic analyzer detection while maintaining high-quality profiles similar to those observed using current methods (i.e., >90% full profiles using 3100 rfu threshold with 100% concordant alleles, comparable inter-locus peak balance, PHR $^350\%$, stutter $\leq 20\%$, pull-up $\leq 20\%$, and no -A).

This presentation will impact the forensic science community by sharing methodologies to significantly improve sample-processing efficiency without the need to purchase new instrumentation or costly reagents. These improvements are robust and can be implemented in a variety of laboratory settings.

The goal of this project was to reduce processing time without incurring significant added costs via a multi-layered overhaul undertaken in the following order: (1) develop a quicker detection method for the 3130xl Genetic Analyzer using an alternative polymer/array length combination; (2) develop fast PCR protocols for various Short Tandem Repeat (STR) primer sets using shorter amplification parameters, low volume reactions, and standard non-fast thermal cyclers; and, (3) normalize an extraction process using the ChargeSwitch® Forensic DNA Purification Kit such that a small range of yields are consistently obtained, thereby eliminating the need for quantification and dilution prior to amplification.

First, alternative polymer and capillary array length combinations were evaluated and compared to the standard POP-4™/36cm array detection method employed for STR detection on a 3130xl Genetic Analyzer. Detection time for standard 4-dye and 5-dye STR amplification kits was reduced to ~25 minutes using POP-6™/22cm array by modifying numerous injection parameters. STR profile quality was evaluated via concordance, dropout, peak height, resolution, ILS sizing quality/migration, pull-up, -A, and background noise. Validation for 4-dye and 5-dye amplification kits consisted of ~200 samples per dye set and studies included precision, resolution, injection time, and comparison to POP-4™/36cm array detection. POP-6™/22cm array detection achieved 0.5bp precision for up to 250bp fragments and 1bp resolution, which are the advertised specifications for POP-4™/36cm array detection.

Next, four fast PCR reagents were evaluated using the Identifiler® primer set, including AmpliTaq® Gold Fast PCR Master Mix, KAPA2G™ Fast Multiplex PCR Kit, SpeedSTAR™ HS DNA Polymerase, and Type-it® Microsatellite PCR Kit. Profiles were evaluated via concordance, dropout, peak height, inter- and intra-locus peak balance, stutter, pull-up, -A, specificity, and background noise. In addition to profile quality, amplification time, reagent cost, and ease of PCR setup were also taken into account. Using these criteria, KAPA2G™ Fast Multiplex PCR Kit was selected to develop and validate fast protocols for the Identifiler®, Identifiler® Plus, and PowerPlex® 16 HS primer sets, using reaction volumes ranging from 3µl to 6µl and two non-fast thermal cyclers (384-well Veriti® and 96-well GeneAmp® PCR System 9700) with amplification times ranging from 43 to 51 minutes. Fast PCR validations consisted of ~200 samples and studies included sensitivity, reproducibility, precision, stochastic, comparison to standard PCR, and contamination. Sensitivity ranged from 0.1875ng to 3.0ng, but optimal DNA input ranged from 0.375ng to 1.5ng for each primer set and amplification volume.

Lastly, extraction normalization for the ChargeSwitch® Forensic DNA Purification Kit included a reduction in the quantity of magnetic beads used per sample and targeted final concentrations of ~0.42ng/µl to 1.63ng/µl for 3µl amplifications and ~0.21ng/µl to 0.83ng/µl for 6µl amplifications. As a result, the need for quantification and dilution of each sample prior to amplification was eliminated. It should be noted that this approach is only acceptable for reference samples (see Standard 9.4 of the 2011 Federal Bureau of Investigation (FBI) Quality Assurance Standards). Validation of the new process (normalized extraction plus fast PCR amplification and POP-6™ detection) included >200 samples and studies included reproducibility, precision, comparison to the current process, and contamination. Profiles were evaluated as described above and were as good as those obtained using the current process.



Criminalistics Section - 2015

Implementation of this or a similar approach reduces start-to-finish processing time to approximately a single workday. Furthermore, results from these studies indicate that a significant reduction in sample processing time is possible without the need to purchase costly instrumentation or reagents and will enable a laboratory to decrease their number of instruments needed to maintain their sample throughput or, alternatively, to increase sample throughput by maintaining the same number of instruments.

Forensic DNA, Fast PCR, Quicker 3130xl Detection



B147 Microtrace to Nanotrace: Extracting Information at Increasingly Smaller Length Scales

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After attending this presentation, attendees will understand that presently the majority of trace evidence examinations focus on features and particles larger than 10 micrometers in size. Highly engineered and naturally occurring particles in the low micrometer- to nanometer-size range surround us and are seeing more use each year.

This presentation will impact the forensic science community by providing a primer to the forensic science community on the potential value, analytical approaches, and concerns that must be addressed when approaching particles at this length scale.

In the spirit of this year's conference theme, "Celebrating the Forensic Science Family," this double-feature, father-son presentation will discuss the benefits, significance, and, ultimately, the need to find and extract information from increasingly smaller amounts of trace evidence. Presented from two perspectives, one couched in the rich history of forensic science and its founders, the other taken from the exciting potential in new and higher-resolution methods, both authors arrive at common ground in recognizing the importance of visual evidence as seen through the microscope.

For more than a century, scientists have exploited the value of small particles through trace evidence. While hardly a novel concept in itself, three factors have caused us to explore and consider its significance in terms of increasingly smaller features and particles in the context of trace evidence. The first is a consequence of the *CSI*-effect. Savvy criminals are often aware of trace evidence and, in some instances, make active efforts to minimize these contacts. Secondly, as society enters the age of nanotechnology, highly engineered particles, layers, and features of materials are becoming increasingly smaller while, at the same time, becoming more complex. As paint layers reach the sub-10 micrometer range, multilayer film layers reach the nanometer scale, and free nanoparticles begin to find regular commercial use in a wide range of consumer products such as cosmetics, glass, fibers, and paint. Finally, robust and practical microanalytical methods of light and electron microscopy combined with vibrational micro-spectroscopy provide the means by which these particles and features can be detected, identified, and compared.

The practical task then becomes searching for such particles and features, finding them, and finally conclusively identifying them. This is hardly trivial given their size, which may be unresolvable by stereomicroscopy alone. For example, microspheres of silica, used in many cosmetic formulations, are only a few 10s of micrometers in size. While not resolvable by stereomicroscopy, these isotropic spheres could be mistaken for an immiscible phase in a microscope slide preparation. Once found and recognized, they must be characterized to extract specific information about size, composition, and nano-morphology. Finally, the new questions of contamination, source, and significance arise in this new realm of materials. For example: Is this layer really a layer? When was this population of nanoparticles introduced? What is the potential for transfer? New, or at least more, vigilant checks for cross-contamination must be considered when working at these length-scales.

While some materials and the feature sizes are novel, the approach is in many ways a continuum of current practices, simply modified to work at increasingly higher resolutions. This presentation will explore the practical application of the approach discussed above through various casework examples from the realms of paint, fibers, cosmetics, dust, and soils to illustrate considerations relevant to the exploitation of increasingly smaller scales of trace evidence.

Ultimately, this study suggests that many existing methods common to most trace evidence laboratories can be better utilized to approach these smaller particles and features while other technologies in microanalysis are becoming (or have become) practical for application to this new realm of trace evidence.

Nanotrace, Microscopy, Trace Evidence



B148 The Investigation of Potential Mechanisms for the Formation of Postmortem Hair Root Bands: A Detailed Microscopical and Ultrastructural Analysis

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After attending this presentation, attendees will better understand the microscopical and ultrastructural changes associated with hair displaying a Postmortem Root Band (PMRB).

This presentation will impact the forensic science community by explaining how this research investigates potential mechanisms for the formation of PMRBs. These results may help establish a more scientifically rigorous framework in which to discriminate between hair with PMRBs and antemortem hair with similar microscopic characteristics.

A PMRB is a distinct microscopic feature that occurs in the pre-keratin region of anagen and early catagen hairs derived from deceased individuals.^{1,2} The appearance of this dark band has been shown to be the result of diffuse light scatter at the interface between the cells of the hair shaft and large elongated gas-filled void spaces.¹ The presence of these void spaces is the underlying basis for the observed PMRB.

The recognition of root banding in hair evidence can potentially provide valuable information concerning crime scene reconstructions; however, interpretations of PMRBs have recently been challenged in two high-profile criminal court cases.^{3,4} One of the underlying issues used to question the validity of interpreting hairs displaying PMRBs is that the mechanism of band formation is not known. To help shed light on this issue, several recent studies have investigated the potential to produce the characteristics of PMRBs in antemortem head hairs through various environmental conditions.⁵⁻⁸ Two of these studies report that some characteristics of PMRBs can be reproduced by environmental factors; it is cautioned that inexperienced hair examiners may falsely identify an environmentally degraded hair as a hair displaying a true PMRB.^{6,7}

The goal of this research is to investigate the potential mechanism for the formation of PMRBs. A better understanding of this mechanism is essential to establish a more scientifically rigorous framework in which to discriminate between hairs with PMRBs and antemortem hairs with similar microscopic characteristics. In this study, detailed observations were made using high-resolution images of ultramicrotome sections of head hairs with known PMRBs. Microscopical analysis of the banded regions indicate that the appearance of the PMRB is due to the degradation of the chemically and mechanically labile, non-keratin Intermacrofibrillar Matrix (IMM) in the pre-keratin region of anagen hairs. In addition, PMRB formation is confined to the cortex region of the hair shaft; there is no observable damage to the layers of the cuticle even at ~50,000x magnification.

In an attempt to further investigate potential mechanisms of PMRB formation, antemortem anagen head hairs were subjected to several conditions (e.g., pH series, protease digestions, buffer solutions) that may affect the IMM. The results from these *in vitro* studies indicate that some microscopic characteristics of PMRBs can be replicated. This is most readily demonstrated when anagen hairs are subjected to slightly alkaline (pH 7-8) aqueous ammonium salt solutions. The banding that forms in these hairs appears, at both the light microscope and scanning electron microscope scale, to be very similar to PMRBs. Ammonium and ammonia are viable causative agents because concentrations of these compounds increase rapidly in the decomposing body due to degradation of protein through various autolytic and bacterial processes.⁹ These results provide valuable insights that may assist in uncovering the mechanism for the formation of PMRBs.



Criminalistics Section - 2015

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Postmortem Hair Root Band, Hair Microscopy, Trace Evidence



B149 Analysis and Discrimination of Colored Pressure-Sensitive Tape Backing by Microspectrophotometry

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The goal of this presentation is to present the results from a research project of absorbance microspectroscopy of red and blue pressure-sensitive tape backings.

This presentation will impact the forensic science community by showing the potential of Microspectrophotometry (MSP) as a method for differentiation of colored tape backings.

Tape is often used in the commission of a crime. Electrical tape can be used in improvised explosive devices while duct tape is often used to bind victims. Pressure-sensitive tape contains several components including, at minimum, an adhesive layer and a backing layer. A variety of colored and patterned tape backings is becoming more readily available. This variety of colors may offer another area of discrimination in the forensic analytical scheme.

MSP is the analysis of color in the visible range on a small scale. MSP is a well-accepted technique used in the analysis of trace evidence such as paint and fibers. Very little literature has been found on the MSP analysis of colored tape backings and no literature was found on how discriminating the MSP could be with tapes of the same color. The current study was performed to evaluate the usefulness of MSP to be used in conjunction with other analytical schemes in the forensic analysis of colored tape backings.

This study involved two different colors (red and blue) of pressure-sensitive tape in a variety of types (duct tape, electrical tape, and miscellaneous tape). Most tapes were purchased at local stores and were sold for general use. In this study, 18 red duct tape samples, 18 blue duct tape samples, 11 blue electrical tape samples, 8 red electrical tape samples, 12 red miscellaneous tape samples, and 5 blue miscellaneous tape samples were analyzed in this study.

The project was designed with the following objectives in mind: (1) to determine if the MSP could discriminate similar colored tape backing; (2) to determine if the MSP would add to the analysis scheme of physical characteristics and Fourier Transform Infrared (FTIR) analysis; and, (3) to determine the inter-variability of MSP results in a single roll of duct tape and electrical tape.

Tape samples were cross-sectioned using a microtome and analyzed using the CRAIC 20/20 PV™ MSP in replicates of five, minimally. Tapes were only compared within their same color and tape type groups and blind replicate samples were also included in the comparison groups. In addition, the same tape samples were evaluated based on physical characteristics (size, backing structure, adhesive color, and scrim, if applicable) and Infrared (IR) spectroscopy. The results of the discrimination of colored tape backings by MSP and how MSP adds additional discrimination to the traditional analysis scheme (physical characteristics and IR) will be discussed.

Duct Tape, Electrical Tape, MSP



B150 Quantitative Algorithm for Digital Comparison of Torn and Cut Duct Tape

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After attending this presentation, attendees will better understand minimizing human contextual bias via a quantitative imaging algorithm and corresponding mathematical methods to extract the edge profiles of torn and cut duct tape samples.

This presentation will impact the forensic science community by presenting information about a quantitative algorithm for the digital comparison of torn and cut duct tape.

The National Research Council (NRC) established a need at the national level for the validation of forensic science methods. Currently, duct tape end matching is based on human judgment with no quantitative criteria for identification. In this research, the needs of the forensic science community are met by minimizing human contextual bias with a quantitative algorithm. The detected edges of the exemplar and an arbitrarily large number of test samples are algorithmically subtracted from one another. The resulting residuals are then used to calculate the Sum of Squared Errors (SSE), a succinct metric that allows quantitative comparison of possible matches. A best or “most likely” match is determined by identification of the match with minimal SSE. The digital results are compared to a prior study of the same set of duct tapes that were visually assessed by a group of three researchers as part of an error determination study, thus providing a quantitative estimate of the respective error rates.

The MATLAB® software platform is used to code a series of mathematical functions in order to extract useful information from an image or a series of measurements. This research uses MATLAB® to obtain an edge profile of the duct tape image and performs analysis on the data. A digital image of the duct tape is collected using a high-resolution scanner at 1,200 Dots Per Inch (DPI). A digital profile of the tape is developed using the focused tear region and the parallel edges of the duct tape. The software performs a series of automated tasks. First, the software takes the selected image, sets threshold levels, and converts pixels to a binary image. Second, the software performs an ad hoc smoothing mechanism that removes the yarns by using a morphological operation that conducts erosion and dilation in order to remove the noise associated with the yarns. Last, the software converts the coordinate points into a graphical format and compares the edge profile to other edge profiles with a residual calculation that assesses the degree of difference along the focused tear region. The results are displayed on a bar and color map graph showing the difference of the SSE values for a matching piece of duct tape in comparison to the other duct tape pieces in the database. The user examines the residual calculations and determines whether one pair is quantitatively a better match than other pairs examined. The results establish that if other pairs of known matches have SSE values in the same range, it strongly suggests that it is the correct match. The duct tape database comprised of 2,200 paired duct tapes requires the analogies of 4,400 scanned duct tape images, making 19,360,000 inter-comparisons. Using this quantitative mathematical algorithm, the capability of the algorithm to identify matching specimens in sets of 200 duct tapes of a particular brand, to look at overall residue values by themselves as an indication of uniqueness, and to quantify the number of false positives and false negatives will be discussed. This study will compare and contrast the results with the findings of prior researchers who conducted a manual comparison of these same duct tape specimens. This prior study was presented at the 2011 American Academy of Forensic Sciences meeting by McCabe et. al.¹ The end product is a quantitative and statistically rigorous guideline for end-match comparison.

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Duct Tape, Physical Matching, Image Analysis



B151 Comparisons of Multivariate Preprocessing for Forensic Discrimination of Fibers by UV/Visible Microspectrophotometry

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The goal of this presentation is to present research studies to validate the forensic comparison advantages of first derivative preprocessing for comparison of Ultraviolet/Visible (UV/Vis) spectra of fibers. The effects of first derivative processing will be demonstrated with clear examples of improved discrimination between selected fibers and by improved classification accuracies using multivariate statistics.

This presentation will impact the forensic science community by illustrating how performing multivariate statistics on derivative spectra can improve discrimination of cotton fibers over other methods of spectral preprocessing for forensic comparisons. Significant increases in discrimination of fibers with mostly flat spectra with small changes in absorbance are possible using derivative spectra. Direct-dyed cotton fibers are one class of fibers that would seemingly benefit significantly from utilizing derivative spectra since these fibers tend to have distinctively low changes in absorbance. Although no single method of preprocessing is best for all types of spectra, the analyst must show caution when selecting cases in which derivatives should be used.

Determining which analytical method will have the highest discrimination power for trace evidence examinations is important to forensic laboratories in terms of allocation of time and resources. The importance of statistical methods for evaluation of trace evidence analysis in forensic science has also been of recent focus.¹

Color plays a critical role when analyzing natural fibers such as cotton in forensic investigations. UV/Vis Microspectrophotometry (MSP) is a non-destructive technique capable of providing objective color measurements on fibers in the form of absorption or transmission spectra; however, forensic fiber examinations can be hindered by spectra with little detail or points of comparison. Wiggins originally suggested that derivative preprocessing can enhance comparisons in UV/Vis spectra regard of cotton fibers.^{2,3} Although derivative preprocessing has been used for many years in analytical chemistry for the various applications described, it has not been widely utilized in forensic applications.^{3,4}

To validate this suggestion, multivariate classification on spectra of reactive, direct, and vat-dyed cotton fibers has been performed. These spectra were preprocessed using multiple methods including baseline correction, normalization, and first and second derivatives. Principal Component Analysis (PCA) followed by linear discriminant analysis (LDA) was employed to estimate classification accuracy.⁵ Direct-dyed fibers exhibited almost featureless and low-absorbing spectra compared to those of reactive and vat-dyed fibers. As a result, classification accuracies for direct-dyed fibers were lower than those calculated for reactive and vat-dyed fibers. The results of this study show that derivative spectra can significantly enhance classification accuracy when analyzing spectra with only subtle features such as those seen with direct-dyed cotton fibers. No single method was best for all classes of fibers in the study and the shapes and intensities of the curves are important when determining if derivative calculations are auspicious.

The generality of this conclusion was confirmed using UV/Vis spectra from more than 400 dyed textile samples of cotton, acrylic, nylon 6,6, and polyester. All spectra were subjected to first derivative preprocessing and classified using PCA/LDA. Leave-one-out cross validation was used to test the discrimination of fiber spectra in each color and polymer group. The high discriminating power of UV/Vis spectra for fibers was further validated by correct classification for 89.50% with this diverse group of spectra from four different textile classes.



Criminalistics Section - 2015

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Fibers, Spectra, First Derivative



B152 Development of a Microspectrophotometric Spectrum Database for Comparison of Casework Textile Fiber Samples to Motor Vehicle Interior Fabrics

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The goal of this presentation is to focus on a recent cold case investigation in Western Australia in which a textile fibers database was utilized for data interpretation and ultimately led to the discovery of potential links between fibers recovered from victims and motor vehicle interior fabric sources. This presentation will highlight the importance of the effective organization of large volumes of textile fiber data for forensic intelligence and evidentiary purposes and will include specific case examples.

This presentation will impact the forensic science community by illustrating the use of a casework fibers database containing motor vehicle interior fiber Microspectrophotometric (MSP) spectra as a powerful investigative tool. Victims of homicide are often transported in a motor vehicle prior to their death and/or prior to disposal of the body. Textile fibers recovered from motor vehicle interior fabrics may exhibit MSP spectra with characteristics unlike those of clothing fibers and may be used to link an offender, victim, or scene to a motor vehicle.

The database developed at ChemCentre is currently populated with more than 7,900 fiber samples, including approximately 900 samples from motor vehicle interior fabrics as well as casework and validation samples and other exemplar materials. The database allows the user to perform objective MSP spectral comparisons by calculating modified Pearson correlation coefficients and to visually compare images of fibers acquired via microscopy. The modified Pearson method of correlation was evaluated using repeat samples collected from more than 40 garments of various fiber types and by re-examining data from previously completed cases and proficiency trials.

An interpretation approach utilizing the database has been developed in response to an investigation which contained more than 4,400 fiber samples and no control garments. The requirement was to investigate potential links between multiple victims by searching for common sources of transferred fiber evidence. Therefore, fibers of any color or type were of potential evidentiary value. The database approach highlighted critically important fibers (those with close MSP spectral matches to large numbers of other fibers) and used these to form groups of fibers with corresponding properties. Groups were then confirmed via comparison microscopy (in brightfield and fluorescence modes). This approach eliminates the need to investigate each fiber individually and minimizes the number of comparisons required via comparison microscopy, allowing for efficient and effective handling of large fiber cases. The database has also been used for objective fiber comparison in routine casework in concurrence with standard fibers methodology.

It is envisioned that future development of the database methodology will include collation of casework fiber samples and the formation of large collections of exemplar materials (primarily motor vehicle interior fabrics) across jurisdictions.

Textile Fibers, Database, Microspectrophotometry



B153 Examination of Statistical Methods for Forensic Analysis of Highly Similar Absorbance Spectra From Textile Fibers

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After attending this presentation, attendees will understand the application of objective statistical methods of analysis for the comparison of highly similar textile fibers using microspectrophotometry.

This presentation will impact the forensic science community by providing error rates for the methods described.

A question for fiber analysis with regard to forensic science is: can a Questioned (Q) fiber be associated with a Known (K) source or not? When the Q and K fibers exhibit obvious differences in physical, optical, and chemical characteristics, fiber discrimination by traditional methods is straightforward. Issues may arise when the Q and K fibers originate from highly similar sources which may lead to false non-discrimination decisions during analysis. The goal of this research is to investigate the rates of discrimination between fibers of sources that are highly similar in color. Principal Component Analysis (PCA) was utilized for dimension reduction since this study deals with large multivariate datasets. Discrimination was based on a combination of the score and orthogonal distances at a specified cutoff value.

The samples for this study originate from three different datasets. The first dataset consists of six 10-gram swatches purchased from and dyed on-site by a third party. The fabric samples include two identical swatches of nylon, two identical swatches of acetate satin, and two identical swatches of acrylic. The dyestuffs were chosen in pairs based on molecular structure. Dyes pairs include ACID BLUE (AB) 25 and 41, Disperse Blue (DB) 3 and 14, and Basic Green (BG) 1 and 4. Nylon swatches were dyed with AB 25 and 41, respectively; acetate satin swatches were dyed with DB 3 and 14, respectively; and acrylic swatches were dyed with BG 1 and 4, respectively. A total of ten fibers were sampled from different areas of each of the swatches, and 15 absorption spectra were collected from each fiber via microspectrophotometry. The individual spectra collected from each fiber were averaged. The second dataset consists of five commercially available blue acrylic yarns purchased from local craft retailers. Yarns that are visually similar with regard to color and shade were purchased. Two pairs of yarns shared the same brand name but were labeled as different shades of the same color as specified by the manufacturer. Dye composition information is unknown for the yarn samples. For the yarns, five fibers were sampled from each and 30 spectra were collected from each fiber.¹ Again, the individual spectra from each fiber were averaged. The third dataset consists of six 100% cotton denims purchased from local craft retailers. Again, dye concentration information for denim samples is unknown. From each of the denims, eight dyed fibers were sampled and 15 spectra were collected along the length of each fiber. Averaged spectra were calculated for each fiber.

Averaged spectra were subjected to PCA for dimensionality reduction. Either the number of Principal Components (PCs) that retain at least 95% of the variance of the original dataset or the first two PCs were kept for further statistical calculations. Hold-one-out cross validation comparisons were performed for this study with regard to same sample (within source) and different sample (between source) comparisons. When a Q spectrum is projected into the K principal component space a score value is calculated based on the score and orthogonal distance, the respective cutoff values, and an optimization parameter, γ , which ranges from zero to one. The Q spectrum is discriminated from the K spectra when the score value is greater than one.

Optimized results for the test swatches are as follows: same sample-7.5% discrimination; different sample-100% discrimination. Results for the yarns are as follows: same sample-16% discrimination; different sample-100% discrimination. Results for the denims are as follows: same sample-10.7% discrimination; different sample-76.5% discrimination.

This work was supported in part by the National Institute of Justice, Office of Justice Programs, award 2011-DN-BX-K553. The content of this publication does not necessarily reflect the position or the policy of the United States Government and no official endorsement should be inferred.

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Fiber Analysis, Microspectrophotometry, Chemometrics

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B154 Identification of Artificially Aged Silk at the Molecular Level

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After attending this presentation, attendees will understand how molecular biomarkers can be used to determine the age of historical silk textiles; they will also gain insight into how these same biomarkers can be used to detect forgeries.

This presentation will impact the forensic science community by speaking to the importance of art forensics in the preservation of cultural heritage, the detection of forged artworks, and the need to further understand our history. Many museums' artifacts are composed of or contain proteinaceous materials such as silk, wool, parchment, wood, etc. The research presented focuses on the molecular biomarkers of natural ageing in museums' proteinaceous specimens and how to use these markers to identify the age or the authenticity of these artifacts. Among the examples discussed will be some of the Iranian Buyid textiles in the United States that contain a large amount of fake ancient silk.

Shortly after the 1925 excavation of a number of genuine silk articles at the Bibi Shahr-Banu site south of Tehran, Iran, the market was flooded with forged Bibi Shahr-Banu silk textiles. Museums in the United States and Europe bought many examples, both genuine and fake; later, the authenticity of many of the specimens was questioned, resulting in a decades-long controversy concerning Buyid silks. Today, many believe that a vast number of these artifacts are fake; however, since no scientific method was available, the claims on the forgery nature of these silks were mostly based on speculation rather than sound scientific methods. The purpose of the research presented is to develop molecular level biomarkers for scientific identification of bona fide silks and forgeries using separation, mass spectrometry, and proteomics.

The degradation of silk — both natural and artificially induced — has been studied using X-ray diffraction, Ultraviolet/Visible spectrometry, and Fourier transform infrared spectroscopy, among others. Mass spectrometry is an ideal technique for studies of the biomarkers of degradation at molecular levels. In addition, it is a highly sensitive technique which allows species identification as well as post-translational modifications of proteins at low microgram levels. The high sensitivity of mass spectrometry allows for the identification of biomarkers of aging with minimal effect to the artifacts, which is essential for the analysis of precious museums' artifacts. The molecular markers of natural aging and degradation of proteins studied for this research include racemization, deamidation, oxidation, truncation, and amino acid conversion. After comparing these biomarkers of aging for the Buyid silk samples, the physical and chemical methods that could have been used to produce the forged silk were studied. For example, it was observed that the Bibi Shahr-Banu forgeries yield unusually high levels of racemization, and this could hold true for other post-translational modifications; this is also currently under study.

This presentation will address how biomarkers can indicate age or forgery as proven by the study of genuine and forged textiles and by the re-creation of forgery methods. It is hoped that attendees will learn the importance of understanding such mechanisms in the preservation of culture and the detection of art crimes.

Silk, Forgery, Mass Spectrometry



B155 Frequency of Occurrence of Original Equipment Manufacturer (OEM) Automotive Refinishes Using the Paint Data Query Database

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The goal of this presentation is to provide a basis for determining the potential significance in observing an OEM refinish in automotive paint comparisons.

This presentation will impact the forensic science community by illustrating how awareness of the rate of occurrence of OEM refinishes will assist paint examiners in determining the strength that may be assigned to conclusions in comparative associations. The rate observed in 1,000 samples will be provided along with the number of OEM topcoats observed within each refinish, which can also affect significance.

In recent years, the Paint subgroup within the Scientific Working Group for Materials Analysis has attempted to develop language that would convey significance of association in comparative paint examinations. The strength of association in reporting a fracture match or the discrimination of paint chips with obvious differences in physical or chemical characteristics can be conveyed using relatively straightforward text. Likewise, the rationale for associating a standard four-layer OEM paint chip with a known vehicle(s) of corresponding layer construction and chemical compositions can be succinctly described as long as appropriate caveats are in place to alert the reader to the existence of other vehicles also painted in the same manner.

Within the extremes of identification and elimination and somewhat proximal to the confines of the “middle road,” there exists the circumstances of associations that may be considered stronger or weaker than the “norm.” An architectural refinish layer on an automotive paint chip would be unusual. The absence of primers in a partial layer system paint chip transfer would weaken the strength of association that could be conveyed. Within these further classifications, where would an analyst place the significance of association of an OEM refinish paint chip comparison between a known source and a questioned paint chip?

To address this circumstance, the frequency of occurrence of an OEM refinish must be considered as well as the number of layers of refinish that are present between the comparison samples. Moreover, the number of refinish layers that are permissible in an OEM repair should also be known.

To gain some knowledge of the frequency with which OEM repairs occur, the physical samples used to populate the Paint Data Query (PDQ) database were assessed for OEM refinishes. Approximately 1,000 samples representing model years 2000-2013 were microscopically examined. Samples containing refinish topcoat layers (e.g., clear/basecoat layers over a typical four-layer OEM layer system) were noted. Visual indicators of basecoat consistency included comparable appearance with respect to color, flake distribution in a metallic finish, number of layers (e.g., tri-coat finishes), relative layer thicknesses, and thickness uniformity across the layer. The clear coat layers of the first and last topcoats were then analyzed by Fourier Transform Infrared (FTIR) spectroscopy to confirm the consistency of an OEM binder formulation between these systems.

The frequency of occurrence results for this study were on par or lower than reported industry expectations or standards for OEM refinish of topcoat systems. Further, the greatest number of OEM topcoat repairs observed was one; however, up to three topcoat OEM refinishes were observed on vehicles by two different manufacturers. All manufacturers are not equally represented in PDQ and not all were represented in the population of OEM refinishes observed nor was the rate of OEM repair correlated to a particular plant or model.

This study was intended to highlight the need for interpretative statements in comparative examinations. While some comparisons of mass-produced manmade materials are straightforward, it is not uncommon to encounter samples that require further discussion to strengthen or weaken the degree to which the items may be considered to be “associated.” It is hoped this work will promote further discussion with respect to the inclusion of interpretative language in comparison reports.

OEM Refinishes, Interpretation, Paint Data Query



B156 Forensic Analysis of Hoax Powders Using Morphologically Directed Raman Spectroscopy

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After attending this presentation, attendees will understand the advantages of using Morphologically Directed Raman Spectroscopy (MDRS) for the identification of hoax powders commonly employed in white powder attacks. In addition, attendees will better understand MDRS and how it can benefit the forensic science community.

This presentation will impact the forensic science community by providing criminalists with a new analytical tool for the identification of white powders commonly used in fake bioterrorism attacks.

In the wake of the September 11th terrorist attacks, there was an influx of white powder events, both real and hoax, throughout the United States. The most infamous of these white powder events was the Amerithrax attacks in the fall of 2001 that left 17 people sick and five dead from anthrax sent via anonymous letters containing white powders. Since these attacks, the Federal Bureau of Investigation and the United States Postal Inspection Service have responded to thousands of white powder events. More often than not, the attacks do not contain any toxic materials and are carried out for the sole purpose of causing terror and damaging infrastructure. These fake bioterrorism agents consist of white powders that come from a variety of common commercial sources and can be blended to further complicate the analysis and identification of these samples.

Artificial sweeteners are commonly employed hoax powders. These blends are simple mixtures of a sweetening agent and bulking material. Sweetening agents are several times sweeter than a comparable amount of table sugar. Consequently, artificial sweeteners require a bulking agent or filler such as dextrose to increase the volume of the blend and to mitigate any undesirable taste brought on by the sweetening agent. Flour, chalk, table sugar, and other colorless powders are also commonly used in these attacks. In addition to the chemical differences of the sample, there are size/shape variations that can be used to differentiate the powders from one another at the particle level.

MDRS is an ideal tool for the investigation of suspicious white powders because it combines chemical identification with particle size and shape analysis. Raman spectroscopy is a useful technique in forensic science for determining molecular chemistry because it is rapid, reliable, does not require contact with the sample, and is non-destructive. Thus, Raman spectroscopic analysis is ideal for the analysis of suspicious white powders; however, the high volume of the filler in sugar substitutes has a tendency to drown out the Raman signature of the sweetening agent when doing a traditional bulk analysis, making detection of the sweetening agent and identification of the brand of sweetener impossible. Raman microspectroscopy can be used to overcome this by analyzing and identifying individual particles of the white powder. Particle morphology and size information are also valuable for differentiating artificial sweeteners that are mixtures of the same components. Automatic image analysis takes the subjective element out of the measurement of particle size and morphology. It also makes the process more rapid than counting and measuring the individual particles within a greater mixture. When Raman microspectroscopy is paired with automatic image analysis of the particles, physical and chemical information about the components of the mixture can be obtained which can be used for discrimination and brand identification.

This presentation details the use of MDRS for the analysis for hoax powders. MDRS can be used for analysis at the individual particle level, making it ideal for identifying compounds that are mixed with bulking agents or in a blend. MDRS is capable of comparing both the concentrations and identities of samples in addition to collecting data on size distribution of the particles. Individual size and shape distributions can also provide information based on excipient particles that could be used for source attribution. MDRS is a useful tool for the analysis of hoax powders at the particles level that enables criminalists to rapidly and reliably determine the composition of these mixtures to aid in the investigation of white powder attacks.

Morphologically Directed Raman, Particle Size Distribution, Hoax Powders



B157 Quantitative Mapping of Post-Blast Nitroglycerin Residues on Pipe Bomb Fragments Using Total Vaporization-Solid Phase Microextraction-Gas Chromatography-Mass Spectrometry (TV-SPME-GC/MS)

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After attending this presentation, attendees will understand the value of quantitating explosive residue on pipe bomb fragments. A new technique will be used for quantitation, TV-SPME-GC/MS, which has several advantages over other methods. The validity of residue distribution mapping will also be discussed.

This presentation will impact the forensic science community by outlining how quantitating explosive residue using a novel technique and diagramming its dispersal can be valuable to both crime scene investigators and laboratory analysts examining pipe bomb fragments. By knowing the concentration of residue components, the efficiency of analytical techniques can be optimized. This in turn will maximize throughput and evidence turnaround time. In addition, if the dispersal of residue indicates any trends, this information can heighten the understanding of the explosion process, such as the progression of deflagration.

Although residue from the explosive filler in a pipe bomb is routinely found on the post-blast container fragments, the amount of this residue is not quantified. The obvious reason for this is that the legal question at hand is identification of the explosive, not its concentration; however, there is value to understanding the distribution of explosive residue on device components and disseminating that knowledge to crime scene personnel and forensic analysts. In particular, such “residue mapping” would provide general guidance as to what fragments may be more likely to contain high levels of residue. Additionally, the distribution of residues would also shed light on the process by which the explosive filler deflagrates, resulting in the ultimate failure of the device container.

In this study, TV-SPME-GC/MS was used to identify nitroglycerin, diphenylamine, and ethyl centralite, which are components found in Double-Base Smokeless Powder (DBSP). Traditional headspace SPME involves a three-phase system consisting of the sample, either in solid or liquid form, the headspace, and the fiber; however, by completely vaporizing the sample, the partitioning between the liquid and headspace is eliminated, eliminating matrix effects and increasing sensitivity. Another benefit of this technique is minimal sample preparation — any solid and/or non-volatile components do not enter the headspace. Additionally, significantly higher volumes of liquid extracts can be analyzed as compared to liquid injection (e.g., 70 μ L). Prior to analysis, fragments were extracted under agitation using methylene chloride or acetone. Extracts were then transferred directly to SPME vials for analysis. SPME parameters were optimized using an experimental design with final values of 20 minutes for extraction time and 60°C for extraction temperature. This technique was applied to five devices constructed from galvanized steel pipes as well as three Polyvinyl Chloride (PVC) pipes, all filled with DBSP. Results indicate the majority of nitroglycerin was located on the endcaps for the galvanized devices, which correlates with the portion of the device that fails first during an explosion. On average, 1.0 \pm 0.7mg of nitroglycerin was recovered from the galvanized devices. According to manufacturer information, the nitroglycerin content in the DBSP used was between 4% and 40%. This indicates that DBSP is quite efficient, with less than 0.05% of the original nitroglycerin remaining on the container fragments.

SPME, Explosives, Nitroglycerin



B158 Assessing the Forensic Utility of Particle Morphometry for the Characterization of Aluminum Powders in Explosives

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After attending this presentation, attendees will better understand the strengths and limitations of morphometry as a method for the characterization and discrimination of aluminum fuels for explosive compositions.

This presentation will impact the forensic science community by discussing this pilot project that is the first published study to demonstrate the application of aluminum particle morphometry as a quantitative method for the characterization of explosives, which may provide valuable prosecutorial evidence as well as lead identification for forensic investigations.

Aluminum (Al) powder is commonly used in the pyrotechnics and explosives industries where it functions to increase the heat of explosion when mixed with other metallic powders, oxidizers, and explosive components.^{1,2} The performance of Al powder as a fuel for explosives depends on powder particle shape and grain size.² In addition, Al powder is widely used in the illicit manufacture of improvised explosive devices as well as improvised explosives.³ Detailed protocols for making and acquiring Al powder are widely available in the “underground” literature and numerous internet web pages.^{4,5} Very little technical knowledge and machinery is required for making Al powders that are of sufficient grade (i.e., particle size range) to be useful for producing explosive mixtures. Owing to the widespread use and the numerous methods for the production of Al powder, there is the potential for significant variability in particle morphology and size distribution. The goal of this research is to investigate the utility of particle morphometry using automated techniques as a metric to discriminate between samples of Al powder.

Approximately 30 samples of Al powders and pastes were acquired from known legitimate manufacturers, several seized explosive devices, and produced in-house from aluminum flake-containing commercial spray paint and by grinding Al foil. Several of the Al powder samples derived from the devices required isolation from the explosive physical mixtures; isolation involved simple water extraction, manual separation, or mild acetic acid digestion (to remove carbonates without digesting the Al particles). Powder samples were subsequently wet-sieved using disposable polyester mesh of nominal sizes 250 μ m, 100 μ m, 50 μ m, and 10 μ m in order to assess within sample variability of size fractions, as well as to produce microscope slide preparations of consistent thickness for imaging. Transmitted light microscope images of the Al powder particles were acquired; images were subsequently batch-processed using commercial image analysis software and customized code. Dimensional analysis was calibrated using a National Institute of Standards and Technology-traceable stage micrometer; polystyrene spheres of 100 μ m, 50 μ m, and 10 μ m were used as secondary standards to assess linear calibration. Each image was converted to a binary image and pre-filtered using a high-pass filter to enhance edge detection. The following metrics were measured for each particle within each image field of view: area, aspect ratio, perimeter, roundness, mean diameter, radii (maximum and minimum distance from particle centroid to edge), and radius ratio. The large multidimensional datasets were analyzed using commercial and open-source statistical packages. The results from several multivariate statistical methods will be presented.

Preliminary results from this pilot study show that commercially manufactured Al powders (flake and atomized spherical grains) have a significantly narrower particle size distribution than in-house-produced powders. In addition, the spherical nature of the known atomized melt samples allows for rapid recognition of an industrial-scale manufacturer. The results from this research will provide a foundation in which to investigate further the variability that occurs within and between manufactured Al powders over production timescales.



Criminalistics Section - 2015

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Aluminum Powder, Automated Image Analysis, Explosives



B159 Classifying Aged Explosives to Determine Source

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After attending this presentation, attendees will understand how explosives recovered over the past 25 years from different regions of the world were characterized so that classification based upon source was achieved. Detection and identification of explosives and their residues are important functions that are routinely performed by forensic scientists in the field and in the laboratory. It has become important to extend the testing of these samples to include methods for determination of sample source. Determination of source may be important from an investigative perspective. This information may also be used in a court of law to classify or even individualize a sample.

This presentation will impact the forensic science community by describing the best method for testing these samples. Methods used to source explosives are different from methods used for identification. This presentation will discuss the methods and analytical approaches used, providing attendees with the background needed to effectively perform this testing in their laboratories.

Sourcing explosives is an important capability. Although identification of the explosive is necessary, in many instances, investigators are aware of the type of explosive used during a bombing very early in an investigation due to various on-scene indicators. In these instances, a more important role of the forensic scientist is to determine the source of the explosive. One way in which sourcing may be achieved is through the detection and identification of physical and chemical markers present in the sample. These markers may provide insight not only about the sample's manufacturing process, but also about the sample's history including source or origin and storage conditions. Determination of individuals or organizations responsible for providing explosives used will allow the investigator to successfully identify, apprehend, and adjudicate responsible parties.

In this study, explosives recovered from various bombings in the 1990s and 2000s were analyzed using various analytical methods. Classifications of these samples based upon chemical and physical markers present in the sample were performed. Taggants and other identifiers present in very low concentration (<0.1%) were detected and identified in samples that were more than 20 years old. Sampling and methods were evaluated and optimized so that a complete characterization and classification of each sample was performed.

The results of this research will be presented. Explosives analyzed include SEMTEX[®], dynamite, C-4, PENTEX[®], tetryl, and DETASHEET[®]. These explosives were from seizures in different regions of the world. Methods including light microscopy, infrared spectroscopy, Raman spectroscopy, and gas chromatography/mass spectrometry will be covered. Method features and limitations will be discussed.

Explosives, Taggants, Sourcing



B160 Smokeless Powders Database at the National Center for Forensic Science

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The goal of this presentation is to develop an internet-accessible database comprised of analytical data for smokeless powder samples of the commercially available single-base and double-base designations.

This presentation will impact the forensic science community by providing a demonstration of the database which contains several hundred records of smokeless powder samples which are currently, or were previously, available for purchase.

Smokeless powders are low-explosive propellants in military and civilian ammunition which are commonly used in pipe bombs and other improvised explosive devices. There are three types of smokeless powders, namely single-base, double-base, and triple-base. The smokeless powders are distinguishable based on their primary energetic materials. Single-base powders contain nitrocellulose as their primary energetic material; double-base powders contain nitroglycerin in addition to nitrocellulose, and triple-base powders contain nitroguanidine in addition to nitrocellulose and nitroglycerin. In each powder type, there are a number of additional constituents which function as plasticizers, stabilizers, opacifiers, flash suppressants, and deterrents. The smokeless powders are available in a variety of shapes, namely: ball, flattened ball, cylinder (tubular), disk, and lamel. Of the three types, single- and double-base powders are readily procured from sporting goods retailers.

In 2009, the National Center for Forensic Science (NCFS) in collaboration with the Explosives Committee of the Scientific/Technical Working Group for Fire and Explosions developed the smokeless powders database which consists of a compilation of data generated from the analysis of the commercially available smokeless powder samples using techniques such as stereomicroscopy, Fourier Transform Infrared (FTIR) spectroscopy, and Gas Chromatography/Mass Spectrometry (GC/MS). The database is comprised of smokeless powder data contributions from a number of sources including NCFS, the Federal Bureau of Investigation, and the Netherlands Forensic Institute. The database contains physical and chemical descriptions of the powders including powder morphology (shape), dimensions, distinguishing features, and the main chemical components for each sample record. The database is searchable by the physical and chemical parameters and returns a list of potential candidates including source information, micrographs of the powders and bulk sample containers, physical measurements, and GC/MS and Attenuated Total Reflectance (ATR)/FTIR data.

The National Institute of Justice recently awarded funds to NCFS for the purchase and analysis of smokeless powders and the subsequent upload of their analytical data. The newly acquired samples will also be distributed to a number of American Society of Crime Laboratory Directors-accredited laboratories as a reference collection. In addition, the explosives committee which oversees maintenance of the database is receptive to contributions of smokeless powder data from national and international sources. The database serves as a resource for law enforcement agencies and will aid in the characterization, classification, and comparison of smokeless powder samples.

This work was supported in part by the National Institute of Justice, Office of Justice Programs, award 2013-R2-CX-K008. The content of this publication does not necessarily reflect the position or the policy of the United States government, and no official endorsement should be inferred. Support is also acknowledged from the University of Central Florida, National Center for Forensic Science, a State of Florida Type II Research Center.

Smokeless Powders Database, Single-Base, Double-Base

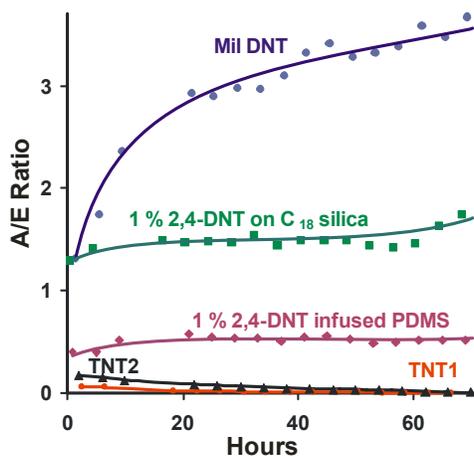
B161 Odorant Measurements and New Materials for Canine Training

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The goal of this presentation is to bring scientific rigor to canine training through measurements and training aid materials.

This presentation will impact the forensic science community by explaining how training aid materials were devised that provide the scent profile of real explosives and how this may improve court acceptance of forensic working dog evidence.

Canines achieve very high sensitivity for detecting military and improvised explosives using the volatile signatures of these materials. Solid-Phase Microextraction (SPME) coupled with techniques such as Gas Chromatography/Mass Spectrometry (GC/MS) is typically used for analysis of such vapor profiles; however, SPME/GC/MS measurements suffer from irreproducibility as a result of fiber-to-fiber absorptive differences as well as drift in the absorptive capacity and MS detector response. This limits the potential to characterize equitably the vapor profile of different samples or monitor the vapor-time profile of a given sample. The National Institute of Standards and Technology (NIST) has introduced an approach that permits the reliable characterization of the vapor profile of a sample relative to the saturated headspace of the target analyte. To achieve reproducibility, the SPME fiber will first sample from an “internal standard” and then sample the test or calibrant sample. This approach, Solid-Phase Microextraction/Externally Sampled Internal Standard (SPME/ESIS), improves the reproducibility of SPME/GC/MS by more than an order of magnitude.¹ SPME/ESIS has been applied to determine the vapor-time profile of a number of explosives, volatile components in explosives formulations, and as a screening tool for the development of training aid materials for canine detection.²



A particularly promising approach to training aid fabrication is “infusing” the volatile components of explosives into a polymer matrix (Polydimethylsiloxane (PDMS)). This approach provides a reliable vapor release and renders explosives inert. Infusion and vapor release were tested for Triacetone Triperoxide (TATP) (an improvised explosive), 2-ethylhexanol (a plastic explosives odorant), and 2,4-dinitrotoluene (DNT) (the volatile odorant in TNT). The results for a simulated training aid for DNT is shown in the figure. After a short induction period, constant release of the DNT from the infused PDMS (pink trace) was demonstrated over 70 hours using the SPME/ESIS technique by the consistency of the A/E ratio.

Infusing the odors of TATP, Composition C-4, and Semtex into PDMS is also providing promising results for canine detection. Bomb dogs trained on these explosives alert 80% to 100% of the time when presented with the infused PDMS simulants.

The design of the training aid container using a novel simulated canine nose has also been evaluated. This anatomically-correct nose “sniffs” with the known velocities of large dogs. A means of capturing and analyzing the collected odorant is also incorporated into the system, permitting a quantitative measure of the performance of the training aid design.

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Criminalistics Section - 2015

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Canine Detection, Training Aid, Quantitative Odor Measurement



B162 Modern Challenges in Fire Debris Analysis

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After attending this presentation, attendees will understand current issues faced by fire debris chemists. This will include issues classifying ignitable liquids, identifying matrix interferences, non-routine analysis, and incendiary devices.

This presentation will impact the forensic science community by serving to help fire debris chemists identify issues they may face in more complex casework.

There has been extensive literature addressing the challenging nature of fire debris analysis. This presentation serves as a continuation and expansion of the literature by providing several examples of such challenges encountered during research or casework. The range of current issues faced by fire debris examiners is vast and extends to neat liquids, debris or matrix effects, and other non-routine samples. Though the majority of liquid samples encountered in the analysis of ignitable liquids are easily identified, some liquids are difficult to classify using the widely recognized and accepted American Society for Testing and Materials classification protocol. This presentation provides several examples of such liquids. Additionally, the presentation examines the variations observed in the alkane, indane, and polynuclear aromatic content among several geographically diverse gasoline samples. Another issue addressed in this presentation, which has been discussed previously in the fire debris community, are the limitations in identifying specific products. These limitations is due both to the change in formulation of a product over time as well as different products with nearly identical chemical compositions. These limitations should be considered in cases where an investigator requests the identification of a specific source of an ignitable liquid beyond the ignitable liquid class.

Matrix effects of further concern to the fire debris examiner include inherent petroleum products and microbial degradation. These two issues have been previously studied in the literature; however, this presentation expands on both. Inherent petroleum products are widespread and should be well understood by all fire debris chemists. As such, several examples are reviewed such as isoparaffinic products in various plastic products, limonene in tires, and gasoline in shoes.

In recent years, fire debris chemists have become increasingly responsible for additional analyses, which may require alternative sample preparation techniques and instrumentation. Among these analyses are alternative fuels such as E85 and biodiesel, vegetable oils, lubricating oils, and petroleum greases. Alternative fuels can typically be identified using general fire debris analytical procedures and their prevalence in casework is increasing. Vegetable oils may be of interest due to the propensity of some oils to self-heat and potentially cause ignition of surrounding fuel. Analysis of vegetable oils requires conversion of the fatty acids in the oil to methyl esters and analysis by a Gas Chromatograph/Mass Spectrometer (GC/MS) equipped with a specific column not commonly used for ignitable liquid analysis. Lubricating oils and petroleum-based greases are an extension of petroleum-based ignitable liquids, but much less volatile. These products may be identified using a standard GS, but can be further characterized, and possibly compared, using a high-temperature GS.

Unconventional ignition methods are often incorporated into improvised incendiary devices and as such are very wide ranging. Some examples that have been encountered include flares, thermite mixtures, and improvised napalm. These types of analyses typically require additional instrumentation outside of the typical GC/MS such as infrared spectrometry, X-ray powder diffraction, and scanning electron microscopy. This presentation hopes to provoke discussion of these various issues as it is important to keep an open dialog among current fire debris examiners.

Fire Debris, Ignitable Liquids, GC/MS



B163 The Effects of Burning and Mold Growth on the Analysis of Fire Logs

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After attending this presentation, attendees will better understand the different fuel types in fire logs and the effects burning and mold growth will have on extraction, analysis, and comparison.

This presentation will impact the forensic science community by raising awareness of the change in fuel type of many fire logs from petroleum-based waxes to vegetable oils. This change in fire log composition means that different methods must be used to analyze fire log evidence, specifically the extraction method and instrument parameters. Fire debris analysts who receive fire log samples will also need to take into consideration the effect fire and mold growth have on the composition of the logs.

Artificial fire logs have become a popular substitute for wood to burn in fireplaces due to the fact that they are readily available, easier to light, and will burn unattended for longer periods of time. These fire logs are typically manufactured by combining a cellulosic material, such as sawdust or wood particles, with a combustible binder. The binder or fuel typically has been a petroleum (paraffin) wax; however, in recent years, many manufacturers have switched to using vegetable oils instead of petroleum waxes. While research has been published regarding vegetable oils and spontaneous ignition as well as the analysis of fire logs containing petroleum-based waxes, none has yet been published concerning the analysis of vegetable oils in fire logs.

Vegetable oils are primarily composed of triglycerides, which consist of three fatty acids attached to a glycerol backbone. The fatty acids in vegetable oils are typically unsaturated straight chains consisting of an even number of carbon atoms. Fatty acids are not well-resolved on a typical fire debris non-polar column but can be derivatized into methyl esters and analyzed on a polar column for best separation.

The Bureau of Alcohol, Tobacco, Firearms and Explosives laboratory received evidence from a case in which it was suspected that a fire log was used to initiate a fire. Numerous samples contained vegetable oil-based fuels and exhibited mold growth. Microbial degradation of petroleum products in soil and building materials has been reported. This degradation makes the classification of the petroleum products nearly impossible. Although the vegetable oil-based fuel in the case samples could be identified, apparent microbial degradation made the comparison of questioned and known samples more difficult.

This study was designed to determine the effects that both burning and mold growth have on the composition of the fire log. A total of 34 fire logs, varying in brand and type, were cut into approximate thirds. One third were analyzed intact and served as the clean, unburned sample. One third were burned until charred, extinguished by smothering the flame, and subsequently analyzed. The final third were burned until charred, extinguished with water, and stored in metal cans outdoors for a month in order to allow mold growth before analysis.

The fire logs were extracted in pentane and the extracts were analyzed by High Temperature Gas Chromatography-Mass Spectrometry (HTGC/MS) to identify any petroleum-based waxes present. The same pentane extract was then derivatized using a base-catalyzed reaction to form Fatty Acid Methyl Esters (FAMES) and run on a GC/MS with a column specific for FAMES in order to determine whether the fuel contained vegetable oils. GC/MS data of the intact fire logs indicated that a majority contained petroleum-based waxes and vegetable oils, thus requiring the use of both instruments. Chromatographic differences in the petroleum and FAME compositions of the intact, burned, and burned/molded samples were documented through visual comparisons and peak area ratios. While petroleum waxes appeared unaffected by fire and mold, vegetable oils showed significant changes in composition.

Fire Logs, GC/MS, Vegetable Oils



B164 Investigation of Body Products on Worn Clothing Found at a Fire Scene Conflicting With Ignitable Liquid Residue Identification by Gas Chromatography/ Mass Spectrometry (GC/MS)

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After attending this presentation, attendees will be aware of the chemical interferences caused by body products on worn clothing and the associated difficulties with classifying Ignitable Liquid Residues (ILRs) using GC/MS.

This presentation will impact the forensic science community by informing fire debris analysts about the extent of the interference to the identification of ILRs that is caused by body products on worn clothing and how it can cause misinterpretations and misclassifications.

The question of whether cosmetic and medicinal body product deposits on clothing as well as the composition of clothing are being mistaken for ignitable liquids is of considerable importance for criminalists in forensic science laboratories. This research investigated the magnitude of the impact that body products (such as body oil, moisturizers, and perfume) on worn clothing have on ILR identification in fire debris. Body products can have similar chemical profiles to ignitable liquids as a result of comparable chemical compounds that are found in both sources. Consequently, body products on worn clothing could potentially cause difficulties for practitioners when classifying ILRs present in fire debris according to the American Society for Testing and Materials (ASTM) method E1618 (“Standard Test Method for Ignitable Liquid Residues in Extracts from Fire Debris Samples by Gas Chromatography/Mass Spectrometry”).

Worn clothing samples of varying fiber content (cotton, polyester, lycra-spandex, and a blend) were collected from three individuals who had applied a body product provided for them before wearing the clothing item. The clothing was worn over a period of 12 hours, prior to collection. Passive headspace concentration by Activated Charcoal Strips (ACS) was performed according to ASTM method E1412 (“Standard Practice for Separation of Ignitable Liquid Residues from Fire Debris Samples by Passive Headspace Concentration With Activated Charcoal”), heating the cans at 80°C for 16 hours followed by desorption of the ACS with carbon disulfide (CS₂). The samples were then analyzed by GC/MS with a quadrupole mass analyzer and the chromatograms were evaluated as to whether the residues could be misidentified as belonging to a class of ignitable liquids.

A preliminary study was performed prior to conducting the experiment with human subjects. It consisted of subjecting various body products to the ASTM E1412 and E1618 analytical methods. Results from the analysis of the neat samples showed that several body products could be mistaken for ILRs. Some products had chromatograms that showed similar visual patterns to ignitable liquids but showed differences in the mass spectral peak identifications. The results of this preliminary study were used to select the body products used in the human trials. In addition, unworn clothing samples were analyzed and the resulting chromatograms revealed compounds that could also cause difficulties with ILR classification. Additionally, it was found that materials used to preserve the clothing should not be ignored. These results highlight the complexities involved with identifying ILRs by demonstrating both the possible misclassifications and masking of ILRs that can occur due to the interference of body products and clothing.

This research has important implications for criminal justice because the identification of an ILR on clothing could result in an innocent person being charged with an arson crime or, in contradiction, present misleading data that would allow the guilty to walk free. Thus, it is critical to be able to differentiate interfering substances on substrates from ILRs when analyzing fire debris.

Fire Debris, Ignitable Liquids, Substrate Contribution



B165 Identification of Triglycerides in Pristine and Degraded Vegetable Oils and Fats in Fire Debris Extracts by Liquid Chromatography/Mass Spectrometry

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After attending this presentation, attendees will understand a novel application of Liquid Chromatography/Mass Spectrometry (LC/MS) to the forensic analysis of vegetable oils and fats in fire debris samples.

This presentation will impact the forensic science community by presenting an LC/MS technique that will provide a method for directly identifying triglycerides in fire debris extracts without prior derivatization. Triglycerides are the principle components of vegetable oils and fats. Identification of triglycerides was found to be possible for vegetable oils and fats that had been used for cooking and/or exposed to fire conditions.

Triglycerides are the principle components of vegetable- and animal-based oils and fats. Analysis for triglyceride-based oils and fats provides valuable information in a range of fire investigation scenarios, including kitchen fires, household fire deaths, and cases of spontaneous combustion that involve oily rags.

As triglycerides are large molecules that are not sufficiently volatile to be analyzed directly by GC-MS, many laboratories derivatize triglycerides extracted from fire debris to their corresponding Fatty Acid Methyl Esters (FAMES). FAMES are readily separated and identified by GC/MS. In October 2013, the American Society for Testing and Materials (ASTM) International published a new standard test method for "Extraction and Derivatization of Vegetable Oils and Fats from Fire Debris and Liquid Samples with Analysis by Gas Chromatography/Mass Spectrometry" (ASTM E2881-13). Unfortunately, it is not possible to specifically confirm the presence of cooking oils and fats using this method as resulting FAMES may originate from free fatty acids in the substrate and not from triglyceride-based oils and fats. The ASTM test method specifically cautions about forming this conclusion in section 4.2.1.

In order to confirm the presence of vegetable oils and fats, a method is needed that allows separation and identification of mixtures of intact triglycerides in fire debris extracts. Analysis of triglycerides by Liquid Chromatography-Mass Spectrometry (LC/MS) has been widely reported in food industry publications; however, these studies typically focus on pristine/unused cooking oils. In fire debris samples, cooking oils may be degraded by exposure to air, usage in cooking, and exposure to fire conditions. The purpose of this study was to determine whether or not mixtures of triglycerides could still be identified in fire debris extracts after the vegetable oils and fats had been used for cooking and/or exposed to fire conditions.

A total of 48 vegetable- and animal-based oils and fats were purchased at local supermarkets, including multiple brands of each type of oil or fat wherever possible. Each of these products was analyzed in their pristine state by LC/MS using Multiple Reaction Monitoring (MRM). Representative samples of each of these oils and fats were then placed in clean, unlined paint cans and heated with a Bunsen burner to their: (1) smoke point; (2) non-piloted ignition point; and, (3) self-extinguishment point. This was based on a method developed by the State of Florida Bureau of Forensic Fire and Explosives Analysis. Each of these burns was completed for neat liquids and liquids spiked onto cotton fabric or wood. Resulting residues were extracted with hexane and analyzed by LC/MS using MRM. Four oils with high smoke points (canola, peanut, soybean, and sunflower) were further subjected to six deep-fry cooking cycles using a commercial deep fryer. These cooked oils were subsequently analyzed by LC/MS to determine the effect of cooking. Cooked oils were then burned as per the method described above to determine the effect of fire conditions on oils that had previously been used for cooking.

Mixtures of four or more triglycerides were readily identified using LC/MS in all samples that had undergone cooking and/or burning. Differences in triglyceride peak ratios were minimal, and in many cases the degraded oil was indistinguishable from pristine/unused oils. In samples where peak ratio differences were observed, more degradation was observed on the more highly unsaturated triglycerides.

As mixtures of multiple triglycerides remained identifiable in all degraded samples in this study and the peak ratios of degraded samples were similar to pristine/unused oils, LC/MS analysis of triglycerides has been demonstrated to be an effective and reproducible method for the identification of cooking oil residues in fire debris extracts.

Vegetable Oils and Fats, Triglyceride Analysis by LC/MS, Fire Debris



B166 Analysis of Arson Fire Debris by Low Temperature Dynamic Headspace Adsorption Porous Layer Open Tubular (PLOT) Columns

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After attending this presentation, attendees will have an understanding of: (1) the general metrology of PLOT-cryoadsorption (cryo); (2) the advantages of PLOT-cryo for fire debris sampling; (3) the ability to use PLOT-cryo in the field; and, (4) the use of high throughput PLOT modules for rapid analyses.

This presentation will impact the forensic science community by providing familiarity with the evolving scope of application afforded by PLOT-cryo.

Many Ignitable Liquids (IL) can be used to start an arson fire, the most common being gasoline, diesel fuel, kerosene, charcoal lighter fluid, paint thinners, and solvents; however, many less-common fuels have been used as well. Attention is even being paid to the new alternative fuels such as biodiesel fuel as potential ILs. Forensic scientists must routinely identify and characterize the accelerant or IL in a credible, defensible manner. The analysis of fire debris for the presence of residual IL has long been an accepted and routine aspect of arson investigations and the techniques available for such analyses have evolved. The nature of ILs as multi-component, moderately volatile fluids makes Gas Chromatography (GC) the most important and widely used method for fire debris analysis and the majority of liquid residue analyses performed in forensic laboratories utilize GC with some combination of detectors and peripherals. The most common is GC with mass spectrometry as the detector. In practice, the use of a single quadrupole mass filter is most common; however, tandem mass spectrometric methods have been used as well. Once presented to the instrument, the analysis of a sample is usually straightforward. There is a challenge in rapidly and reliably obtaining a sample for analysis from the vapor headspace of fire debris collected at the scene.

In this presentation, results of the application of PLOT-cryo to the analysis of ignitable liquids in fire debris are presented.¹ This study tested ignitable liquids, broadly divided into fuels and solvents (although the majority of the results that will be presented were obtained with gasoline and diesel fuel) on three substrates: douglas fir, oak plywood, and nylon carpet. It was determined that PLOT-cryo allows the analyst to distinguish all of the ignitable liquids tested by use of a very rapid sampling protocol and performs better (more recovered components, higher efficiency, lower elution solvent volumes) than a conventional purge-and-trap method. This study also tested the effect of latency (the time period between applying the ignitable liquid and ignition) and tested a variety of sampling times and a variety of PLOT capillary lengths. Reliable results can be obtained with sampling time periods as short as three minutes and, on PLOT capillaries, as short as 20cm. The variability of separate samples was also assessed, a study made possible by the high throughput nature of the PLOT-cryo method. It was also determined that the method performs better than the conventional carbon strip method that is commonly used in fire debris analysis. Variations of the PLOT-cryo method to provide for high-sample throughput and portable operation in the field will also be described.²⁻⁵

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PLOT-Cryoadsorption, Fire Debris, Ignitable Liquids



B167 Application of Likelihood Ratios in Fire Debris Analysis

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The goal of this presentation is to discuss a statistical approach to classification of fire debris as positive or negative for the presence of ignitable liquid residue and the determination of a likelihood ratio for the two competing hypotheses. Conversion of the likelihood ratio to a verbal scale expressing support for one hypothesis relative to the other will also be discussed.

This presentation will impact the forensic science community by showing the benefit of exposure to concepts that are fundamental to the evaluation of forensic evidence.

Likelihood ratios can be understood in the framework of receiver operating characteristic curves, probability density distributions, and Bayesian decision theory. A likelihood ratio allows the forensic scientist to evaluate evidential value under two competing hypothesis in the absence of information regarding prior probabilities, which are often unavailable. The magnitude of the likelihood ratio can be converted to a verbal scale that expresses the strength of evidential support for the two competing hypotheses. Evett has proposed a verbal scale of support as defined by the following terms on the indicated likelihood ratio intervals: “none” (0, 1); “limited” (1, 10); “moderate” (10, 100); “moderately strong” (100, 1,000); “strong” (1,000, 10,000); and, “very strong” (>10,000).¹ In the event that prior probabilities are available, posterior odds can be determined.

One example that demonstrates these concepts is the classification of fire debris as positive or negative for the presence of ignitable liquid residue. The likelihood ratio under the hypothesis that a sample is positive (H+) or negative (H-) for ignitable residue, given some Evidence (E), is defined as the ratio of class-conditional probabilities — $P(E|H+)/P(E|H-)$. The underlying statistical model is based on a set of extracted ions from total ion spectra of ignitable liquids.² The total ion spectra for ignitable liquids and substrate pyrolysis products were taken from the Ignitable Liquids Reference Collection and the Substrates Database and computationally mixed to produce a balanced set of 3,000 total ion spectra for samples that were defined to be positive or negative for ignitable liquid residue.³ Principal component analysis was performed on the data set for feature extraction and a support vector machine with radial kernel function was trained on the resulting scores and tested by ten-fold cross validation with 20% hold-out.

As a hard classifier, the optimum model had a cross-validation accuracy of 90% (i.e., 275 of 300 ignitable liquid samples were correctly classified and 262 of 300 substrate samples were correctly classified). The probabilities of class membership were obtained from the support vector machine model using a flat prior probability and the strength of support for the competing hypotheses was determined from the likelihood ratio. Using Evett’s verbal scale, of 300 ignitable liquid containing samples, the likelihood ratio for one sample showed strong support for the presence of ignitable liquid residue, 36 showed moderately strong support, 143 showed moderate support, 95 showed weak support, and 25 showed no support. Using the same verbal scale, of 300 substrate samples, the likelihood ratio for three samples showed moderate support for the presence of ignitable liquid residue, 35 showed weak support, and 262 showed no support. Results from further testing with other models will also be reported.

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Fire Debris, Likelihood Ratio, Statistics



B168 Detection and Quantitation of Polydimethyl Siloxane Using Liquid Chromatography/Mass Spectrometry

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After attending this presentation, attendees will understand and be able to implement a method for the detection and quantitation of Polydimethyl Siloxane (PDMS), a common condom lubricant.

This presentation will impact the forensic science community by providing a successful validation that can result in a more robust and comprehensive analysis of chemical extracts which contain PDMS.

This presentation will cover a validation study for the use of a new methodology in the analysis of forensic samples related to sexual assault investigations. This successful validation will result in a more robust and comprehensive analysis of chemical extracts which contain PDMS.

Examination of items of evidence from sexual assault crimes may in some instances require the detection and comparison of materials from condoms and sexual lubricants used in the commission of the crime. Improvements in the ability to detect and discriminate these types of materials can lead to more effective processing of sexual assault casework. A common condom lubricant, PDMS, is a very stable polymer with several potential sources including, but not limited to, lotions, cosmetics, machining greases, and lubricants. As such, trace amounts of PDMS may be present on items of evidence from environmental contamination. Typical analyses targeting PDMS are qualitative in nature and do not allow for the discrimination of trace background levels from more concentrated lubricant stains.

The quantitative analysis of PDMS was conducted by normal phase Liquid Chromatography/Atmospheric Pressure Chemical Ionization-Mass Spectrometry (LC/APCI-MS) with Multiple Reaction Monitoring (MRM). LC/APCI-MS (MRM) analysis (limit of detection $1.7\text{ ng } \mu\text{L}^{-1}$; limit of quantitation $3.5\text{ ng } \mu\text{L}^{-1}$).

Cuttings from a collection of new and well-worn underwear were analyzed by this method to establish the expected background levels of PDMS in garments. The global average background concentration of PDMS in new and freshly washed but unworn undergarments was determined to be $6 \pm 5\text{ } \mu\text{g cm}^{-2}$. A sampling of well-worn and washed men's and women's undergarments yielded a global average PDMS concentration of $4 \pm 2\text{ } \mu\text{g cm}^{-2}$.

PDMS concentrations in a variety of lotions, condoms, and personal lubricants were determined to assess the contribution of each product to a potential stain or background. All lotions studied here were found to contain less than 1.0% PDMS by mass. Condom residue varied in composition based on lubricant type; spermicidal and unlubricated condoms did not typically contain PDMS. Condoms labeled as lightly lubricated were nearly 100% PDMS by mass. The average for other lubricated condoms was 29% PDMS by mass, but ranged from 10% to 50% PDMS by mass. Only one personal lubricant studied here was found to contain PDMS (41% by mass).

The findings from this study show that underwear background PDMS levels are not significant when compared to the PDMS content of condom lubricants.

The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

Condoms, Siloxanes, Mass Spectrometry



Criminalistics Section - 2015

B169 2015 Update From the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG)

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After attending this presentation, attendees will be informed concerning SWGDRUG activities during 2014 and be updated on current and future projects.

This presentation will impact the forensic science community by providing the latest information pertaining to SWGDRUG activities, current projects, and resources provided to seized-drug analysts and laboratories.

SWGDRUG was formed in 1997 as a joint effort between the United States Drug Enforcement Administration Office of Forensic Sciences and the Office of National Drug Control Policy. **The mission of SWGDRUG is to recommend minimum standards for the forensic examination of seized drugs and to seek their international acceptance.**

SWGDRUG recommendations are available to the general public via the group's website (www.swgdrug.org). They include a code of professional practice for drug analysts as well as recommendations in the areas of education and training, methods of analysis, and quality assurance. Recently, the SWGDRUG core committee revised its recommendations pertaining to the use of reference materials to address laboratory needs during the verification of new reference materials.

SWGDRUG continues to provide the forensic community with its Mass Spectrometry (MS) and Infrared (IR) spectroscopy libraries, both of which continue to be regularly updated. Version 2.1 of the MS library was made available June 3, 2014, and currently contains more than 1,880 spectra obtained under electron ionization conditions. Included in the library are many of the recently encountered synthetic cannabinoids, substituted cathinones, and hallucinogenic phenethylamines. Version 1.1 of the IR library was made available May 30, 2014. This library contains more than 150 compounds obtained under attenuated total reflectance conditions. Both of these libraries are available in various instrument formats and can be downloaded from the SWGDRUG website and into laboratory instruments. Feedback from analysts and library users continues to be highly positive. The library will continue to be updated on a regular basis and contributions from the forensic community are strongly encouraged.

In an effort to continue providing the seized-drug community with a variety of tools, SWGDRUG also continues to publish drug monographs containing **detailed information and analytical data for reference materials**. During the last two years, more than 210 monographs have been added, containing data from reference materials **which have been analyzed, verified, and authenticated by the Drug Enforcement Administration Special Testing and Research Laboratory**. **These monographs, available via the SWGDRUG website, are intended to be used for the verification of acquired reference materials.**

With the goal of assessing the current controlled substance analogue issue, SWGDRUG has drafted a document containing general recommendations regarding analogues and structural class determinations. A final version of this document will be posted on the SWGDRUG website during 2014. The document emphasizes the need for understanding how analogues and structural classes are legally defined in different jurisdictions prior to analysts rendering opinions about a substance's classification.

This presentation will also discuss future group directions resulting from the establishment of the National Institute of Standards and Technology's (NIST's) Organization of Scientific Area Committees and current SWGDRUG projects, including the development of a supplemental document to assist laboratories in the estimation of the uncertainty associated with net weights obtained via extrapolation exercises.

The SWGDRUG core committee is comprised of representatives from federal, state, and local law enforcement agencies in the United States, Canada, Brazil, Great Britain, Germany, Austria, Switzerland, Australia, and Singapore. The following forensic organizations are represented: the European Network of Forensic Science Institutes (ENFSI), the Academia Iberoamericana de Criminalística y Estudios Forenses (AICEF), the Asian Forensic Science Network (AFSN), and the United Nations Office on Drugs and Crime (UNODC). Core committee members also include forensic science educators and representatives from forensic science organizations across the United States, the American Society of Crime Laboratory Directors (ASCLD), ASTM International, and NIST.

Controlled Substances, SWGDRUG, Drug Analysis



B170 Synthetic Drug Trends in the United States

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This goal of this presentation is to increase attendees' understanding of the synthetic drug market in the United States by illustrating the synthetic cannabinoid, substituted cathinone, and hallucinogenic phenethylamine seizure trends tracked by the Drug Enforcement Administration (DEA).

This presentation will impact the forensic science community by increasing the forensic, enforcement, and legislative communities' understanding of the current trends and flow of synthetic drugs within the United States in order to be better prepared to fight the synthetic drug epidemic.

By employing a variety of sources, information was obtained regarding drug exhibits analyzed by the eight DEA chemistry laboratories and various state and local forensic laboratories. Among the information maintained by these sources are seizure details and analytical results, which can be used to compile drug intelligence, detect the appearance of new drugs of abuse, and monitor drug trends. Data were collected for this analysis through a query of archived seizure and analysis information compiled by these sources. The information targeted in this query included the date and location of the seizure, brand names of the products seized, and substances identified during the chemical analysis. This information was compiled to construct a nationwide trend analysis of the synthetic drug market.

This study utilizes numerical data from various sources of information to track overall trends in the synthetic drug market. In addition to a breakdown of nationwide DEA synthetic drug seizure data, a number of trends will be investigated. This includes the effects of federal legislation on the number of seizures of a particular synthetic drug, as well as tracking of brand names to observe the change in active ingredients over time. Geographic trends such as location of the DEA's first encounter of a substance and prevalence of compounds over a geographic area are also explored. Trends in the use of adulterants and diluents are examined within the scope of geographical area and brand name.

Since the introduction of synthetic cannabinoids and substituted cathinones to the United States' illicit drug market in the late 2000s, the synthetic drug market has exploded. These products offer unique challenges to the entire criminal justice system, in part due to the rapid speed at which the suppliers alter these compounds to circumvent current drug legislation. The DEA Special Testing and Research Laboratory has encountered approximately 300 new psychoactive substances since they first emerged in the United States in 2009. In addition to understanding the past and present synthetic drug situation, the trend information presented may be used to predict what compounds could be popular next.

Synthetic Drugs, Trends, Designer Drugs



B171 Collection and Analysis of Fire Debris Evidence to Detect Methamphetamine, Pseudoephedrine, and Ignitable Liquids in Fire Scenes at Suspected Clandestine Laboratories

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The goal of this presentation is to describe investigative tools to collect and detect methamphetamine and its precursors in fire debris evidence.

This presentation will impact the forensic science community by providing knowledge and understanding to attendees and investigators about the possibility of collection and laboratory examination of clandestine laboratory evidence at fire scenes. Implementation and application of ignitable liquid and methamphetamine detection methods will strengthen investigations and assist in determining the origin and cause of the fire.

The “One-Pot” clandestine methamphetamine production method involves the combination and use of highly reactive and flammable materials. Individuals attempting this method are creating clandestine laboratories within residences or other occupied structures and the likelihood of a subsequent fire puts anyone nearby at risk. In some jurisdictions, a fire caused by the production of methamphetamine falls under a first-degree arson statute, which can involve a much longer prison sentence when compared to an illicit drug production penalty. The ability to detect methamphetamine and the “One-Pot” precursors in the fire debris samples would strengthen the fire investigation. Although a positive detection does not guarantee methamphetamine production, the combination of the presence of an ignitable liquid and drug detection, within the totality of circumstances, has the ability to indicate the presence of a clandestine laboratory. The research was designed to answer the following questions: (1) can evidence collected at a suspected methamphetamine laboratory fire be analyzed for ignitable liquids and for the presence of methamphetamine and its precursor, pseudoephedrine; (2) would more severely charred or burned samples show a decrease in sensitivity in the analytical detection process for methamphetamine and/or pseudoephedrine; (3) are certain sample types (carpet, wood, wipes, etc.) more likely to contain the drugs of interest; and, (4) if the method for methamphetamine and/or pseudoephedrine works with advanced technologies like Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS), is there a way for it to work with existing resources in most laboratories, such as Gas Chromatography/Mass Spectrometry (GC/MS)?

To test these questions, “One-Pot” methamphetamine reactions were carried out in a safe environment and the liquid and solid products were separated. The solid waste (sludge) and solvents were then transported to a fire research facility and the “One-Pot” methamphetamine products were used to recreate a series of fires. Small burn cells were constructed and used to represent a residential environment, then the simulated “One-Pot” laboratories were burned in each burn cell. Based upon the original placement of the “One-Pot” material and observations made during the burning process, several fire debris samples were collected from each cell, including wall wipe samples, plastic bottle remnants, wood, and carpet. Each sample was analyzed for ignitable liquids using passive headspace concentration with charcoal strips and GC/MS. Following ignitable liquid analysis, fire debris samples were extracted with LC/MS/MS running buffer and subjected to LC/MS/MS analysis to detect methamphetamine and pseudoephedrine in the fire debris samples. Additionally, the fire debris samples, charcoal strip extracts, and preserved charcoal strips were provided to local law enforcement laboratories. The preserved charcoal strips were extracted with methanol and the extract was analyzed for methamphetamine using GC/MS with positive results. This work demonstrates that fire debris analysis extraction methods can prove the presence of clandestine methamphetamine laboratories that result in arson fires.

Clandestine Laboratories, Ignitable Liquid, Methamphetamine



B172 TLC-SERS of Controlled Substances: Colloid Optimization and Drug Expansion

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After attending this presentation, attendees will understand the emerging hyphenated Thin-Layer Chromatography with Surface-Enhanced Raman Spectroscopy (TLC-SERS) technique and how it can be used to identify drug mixtures.

This presentation will impact the forensic science community by expanding the use of TLC-SERS for the analysis of controlled substances.

The purpose of this research was to expand previous research on the identification of controlled substances using TLC-SERS. TLC-SERS is a hyphenated technique where a mixture is first separated using TLC followed by SERS of the TLC plate. A noble metal colloid is deposited on the resultant TLC spot and SERS spectra are directly collected. This combined technique has the potential to benefit the forensic science community because it requires less sample, time, and money when compared to other methods of analysis. In addition, it adheres to the standards for positive drug identification established by the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG).

The first goal of this research was to find a colloid that provided the most enhancement of the Raman signal for various controlled substances. Cocaine, methamphetamine, diazepam, and codeine as 4mg/ml solutions in methanol were used to evaluate the various colloid preparations. A number of both silver and gold colloids were tested and evaluated. Individually, the drugs were drawn up the TLC plate using a mobile phase of 9:1 chloroform:methanol. The plates were allowed to dry, then 20 μ l of the desired colloid was added. The resulting spectra were evaluated on uniformity across sampling, peak height, shelf life of colloid, ease of preparation, and cost of starting materials. The results of this investigation determined that silver colloids worked better than gold colloids. The best colloid was a preparation of a silver colloid first made by White and Hjortkjaer that has the largest SERS enhancement, has a long shelf life, is reproducible, and easy and fast to produce.¹ This silver colloid can be prepared in only a few minutes and has a reported shelf life of more than a year. The second goal of this research was to expand the number and types of controlled substances that can be identified using TLC-SERS. In addition, lower limits of detection were established for each drug. The controlled substances selected for inclusion in this part of the project were determined to be heavily used or on the rise by the 2012 United Nations Office on Drugs and Crime (UNODC) World Drug Report.² The drugs analyzed included heroin, methadone, morphine, codeine, fentanyl, buprenorphine, desomorphine diazepam, GHB, flunitrazepam, phenobarbital, temazepam, methamphetamine, amphetamine, cocaine, mephedrone, MDPV, MDMA, and ketamine. Methanol solutions of each drug, along with drug mixtures, were made and spotted on TLC plates. Following separation, 20 μ l of the optimized colloid were added to each TLC spot and SERS spectra were collected and analyzed.

TLC-SERS spectra are reproducible and interpretable, thus this research demonstrated that TLC-SERS is a successful method for the separation and identification of a wide range of drugs and drug mixtures. Coupling TLC with SERS is a convenient way to reduce the amount of material, equipment, and time needed for controlled substance analysis when compared to current methods and conforms to the standards set forth by SWGDRUG. This is a potentially valuable on-site technique since TLC is a relatively quick and easy technique and the Raman technology has recently become portable. The added sensitivity of the SERS allows for the possibility of very small sample size.

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Controlled Substances, TLC-SERS, Colloids



B173 Detection of Drugs in Pharmaceutical Preparations Where Diversion Can Occur by Direct Analysis in Real-Time AccuTOF™-Mass Spectrometry (DART®-MS)

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The goal of this presentation is to inform the forensic community about a novel use of Direct Analysis in Real-Time AccuTOF™-Mass Spectrometry (DART®-MS) for screening of pharmaceutical preparations in cases of suspected drug diversion or where diversion may easily occur.

This presentation will impact the forensic science community by providing a technique for rapid identification of the contents of pharmaceutical preparations where drug diversion can occur.

Drug diversion in healthcare facilities is a multi-victim crime. Harm can come to patients, the healthcare worker, and the employer. Absence or dilution of an intended drug could result in patients receiving substandard care from their healthcare provider and may cause undue pain or anxiety in their treatment.

DART®-MS is a relatively new ionization technique that is being used to analyze a wide variety of compounds and matrices. DART®-MS offers the advantages of direct sample examination without the need for a vacuum system, minimal or no sample preparation, and high sample throughput. The use of a time-of-flight mass analyzer allows for accurate mass measurements for compounds present in or on the substrate.

A DART®-MS library was created using 20 common parental pharmaceutical preparations. These included typical surgical analgesic and anesthetic mixtures of controlled substances. Two hundred randomly selected pharmaceutical preparations were obtained from the Forensic Industrial Environmental Research and Metabolism (FIRM) Laboratory at Medical College of Virginia Hospitals at Virginia Commonwealth University. Specimens for this study were pharmaceutical preparations from “show cause testing” and routine analyses for possible diversion. These pharmaceutical preparations were screened using a DART® ion source coupled to a JEOL JMS T100LC AccuTOF™ MS operating in positive-ion mode with the following parameters: the ion source was helium gas, operated at a flow rate of 2.0L/min, a gas heater temperature of 300°C, a discharge electrode needle at 4,000V, electrode 1 set at 150V, and electrode 2 set at 250V. The resolving power of the MS was 6,000FWHM. Measurements were taken with the ion guide peak voltage at 800V, reflectron voltage at 900V, orifice 2 set at 5V, ring lens at 3V, and orifice 1 temperature at 300°C. The measurements were taken using the function switching method, allowing for collection every 0.25 seconds at the orifice 1 voltages of 20V, 30V, 60V, and 90V. The measured mass range was from 40Da to 1,000Da. The DART®-MS results were compared to both the created pharmaceutical preparations library and the National Institute of Standards and Technology library. The drug or drugs detected in each specimen was then confirmed by High-Performance Liquid Chromatography (HPLC) using a previously published method.¹

The DART®-MS was determined to have the ability to screen for and identify the main active drug or drugs in these pharmaceutical preparations by using the created pharmaceutical preparations library. The DART®-MS was also able to identify many of the excipients in the preparations. The DART®-MS analysis of the 200 pharmaceutical preparations versus the HPLC analysis of the drugs found in the pharmaceutical preparations resulted in an excellent correlation.

Reference:

1. Wolf CE and Poklis A. A rapid HPLC procedure for analysis of analgesic pharmaceutical mixtures for quality assurance and drug diversion testing. *J. Anal. Toxicol.* 2005;29:711-714.

DART®-MS, Diversion, Pharmaceutical Preparations



B174 Quantification of Controlled Substances in Simulated Samples Using Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR) and Principal Components Regression

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After attending this presentation, attendees will understand how ATR-FTIR spectroscopy can be used in conjunction with multivariate statistics to develop and evaluate models for the quantification of controlled substances in submitted samples.

This presentation will impact the forensic science community by providing a rapid and non-destructive method to quantify controlled substances in samples using ATR-FTIR and multivariate statistical procedures.

ATR-FTIR is widely used for the analysis of submitted samples suspected to contain controlled substances. The ATR sampling accessory minimizes sample preparation and is relatively non-destructive, while FTIR is a Scientific Working Group for the Analysis of Seized Drugs Category A technique, enabling definitive identification of controlled substances in submitted samples.

In this research, Principal Components Regression (PCR) was investigated as an additional tool to aid in identification and quantification of controlled substances in sample mixtures analyzed by ATR-FTIR. PCR is a multivariate statistical procedure that combines Principal Components Analysis (PCA) and multiple linear regression. The former is used to calculate scores for the samples that are then used in the latter to generate a calibration curve that can be used for quantification purposes.

To test the utility of PCR for this application, two-component sample mixtures containing either amphetamine or methamphetamine as the controlled substance and caffeine as a cutting agent were prepared, containing 20%-100% (w/w) controlled substance, in 20% increments. These mixtures were used as the training set for the PCR model. A second set of mixtures was prepared, again using amphetamine and methamphetamine as the controlled substance and caffeine as the cutting agent. Mixtures in this set ranged from 10%-90% (w/w) controlled substance, in 10% increments. This set was used as the test set to evaluate the PCR model. All sample mixtures were then analyzed in triplicate by ATR-FTIR.

Prior to statistical analysis, data pretreatment procedures are often necessary to ensure that variation observed among the samples is chemically relevant. Examples of pretreatment procedures for spectral data include baseline correction, smoothing, scatter correction, and normalization, all of which were investigated in this research. The pretreated data were subjected to PCA and the effect of the pretreatment was evaluated based on the change in the association of replicates in the PCA scores plot compared to the untreated data. For this data set, the greatest improvement in the association of replicates was observed after applying a log transformation, followed by the baseline correction and smoothing functions available in the instrument software, and finally, standard normal variate normalization.

To develop the model, PCA was first performed on the pretreated training set data to generate scores and loadings matrices. The number of Principal Components (PCs) to include in the model was investigated using leave-one-out cross validation. The optimal model retained seven to nine PCs, with validation errors ranging from 2%-5%, indicating acceptable model performance. Multiple linear regression was then performed using the scores and PCs, in conjunction with the percent concentration of controlled substance, to generate a calibration curve. The resulting calibration curve had a correlation coefficient (r) of 0.98, indicating strong correlation between the predicted and actual concentrations of each controlled substance in the training set.

The performance of the optimized PCR model was evaluated based on the error in the predicted concentrations for the sample mixtures in the test set. Prediction errors ranged between 4% and 7%, which demonstrated the potential of PCR for quantification using spectral data. Thus, the ability to quantify controlled substances by applying PCR to spectral data collected using ATR-FTIR provides additional discriminating information about the samples.

ATR-FTIR, Controlled Substances, Multiple Linear Regression



B175 Solid State Nuclear Magnetic Resonance (NMR) of Street Drugs — The New Presumptive Drug Test

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After attending this presentation, attendees will understand the effectiveness of NMR analysis in relationship to preemptively identifying unknown street drugs.

This presentation will impact the forensic science community by offering a new method of preemptively determining the identity of an unknown compound.

Solid state NMR has proven itself to be an interesting new tool for obtaining quick, easy, and inexpensive NMR spectra for research and instructional purposes. It can also be used to attain spectral profiles on pure samples of drugs that produce “legal highs.” This work attempts to create an NMR database of synthetic compounds known to be in use recreationally or analogs thereof. Such a library would be useful to those working in crime laboratories to identify unknown chemical compounds found at crime scenes. This test is more accurate than a color indicator and is easier to administer than a traditional mass spectrometric analysis. A single test requires only 20 minutes and is very cost effective.

Major classes of compounds tested include psychedelics, cathinones, and cannabinoids. Standard samples were provided by Cayman Chemical and street samples were provided by the Pennsylvania State Police. These street samples were mostly mixtures of compounds, most notably XLR-11, PB-22, and AB-Fubinaca. Compounds were dissolved in the appropriate deuterated solvent and centrifuged to insure the compounds completely dissolved. Any sample that crashes out of solution during injection could prove dangerous to the inner line of the instrument. The line was purged before and after each test with the corresponding solvent, then the instrument was shimmed with deionized water.

The standards’ spectra are comparable to those of the samples. An interactive library will be compiled so a drug profile can be quickly compared against a library and a presumptive identity can be established. This method will not be meant to definitively identify an unknown compound but it can aid in rapid presumptive analysis so that follow-up analyses are more targeted.

Since NMR measures excitation in proton nuclei and not fragmentation patterns, a consistent result of a given compound is not always guaranteed; however, NMR will consistently give a general profile of a given compound. If there is no discrepancy in shimming and solvent choice, NMR can be used to compare spectral similarities among unknown compounds. A quick and easy presumptive test for drugs, provided by the solid-state NMR, can be useful to save time on a more in-depth instrument and accurately identify at least the class of compound of a known substance.

NMR, Cathinones, Presumptive Test



B176 Elemental Analysis of Presumptive Clandestine Laboratory Evidence Using Laser-Induced Breakdown Spectroscopy (LIBS)

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The goals of this presentation are to introduce the fundamentals of LIBS, define what is needed for trace element identification, and explain how it can be incorporated into the elemental analysis of clandestine laboratory evidence.

This presentation will impact the forensic science community by explaining the advantages of continuing to integrate LIBS in trace elemental analyses and explaining the potential use of the LIBS instrument in forensic cases.

Clandestine laboratories have been a rapidly growing problem throughout the United States. The ease of obtaining the ingredients to manufacture methamphetamine is a growing concern; therefore, analytical methods for the detection of these ingredients are critical. Numerous elemental analysis techniques currently exist in forensic science; however, LIBS is a rapidly expanding technique. LIBS uses a low-energy laser pulse in conjunction with lenses to generate a plasma when the laser beam interacts with the sample surface. A spectrum is generated that displays atomic and ionic spectral emission lines unique to the elements present. This spectrum is essentially a “fingerprint” of the respective elements present in the sample. The main objective of this research was to develop and optimize a method to identify elements commonly found in clandestine laboratory evidence. This was accomplished by examining the changes in the data caused by varying parameters of the instrument including: spot size, number of shots, spectrometer delay, repetition rate, laser intensity, accumulation of data, and gas flow. Helium and argon were used to optimize elemental excitation. The elements included in this study are commonly encountered in the methamphetamine manufacturing process including: sodium, sulfur, chlorine, red phosphorus, iodine, and lithium. An advantage of LIBS is that it can detect lighter elements, such as lithium, which is not possible with scanning electron microscopy-energy dispersive spectrometry.

The samples were evaluated during method development and optimized for each instrumental parameter. The data were reviewed for peak shape, peak abundance, and the presence of atomic and ionic spectral lines. Sample preparation techniques were also compared for each sample including: embedding in nail polish, using double-sided adhesive tape, and the use of adhesive dots. Three replicates for each sample preparation technique were examined in triplicate to observe homogeneity of the sample preparation technique as well as matrix effects. Once the optimal sample preparation technique was established, a large number of samples for each element were analyzed in triplicate to evaluate reproducibility and determine a potential threshold for elemental identification. The optimized methods for each element as well as identification threshold levels were also applied to mock case samples.

The neat samples were successfully optimized for each instrumental parameter. The intensity of the spectral lines for sodium, red phosphorus, and lithium were detected in an ambient atmosphere, while helium was needed to increase the spectral line intensities for sulfur, chlorine, and iodine samples. Some of the elements of interest displayed spectral line overlaps, and in some cases, the adhesive/sample preparation technique demonstrated interferences. A minimum of three to four spectral lines were used to determine if an element was present. Overall, LIBS has the potential to be a powerful technique for forensic trace elemental analysis.

LIBS, Methamphetamine, Clandestine Laboratories



Criminalistics Section - 2015

B177 You Got DNA From What?

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After attending this presentation, attendees will be informed regarding interesting and challenging samples from which their peers were able to obtain interpretable DNA profiles.

This presentation will impact the forensic science community by demonstrating that their forensic biology peers have great senses of humor.

The second annual You Got DNA From WHAT? session will once again allow attendees to gather and pay homage to how amazing DNA is and to reflect on the fact that DNA has been extracted from SO many novel — and interesting — items. As testing sensitivities improve and technologies continue to change, one constant is the need for intuitive analysts to properly sample items of evidence. This session gives those intuitive DNA dudes and dudettes an opportunity to present brief PowerPoint® slide-based synopses on unique and challenging evidence samples. This event will allow DNA analysts to: (1) boast of their DNA success; (2) discuss challenges in obtaining results from a particular item of evidence; (3) ponder how they might improve on their method in the unlikely event they ever encounter this type of evidence again in their lifetime; (4) gloat vis-à-vis their analytical superiority; and, (5) figure out how to one-up last year's presenters!

This 2nd annual Friday evening You Got DNA From WHAT? session, the brainchild of Criminalistics Section Fellow Daniel Petersen, is intended to be informal, entertaining, and informational (in that order!). The list of You Got DNA From WHAT? speakers, as well as the subject matter of each presentation, will be kept under wraps until the last minute to keep attendees wondering.

If you've ever found yourself at loose ends on the Friday night prior to the meeting wrapping up on Saturday and you've ever made this exclamation, or wish you had, this session is for you!

DNA, Unique Samples, Sensitivity



B178 Improving Methods for the Recovery and Analysis of Touch DNA From Fingerprints at Crime Scenes

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After attending this presentation, attendees will better understand the most effective method of recovering DNA from touched or handled items at crime scenes for the purpose of human identification. The presentation will guide the audience through the optimum swabbing technique, the best swabbing media, and describe the process of direct Polymerase Chain Reaction (PCR) with the extraction step omitted.

This presentation will impact the forensic science community by highlighting the benefits of direct PCR in a forensic context and making the audience aware that an extraction step is not always beneficial for processing touch DNA swabs. This method reduces time and labor costs and minimizes the risk of contamination while increasing the likelihood of obtaining a meaningful profile for interpretation. The methodology described can easily be adapted into mainstream forensic practice.

The ability to generate a DNA profile from a fingerprint for the purpose of human identification will have significant implications for solving a broad spectrum of criminal investigations, ranging from theft to crimes of violence. DNA retrieved from fingermarks deposited by touch (referred to as “touch” DNA) is often degraded, limited in quantity, and may comprise elements that co-extract with the DNA and hinder subsequent amplification. There is a limit of sensitivity that still precludes many items touched at a scene from generating a useable DNA profile, despite their potential importance in a criminal investigation. Examples of these sample types include triggers, steering wheels, bullet cartridges, and handles of knives. In many criminal cases, the ability to retrieve the maximum amount of DNA from “touch” DNA samples is of paramount importance and crucial to resolving the case.

The first DNA profile generated from a fingerprint was reported over a decade ago and revolutionized forensic science. In spite of this, recent research demonstrates an extremely low success rate (5%-6%) using standard methodology in terms of generating a profile from “touch” DNA sources that is deemed suitable for identification purposes. This highlights the need for improved methodology. A novel method that routinely generates meaningful DNA profiles from latent fingermarks for the purpose of human identification is reported here. Depending on the type of short tandem repeat DNA profiling kit used, the success rate is 62% to 70% and is not dependent on an enhanced PCR cycle number. Its novelty that will be discussed involves an optimized swabbing technique and detergent media, omission of a DNA extraction process, and the addition of PCR facilitators to the reaction vessel. By using a direct PCR approach, full DNA profiles from fingerprints that have been deposited only 15 minutes after a person has washed his/her hands can routinely be generated.

A total of 34 people washed their hands to remove external DNA and after only 15 minutes deposited fingermarks from all digits of their dominant hand onto a plastic substrate. Nylon fibers pre-moistened with heated detergent were used to collect any DNA from the substrate and then placed directly into the amplification reaction tube. From the 170 fingermarks tested, only four DNA profiles (<1%) failed to yield DNA and 116 DNA profiles (68%) were recorded with sufficient data to be used in DNA databases. This finding demonstrates a 62-fold difference between standard methodology and the improved DNA-capture method.

The method has since been applied to casework to generate a DNA profile from a single fingermark deposited on a drug wrap, illustrating the potential for significant impact on forensic practice and criminal investigations. The process described is simple, elegant, and should readily gain general acceptance into legal systems. This will allow meaningful DNA profiles to be generated routinely from touched items where current forensic practice has little or no chance of generating a DNA profile.

DNA Profiling, Touch DNA, Direct PCR



Criminalistics Section - 2015

B179 Assessment of Fingerprints for Forensic Short Tandem Repeat (STR) Analysis

Lana Ostojic, MS, OCME of NYC, 421 E 26th Street, New York, NY 10016*

After attending this presentation, attendees will understand the challenges of working with fingerprints. The presentation will focus on the evaluation of cellular and DNA content of a single fingerprint and the ability to produce Combined DNA Index System (CODIS)-eligible STR profiles.

This presentation will impact the forensic science community by providing information on investigative values of fingerprints as forensic DNA samples.

Touched or grabbed items, such as handles of weapons, tools, or other objects with no apparent biological staining, can be used as evidence in investigations in a wide variety of criminal cases, including homicides, sexual assault, and property crimes; however, there are some fundamental difficulties when working with samples collected from touched objects, including variability in quantity and quality of extracted DNA. Fingerprints can result in little-to-no DNA but also in DNA profiles that are suitable for upload to forensic STR databases. Importantly, sometimes fingerprints are the only available source for forensic DNA testing. Obtaining high-quality DNA profiles that can be used for CODIS from the aforementioned types of evidences has a tremendous potential in the investigation of a wide variety of criminal offenses.

In a longitudinal study, the New York City Office of Chief Medical Examiner Department of Forensic Biology collected more than 700 fingerprints. Unrelated volunteers provided several series of fingerprints on separate days. Sample collection was performed under similar conditions to minimize variations. Following deposition, the fingerprints were assigned a quality score of one to five (sparse to dense shedding), aided by the use of an Olympus® SZX-16® stereomicroscope. Individuals differed in their shedding scores; however, most fingerprints scored three or four. A Chi-square test on the data confirmed that it is not possible to classify people as good or bad shedders. Nevertheless, some individual's scores tended to be slightly lower, enabling these persons to be described as "bad shedders" relative to individuals who's scores tended to be a little higher. A Chi-square test also revealed no differences between left and right hand regarding the shedding score, indicating that shedding is independent of hand dominance or how frequently one hand is used over the other.

The laboratory further investigated whether there is a correlation between shedding score and the amount of DNA recovered. Linear regression showed that one unit in shedding score is associated with a 2.24pg/μl increase in the amount of DNA recovered; however, this is not significant ($p=0.20$) indicating shedding score is not helpful in predicting DNA amount. The laboratory also investigated the correlation between shedding score and completeness of the DNA profile obtained. Linear regression showed that each unit of shedding score was associated with a 4.2% increase in the percent profile obtained and this was significant ($p=4.8 \times 10^{-4}$); however, because of the great variability in profile quality, shedding score alone was not a reliable predictor of profile quality. A possible explanation for this could be that many deposited cells in a fingerprint may not be nucleated, plus cell flakes may or may not be carriers for extracellular DNA which is not visible microscopically.

Most of the STR profiles obtained from the fingerprints were partial. Profiles that were at least 70% complete were considered as valuable, since such profiles could be used to search databases. The probability to obtain such profiles from a single fingerprint varied, but was approximately 50%. In conclusion, a fingerprint can be considered as a potential source of DNA for forensic identification.

Fingerprints, STR Profiles, Touched Items



B180 Obtaining STR-Quality Touch DNA From Archived Latent Fingerprints

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After attending this presentation, attendees will understand the importance of collecting and extracting touch DNA evidence from each contact surface for both freshly collected and archived fingerprints.

This presentation will impact the forensic science community by helping attendees to better understand the fragility of such evidence and recognize the need to develop a successful protocol for extracting and typing the highest-quality DNA possible from these types of DNA sources.

Customary collection methods for latent fingerprints discovered at crime scenes involve powder dusting and tape-lifting the prints, followed by attaching the fingerprint-containing adhesive to paper backing cards for storage, thus forming an archived latent fingerprint. Ultimately, this approach allows the crime scene investigator to preserve the latent print(s) but also sandwiches valuable touch DNA evidence between the adhesive and paper surfaces; however, it is uncommon for forensic laboratories to extract DNA that has been stored in this manner due to low success rates and minimal research on DNA extraction methods for archived fingerprints. Further, it is uncommon for investigators to physically collect fingerprints deposited on paper surfaces after on-scene enhancement and photography, leaving valuable trace DNA behind.

In this study, the goal was to determine if it would be possible to obtain sufficient high-quality DNA for successful Short Tandem Repeat (STR) amplification from archived fingerprints. Eight sets of fingerprints were collected from both glass and paper surfaces from five volunteers. From these, multiple variables were tested and compared, including freshly collected samples versus aged samples, glass deposition versus paper, black magnetic enhancement versus standard black dusting powder, and recovery of DNA from the adhesive surface of the archived sample versus the paper side. Two different silica-based solid phase column DNA extraction methods were tested. Following extraction, all samples were quantified using a real-time Polymerase Chain Reaction (PCR) -based human-specific assay and STR loci were amplified and analyzed using standard methods. As expected, “fresh” fingerprint samples yielded higher DNA concentrations ($0.175\text{ng}\pm 0.3\text{ng}$) than “aged” fingerprint samples ($0.126\text{ng}\pm 0.3\text{ng}$). Enhancement powder used did not affect DNA yield, as samples visually enhanced with black magnetic dactyloscopic powder yielded approximately the same DNA yield ($0.166\text{ng}\pm 0.3\text{ng}$) as samples enhanced with standard black dusting dactyloscopic powder ($0.136\text{ng}\pm 0.3\text{ng}$). Furthermore, STR-quality DNA was obtainable from both the adhesive sides (avg. $0.356\text{ng}\pm 0.38\text{ng}$) and paper sides (avg. $0.211\text{ng}\pm 0.27\text{ng}$) of archived fingerprints. This data demonstrates two important findings. First, quantifiable touch DNA is obtainable from latent prints left on paper surfaces, and, thus, latent fingerprints deposited on paper should be collected from crime scenes for potential DNA analysis after on-scene enhancement and photography. Second, both the adhesive and paper surfaces of an archived fingerprint card should be processed for DNA analysis rather than processing only the adhesive side, the latter of which is standard protocol for labs attempting analysis of this sample type.

Three DNA collection methods were also evaluated: direct cuttings, double-swab technique with lysis buffer as the diluent, and single-swab technique with ultrapure water as the diluent. Of these methods, the double-swab technique yielded three times more DNA ($0.497\text{ng}\pm 0.45\text{ng}$) than the other two collection methods. Interestingly, none of the samples showed signs of PCR inhibition, regardless of chemical treatment for fingerprint enhancement; however, only samples collected using the single-swab technique ($n=5$) yielded STR profiles. Of these samples, 60% yielded near-complete profiles; however, all samples showed electropherogram data indicative of DNA degradation.

The results of this study show that it may be possible to obtain STR-quality DNA from archived, paper-backed latent fingerprints. While these results are encouraging, it is well known that outdated methods for collecting latent prints often did not include the use of gloves or other personal protective equipment and that fingerprint brushes are/were often used for multiple collections without cleaning. Thus, future studies will include thorough contamination and source attribution studies as well as an evaluation of the effects of brush reuse for collection of latent fingerprints.

Touch DNA, DNA Extraction, Archived Fingerprints



B181 Non-Destructive Biological Evidence Detection and Collection Using the ESDA®-Lite

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After attending this presentation, attendees will understand the basic operating principles of collecting human DNA evidence using the Electrostatic Detection Apparatus (ESDA®)-lite. Additionally, attendees will learn of the potential forensic applications of utilizing alternative collection methodologies such as dry-swabbing and the ESDA technique for non-destructive biological evidence detection and collection from paper substrates.

This presentation will impact the forensic science community by providing a confident approach to non-destructive biological evidence sampling from paper substrates. This technique would allow forensic DNA analysts to detect and collect biological materials from an evidentiary item without damaging its structural integrity and/or interfering with any subsequent examinations. Non-destructive collection of biological samples for DNA processing would preserve the physical integrity of evidentiary items to allow for more thorough evaluations by other forensic disciplines subsequent to DNA processing. The additional information gained from items processed in this manner could aid in the conviction or exoneration of individuals associated with evidentiary items containing touch DNA, such as questioned documents, entry-point surfaces, clothing, and other handled items. Evidence processed in a non-destructive manner would also remain available for future evaluations that could prove pivotal to the outcome of a cold case investigation and/or criminal retrial. All of the tools and techniques suggested are either relatively inexpensive or are already available in crime laboratories and could easily be incorporated into standard laboratory operating procedures.

The ESDA®-lite was systemically evaluated for its ability to non-destructively collect DNA from latent fingerprints deposited on various paper substrates for Short Tandem Repeat (STR) DNA profiling. Fingerprints were deposited on a variety of paper substrates that included resume paper, cotton paper, magazine paper, currency, copy paper, and newspaper. A total of 162 samples were prepared. For each collection technique, 54 latent fingerprints were sampled. Three DNA collection techniques were performed: ESDA collection, dry swabbing, and substrate cutting. Efficacy of each collection technique was evaluated by the quantity of DNA present in each sample and the percent profile generated. For each collection technique and substrate type, the DNA quantities were averaged across all three donors (Table 1). Large standard deviations were observed due to the wide range of DNA quantities deposited by the different fingerprint donors. Across all substrates, full and high partial profiles were generated for 65% of the samples collected with the ESDA technique, 93% of the samples collected via dry swabbing, and 52% of the samples collected with a destructive collection technique. Profiles in which 70% of the alleles (22 of 32 alleles or 11 loci) were obtained were considered to be high partial profiles.

Table 1: DNA quantity (ng) present in fingerprint samples collected via the ESDA, dry swabbing, and destructive (cutting) collection techniques.

Paper Substrate	Mean DNA Quantity (ng)		
	ESDA	Dry Swabbing	Destructive
Resume	0.367 ± 0.601	0.521 ± 0.348	0.061 ± 0.097
Cotton	0.217 ± 0.264	0.547 ± 0.519	0.000 ± 0.000
Magazine	0.332 ± 0.308	1.049 ± 1.186	0.130 ± 0.149
Currency	0.320 ± 0.696	0.623 ± 0.319	0.187 ± 0.184
Copy	0.170 ± 0.185	0.798 ± 0.460	0.550 ± 0.881
Newspaper	0.170 ± 0.226	0.267 ± 0.245	0.677 ± 0.453

Both the ESDA and dry swabbing non-destructive sampling techniques outperformed the destructive methodology of substrate cutting. A greater number of full profiles were generated from samples collected with the non-destructive dry swabbing collection technique than were generated from samples collected with the ESDA; however, the ESDA also allowed the user to visualize the area of interest while non-destructively collecting the biological material. The ability to visualize the biological material made sampling straightforward and eliminated the need for numerous, random swabbings/cuttings. Based on these results, the non-destructive ESDA collection technique has great potential for real-world forensic implementation.

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Criminalistics Section - 2015

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DNA Typing, Non-Destructive Collection, DOMEX



B182 Examination of Rapidly Mutating Y-STR Loci for Increased Resolution of Common Haplotypes Using a Large Multiplex Kit

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The goal of this presentation is to introduce attendees to a set of rapidly mutating Y-chromosomal Short Tandem Repeat (Y-STR) markers in a commercially available multiplex kit. Population statistics on the specific loci examined and their utility for discriminating closely related males will be presented.

This presentation will impact the forensic science community by introducing the usefulness of a set of rapidly mutating Y-STR loci in a commercially available kit to increase discrimination among closely related males.

Y-STR testing has become an important tool for forensic investigations, especially in DNA mixture samples where low-level male DNA is mixed in a high female DNA background. Until 2014, only two commercial kits of 17 and 23 Y-STR markers were available for the forensic community that provide relatively high discrimination among unrelated individuals, with a discrimination capacity greater than 97% (National Institute of Standards and Technology (NIST), unpublished data).^{1,2} Given the haploid nature of the Y-chromosome, a match between the evidence and the accused is evaluated in terms of how frequently the haplotype is observed in a relevant database. In addition to the limitation of Y-STR statistical results restricted to the size of the database, the current set of Y-STR markers are limited at separating related males such as fathers, sons, and brothers.

Recently published Rapidly Mutating (RM) Y-STR loci with mutation rates from roughly 1% to 7% per meioses were evaluated at NIST.^{3,4} To assess the utility of RM Y-STR markers for casework, NIST has evaluated the Yfiler® Plus kit released for commercial use in 2014. The Yfiler® Plus kit includes seven rapidly mutating markers intended to increase discrimination among closely related individuals. The evaluation used a set of unrelated population samples to determine population genetics parameters, such as haplotype diversity, and a set of father-son samples to determine the usefulness of the markers for distinguishing related males.

NIST population samples of more than 600 unrelated individuals in three United States groups, Caucasian, African American, and Hispanic, were initially tested with the Yfiler® Plus kit.⁵ Subsequently, nearly 400 father-son samples among United States Caucasians, African Americans, Asians, and Hispanics were also tested.⁶ All samples had been previously typed using the original two commercially available forensic Y-STR kits.^{1,2}

As a result of the testing, NIST found that the RM Y-STR markers provided increased discrimination and variation among common haplotypes unresolved using the original commercially available Y-STR kits. Additional Y-STR loci, especially from rapidly mutating markers, can be useful for increased discrimination among closely related males; however, caution should be considered in the interpretation of these markers when discrepancies occur among close relatives that could lead to incorrect exclusions based upon a pre-determined number of mismatches (e.g., in missing person cases).



Criminalistics Section - 2015

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Rapidly Mutating, Y-STR, Y-Chromosome



B183 Inferring Bloodstain Age With a Mobile Spectrometer for Forensic Crime Scene Investigation

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After attending this presentation, attendees will better understand the evaluation of aging bloodstains using optical spectrometry to develop an application for a smartphone-like device capable of predicting the age of a bloodstain based on a picture captured with a camera phone. Data obtained from an Ocean Optics® spectrometer was compared to data obtained with a portable mobile spectrometer to determine the mobile device's ability to quantify bloodstain age.

This presentation will impact the forensic science community by providing preliminary data for the development of a tool for crime scene investigators who need field-ready, hand-held forensic solutions to allow them to process, prescreen, triage, and analyze forensic evidence in the field.

The application is based on the principle of reflective spectroscopy. Spectral reflectance of a bloodstain changes over time, as the blood itself undergoes changes in its chemical composition while exposed to the environment. By analyzing how the spectroscopy data changes as blood ages, the data may be characterized in order to create a smartphone application. The smartphone application would be used together with a modular attachment used to record the spectral reflectance and analyze the data to predict the age of the bloodstain. Spectroscopic measurements of bloodstains of varying ages from zero hours to 1,400 hours were taken with two different devices: (1) a commercially available, laboratory-grade spectrometer (Ocean Optics®) using its respective analysis software; and, (2) a smartphone with an attached module that uses the camera to acquire a dispersed optical spectrum. The devices were set up adjacent to one another and measurements of one sample at a time were taken by each device. Samples consisted of bloodstains, approximately 2.5 centimeters in diameter. The samples were collected from a single individual via a lancet prick on a finger (Institutional Review Board-approved procedure). Once the finger was pricked, it was touched directly to a square of white cotton cloth until the 2.5 centimeter diameter was obtained. The samples were then placed, ten at a time, on a rotating platform that moved under the two collecting devices. The platform, accompanied with a Light-Emitting Diode (LED) light source, operated on a timer so that spectra data could be collected on each sample at specific time intervals. A blank white cloth was included to normalize the reflectance data. The platform rotated one sample at a time, paused for 30 seconds at the new location, a measurement was taken by both devices, and rotated once again. At 25 seconds into the pause, the LED light turned on, each device took a measurement, and the light turned off before the platform rotated again. This was done for two complete rotations of each sample set. This allowed each of the ten samples to be measured every five minutes.

Analysis of the data has resulted in the development of a method that allows for the prediction of the age of a bloodstain based on the spectroscopic measurements with an inference error that increases with the age of the stain. Based on prior studies and on data generated in this project, an algorithm was developed that can process data in real-time on a smartphone. Results suggest that the processing algorithms are promising for use in crime scene analysis to distinguish samples of differing age. Preliminary results are based on 318 spectra, for which confidence intervals at 95% or greater were calculated, based on the t-distribution, to determine if samples of varying age could be reliably distinguished when assuming a constant temperature and humidity over the measurement conditions. The following statements can be made with a 95% confidence interval from current data: a bloodstain <1.5 hours old can be identified as younger than a >5.0-hour-old stain; a <5.0-hour-old stain can be identified as younger than a >24-hour-old stain; a stain <24 hours old can be identified as younger than a stain >20 days old. The algorithms are being refined to increase accuracy and to provide greater robustness against variations in temperature and humidity. Accuracy in predicting the absolute age of the bloodstain will be discussed using more data points. Further analysis and development will enable the production of a field-ready smartphone application that can quickly help prioritize sample collection and processing, resulting in increased efficiency at the crime scene.

Bloodstain, Mobile, Spectrometer



B184 Increasing Discrimination of Degraded DNA Using Quantifiler® Trio With the Ion Personal Genome Machine® Sequencer

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After attending this presentation, attendees will learn how Single Nucleotide Polymorphisms (SNPs) and Next Generation Sequencing (NGS) technologies can improve the analysis and identification of degraded DNA samples.

This presentation will impact the forensic science community by presenting a possible complementary solution on the use of NGS in degraded DNA casework analysis. Attendees will observe that the Probability of Identity (PI) values provided by partial STR profiles will be further augmented by PI values from SNPs.

Forensic casework analysis that involves degraded or compromised DNA samples in trace amounts often shows allele dropout at larger molecular weight loci, which results in partial profiles that may have limited or no probative value. It is beneficial to have accurate DNA quantification data to enable forensic analysts to critically evaluate how to select the appropriate downstream STR technologies in order to obtain the most probative results with challenging casework samples. At the threshold that STR kits cannot provide complete profiles anymore, forensically informative SNPs can provide the additional discriminatory power to complement partial or no STR profiles produced by Capillary Electrophoresis (CE). The HID-Ion AmpliSeq™ Panels were developed to provide an alternative and complementary approach to current human identification STR technologies, as well as utilize NGS high-multiplexing capabilities.

The HID-Ion AmpliSeq™ Identity Panel consists of 90 autosomal, of which 85 are unlinked, and 34 upper Y-clade markers. These Identity Informative SNPs (IISNPs) were selected due to their high heterozygosity and low population heterogeneity. Using an input of pristine DNA, the combined 85 unlinked IISNPs provide a random match probability of 10^{-33} in most parts of the world. The 34 upper Y-Clades were selected from the Y-Phylogenetic Tree.¹

The HID-Ion AmpliSeq™ Ancestry Panel consists of 55 Kidd and 123 Seldin markers.^{2,3} These Ancestry Informative SNPs (AISNPs) were selected to infer ancestry from the major eight global regions. These AISNPs are highly supported by Kidd's ALFRED database, which contains a considerable amount of allele frequency variation and population statistics.⁴

This study intends to evaluate data to determine how to proceed with sample processing for degraded DNA samples. Using a human male genomic DNA, PB001, varying degrees of degradation were produced with a combination of both mechanical and enzymatic shearing techniques. Then, utilizing the Quantifiler® Trio DNA Quantification Kit, a Degradation Index (DI) was produced to evaluate the integrity of the degraded PB001. The DI is a ratio of the Small Autosomal (SA) target concentration to the Large Autosomal (LA) target concentration in the stock. The SA locus is 80bp in length while the LA locus is 214bp in length. For four levels of PB001 degradation — none (control), low, medium, and high — the observed DIs were 0.9, 3.4, 37.3, and undefined, respectively. The DI for the highly degraded sample was undefined because there wasn't enough LA.

A correlation was observed between DI and probative value obtained from CE STR and Ion PGM™ System SNP results. Using the GlobalFiler® kit, the PIs for Africans, Asians, Caucasians, and Hispanics for the sample whose DI was 37.3, were 7.14×10^{-3} , 1.01×10^{-2} , 9.201×10^{-3} , and 1.22×10^{-2} , respectively. For the SNP panels for the same sample, the PIs for Global, Asian, Ad-Mixed American, African, and European were 2.21×10^{-14} , 1.89×10^{-13} , 2.41×10^{-14} , 3.42×10^{-13} , and 1.35×10^{-14} , respectively. The control, low, and medially degraded samples did not lose any SNPs. A plot of PI vs. DI was prepared to illustrate the best downstream sample processing for samples identified with varying levels of degradation.

This work demonstrates that the HID-Ion SNPs offer complementary discrimination power to CE STR data for samples that would be considered “poor” quality for STR processing in a casework laboratory based upon their DI value.



Criminalistics Section - 2015

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Degraded DNA, Next Generation SNP Sequencing, Quantification



B185 Single-Step Photobleaching: A PCR-Free Alternative to Typing Low-Copy Number Templates

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After attending this presentation, attendees will better understand photobleaching, a single molecule technique that allows examination of the individual DNA molecules (rather than ensemble measurements) in a sample. In addition, attendees will realize that this strategy is advantageous for samples with too few molecules for Polymerase Chain Reaction (PCR) and that photobleaching experiments are not subject to the stochastic variation that is typical of PCR, particularly when Low-Copy Number (LCN) templates are amplified.

This presentation will impact the forensic science community by educating them on alternative strategies in dealing with LCN templates. Specifically, the goal is to stress that single molecule techniques, such as photobleaching, examine individual DNA molecules in a sample rather than making ensemble measurements as with PCR. In addition, the presentation emphasizes that by attaching genomic DNA to a glass surface, the same individual DNA molecule can be repeatedly measured. Finally, the forensic science community should realize that by using this technique, which is non-destructive, it should be able to recover the sample after performing these experiments, making it available for traditional PCR analysis if need be.

STR typing and next generation sequencing technologies rely upon PCR-based methods for the exponential amplification of DNA markers; however, the PCR process is known to have its limitations with regard to DNA that is degraded or is low in copy number. While the latter results in stochastic variations that are difficult for forensic investigators to interpret, the former can prevent PCR amplification altogether, particularly when targeting larger-sized fragments. Therefore, in this study, these issues are avoided by completely bypassing the PCR process. Instead, well-established single molecule-based approaches, which are sensitive enough and have the resolution (within one nm) to examine individual DNA molecules within forensically challenged samples, are utilized. The single molecule techniques also provide the added benefit of being reproducible, as long as the DNA of interest is anchored to the glass surface.

Specifically, single-step photobleaching was performed, a single molecule method that enables determination of the number of STR repeats at the TPOX locus by directly counting the number of steps in the fluorescence signal as the dye molecules are destroyed over time by exposure to light. The DNA molecules utilized for these experiments were generated by annealing primers and fluorescently labeled eight bp oligonucleotides to various single-stranded DNA templates, consisting of 6, 8, 12, and 16 tetranucleotide repeats flanked by two primer binding regions. The annealed double-stranded DNA construct was ligated using a previously published DNA ligation protocol. Upon completion, the size of each of the ligated DNA templates was confirmed by Capillary Electrophoresis (CE) on a 3700xL genetic analyzer. Following CE, the ligated products were diluted to single molecule concentration (10 to 100 picomolar) and then immobilized onto separate coverslips that were functionalized with poly-L-lysine. Each coverslip was subsequently examined by (TIRF), a popular single molecule technique that illuminates only those molecules present at the water-glass interface, and monitored until all the dyes in the region of interest had been bleached. Using this approach, this study was able to visualize two, three, five, and seven distinct steps when imaging DNA templates consisting of 6, 8, 12, and 16 tetranucleotide repeats, respectively. To confirm these results, the ligation reactions were performed again using eight bp oligonucleotides tagged with five nm gold nanoparticles and the products imaged via Transmission Electron Microscopy (TEM). As with the TIRF approach, the TEM images also revealed clusters of two, three, five, and seven beads using the abovementioned template sequences. Altogether, this data illustrates that the combined ligation/TIRF approach is a promising mechanism for determining the number of STR repeats at a particular locus; however, further work is required to be able to covalently (versus electrostatic interactions) attach the DNA to the surface of the glass. In doing so, this research will be able to examine genomic DNA, melt it, wash off the complementary strand, and repeat the experiment a number of different times for consistency. Although this process will enable generation of a genetic profile in high-profile cases where the DNA evidence is limited or compromised, the resolution will be lower than with traditional STR typing technologies. Additionally, it is hoped that by refining the attachment strategies the genomic DNA will be recoverable from the coverslip, making it available for traditional PCR analysis if so desired.



Criminalistics Section - 2015

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PCR-Free, Low-Copy Number, Single Molecule



B186 Development of a Novel DNA Phenotyping System Using Genome-Wide SNP Data

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After attending this presentation, attendees will: (1) learn the differences between traditional DNA analysis using Short Tandem Repeats (STRs) and modern DNA genotyping using Single Nucleotide Polymorphisms (SNPs); (2) understand how the genetic content of DNA encodes the observed variations in human appearance; (3) gain an appreciation for how these genetic variants can be used to predict traits (phenotypes); and, (4) learn about a new methodology for producing predictive models for forensic traits from genome-wide SNP data.

This presentation will impact the forensic science community by presenting a novel methodology for the discovery of significant SNPs for forensic traits and the development of those SNPs into predictive models that can be used for DNA phenotyping or kinship analysis.

Traditional forensic DNA analysis uses STRs to match an individual to a DNA sample by testing crime scene DNA against known suspects or a DNA database, such as the Combined DNA Index System. When this fails to yield a match, alternate approaches are required to generate leads from a DNA sample. DNA phenotyping refers to the use of SNPs, DNA variants that code for differences between individuals, to predict an individual's appearance based only on his or her DNA. Prior work on DNA phenotyping has been limited to using only a few SNPs which have been identified in the literature as being individually significant for prediction of eye and hair color and ~100 SNPs to calculate large-scale ancestry (i.e., African, European, or East Asian). Presented here are the results of work to build a novel DNA phenotyping system, developed under funding from the United States Department of Defense, that employs thousands of SNPs for the prediction of complex traits, resulting in improved prediction accuracy over a broader phenotypic range. This system can be applied to individuals from any ethnic background, even admixed individuals.

The predictive models were built using genome-wide genotype data from more than 2,500 subjects for eye color and hair color and from more than 500 subjects for skin color and freckling. Each subject's proportional ancestry in seven global populations (Africa, Middle East, Europe, Central Asia, East Asia, Oceania, and America) was calculated by comparing more than 20,000 SNPs, carefully selected from across the genome, against a set of more than 2,200 background subjects from known populations. This approach can detect even low levels of admixture. Within-continent ancestry (e.g., Northeast vs. Northwest Europe) and the principal components of ancestry were also inferred. These values were then used as covariates for the discovery of significant SNPs associated with each forensically relevant pigmentation phenotype. SNP discovery was performed using advanced data mining techniques that search not only for SNPs that individually contribute to phenotype, but also those that interact in a non-additive (epistatic) fashion. This was achieved using a custom distributed implementation of the Multifactor Dimensionality Reduction (MDR) algorithm.

Using the SNPs discovered during data mining as well as ancestry SNPs, predictive models were constructed using advanced machine-learning algorithms. These techniques allow non-linear variable combinations and are not negatively impacted by the inclusion of extraneous variables. The entire mining and modeling process was performed within a ten-fold cross-validation framework to allow all of the data to be used to build a model while still allowing for accuracy evaluation using out-of-sample predictions. A statistical procedure for evaluating confidence has been developed, which calculates a consistency value for each new prediction for each possible category (e.g., red, blond, brown, and black hair color). Each prediction is presented with a measure of confidence as well as a list of trait categories that can be excluded with very high confidence. DNA from an unknown subject can be run through these predictive models to produce a physical profile. Blind validation testing has been performed for ancestry, eye color, hair color, and skin color on 24 subjects (Table 1) from a range of ethnic backgrounds (European, African-American, Central Asian, and Middle Eastern). The final system has been successfully tested using as little as two ng of extracted DNA.

Table 1: Results of blind validation testing. For each trait, the average consistency for the absolute correct category and the frequency at which the absolute correct category was found to have the highest consistency (two very conservative estimates of accuracy) are reported.

Trait	Average Consistency for Absolute Correct Category	Frequency of Highest Consistency for Absolute Correct Category
Eye Color	76.1%	84.8%
Hair Color	67.7%	95.7%
Skin Color	56.0%	82.6%



Criminalistics Section - 2015

A method to determine the degree of relatedness between two subjects using genome-wide SNP data was also developed. This model uses hundreds of thousands of SNPs to ascertain the precise level of similarity between two individuals' genomes. This method has >90% accuracy up to third-degree relatives and can distinguish up to sixth-degree relatives from unrelated pairs with >95% accuracy. Validation results for both the DNA phenotype and kinship inference systems will be presented, along with plans for future enhancement of the underlying processes and software.

SNP, DNA Phenotyping, Kinship Inference



B187 Use of Likelihood Ratios to Unlock Information in Various Short Tandem Repeat (STR) Kits' Threshold Validation Data Sets With the Use of Lab Retriever

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After attending this presentation, attendees will better understand using likelihood ratio software with their own internal validation data sets to gain new knowledge about genotyping systems as well as the effects that genetic analyzer injection time has on the likelihood ratio.

This presentation will impact the forensic science community by increasing the understanding of likelihood ratios and by explaining how increasing injection time affects the amount of information gathered and the probability of drop-out.

Internal validations of PowerPlex® 16 HS, PowerPlex® Fusion, and Identifiler® Plus amplification kits each produced large data sets containing five identical amplification replicates, with each sample injected at one, five, and ten seconds on a genetic analyzer. These validation data sets were utilized to obtain threshold values for the laboratory's interpretation guidelines. An empirical analytical threshold was derived by examining multiple negative controls for the highest non-artifactual peak, then multiplying the height of that peak by two. Lab Retriever, a freely available software program for the calculation of likelihood ratios, was used to calculate likelihood ratios of the validation sensitivity and mixture samples. This software program incorporates a Probability of Drop-Out, $P(Do)$, via an easy-to-use interface. Although a default $P(Do)$ calculator is readily available with the Lab Retriever software, it was desirable to determine $P(Do)$ calculators specific to each amplification kit and injection time for use in the laboratory, based on the laboratory's existing data. Using the validation data sets generated, along with calculations computed with Logic Drop, a logistic regression tool created in R-code, a system-specific algorithm was generated. This information was formatted into a user-friendly calculator, which was used to determine the $P(Do)$ for each sample in this study, based on the kit in which the sample was amplified and the injection time of the sample. The table below outlines values obtained from Logic Drop for the $P(Do)$ calculator for each kit studied:

	PowerPlex® 16 HS			AmpFISTR® Identifiler® Plus			PowerPlex® Fusion		
	1 Sec	5 Sec	10 Sec	1 Sec	5 Sec	10 Sec	1 Sec	5 Sec	10 Sec
Y-Intercept	7.2946	4.2663	3.0564	5.4327	4.2269	4.0238	7.0957	1.9859	0.9999
Slope	-0.071	-0.036	-0.026	-0.067	-0.049	-0.039	-0.104	-0.026	-0.016

In addition, $P(Do)$ was calculated by hand by dividing the number of alleles observed by the number of alleles expected and was compared to the $P(Do)$ calculated based on Logic Drop values. Likelihood ratios for single-source samples, two-person mixtures, and three-person mixtures were calculated for each race (Caucasian, African American, and Hispanic) and injection time (one, five, and ten seconds) to determine if there was any statistical difference between them. An analysis of variance was calculated from that data that indicates that increasing injection time does not provide a statistically significant increase in information as is commonly believed. Significant differences were only observed between one-second and ten-second injection times for an input DNA concentration of 250pg using the AmpFISTR® Identifiler® Plus typing kit. This information begs the question of whether increasing the injection time with the hopes of increasing the information in the sample will be beneficial.

Likelihood Ratio, Lab Retriever, Probability of Drop-Out



B188 Fabric Phase Sorptive Extraction Media: A Highly Effective Forensic Sample Collection and Storage Device

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After attending this presentation, attendees will understand the working principle, advantages, and potential applications of the recently developed sample preparation technique known as Fabric Phase Sorptive Extraction (FPSE) in preparing forensic samples for instrumental analysis. The preparation capabilities of FPSE include extraction as well as storage.

This presentation will impact the forensic science community by proving that FPSE media can be used for the extraction of samples of forensic interest. Use of FPSE also reduces the collection steps involved in performing an extraction, therefore reducing the expensive labor and supply costs associated with collecting the sample from the field/crime scene and transporting it to the analytical laboratory before instrumental analysis begins.

FPSE has been shown to be a simple and more effective alternative to traditional, commercially available extraction techniques for preparing forensic samples for instrumental analysis and highly effective in extracting illicit drugs, nitroaromatic explosives, and other trace organic compounds of forensic significance directly from biological/environmental samples. The current study illustrates the application of FPSE in extracting and retaining eight compounds of interest from water. These compounds are: 3,4- and 3,5-dimethylphenol (compound class: phenols); Diphenylamine (DPA) and 2-Nitrodiphenylamine (NDPA) (compound class: amines); benzophenone and t-chalcone (compound class: ketones); and, phenanthrene and anthracene (compound class: Polycyclic Aromatic Hydrocarbons (PAHs)).

The two selected PAHs were included by the United States Environmental Protection Agency (U.S. EPA) in their Priority Pollutants List because of their carcinogenic properties. 3,4-dimethylphenol, 3,5-dimethylphenol, NDPA, and DPA are toxic to humans and wildlife which makes them relevant in the fields of environmental and criminal forensics. Benzophenone derivatives, such as benzophenone-3 which is commonly used in sunscreen products, have been found to be toxic and present serious ecological risks. Benzophenone and t-chalcone were chosen to represent ketones, which is a prevalent functional group in chemistry. Various methodologies have been developed and published over the years for sample preparation for the chemical analysis of these compounds. These methodologies have been based on techniques including, but not limited to, Solid Phase Extraction (SPE), Liquid-Liquid Extraction (LLE), Solid-Phase Microextraction (SPME), and Stir Bar Sorptive Extraction (SBSE).

Methods involving sample preparation by FPSE were developed for these eight compounds with each class treated separately because of the wide range of chemical properties within this group. The effects of extraction and desorption parameters such as extraction volume, extraction time, ionic strength, stirring rate, desorption time, and desorption solvent system on the extraction/desorption efficiency were investigated and optimized. FPSE was coupled to high-performance liquid chromatography with ultraviolet detection. The developed method was used for the determination of analytical merits: intra-day and inter-day repeatability, linearity, limit of detection and limit of quantitation. This method was also applied to extracting these compounds from spiked samples of water from a local pond and from reclaimed water. The optimized and validated FPSE methods were combined for extractions of the aforementioned group of eight compounds. Once these were extracted, the FPSE media were stored under refrigeration and desorption was done more than a month later with no loss of analytes.

FPSE addresses some important shortcomings of conventional extraction and microextraction techniques. For instance, extractions from pond and reclaimed water were done without any sample pretreatment such as filtration or centrifugation of the sample prior to extraction. FPSE utilizes a small flexible media coated with sol-gel hybrid organic-inorganic polymeric material as the extraction sorbent. The sol-gel coating process results in a highly porous polymeric network chemically bonded to the substrate surface. The hybrid material inherently possesses high thermal, chemical, and mechanical resistance as well as high specific surface area. Comparing to the primary contact surface area of 1,000mm² for FPSE vs. 100mm² for SBSE vs. 20mm² for SPME, FPSE is superior which translates into greater extraction efficiency of either of these techniques. The high primary contact surface area of FPSE media increases the probability of the successful sorbent-analyte interaction for an effective analyte extraction, resulting in fast extraction equilibria along with high preconcentration factor.

Retentive Characteristics, FPSE, Forensic Evidence

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B189 Investigating the Use of Illicit Drug Smoke Aerosol Residues as Recoverable Trace Evidence

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After attending this presentation, attendees will understand how aerosol residues may provide a valuable form of forensic evidence in drug-related cases that is currently unused.

This presentation will impact the forensic science community by providing the first results of examining third-hand smoke aerosol residues from drug substances other than tobacco cigarettes. This presentation will add to criminalistics and trace evidence research by exploring the potential for expanding the types of drug evidence recovered from scenes when the contraband is not present.

Smoke aerosol residues, or third-hand smoke, from tobacco cigarettes has been highlighted in recent years as a silent and significant threat to the health and safety of indoor environments. These residues are the remnants of second-hand smoke that are deposited onto surfaces such as clothing, car interiors, countertops, tables, and chairs. Studies have demonstrated that these smoke residues and their degradation products on various interior surfaces can be collected and identified in areas of habitual smoking. Where these findings are especially important for health concerns, the studies performed with nicotine and other cigarette smoke alkaloids lend themselves to an untapped source of trace forensic evidence.

In this experiment, three commonly smoked illicit drugs were chosen and examined for their potential as recoverable smoke aerosols from idealized surfaces. Cocaine, heroin, and methamphetamine were made into useable standard solutions and a known mass was deposited onto a substrate and dried for 24 hours. These substrates were heated at various temperatures to volatilize the drug into a vapor and the resulting aerosol was collected either passively or under the influence of suctioning air flow onto a silicon wafer collector surface. The size of the aerosol particles was investigated using particle measurement instruments and the collection substrates were analyzed via electron microscopy to examine the physical state of the aerosol particles. Chemical analysis was performed using high-performance liquid chromatography, electrospray ionization/mass spectrometry, and direct analysis in real-time mass spectrometry to investigate the extent of pyrolytic degradation of each drug as a function of temperature. Experiments were also performed by introducing adulterant or substances in combination with the illicit drug to understand how impure street samples may undergo different volatilization processes.

Particle size measurements showed that greater than 90% of the aerosols generated were 1 μ m or less in size for both illicit and adulterant drugs examined. A trend toward smaller particle sizes was also seen with increasing temperature; however, a slightly larger average size was seen when an illicit drug was mixed with an adulterant. The increase in temperature also increased the concentration of degradation products formed from pyrolysis of the drugs, beginning around 400°C for most drugs examined.

This study examines the first steps toward the recovery of useful forensic evidence in drug-related offenses without having to have a drug sample, or even visible drug particles, present. Future work will expand on the current study pertaining to the illicit drugs studied and various combinations as well as recovery from surfaces encountered in buildings and vehicles and environmental effects.

Illicit Drugs, Residue, Aerosol



B190 Analysis of Phenethylamine Street Drugs for Psychoactive Compounds and Impurities

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After attending this presentation, attendees will understand details concerning the challenges of separation and analysis of the various phenethylamine compounds and how the analysis of the reference materials differs from what is observed in actual street samples. Separation utilizing Ultra-High Performance Liquid Chromatography, coupled with Time-Of-Flight/Mass Spectrometry (UHPLC-TOF/MS) will be demonstrated along with a direct sample analysis approach utilizing Direct Sample Analysis (DSA)-TOF/MS.

This presentation will impact the forensic science community by discussing how discovering the impurities within these compounds and raising awareness of the dangers of consuming drugs from incompetent synthesis may prevent future overdoses and health complications and diminish the market for unsafe illegal drugs.

The purpose of this study is to determine not only the identity of the psychoactive compound(s) and their concentrations in the various street samples but also to determine impurities which may exist from less-than-ideal synthetic procedures likely employed by potential users/manufacturers of “2C-type” drugs. Serious health complications and fatal overdoses have brought phenethylamine designer drug use to the public’s attention. The phenethylamine compounds alone are not believed to be causing the health complications but rather the cause may be impurities within the sample. These impurities may result from the improper technique and inadequate equipment used during illegal synthesis. The substituents on these emerging drugs are constantly changed in order to avoid legal ramifications; however, many of these compounds are Schedule I drugs and, therefore, their analogs are also illegal according to the Federal Analog Act. These compounds are 2C-X-series analogs of mescaline. The name “2C” results from the two carbons in the ethyl chain. These synthetic drugs are marketed as having affects similar to LSD and are typically consumed sublingually via blotter paper. The compounds have psychedelic affects on the 5HT^{2A} receptor in the brain. The compounds have a variety of street names including “N-BOMB,” “Smiles,” and “Bromo-DragonFLY.”

The objective of this research is to qualitatively and quantitatively identify the drugs and potential impurities. Street samples were compared to known standards in order to determine if impurities exist that may be resulting in health complications. The analysis was done using a variety of UHPLC instruments. Liquid chromatography was utilized rather than gas chromatography because the 2C compounds are considerably more reactive than many other recreational drugs. Analysis by gas chromatography has proven troublesome, at best. Additionally, using gas chromatography would require derivatization, which generally utilizes aprotic solvents, which do not allow for dissolution of the 2C compounds. Following UHPLC separation, TOF/MS was employed for compound identification and subsequent quantification. Finally, a separate sample introduction technique (DSA) was coupled with TOF/MS, thus providing various methods of analysis and identification of the targeted drugs and impurities, which will all be compared and contrasted in this presentation.

MS/MS spectra were used to determine the fragmentation patterns; these fragmentation patterns were observed for 28 standards. The first fragment for every 2C compound was a loss of 17amu, representing the loss of the -NH₂ substituent. Compounds belonging to the NBOMe subgroup fragmented into a 121 peak. Halogenated compounds displayed the loss of a halogen group. The mass spectrum for every 2C compound includes a peak at 77m/z, which represents the phenyl ion, and a peak at 91m/z, which represents the tropylium ion. While all the MS/MS spectra for the compounds were similar in fragmentation patterns, they displayed differences that allow the analyst to distinguish which compound is present. Using a high-resolution MS instrument enables an analyst to determine the molecular formula of unknown compounds. The mass spectra generated after UHPLC separation and DSA of a blotter paper street sample were compared with the spectra of the 19 standards. Upon comparison, 25C NBOMe and 25B NBOMe were identified on the blotter paper. This identification was confirmed by comparing mass spectra fragmentation patterns and isotope ratios for chlorine and bromine from the street sample and the standards. Discovering the impurities within these compounds and raising awareness of the dangers of consuming drugs from incompetent synthesis may prevent future overdoses and health complications and diminish the market for unsafe illegal drugs. Because these drugs are emerging substances of abuse, there are no accepted protocols for analysis. DSA allows for a quick screening of seized compounds that can then be analyzed and identified using UHPLC-TOF/MS.

2C, Phenethylamines, DSA-TOF/MS



B191 Rapid Drug Identification in the Field Using Direct Analysis in Real Time (DART®) and a Portable Ion Trap Mass Spectrometer

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After attending this presentation, attendees will understand how a portable ion trap mass spectrometer coupled with DART® can be used for definitive drug analysis in the field. Attendees will learn the capabilities of the instrument, including how the use of multiple-stage tandem mass spectrometry (MS) can be used to differentiate closely related drugs.

This presentation will impact the forensic science community by demonstrating the DART® coupled to a portable ion trap mass spectrometer, a new tool that can provide rapid, accurate analysis of established and newer designer drugs in the field.

Identification and differentiation of the consistently evolving designer drugs being encountered by law enforcement is challenging established techniques in drug analysis. Color tests have been used as reliable presumptive field tests for some time but many newer phenylethylamine derivatives, cathinones, and synthetic cannabinoids yield false negatives when tested with these reagents. This is a particular challenge for law enforcement officials who need rapid characterization of substances purchased in undercover drug buys to validate that the substance is actually a controlled substance. Hand-held Raman spectrophotometers have become commercially available to address this issue and are a good replacement for presumptive color tests; however, they are not specific enough for definitive testing. The development of ambient ionization techniques for MS, including DART® ionization, have made it feasible to obtain mass spectra directly from drug evidence. Steiner and Larson validated the use of DART® coupled with a time-of-flight MS for reliable drug screening in the laboratory.¹ For the purpose of this research, DART® has been coupled with a portable ion trap MS to yield an instrument that can be used for definitive identification of drugs, without sample preparation, in the field.

Initial validation experiments were done in a laboratory setting using pure drug standards. The IonSense® DART® source was coupled with a MassTech® MT Explorer 50 ion trap MS, a self-contained instrument weighing 75 lbs. with dimensions of 12"x17"x20". Numerous drug standards were tested using both a compressed cylinder of nitrogen gas and an air compressor as the gas supply for the DART® source. Using nitrogen as the supply gas, the expected mass spectra and tandem mass spectra were obtained for all drugs tested. Air could be used for analysis of most compounds; however, some drugs, including methamphetamine, did not yield significant signal when air was used. Ozone, formed from the exposure of oxygen to the DART® plasma, was detected around the DART® source when the air compressor was used as the supply. It is likely that drugs that are significantly reactive with ozone were not detected because of their reaction with ozone.

After establishing a preliminary library of mass spectra and tandem mass spectra using drug standards, the instrumentation and a small canister of compressed nitrogen gas were transported to the Osceola County Sheriff's evidence room. The instrument was set up in ~15 minutes and was ready to function after vacuum was established (approximately 30 minutes). Numerous pieces of evidence including a cannabis leaf, "K2," cocaine, heroin, methamphetamine, an oxycodone tablet, and an alprazolam tablet were directly analyzed, yielding accurate results in real-time.

Current work is focused on studying closely related compounds to fully assess the specificity of this technique. The portable ion trap is lower resolution than the time-of-flight MSs previously used for drug analysis with DART® and cannot be used for accurate mass analysis. Additionally, accurate mass analysis cannot be used to differentiate isomers; however, ion traps can perform multi-stage tandem mass spectrometry and all the drugs tested were distinguished using MS2 spectra, except two closely related isomers, 5-APDB and 6-APDB. Currently, MS3 spectra are being acquired to determine further test the specificity of this instrumentation. Several compounds that are isomeric or isobaric with controlled substances and are also expected to yield similar MS2 spectra are being tested to see if for compounds with identical mass spectra (MS1 spectra) and similar MS2 spectra, the MS3 spectra will be sufficiently unique to differentiate them.

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1. Steiner, Robert R. and Larson, Robyn L. Validation of Direct Analysis in Real Time Source for Use in Forensic Drug Screening. *J. Forensic Sci.* 2009: 54: 617-622

Mass Spectrometry, Drug Analysis, At Crime Scene

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B192 Forensic Analysis of Dipyrone in Fentanyl Samples

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The goal of this presentation is to provide attendees with relevant information about a newly emerging trend of fentanyl samples encountered by the Drug Enforcement Administration (DEA). Attendees will also gain an insight of commonly encountered adulterants/cutting agents and the methodologies required for conclusive identification. These findings and methodologies may impact individuals in both the forensic science and toxicology fields.

This presentation will impact the forensic science community by highlighting some of the trends that are seen with fentanyl samples in the DEA Northeast Laboratory.

Fentanyl, N-phenyl-N-[1-(2-phenylethyl)-4-piperidinyl]-propanamide, is a synthetic opioid commonly used in anesthesia both as a pre-anesthetic and postoperatively to control pain.¹ It has an analgesic potency of approximately 100 times that of morphine and 50 times that of heroin.² Due to its potential for abuse, it is a Schedule II drug under the Controlled Substances Act. Illicit drug dealers often encounter difficulty when utilizing fentanyl as an adulterant due to its extremely high potency compared to other cutting agents. In 2013, the DEA Northeast Laboratory experienced a resurgence of heroin exhibits adulterated with fentanyl. This potent combination of dangerous drugs was attributed to an increase in overdose fatalities throughout the northeast region.

In an effort to maximize the effects of the drug, illicit dealers add additional adulterants which have synergistic effects in combination with fentanyl samples. Dipyrone, a methanesulfonic acid sodium salt of aminopyrine, is a relatively new adulterant that has been commonly seen in fentanyl cases in the northeast region. On its own, dipyrone exhibits analgesic effects on the body and is also commonly used to control pain; however, due to its potential fatal side effects, it has been withdrawn from medical use in the United States. Forensic analysis of dipyrone utilizing conventional methodologies such as gas chromatography/mass spectrometry and gas chromatography/flame ionization detector may yield a false positive for the presence of aminopyrine. Electrospray Ionization-Liquid Chromatography/Mass Spectrometry (ESI-LC/MS) has shown to have high discriminatory power and the required sensitivity for the conclusive identification of dipyrone and other adulterants in illicit fentanyl exhibits.

In this study, two ESI-LC/MS methods will be presented for routine confirmation of dipyrone in fentanyl-seized samples. Preliminary results showed that dipyrone and aminopyrine can be confirmed in positive mode ESI based on C18 chromatographic separation and unique fragmentation for each compound. The results also revealed that positive mode ESI will not detect the pseudomolecular ion for dipyrone but will for aminopyrine. In order to detect the pseudomolecular ion, one must employ negative mode ESI-LC/MS. Both methods employed the use of a C18 stationary phase and a quaternary pump.

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Dipyrone, Fentanyl, LC/MS



B193 The Utility of Ultra High-Performance Liquid Chromatography With Time-of-Flight Detection for the Identification of Synthetic Cannabinoids: Part I — The Role of the Separation Technique

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After attending this presentation, attendees will understand some principles of Ultra High-Performance Liquid Chromatography (UHPLC), in particular how to develop a separation for a complex mixture. In addition, attendees will gain insights into the differences between UHPLC and capillary Gas Chromatography (GC) for the separation of synthetic cannabinoids, including structural isomers.

This presentation will impact the forensic science community by clarifying the role of UHPLC in aiding in the identification of highly similar solutes, such as synthetic cannabinoids, that are present in emerging drugs.

Separation techniques are an integral part of forensic drug analysis toolbox for both screening and confirmation purposes. The Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG), which is responsible for setting standards for drug analysis, requires a category A test such as Mass Spectrometry (MS) with an additional test from either category B or C. If a category A method is not used, two uncorrelated techniques from category B and one from category C must be included, from which separation techniques such as capillary electrophoresis, gas chromatography, liquid chromatography, and thin layer chromatography would qualify. In addition, even if a technique such as GC/MS is used, an additional identification test is highly desirable for increased confidence of analysis and quality assurance purposes. Methodology capable of resolving a host of similar compounds is required, especially in the case of emerging drugs. In this vein, UHPLC offers relatively high resolving power and is well suited for this purpose. Besides the ability to separate controlled substances of a particular class of seized drugs, such as the synthetic cannabinoids, the utility of UHPLC depends on the capability of distinguishing between structural isomers and certain stereoisomers. The latter is particularly important since the controlled substances can have both controlled and non-controlled isomers. Distinguishing compounds based on retention time becomes particularly important when the solutes have identical mass spectra. UHPLC-Time Of Flight (TOF)/MS and GC/MS separations are compared for 23 out of the 25 controlled synthetic cannabinoids. For UHPLC reversed phase chromatography with three 2.7 μ m 2.1x 150mm columns containing superficially porous stationary phases, including C18, Phenyl-Hexyl, and pentafluorophenylpropyl (PFP) are used, while for capillary GC a 0.25 μ m 0.25mm x 30m Elite-5MS (equivalent to DB-5, HP-5) capillary column is employed. Superficially porous particle columns (i.e., 2.7 μ m particles) offer UHPLC performance with significantly lower back pressure than fully porous \leq 2 μ m particle columns. For UHPLC "optimum" isocratic or gradient separations are obtained with 0.1% formic acid as the base solvent by varying the amount of acetonitrile or methanol, changing the time of the gradient and varying the temperature. For a gradient separation using a Phenyl-Hexyl column, 19 out of 23 solutes are resolved (resolution \geq 1), including two pairs of diastereomers (CP47, 497 and epi CP47, 497; CP47, 497-C8 and epi CP47, 497-C8) and a pair of structural isomers (JWH-019 and JWH-122) employing acetonitrile as the strong solvent at 35°C. The unresolved solutes are resolved using alternative chromatographic systems, employing either GC or a UHPLC (C18 or PFP stationary phase), which separate 15, 17, and 14 out of the 23 controlled synthetic cannabinoids, respectively.

For the separation of a controlled synthetic cannabinoid JWH-018 and nine of its structural isomers, using the same chromatographic conditions for the 23 solutes above, capillary GC, and the three UHPLC stationary phases Phenyl-Hexyl, C18 and PFP resolved 4, 0, 3, and 3 compounds, respectively. For the four systems investigated, only UHPLC with the PFP stationary phase resolved JWH-018 from the other structural isomers

The degree of orthogonality of the various chromatographic systems is demonstrated using multivariate analysis.

Synthetic Cannabinoids, UHPLC, SWGDRUG



B194 The Utility of Ultra High-Performance Liquid Chromatography with Time-of-Flight Detection for the Identification of Synthetic Cannabinoids: Part II — The Role of the Detection Technique

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After attending the presentation, attendees will understand the advantages and disadvantages of using a Time-Of-Flight (TOF) high resolution mass spectrometer as detector following separation by Ultra High-Performance Liquid Chromatography (UHPLC) as compared to the traditional capillary Gas Chromatography-Quadrupole Mass Spectrometry (GC/MS). Attendees will also be exposed to the differences between classical electron-impact mass spectra and those of electrospray ionization mass spectra with and without in-source fragmentation.

The presentation will impact the forensic science community by further exploring the role of high resolution mass spectrometry in the identification of forensic drugs. Examples will include analyses of emerging drugs such as synthetic cannabinoids and some of their isomers.

The Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) classified mass spectrometry in the category A (highest discriminating power), with the exception of techniques that only produce molecular weight information. The SWGDRUG recommendations do not specifically address the issue of high-resolution mass spectrometry, as it is addressed by other similar groups (e.g., the recommendations of Scientific Working Group for Forensic Toxicology (SWGTOX)). This research tackles the role of high-resolution mass spectrometry in the context of the analysis of synthetic cannabinoids.

Currently, there are 25 synthetic cannabinoids on the list of controlled substances by the Drug Enforcement Administration and more synthetic cannabinoids are likely to become controlled in the future. A mixture of 23 controlled synthetic cannabinoids was subjected to analyses by GC/MS using a 0.25 μ m 0.25mm x 30m Elite-5MS capillary column (equivalent to DB-5, HP-5) and UHPLC-TOF/MS with three 2.1 x 150mm columns packed with 2.7 μ m superficially porous stationary phases (C18, Phenyl-Hexyl, and pentafluorophenylpropyl (PFP)). The analyses were also performed on a mixture of ten positional isomers of JWH018 to assess their identification in potentially complex mixtures.

The mass spectra resulted from the GC/MS experiments showed a high degree of specificity with the exception of diastereoisomers (CP47, 497 and epi CP47, 497; CP47, 497-C8 and epi CP47, 497-C8); however, all chromatographic systems were able to separate these diastereoisomers. The ten positional isomers of JWH018 were also easily distinguishable based on their mass spectra.

The masses measured by UHPLC-TOF/MS experiments were generally within a few ppm of the accurate mass of the synthetic cannabinoids. Measurement of the accurate mass can bring an increased level of confidence to a forensic analysis. Mass spectra using in-source fragmentation brought additional information regarding the analytes; however, the fragmentation pattern did not have the degree of specificity observed in GC/MS experiments. Especially in the case of JWH018 isomers, where the difference between compounds was mostly limited to a side alkyl chain, the mass spectra did not include specific peaks allowing differentiation between compounds. Although all solutes could be uniquely identified by GC/MS, UHPLC-TOF/MS provides a highly orthogonal technique that greatly enhances the confidence in the analysis.

Synthetic Cannabinoids, High Resolution MS, Forensic Drugs



B195 High-Sensitivity Drug Analysis With Optical Isomer Resolution Using Mixed Chiral Selectors

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The goal of this presentation is to discuss the application of mixed chiral selectors to the separation of drugs and their optical isomers using highly sensitive Capillary Electrophoresis/Mass Spectrometry (CE/MS) in approximately 20 minutes.

This presentation will impact the forensic science community by describing how to use this rapid and highly sensitive method to separate and identify isomers of commonly encountered drugs of abuse such as amphetamine and methamphetamine.

The separation of drug isomers is essential since scheduling and sentencing could vary based on which isomer of a compound is present in a sample. Several techniques are currently used for the separation and detection of optical isomers including Gas Chromatography/Mass Spectrometry (GC/MS), Capillary Electrophoresis/Ultraviolet (CE/UV), and Liquid Chromatography/Mass Spectrometry (LC-MS). The drawbacks of these techniques are the use of expensive chiral columns (GC/MS and LC/MS), derivatization of the sample (GC/MS), and low sensitivity and specificity (CE/UV). To address these deficiencies, a highly sensitive CE/MS technique has been developed for the separation and detection of drugs and their optical isomers in about 20 minutes using only 1 nanoliter of the solution. The technique is faster and is almost 1,000x more sensitive than current GC/MS and LC/MS techniques. In addition, it provides base-line separation of the optical and positional isomer in one run. The superior properties of this technique are due to three unique characteristics of this design: (1) the new CE/MS uses a porous tip for interfacing CE to MS. A porous tip allows narrow capillaries (<20 μ m-i.d.) to be interfaced to MS allowing maximum sensitivity under electrospray ionization without introducing any dead volume and consuming only one nanoliter of the sample solution; (2) the use of 18-crown-6 as a complexation reagent in the CE background electrolyte — the sensitivity of the amine-containing compounds are enhanced due to high ionization efficiency of the complexes; and, (3) the addition of the chiral selector (+)18-crown-6-tetracarboxylic acid not only allows for higher sensitivity but also separation of the optical isomers of compounds containing primary amines; however, the optical isomers of secondary amines such as methamphetamine were not baseline separated. To separate all amines, (+)18-C-6-TCA was mixed with several types of cyclodextrins (α -, β -, γ -cyclodextrin) to examine the optimum background electrolyte for CE/MS analysis drugs and their optical isomers. Using a mixture of (+)18-C-6-TCA and β -cyclodextrin was found to baseline separate (\pm)-amphetamine and (\pm)-methamphetamine mixture in less than 20 minutes. To speed up the analysis, this recently developed ultrafast CE-MS is being applied to the analysis of drugs and their positional and optical isomers in approximately 60 seconds.

Using a background electrolyte containing both 18-C-6-TCA and cyclodextrin provides enhanced separation and sensitivity over a background electrolyte of the individual chiral selectors.

Drug, Chiral Separation, CE/MS



B196 Determination of the Stoichiometry in the Modified Ferric Hydroxamate Test for Gamma-Hydroxybutyric Acid (GHB)

*Thomas A. Brettell, PhD**, Cedar Crest College, 100 College Drive, Allentown, PA 18104; and *Karen Lau*, 1689 32nd Street, SW, Allentown, PA

After attending this presentation, attendees will understand how the method of continuous variation and mole ratio method, utilizing Ultraviolet/Visible (UV/Vis) spectrophotometry, can be used for the determination of the stoichiometry of the purple-colored metal-ligand complex formed in the modified hydroxamate test for GHB.

This presentation will impact the forensic science community by providing an understanding of the mechanism and final structure of the complex that forms in the modified ferric hydroxamate test for GHB.

A rapid color test was developed to detect GHB in human urine in 2002. The test involves converting GHB to Gamma-Butyrolactone (GBL) with acid followed by a ferric hydroxamate reaction with a monohydroxamic acid intermediate. A purple-colored complex with a 3:1 stoichiometry of drug ligand to iron metal was proposed and formed at pH 3; however, results of multiple, previous monohydroxamic acid studies contradicted that finding. Two of the previous studies found a 1:1 stoichiometry between a monohydroxamic acid ligand and iron metal ion and that the complex forms the most intense color at approximately 510nm below a pH of 2. The goal of this study was to further investigate the modified ferric hydroxamate test for GHB.

The methods of continuous variation and mole ratio were used to determine the stoichiometry of the purple complex formed in the modified ferric hydroxamate test for GHB. GBL was used as the starting material for research purposes. The monohydroxamate acid intermediate was formed after the addition of 0.5M hydroxylamine hydrochloride in 95% ethanol and 6M sodium hydroxide. The purple complex formed after the addition of iron (III) chloride and an adjustment of pH using 6M hydrochloric acid. UV/Vis spectrophotometry was used for the analysis and absorbance values were collected at 500nm (I_{MAX} of the complex). Studies of pH were performed by adding 0.2 mL and 0.1mL increments of hydrochloric and sulfuric acids to observe at what pH the complex is formed. Concentration studies were also performed to determine figures of merit including Limit Of Detection (LOD), Limit Of Quantitation (LOQ), Linear Dynamic Range (LDR), and molar absorptivity of the complex (ϵ). These studies were also repeated using lactones of different ring sizes, Beta-Butyrolactone (BBL) and Delta-Valerolactone (DVL). The effect of ionic strength by the addition of sodium chloride was also studied. Lastly, the modified ferric hydroxamate test was analyzed using GHB as the starting material.

It was determined that the purple complex formed in the modified ferric hydroxamate test had a 1:1 stoichiometry of drug ligand to iron metal. This stoichiometry was consistent with GBL, BBL, and DVL. The results of the pH study indicated that the complexes do not form until the pH was below 2. This result was achieved using two different acids with two different concentrations. Results from a concentration study for GBL indicated a LOD of $7.7 \times 10^{-4}M$, LOQ of $1.4 \times 10^{-3} M$, LDR of 1.4×10^{-3} - $5.0 \times 10^{-2}M$, and ϵ of 352.2 Liters/mol \times cm. The ionic strength studies indicated that the decrease in pH using an acid, not just the addition of chloride ions, was crucial to the formation of the purple complex. Preliminary results from the application of GHB to the modified ferric hydroxamate test indicated that it might not be necessary to add acid for the last step if enough acid was used to convert a sample containing GHB to GBL.

It is important for forensic chemists to understand the chemistry behind this color test because it reinforces validity of their results. Furthering the research behind the modified ferric hydroxamate reaction for GHB may allow for its future use in crime laboratories.

Stoichiometry, GHB, GBL



B197 An Analysis of Elemental Content in Various Brands of Cigarette Ash by Atomic Absorption Spectroscopy

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After attending this presentation, attendees will understand the value of elemental analysis of cigarette ash when cigarette butts yield no DNA profiles. Attendees will learn that atomic absorption spectroscopy, a less sensitive but less costly instrument, can provide results consistent with those obtained using inductively coupled plasma methods.

This presentation will impact the forensic science community by showing that the analysis of cigarette ash can provide additional information for use at a crime scene.

Very little work exists studying the ash produced by cigarettes to benefit forensic science. This study quantified element concentrations in cigarette ash to investigate the ability to distinguish American cigarettes through four comparisons: inter-brand, intra-brand, menthol/non-menthol, and pack. To obtain cigarette ash, a “smoking apparatus” including a vacuum pump was built to simulate smoking a cigarette. Approximately 100mg of ash was digested in 2mL of concentrated HNO₃ followed by the addition of 2mL of concentrated HCl. Trace metal grade standards were used to generate calibration curves with concentration ranges in parts per million (ppm) using a Buck Scientific® Accusys 211 Atomic Absorption Spectrophotometer (AAS). Three elements (potassium (K), calcium (Ca), and magnesium (Mg)) were chosen due to producing the highest intensity values when analyzed via scanning electron microscopy-energy dispersive spectroscopy. Zinc (Zn) was also chosen based on elements studied in previous similar studies. Using Linear Discriminant Analysis (LDA) and the four previously mentioned metals, comparisons of inter-brand, intra-brand, and menthol/non-menthol cigarettes were performed.

Within the inter-brand study, Parliament® (PM), Newport® (NP), Camel Blu® (CB), and Marlboro® Red (MMR) cigarettes were compared. When the concentrations of each element were plotted against one another, Mg compared to Ca provided the most distinct clustering of points. In an LDA plot, it was found that separation occurred between the clusters of PM, NP, and CB brands. This was consistent with a previous similar study utilizing inductively coupled plasma-atomic emission spectroscopy.¹ Although brand clustering was observed, the LDA model generated was not sufficient for complete discrimination.

In the intra-brand study, Marlboro® Green (MGr), Marlboro® Gold (MG), Marlboro® Blue (MB), Marlboro® Black (MBK), and Marlboro® Smooth (MS) were compared. Element plots showed that, when compared, Ca and K displayed the most clustering among the cigarette brands. This finding supports the results observed in another study comparing element concentrations in cigarette material.² In the LDA plot, there was more overlap observed between brands in the intra-brand study, which was expected; however, the LDA plot indicated that complete discrimination could not be achieved.

For the menthol/non-menthol comparisons, Ca plotted against K displayed the most separation between the two groups. In regard to the pack comparisons, a nested Analysis Of Variance (ANOVA) was performed to determine if the variance seen between packs of the same brand affect the variances observed between different brands. All four elements produced a significant effect on pack variance; however, variance between packs of brands was not enough to affect the variance between brands. Overall, Mg, Ca, and K provided the most information for comparison purposes. Although the results are consistent with previous studies, analyzing the composition of the cigarettes based on the four elements selected does not produce LDA models with the ability to discriminate between brands. The analysis of additional elements could potentially aid in doing so.

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Forensic Science, Atomic Absorption Spectroscopy, Cigarette Ash



B198 Forensic Source Attribution Using Stable Isotopes: Hairs to Humans and Insects to Carrion

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After attending this presentation, attendees will understand how stable isotope ratio measurements can be used to: (1) provide physical and characteristic traits about donors from their hair, such as body mass index and age; and, (2) link different life stages of blow flies to their larval food source.

This presentation will impact the forensic science community by providing examples of objective chemical measurements and statistical classification methods for comparing human hairs with the traits of the donors. In addition, this presentation will provide an example of an instrumental method of analysis for predicting the food (carrion) source of blow fly larvae, pupae, and adult flies.

Although Isotope Ratio Mass Spectrometry (IRMS) has historically been considered a specialized technique requiring significant training and expertise, modern instruments provide such automation and ease-of-use that it is becoming more user friendly and a more common tool for answering a variety of forensic, ecological, geological, anthropological, and environmental questions. In the forensic community, IRMS is already in use in many government forensic laboratories and has passed *Daubert* standards for admissibility in court on many occasions. This presentation provides two studies involving source attribution using IRMS.

In the first part of the presentation, two different methods will be presented to classify and attribute human hair to subject groups such as body mass index, age, and sex. One method uses the absolute abundance of the amino acids in human hair, as determined by derivatization Gas Chromatography/Mass Spectrometry (GC/MS). Using this method, the classification model Fuzzy Rule Expert System (FuRES) can predict age or body mass index with better than 90% and 80% success rates, respectively. The second approach uses bulk and amino acid-specific isotope analysis as input variables for classification. Both methods of amino acid analysis required the analysis of 14 amino acids that were released from the hair following acid hydrolysis. Unlike GC/MS, the liquid chromatography/IRMS measurements did not require derivatization prior to analysis. Statistical techniques such as Canonical Discriminant Analysis (CDA) are used to overlook the covariance of amino acid values between individuals caused by dietary factors and instead highlight the selective differences caused by grouping factor(s) such as age, body mass index, and sex. For example, using leave-one-out cross-validation, CDA is able to predict the body mass index of a donor's hair sample with an approximately 80% success rate. Age group can be predicted with an approximately 87% success rate using leave-one-out cross-validation.

The second part of the presentation uses isotope ratios to link blow fly larvae, pupae, and adult flies to different food (carrion) sources. Ecologists often use isotope ratio analysis to determine the trophic level of organisms and their primary food sources; however, such analyses are rarely interested in linking adult insects to a specific meat source in a forensic context. This study presents a proof-of-concept study to test the hypothesis that adult blow flies can be linked to specific food sources via their stable isotope ratios. The results indicate that whereas carbon does not undergo systematic fractionation between carrion source, larvae, pupae, and adult flies, nitrogen undergoes significant fractionation (toward ^{12}C enrichment) at each lifecycle providing a total enrichment of approximately six per mil relative to the source carrion.

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Hair Analysis, Isotope Ratios, Forensic Entomology



B199 From Plants to Projectiles: New Analytical Approaches to the Utility of Direct Analysis in Real Time (DART®) Technology in Forensic Cases

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After attending this presentation, attendees will better understand two new applications of Direct Analysis in Real Time Mass Spectrometry (DART®-MS) technology in forensic science for the characterization of trace evidence off the surface of spent projectiles as well as the characterization of psychoactive compounds from interesting plant-based drugs of abuse.

This presentation will impact the forensic science community by demonstrating the applicability of DART® technology, a rapid, ambient ionization technique for relevant evidentiary materials in forensic cases from plants to projectiles.

This two-part presentation will address new application areas of DART®-MS: non-destructive characterization of trace evidence from the surface of projectiles and the evaluation of DART®-MS as a rapid analytical technique for the characterization of non-traditional, plant-based drugs of abuse. The purpose of the first part of this study was to examine the feasibility of trace material identification from the surface of spent bullets using DART®. The goals of the second part of this research were to develop methods for the analysis of a wide variety of plant-based drugs of abuse and to apply these methods in an effort to differentiate between multiple strains of seed species.

The examination of trace amounts of intermediate target materials collected on the surface of spent projectiles provides crucial information for trajectory reconstruction. Determining the origin of an unknown material adhered to a spent bullet allows for the identification of intermediate targets the bullet either contacted or penetrated during flight. Although significant information can be obtained, this aspect of trajectory reconstruction is often ignored. The ability of different bullet types to collect trace materials from intermediate targets and the ability to associate these materials to their origin was examined using microscopy and DART®-MS. Full metal jacket, jacketed hollow point, and lead round nose bullets were fired into sheets of five different commonly used building materials. All spent bullets were examined and photographed using a stereomicroscope. The spent bullets were then examined using DART®-MS to determine if any ion profiles generated from the trace materials could be associated with those of the intermediate target building materials through which the bullets were fired. The collection of trace from all five types of building materials was highly dependent on the type of bullet. The trace materials collected produced unique and interpretable ion profiles. Additionally, MS data from four of the five building materials tested matched the MS data generated from trace material collected on bullets from the respective target materials.

For the second part of this study, 12 different seed species reported to have psychoactive effects on the user were obtained and analyzed. Many plant species around the world are known to contain various psychoactive compounds. Due to their effects when consumed, many of these plants are used as a part of religious and ritualistic practices in many different cultures. As with any psychoactive compounds, these plants have the potential to be used in a recreational manner. In the United States, plant-based drugs of abuse, such as marijuana, have become commonly abused substances. Although the federal government currently regulates marijuana, many of the plant materials containing potential drugs of abuse are not regulated and can be purchased legally from various online sources. Physical examination was performed, in which average measurements were obtained to describe the length, width, thickness, and mass of each seed species, followed by DART®-MS analysis. The seeds were prepared for analysis by DART®-MS by grinding to homogenize the seed and embedding the powder onto QuickStrip™ cards. To optimize the method for analysis, three different DART® carrier gas temperatures were investigated for each sample by considering the signal to noise ratio, ion abundance, and presence of the analyte of interest at each source temperature using a single quadrupole mass spectrometer. The analytes detected were then subjected to MSⁿ fragmentation in a quadrupole ion trap mass spectrometer to confirm the identity of the analytes being detected. Fragmentation patterns were then compared to known fragmentation patterns reported within the literature, by other ionization methods such as: chemical ionization, atmospheric pressure chemical ionization, and electrospray ionization.

Forensic Chemistry, DART®-MS, New Approaches



Digital & Multimedia Sciences Section - 2015

C1 Large-Scale, Common-Source Identification of Digital Camera Images

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After attending this presentation, attendees will be aware of a method for large-scale, common-source identification of a digital camera with possibilities to improve the speed of identification.

This presentation will impact the forensic science community by showing that large-scale camera identification is possible on a standard desktop computer in forensically relevant time while still maintaining a high degree of accuracy.

Photo Response Non-Uniformity (PRNU) noise, present in images made by a digital camera, can be used to identify the source camera of an image or link images with a common source. PRNU is caused by imperfections of image sensors during the manufacturing process. Due to these imperfections, each pixel on a camera sensor gives a slightly different signal, even when receiving the same amount of light. PRNU noise is systematic, meaning that it is approximately the same for every image made by a specific camera. Therefore, it can be used to identify the source camera of an image.

Many cases, such as child pornography cases, have large numbers of images (>10,000) from unknown sources. To identify a common source between these images, all the images have to be compared to one another. For example, a case with 10,000 images results in approximately 50 million comparisons. Using the original method, it would take roughly one year of computation time on a desktop computer with a 2.3 GHz quad core Central Processing Unit (CPU). For practical purposes, this is too long; therefore, two methods have been proposed in the past to decrease the computation time of camera identification.

The first method is to reduce the number of pixels used for the comparison of the PRNU patterns. Instead of taking all pixels of an image, only a percentage of the pixels are selected. The second method reduces the bits per pixel to store the data. Both methods reduce the information and, therefore, the computation time needed for the camera identification process. Applying these methods to a case with 100,000 images reduces the calculation time to approximately one day instead of one year; however, these methods were designed for cases where a suspect device is available to generate high-quality PRNU noise patterns.

Experiments on single-image comparison showed that applying these methods results in a low sensitivity: approximately a true positive rate of 0.65 at an experimental false positive rate of zero. To counter the negative effects of reducing the information, an algorithm was developed. This algorithm increases the sensitivity to approximately 98% at a small fraction of the total calculation time.

The idea behind the algorithm is that the PRNU noise pattern becomes clearer when multiple images of the same camera are used to estimate the PRNU noise pattern. The assumption is that the two images with the highest correlation above a certain threshold have a common source. The PRNU noise pattern of these images is then averaged for a clearer pattern. More images are added to this pattern, if their correlation with this averaged pattern is above the threshold. Images that would normally fall below the threshold, even though they are from the same source, are now added to the averaged pattern; while other images (originating from a different source) that were above the threshold by random chance, are now excluded.

This package of methods allows for large-scale, common-source identification on standard desktop computers, available to any forensic scientist, in a reduced amount of time.

This method can be combined with the quantization tables in JPEG files as a pre-selection measure as well as using Graphics Processing Units (GPUs) to improve the speed with larger databases of child pornography cases. Practical results of a large database of images are presented.

Image Comparison, Camera Identification, PRNU



Digital & Multimedia Sciences Section - 2015

C2 Camera-to-Subject Distance and Facial Comparison Examinations

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WITHDRAWN



Digital & Multimedia Sciences Section - 2015

C3 Assessing the Relationship Between Individual Differences and Child Pornography Image Preferences in an Internet Sample of Child Pornography Consumers

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After attending this presentation, attendees will have a better understanding of the relationship between child pornography image preferences and personality characteristics.

This presentation will impact the forensic science community by assessing whether personality characteristics are significantly related to the types of images preferred by internet pornography users. The preferred type or genre of images, rather than the collection itself, may provide researchers with a better understanding of the personality characteristics associated with child pornography use.

Internet child pornography is not only a global epidemic, it is one of the fastest growing crimes in the United States. According to the Federal Bureau of Investigation, the United States has seen a 2,500% increase in the last ten years in the number of child pornography arrests. What drives someone to collect different genres of pornography is unknown; however, one thing is for certain, the collections of child pornography users are vastly different. According to Taylor et al., child pornography should be considered as a wide range of images, which involve different levels of child victimization (e.g., innocent vs. erotic, posing vs. sadistic).¹

In general, research suggests that the majority of offenders are collecting images of young children with higher levels of child sexual victimization (e.g., sexual penetration, sadistic violence).^{2,3} For example, the Child Exploitation and Online Protection Centre reported an increase in the number of non-commercial sources depicting babies and toddlers in child sex abuse images, as well as an increase in the number of images depicting children of different racial backgrounds and locations, such as South America and South Korea.^{4,5} In addition, the United Kingdom's Internet Watch Foundation reported an increase in the number of commercial websites depicting severe forms of child sexual abuse with more than half (69%) of the child victims appearing to be under the age of ten years.⁶ Of those images, 24% appeared to be under the age of six years and 4% of the child victims appearing to be to be under the age of two years.⁶

The preferred type or genre of images, rather than the collection, may provide researchers with a better understanding of the personality characteristics associated with child pornography use; however, this link between individual differences (e.g., extraversion) and types of images (e.g., age of child, sadistic vs. erotic) collected by child pornography users has yet to be simultaneously analyzed.⁷ Therefore, the current study will be the first to assess whether personality characteristics are significantly related to the types of images preferred by internet pornography users. The specific goal of this study was to determine if certain personality characteristics were predictive of certain child pornography image preferences.

This study was conducted electronically using an anonymous, internet-based survey in order to sample child pornography users from the "general population of internet users" rather than the clinical or forensic population. Respondents were solicited from the website Mechanical Turk[®]; research studies have shown Mechanical Turk[®] may be used to obtain high-quality data inexpensively and rapidly from a diverse participant pool and it provides better generalizability than snowball sampling procedures.^{8,9}

For the current study, more than 1,000 respondents completed the anonymous internet-based survey. The results and future implications of the study's findings will be discussed.



Digital & Multimedia Sciences Section - 2015

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Internet Child Pornography, Personality, Child Sex Abuse Images



C4 Forensic Image Comparison of Feet

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The goal of this presentation is to develop a biometric method (observation checklist) for foot comparison via images. On recordings of certain crimes, such as child abuse situations, the face is not always (properly) shown, unlike other body parts such as the feet. In such cases, feet can offer a solution for identifying the perpetrator. Therefore, the need has arisen to develop a method for the identification of people based on images of their feet.

This presentation will impact the forensic science community by providing a framework for foot comparison which can be used in casework.

To be able to evaluate the proposed method, a Netherlands Forensic Institute (NFI) foot database of images (left and right feet) was created. The NFI foot database of images consists of 52 subjects (19 females and 33 males). Eight photographs, four of each foot, were extracted from each subject; the four photographs were from different sides.

During the project, a study was performed to evaluate the potential of using numerical features for the observation checklist. The dispersion was observed to conclude which numerical features would be good individualizing factors. Ten out of sixteen features were dispersed enough to be used in the observation checklist (standard deviation >0.04). The repeatability and reproducibility of the model was studied and it was concluded that the length ratios of the toes have the smallest values. Another factor studied for the applicability of the numerical features was the foot's daily changes; the toes' length ratios have the smaller daily change.

The proposed identification checklist has the potential to be effective on actual cases of perpetrator identification. The accuracy of the tests is always above 90%, with the highest score being 98.34% of the top-side test and the lowest being 90.30% of the poor quality test. Furthermore, it was observed that the use of the proposed method improved the results by an average of 10% compared to not using it.

A probability format was suggested as a means for reporting. As the checklist consists of morphological features which are very distinctive for the comparison and scientists have not found a way to calculate the Likelihood Ratio (LR) from non-numerical features, the needed ratio cannot be estimated; however, a statistical analysis was performed to check the potential of reporting with LR for the suggested numerical features. Again in the bivariate analysis, the combination of toe length ratios gave maximum probability densities compared to the other combinations. In addition, the larger LR for a specific case was calculated on the sole-side image from which six numerical features can be extracted.

To conclude, although the proposed identification checklist has all the potential to be used on actual cases of perpetrator identification from foot imagery, more research is recommended.

Feet Comparison, Image, Biometric



Digital & Multimedia Sciences Section - 2015

C5 Age Estimation of Adolescents and Adults Using the Dimensions of the Eye and Pupil in “Selfie” Photographs

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After attending this presentation, attendees will understand how to use dimensions of the eye and pupil to assess the age of an individual from a digital image.

This presentation will impact the forensic science community by demonstrating the effectiveness of investigating digital images and correlating an age with the photographed individual. This becomes most relevant for individuals in their teens, as they often appear older due to the use of makeup, posing, and filter technologies commonly used to take a picture of oneself, otherwise known as a “selfie.” By analyzing facial features, particularly the eye and pupil regions, the subject has less ability to hinder age estimation based solely on physical appearance.

Institution Review Board (IRB) approval was obtained in order to use human subjects. The reasoning of this project, as well as the safety and privacy of the participants, procedures, consent forms, and storage of data needed IRB approval. The target age group of participants is between 11-19 years old; however, participants not within the age range were accepted. Participants were recruited by word of mouth, personal connections, and e-mail communications. Those under the age of 18 years old were required to obtain parental permission and provide child assent. Those over the age of 18 years old required informed consent. Because there are many variables that influence the functionality of the pupils, such as mood, eye problems, medications, and lighting, images were taken under controlled conditions which include using the same room and lighting.

Each subject was given an Identification (ID) number for the project and asked a series of simple questions detailing his/her mood, medications, and eye-problem history as well as his/her age, birthday, and other demographic information. The illumination of the room was captured with a Dr. Meter® Digital lux meter for each participant, so that the camera settings and metadata from the Nikon® D3100 digital camera and Apple® iPad® iOS® Version 7.1.1 could be compared. Each participant sat 1.5 meters away from the digital camera and a set of two pictures were taken, one with a spontaneous gaze, looking past the camera, and the other with an attentive gaze, looking straight into the camera. A forensic evidence ruler was included in these photographs for accurate calibration of measurements using Adobe® Photoshop® CS5. Finally, the participant was asked to take a picture (a “selfie”) and a short video of him/herself using the iPad®. The distance at which the subject comfortably held the iPad® to perform these procedures was recorded.

The images were downloaded onto a computer for analysis using Photoshop®. Each image was calibrated so the pupillary diameter, area, and interpupillary distance could be determined and compared using formulas given in MacLachlan & Howland.¹ Using the known age of each participant and the age from the formulas, the effectiveness of age estimation can be determined.

Reference:

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Age Estimation, Visual Biometrics, Interpupillary Distance



Digital & Multimedia Sciences Section - 2015

C6 How Accurate is 3D Facial Morphology for Personal Identification?

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After attending this presentation, attendees will be acquainted with the principles of computer-aided personal identification using 3D records of human faces, its technical and methodological imperfections, and novel modifications proposed to currently available algorithms.

This presentation will impact the forensic science community by providing insight into current state-of-the-art forensic facial identification and by introducing novel approaches designed specifically for forensic purposes, developed using the 3D Virtual Model Database of Human Faces.

The increasing accuracy of sensors used for capturing human faces together with advances in computer graphics and vision have had a great impact on the quality of images that are being admitted for forensic examinations. Matching image-based forensic evidence from crime scenes (surveillance videos) with suspects (mug shots, ID photographs) is known to be highly dependent on the image quality, optical distortion, perpetrator's head position, or disguise. Therefore, in many forensic cases, automatic computer-aided systems for facial recognition are of little use as they fail to provide positive (i.e., to identify a person), or negative (i.e., to eliminate a person) results. Currently, new development in 3D technologies has offered solutions to overcome these key limits by allowing 2D-to-3D and 3D-to-3D digital record matching.

Despite that fact that 3D video surveillance systems are being developed and are expected to be widespread in the near future, so far there has not been sufficient scientific evidence on how accurate and reliable it is to utilize 3D records of human faces for the purpose of forensic identification or whether matching 3D digital evidence meets legal requirements that demand the use of quantifiable scientific methods with known error rates. 3D human faces are generally captured by a variety of in-lab equipment — laser- or stereophotogrammetry-based devices, which eventually generate plain or textured photorealistic 3D meshes. Processing and matching two or multiple 3D surface meshes is a challenging task which requires a proper alignment algorithm together with appropriate numerical and visual representations. There are a number of existing algorithms and metrics, such as the Iterative Closest Point (ICP) for aligning two surface datasets or Hausdorff distances for quantifying actual distances between two aligned meshes. In addition, surface-based visualization technique is commonly performed using colormaps and scalar fields.

The present research tested the currently available algorithms for matching 3D faces using a dataset sampled from a large database of high-resolution 3D scans — the 3D Virtual Model Database of Human Faces. This study established a stripped version available throughout the project website; the database contains approximately 2,000 scans acquired for a variety of reasons — studies of individual and age-related human variation, growth and development studies, parent-to-offspring resemblance, etc. Specifically, it contains both subadult and adult individuals scanned repeatedly under controlled and semi-controlled conditions as well as those composing a large reference sample of non-matching individuals at a population level.

To improve the matching process, available algorithms were modified by introducing an iterative process of successive improvement of the average face speed up with KD-trees which allows a better alignment of 3D meshes when a multiple comparison is launched. The magnitude of dis/similarity among aligned meshes was expressed by signed and absolute Hausdorff distances (i.e., the greatest of all the distances from a vertex in reference mesh to the vertex in the compared one and further extended to incorporate vertex to polygon and inter-polygon distances).

The results show that all modifications to the tested algorithms proved to be useful for further development of a forensic 3D facial identification system; however, there are a number of other issues of computational and technical nature that need to be addressed in order to acquire high-match scores in same-person comparison. The purpose of this presentation is to present the issues to the forensic community.

Facial Comparison, 3D Surface Data, 3D Database



Digital & Multimedia Sciences Section - 2015

C7 Effects of Latent Print Processing on Evidence Prior to Digital Evidence Examination

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The goal of this presentation is to provide attendees with an understanding of the effect of latent print processing of evidentiary items that will subsequently undergo digital evidence examination.

This presentation will impact the forensic science community by providing information that will assist laboratories in determining the proper order of examination when evidence items are received that will undergo both latent print processing and digital evidence examination. If latent print processing is found to interfere with successful digital evidence examination, the digital examination will have to be performed prior to the latent print processing.

An increasing number of evidence items are being submitted to forensic laboratories in which both latent fingerprint examinations and digital evidence examinations are requested. During one of these recent submissions in the forensic laboratory, the question arose as to whether latent print processing should occur before the digital evidence examination in order to preserve any possible latent prints deposited on the evidentiary items or if the latent print processing would impede the ability to retrieve digital evidence from the items when they were examined subsequent to the processing. When items of digital evidence are examined, they are required to be powered on or energized in some way, utilizing the delicate electronic connections within the items. It is theorized that these delicate connections could be affected by the chemicals and process that are encountered when they are processed for latent prints, using cyanoacrylate fuming and subsequent dye staining processes.

In order to get a sampling consistent with the different types of items that could be submitted for digital evidence examination, the research included Universal Serial Bus (USB) flash memory drives of different sizes, media cards of different sizes, optical media including compact discs and Digital Video Discs (DVDs), and bare internal hard drives. Each evidence item contained data and a hash value was obtained for each item before any processing was performed. The items were then processed with cyanoacrylate fuming and subsequently a hash value was obtained for each item again. They were then processed with a dye staining process. After the dye stain, a hash value of each item was obtained again. The hash values of the item before any processing, after cyanoacrylate fuming, and after fuming and dye staining will be compared. The effects of the processing will be discussed, including the numbers of successful hash value comparisons that were able to be made at the conclusion of testing.

Digital Evidence Examination, Latent Print Processing, Evidence Processing Order



Digital & Multimedia Sciences Section - 2015

C8 Placing the Suspect “Behind the Keyboard” Through the Application of Handwriting Analysis to MS® Office OneNote® File Content

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After attending this presentation, attendees will have a general understanding of the potential value digitally captured handwritten content may provide in the individualization of digital evidence.

This presentation will impact the forensic science community by providing an additional method for linking an individual to specific digital evidence files and/or devices.

One of the common issues with the examination of Digital and Multimedia Evidence (DME) is individualization of reported data to a suspect. Without additional evidence such as fingerprints, photographic evidence, or witness testimony, placing the suspect “behind the keyboard” of an electronic device may be a daunting task for the DME examiner. This lack of ability to individualize the evidence to an individual, rather than just a “user of the electronic device,” is sometimes provided as an argument toward labeling DME examination as an investigative/intelligence tool rather than as a true forensic science. This presentation was developed from the results of an investigation where handwritten data allegedly captured using a tablet-type electronic device was individualized to the subject under investigation through handwriting comparison.

Evidence was submitted to the United States Army Criminal Investigation Laboratory (USACIL) for digital evidence analysis related to the possession of suspected child pornography. Of the 134 items submitted for analysis, 67 items were subjected to either a full or triaged analysis while the remainder were either non-functional, contained no data, or were remanded back to the submitting agency for either manual review or an additional request for further examination. Of the 67 items examined, 32 contained pictures and/or videos of interest to the investigation and seven items contained additional case-pertinent data. The results of the initial triaged analysis of the evidence indicated one of the largest collections of suspected child pornography in USACIL history.

In addition to the large amount of suspected child pornography, several Microsoft® (MS®) Office OneNote® files containing apparent handwritten content related to fantasized sexual relations with underage persons were observed. Coordination with the submitting agency resulted in a request to have a USACIL document examiner attempt handwriting comparison on the recovered digital data. A preliminary examination of the OneNote® files disclosed that the handwritten entries appeared to be freely and naturally written and contained sufficient detail for comparison. Copies of the files were provided to the submitting agency with instructions to obtain comparable known writing from the suspect. Upon receipt of the known writing samples, a handwriting examination comparing the questioned writing from the OneNote® files to the known writing of the suspect was conducted. The results of the examination identified the suspect as the author of the questioned handwritten entries.

As a result of the joint examination efforts between digital evidence and handwriting examiners, the suspect was conclusively linked to the electronic document and therefore linked to the device. With the development and enhancement of more electronic devices capable of capturing handwritten data such as tablets, touch-screen monitors, and smart phones, the likelihood of finding handwritten data on electronic devices may be increasing. The results of this case study indicate the potential for individualization of digital evidence through handwriting analysis conducted on digitally captured handwritten material. Therefore, these types of files should not be overlooked and a more concerted effort to look for these types of files may be warranted.

The opinions or assertions contained herein are private views of the author and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

Digital Evidence, Handwriting, Individualization



Digital & Multimedia Sciences Section - 2015

C9 The Role of Fantasy in Investigating Online Predation Cases

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After attending this presentation, attendees will be aware of ways to use close examination of internet communications to identify and investigate online predators. The presentation applies principles of hybrid crime assessment to identify pressure points at which the online predator is likely to become dissatisfied with cyberspace and seek to move his or her crimes into physical space. One tool for conducting these investigations is understanding the role of fantasy and how it can be manifested in relation to crimes in cyberspace.

This presentation will impact the forensic science community by providing a new investigative tool for conducting predator investigations that have both online and physical interactions between predators and their victims.

One online dictionary defines predation as, "...a relation between animals in which one organism captures and feeds on others"¹. In cyberspace online predation can have much the same definition; however, feeding on others usually becomes psychological rather than physical. Online predation almost always includes an element of fantasy, whether as a tool for exploitation or as a state of mind in the predator.

Contrary to some popular definitions, victims of online predators are not limited to children. Online predation is virtually always a power issue, often mixed with fantasy, and its targets depend upon the proclivities of the predator. Understanding this combination is useful as an investigative tool. Identifying the role of fantasy in the mind of the predator can assist the investigator in identifying and investigating the suspect.

Fantasy can take two forms. One way to differentiate is to view the fantasy as a way to escape into a world where day-to-day issues lose their importance. Healthy individuals fantasize periodically but generally emerge from their daydreams unscathed and, perhaps, refreshed; however, other individuals — not as mentally healthy — escape into fantasies and may not emerge. Their lives become integral with their fantasies and, at some point, those fantasies may evolve and develop to the point where the individual must act them out as part of his or her daily physical existence. Simply, these people lose the balance between the real world and their manufactured fantasy world. Consequently, they leave clues that can assist in investigating their activities and can also assist in tracing them through cyberspace and into physical space.

Cyberspace is an alternative dimension. It is a parallel universe that gives its denizens the ability to live in a world that meets their criteria and does not offer the normal disappointments of daily physical-world life. The presence of erotic chat rooms, sadomasochistic websites, and virtual worlds addressing other extremes of behavior attest to a market serving fantasies that are somewhat "off the grid." Online pornography has long been a topic for academic study, in part due to its breadth and depth. Deviant behavior is alive and well and has a home in cyberspace.

Humans live three lives: public, private, and secret. In the anonymity of cyberspace, it seems safe to acknowledge and act out the secret life. Investigators can use this behavior to predict physical behavior and, in some cases, predict the point at which it will emerge with dangerous consequences into physical space.

This presentation will explore two cases of online predation: a romance scam and a lengthy (over five years) international cyber stalking. Through examination of internet communications relating to these two cases, attendees will see examples of how online predators signal their unhealthy intentions and give clues about how to locate and investigate them. Both of the predators lived in worlds of fantasy and in both cases their behavior online signaled emergent behavior in physical space. In one case, investigators were successful in bringing the perpetrator to justice while in the other case, the suspect is still at large.

The presentation will discuss the issues involved in fantasy, the two exemplar cases, and, finally, the evidence available to digital investigators following the suspects in cyberspace.

Reference:

1. <http://dictionary.reference.com/browse/predation>

Online Predator, Digital Investigation, Fantasy

C10 3D Superimposition: A New Technique of Personal Identification

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After attending this presentation, attendees will be informed about a new technique of personal identification.

This presentation will impact the forensic science community by presenting a standardized technique for personal identification based on 3D superimposition.

The goal of this presentation is to show an objective, non-invasive, technique for the subject's personal identification through an analysis of recorded images and 3D avatar superimposition.

Personal identification based on 3D digital stereophotogrammetry presents a natural evolution of previous research in this field, the so-called "Parameterized Superimposition (PS)." Total cooperation of the suspect and the structure where the offense was perpetrated are boundaries of the PS technique. Photogrammetry currently provides the most cost-effective 3D capturing system as it is quick, inexpensive, and non-invasive; the equipment necessary for acquisition is easily transportable and offers high reliability. The technique was suitable for capturing facial morphology for clinical and anthropological usage. This technique involved four steps:

Preparatory Phase: The recorder images of a subject were studied and improved. Frames with a better view of the subject's facial landmarks were then chosen.

3D Acquisition Phase: A 3D stereophotogrammetric avatar of the subject's face was created; this phase only required four photos taken simultaneously with a calibrated camera.

Superimposition Phase: This phase was the preparatory phase for the final step. The 3D avatar of the subject's face was carefully spatially oriented in the same position as in the photos and snapshots that were previously taken. During the morphological analysis, the snapshot of the 3D avatar was superimposed on the frame of the subject's picture by a specific software.

Metric Image Analysis: To perform this step, it was necessary to clearly recognize at least five landmarks on the 3D avatar using appropriate software. A 3D parameterized avatar of the subject was created with a stereophotogrammetric technique and the subject's frame was selected and acquired. In the metric image analysis step, a quantitative comparison between the image of the frame of subject's face and the avatar's snapshot was obtained. Objective anthropometric landmarks, such as exocanthions, glabella, and asubspinal point, were marked on the 3D avatar and on the picture of the frame of the subject's face to calculate the distance of the absolute points discerned on those two images.

Results: The absolute and relative distance between the marked points, the perimeters, and the area of the triangles, obtained by connecting the points, and the compactness indices were automatically calculated with a proper program.

Conclusion: The morphologic phase revealed a full overlap between the 3D avatar and the frame's picture. Metric phase revealed that correlation coefficient values, higher than .998, confirm the identification hypothesis. The technique described is objective, repeatable, and not invasive. Technical skills are required, meaning that improvisation is not allowed.

Personal Identification, 3D Superimposition, Anthropology



Digital & Multimedia Sciences Section - 2015

C11 Online Anonymity: Forensic Analysis of The Onion Router (Tor) Browser Bundle

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After attending this presentation, attendees will understand the manner in which The Onion Router (Tor) provides anonymity to its users, some of the vulnerabilities that exist within the software, and a few forensic analysis techniques that are capable of circumventing the secrecy.

This presentation will impact the forensic science community by allowing practicing digital forensic analysts to take away valuable information pertaining to the Tor Browser Bundle (TBB) that may save time in future investigations as attendees will be aware of several methods that will and will not work in the process of gathering pertinent evidence.

Tor is a network of encrypted onion routers that helps to increase the level of anonymity experienced by its users. The security and privacy provided by the TBB was originally intended to protect the communications of the government; however, it is also a facilitator for individuals participating in illicit activities. It was hypothesized that if correct methodology is executed and the data collected is analyzed using pre-existing forensic techniques, then relevant evidence of the browsing history and presence of the TBB may be acquired. To test this, Virtual Machines (VM) were constructed to test four possible scenarios: (1) a machine running only Internet Explorer®; (2) a machine with TBB downloaded but no active use recorded; (3) a machine with TBB downloaded and used to navigate both the internet and Darknet; and, (4) a machine where TBB had been installed, used, then uninstalled. Furthermore, an additional VM was created solely for the purpose of tracking registry changes throughout the course of installing and uninstalling the TBB. It is hoped that beneficial information will become evident by capturing packets while the TBB is navigating to .onion and .com websites, dumping the Random Access Memory (RAM), and comparing versions of the registry from various points of the installation process.

In conclusion, the RAM dump provided several file types from the carved image that linked the web browsing to the TBB and several .onion sites. The registry files demonstrated that deleting Tor does not carry out a full uninstallation, thereby leaving artifacts behind. Lastly, the packet capture proved that Tor traffic is very different in appearance and content than that of a standard web browser.

Each method employed reaped beneficial artifacts proving that the TBB does not provide complete anonymity. These techniques would for the most part only be applicable in a proactive network forensics environment using a remote process to monitor a suspect's activity. Therefore, it is less likely that these methods would be used in a digital forensics lab that receives evidence from a crime scene. Naturally, the digital forensic community will remain persistent in their quest to refine an applicable technique that will adequately gather incriminating evidence from a hard drive subsequent to collection; however, without more advanced technology and abundant resources like those available to government agencies such as the Federal Bureau of Investigation (FBI) and the National Security Agency (NSA), digital analysts will be hard pressed to find a reliable method of breaking through the anonymity provided by the TBB for confiscated hard drives and associated digital evidence.

Anonymity, Forensic Analysis, Tor Browser



Digital & Multimedia Sciences Section - 2015

C12 Fingerprint Replication Utilizing Latent Fingerprints for Conducting Forensic Analysis on Mobile Devices With Biometric Security

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After attending this presentation, attendees will have a better understanding of biometric security, specifically fingerprint recognition, in personal digital devices, a technical understanding of the device's specific operations, and of the means for circumventing the device's biometric security to conduct a digital forensics analysis.

This presentation will impact the forensic science community by providing a supplemental means to collect digital evidence from personal devices that are protected with biometric security systems. Current techniques for data extraction on mobile devices include brute forcing pin codes, software security bypass via custom boot-loader, and physical data extraction using a Joint Test Action Group (JTAG) connection; however, these methods are not always implementable under the following conditions: (1) the device allows a limited number of pin code entries before the device locks itself or wipes the data; (2) the operating system does not have a known security bypass; or, (3) the data on the device is encrypted rendering a physical data extraction useless.

The goal of this project is to develop techniques to visualize a latent print from the surface of a mobile device; this print can then be used to develop a mold that can reconstruct the user's print. After using fingerprint powders to lift a print from the device surface, a digital image of the print was created using either a scanner or camera. This image was imported into an image processing software package in order to use the color range selection tool to extract the print from the background. The threshold tool was then used to enhance the print's clarity from the background while converting the image to black and white.

After creating a viable image, a mold was created using either the Printed Circuit Board (PCB) or 3D printing method. Of these two methods, the PCB method is the low-tech, low-cost method to create the mold. This method involves printing the enhanced image with a laser-jet printer to a transfer medium. Using a heat source, the image is transferred from the transfer medium to a copper-clad board. Once the image has been transferred, ferric chloride is used to etch the excess copper from the board, leaving behind the copper concealed by the image transfer. The 3D printing method is more expensive due to the materials and equipment utilized. After the enhanced image is obtained, it must be extruded into a 3D image using a Computer-Aided Design (CAD) program. This is then printed with a 3D printer to create the mold. Using the mold created from either method, the fingerprint can then be reconstructed with a material that mimics the conductivity and pliability of human skin.

This study indicates that the use of gelatin, ballistics gel, and Elmer's® white glue are viable candidates that can be used in the process of reconstructing the fingerprint as each of these substances is recognized by the device's scanner. Currently, research is in progress to refine the methods used to create the molds and to enhance the extracted fingerprint image to produce a more accurate reproduction. This provides additional evidence, not present in the digital examination, but likely to be very useful in an investigation that includes mobile devices.

Biometric Security, Digital Forensics, Fingerprint Reconstruction



Digital & Multimedia Sciences Section - 2015

C13 Implications for Digital Forensics Investigations of the United States 2nd Circuit Ruling Upholding Deletion of Non-Responsive Computer Files: A United States and European Union/Germany Perspective

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The goals of this presentation are to discuss ruling impacts and to describe current research in the United States and the European Union/Germany addressing selective suppression.

After attending this presentation, attendees will better understand the results of research into technical and legal issues arising from this landmark decision. This has significant technical and legal implications for all digital forensics investigations.

In *United States v. Ganas*, June 2014, the Second Circuit held that the government has a duty to delete or return non-responsive data it had previously seized through a valid search warrant, raising the bar for what the government must do post-seizure of digital evidence.¹ Attendees will learn the results of this research into technical and legal issues arising from this landmark decision. This has significant technical and legal implications for all digital forensics investigations.

This presentation explores the implications of this ruling from the technical and legal perspectives of experts in the United States and the European Union and of members of a digital forensics research collaboration between the University of Washington and Fraunhofer Institute Darmstadt. This study also discusses comparative selective suppression procedures used in Germany.²⁻⁵

Technology and policy are often on a collision course; this ruling is an example. The technical challenges are many: (1) how do we delete non-responsive data from disk images without violating the integrity of responsive files; (2) what do we do with hash values and digital signatures; (3) how do we edit video and audio files to show that all retained parts correspond to their respective parts from the original file; (4) how do we create signatures that show which parts were deleted; and, (5) how do we delete parts of a database without violating relevant data or complete databases with mainly non-responsive information that might not be retained at all?

Additional legal/policy concerns include: (1) what procedures will the government use to delete non-responsive data; (2) whose responsibility will it be to perform the deletion; (3) must the government verify the deletion and, if so, how can the deletion be verified; (4) how long can a non-responsive file be retained before it becomes an unreasonable seizure; (5) how does this interact with rights that criminal defendants have on appeal; (6) what is considered a “document;” and, (7) who decides what is non-responsive?

Although the government argued that selective deletion from an imaged hard drive would be impractical, the court stated that it was not entirely convinced: “But even if we assumed it were necessary to maintain a complete copy of the hard drive solely to authenticate evidence responsive to the original warrant, that does not provide a basis for using the mirror image for any other purpose.” This begins to imply what the court thinks authenticity may entail, but raises the additional questions described above.

Similar to the United States, in Germany, infringement due to search-and-seizure measures is justified only on the condition that they are in accordance with the German Criminal Procedure Code, securing data always requires the object in question to be of significance for the investigation.⁶ To determine evidentiary significance, off-site reviews are permitted pursuant to the German Criminal Procedure Code.⁷ As soon as a decision is made, these documents/data must be returned or deleted; however, German authorities recognize that selective suppression was “impractical,” respectively “technically impossible,” due to harming the validity of a mirror image.¹

In contrast, securing storage media in Germany is enshrined in the constitutionally guaranteed “right on informational self-determination” and the “guarantee of the confidentiality and integrity of IT-systems”; both rights derive from the general “right of freedom” in connection with the “guarantee of human dignity” of the German Basic Law.^{8,9}

This study explores the technical and legal research on selective suppression. While the technical team explored a technical solution to the questions raised in this presentation, it was discovered that German procedures for off-site review and their views on rights regarding digital information have relevance to questions raised by the 2nd Circuit decision.¹ These will be discussed and reviewed anticipating that the discoveries and insights in this study may be helpful to those adapting their digital forensics investigations to updated decisions.



Digital & Multimedia Sciences Section - 2015

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2nd Circuit Ruling, Selective Suppression, Digital Forensics



Digital & Multimedia Sciences Section - 2015

C14 Modeling Digital Autopsies on Medical Autopsies

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After attending this presentation, attendees will be able to reflect on tests for “normality” during digital forensic examinations to exclude possible alternative explanations that may fit the facts in a case.

This presentation will impact the forensic science community by increasing reliability of findings in certain cases.

Forensic examination is at the core of digital forensics practice. Examination uses science to provide an answer to a question that is relevant for legal or related purposes. The question may entail association, event reconstruction, or some other insight from forensic evidence. Once an answer x is obtained, the question can be reformulated in the form: Is x true? The outcome of such an examination then is a (qualified) *yes* or *no* (or *unable to determine*). The need to qualify the *yes* or *no* relates to the level of certainty with which the question can be answered.

Digital forensics certainty (or error rate) remains problematic.^{1,2} The premise of this presentation is that certainty is increased when alternative explanations have been eliminated. Digital forensic examination research often focuses on specific technologies that are subject to change — reducing certainty. This study suggests that more technology-neutral research needs to be done as a precondition for expressing confidence.

Unifying forensic science may be unrealistic, but metaphors from the medical sciences are common in digital forensics (e.g., the notion of a dead examination or the Autopsy Forensics Platform).³ Conducting a thought experiment to explore a mapping between medical autopsies and digital forensic autopsies is therefore prudent.

The first striking similarity considered in this presentation is the crisis experienced in medicolegal death investigations in the 1800s due to the variety of (questionable) methods used. Rudolf Virchow is credited with establishing a method to conduct autopsies that met scientific standards and that subsequently became the standard protocol.⁴ Arguments about the “scientificness” of digital forensics continue and are raised in the presentation.

One of the characteristics of the Virchow protocol is that the entire body is examined irrespective of the presumed cause of death. It avoids confirmation bias. The study demonstrates the general acceptance of this (unintuitive) imperative. The internal examination consists of a systematic removal of the organs; each organ is inspected to establish its consistency with expected (“normal”) characteristics. Similar verification is a significant difference between autopsies in these disciplines. Another significant difference is the nature of findings. These differences and the validity of transferring imperatives from one discipline to the other are considered in the study.

Is it possible (and meaningful) to “remove organs” from digital artifacts and express an opinion on the normality of such “organs”? The digital equivalent of an organ is a system, subsystem, or an application (henceforth, system). The National Institute of Standards and Technology (NIST) National Software Reference Library already catalogs hashes of files occurring “normally” in many systems. This experiment requires more: it needs a version of a system consisting of a number of “known” files. A database with at least two sets per system is required — a set of file hashes for a minimal installation as well as a similar set for a full installation of a system. Details and relevant logic of which files should be included forms part of the presentation. In such a database, for any system S_i , the set of hashes corresponding to a full install will be denoted by $\uparrow S_i$ and $\downarrow S_i$ will be used for the minimal case. Assume that F is the set of hashes of all found files. Then $FS_i = \{f \in F \mid f \in \uparrow S_i\}$ is a potential system (or “organ”); if $\downarrow S_i \subseteq FS_i$ then FS_i may be considered “normal.” It also means that every $f \in FS_i$ has been accounted for. Shared files need special consideration. This presentation will argue that “normality” of files not included in the hash sets implies syntactic correctness. Additional criteria for some excluded categories apply.

The proposed approach verifies “normality” of system anatomy beyond the primary focus of the examination. This may be extended to physiological “normality” — where cause and effect, and, hence, valuable evidence may be revealed.

This presentation includes some observations about practical difficulties in identifying the “organs” from a small proof-of-concept experiment.



Digital & Multimedia Sciences Section - 2015

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Digital Autopsy, Normality in Digital Forensics, Science of Digital Forensics



Digital & Multimedia Sciences Section - 2015

C15 Exploring Myths in Digital Forensics

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The goals of this presentation are to explain the evolution of digital forensics as a practice and to explore the historical rationale of some of the “myths” leading to the “best practices” in digital forensics and why many may no longer be relevant given today’s technologies.

After attending this presentation, attendees will have a better perspective of applying admissibility tests to digital evidence.

Digital forensics — née *computer forensics* — is one of the newer forensic science sections in the American Academy of Forensic Sciences (AAFS), having been established in 2008. After attending this presentation, attendees will understand some of the fundamental differences between digital forensics and the more traditional forensic sciences. Among these differences is the historical way in which digital forensics has evolved as a science and a field of practice. Appreciation of these differences may contribute to the conversation surrounding the current work of the National Institute of Justice and the National Institute of Standards and Technology as they try to codify a definition of the science of digital forensics.

Unlike the traditional forensic sciences, digital forensics investigations and methodologies were originally developed 30 years ago by computer-savvy users and practitioners rather than by the computer science community. The development of computer forensics as a discipline and field of study was very ad hoc in the 1980s and 1990s; indeed, there were very few computer forensics examiners who were not in the law enforcement community during that era. Furthermore, computer forensics courses and curricula in higher education were practically non-existent 15 years ago.

The acceleration of change in computer technology over the last 25 years has resulted not only in changing digital forensics hardware and software tools and methodologies, but also in big changes in the very technology that holds the evidence, ranging from floppy disks and hard drives to smartphones and solid-state memory devices. Many of the “best practices” of the 1990s are actually irrelevant with today’s technologies, yet are still taught in training and education programs today. Decisions based upon the adherence to the myths might cause investigators or prosecutors to not introduce probative evidence at trial, falsely believing that such evidence would not withstand a challenge in court; alternatively, a challenge might be successfully mounted due to a lack of understanding of true best practices.

This study will introduce several of the long-held computer forensics myths and discuss their place in the modern practice of digital forensic science.

Digital Forensics, Best Practices, Evidence



Digital & Multimedia Sciences Section - 2015

C16 Forensic Investigation of Crimes Committed by Unmanned Systems

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After attending this presentation, attendees will appreciate the potential of unmanned systems for the perpetration of crimes and will appreciate the problems they pose for forensic investigations.

This presentation will impact the forensic science community by introducing the threat posed by the use of unmanned systems, to include robots and drones, to commit crimes and the lack of preparedness by the forensic community to address it.

After centuries of mythology and conceptualization, robots are suddenly among us and they are rapidly proliferating. They can walk, swim, and fly. They are inexpensive and easy to operate. As with all machines, they are, at least for now, extensions of their human controllers.

As with any technology, today's robots, commonly referred to as drones, will be embraced by voyeurs, stalkers, child molesters, blackmailers, criminals, smugglers, and terrorists. Drone-assisted crimes have already surfaced: a plan for a model airplane carrying explosives to the Pentagon was thwarted, a quad-copter carrying contraband was detected scaling a prison wall, and a submarine carrying drugs to shore was captured; however, it is likely that many similar crimes have gone undetected.

There is every reason to believe that forensic investigators will soon be asked to conduct examinations of drones that were involved with significant accidents or crimes. Investigation of such cases, where the human perpetrator was *in absentia*, pose daunting challenges that will require special methodologies.

How will such an examination proceed? Unless the robot or drone is captured or found, there will be no fingerprints or biologic stains at the scene. Instead of footprints, there may be some sort of tread marks or, in the case of an aerial drone, perhaps no markings at all. Evidentiary procedures and even longstanding investigation paradigms (such as placing the perpetrator at the scene of the crime) are disrupted by drones. A digital examination of the drone may yield some important data. Perhaps a digital trail had been left on nearby cell phone towers. The drone may have a photo or video camera on board that may yield valuable data, but so might an examination of its mechanical systems or a chemical analysis of Chemical, Biological, Radiological, and Nuclear (CBRN) trace evidence. Even the "behavior" of the drone might reveal clues about its operator.

One can think of drone-assisted aggression as cybercrime that transcends beyond the limitations of the digital spectrum: it deploys a physical platform that can move, act, and react. An analogy can also be made to traditional murder-for-hire crimes, where another human is used as a *tool* to perpetrate the crime — only here, a machine is contemplated instead of a person. Analysis of cases involving human surrogates may provide insights for forensic investigators.

Sales of aerial drones account for approximately 80% of the unmanned systems market and nearly all of the media attention. The focus of drone manufacturers is transitioning from military to domestic markets as the Federal Aviation Administration readies the skies for commercial drone traffic (likely to be widespread by 2016), which they believe will reach 15,000 units by 2020. Sales to hobbyists, who can currently fly drones that weigh under 55 pounds, are reaching thousands of units per month.

With the dramatic increase in domestic drone use, it is reasonable to assume a corresponding rise in drone misuse (accidents) and abuse (crimes), many of which will be embedded in criminal and civil investigations and court cases. Yet, despite the enormous implications for the forensic science community, no references to presentations or papers written on this subject by forensic scientists have been found. Thus, it is a reasonable conclusion that the forensic community is ill-prepared to deal with the many threats presented by drones. Possible approaches to investigation of these crimes will be discussed.

Drones, Robots, Unmanned Systems



Digital & Multimedia Sciences Section - 2015

C17 Cloud Computing Forensic Science Challenges

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After attending this presentation, attendees will have a better understanding of some of the main challenges faced by forensic investigators attempting to identify, collect, analyze, and interpret digital evidence residing in cloud computing environments.

This presentation will impact the forensic science community by describing research performed by the National Institute of Standards and Technology (NIST) Cloud Computing Forensic Science Working Group, which was established to aggregate forensic science challenges in the cloud environment and to develop plans for measurements, standards, and technology research to mitigate the challenges that cannot be handled with current technology and methods.

The cloud exacerbates many technological, organizational, and legal challenges already faced by digital forensics examiners. Several of these challenges, such as those associated with data replication, location transparency, and multi-tenancy, are somewhat unique to cloud computing forensics.

The Group plans to prioritize the challenges enumerated. For high-priority challenges, gaps in technology and standards will be determined, resulting in a roadmap for addressing the challenges.

The challenges this study has aggregated are categorized into the following groups:

Architecture (e.g., diversity, complexity, provenance, multi-tenancy, data segregation, etc.) — Architecture challenges in cloud forensics include dealing with variability in cloud architectures between providers; tenant data compartmentalization and isolation during resource provisioning; proliferation of systems, locations, and endpoints that can store data; accurate and secure provenance for maintaining and preserving chain of custody; infrastructure to support seizure of cloud resources without disrupting other tenants; etc.

Data Collection (e.g., data integrity, data recovery, data location, imaging, etc.) — Data collection challenges in cloud forensics include locating forensic artifacts in large, distributed, dynamic systems; locating and collecting volatile data; data collection from virtual machines; data integrity in multi-tenant environments where data is shared among multiple computers in multiple locations and accessible by multiple parties; inability to image all the forensic artifacts in the cloud; accessing data of one tenant without breaching the confidentiality of other tenants; recovery of deleted data in a shared and distributed virtual environment; etc.

Analysis (e.g., correlation, reconstruction, time synchronization, logs, metadata, timelines, etc.) — Analysis challenges in cloud forensics include correlation of forensic artifacts across and within cloud providers; reconstruction of events from virtual images or storage; integrity of metadata; timeline analysis of log data including synchronization of timestamps; etc.

Anti-Forensics (e.g., obfuscation, data hiding, malware, etc.) — Anti-forensics are techniques used specifically to prevent or mislead forensic analysis. Challenges in cloud forensics include the use of obfuscation, malware, data hiding, or other techniques to compromise the integrity of evidence; malware may circumvent virtual machine isolation methods; etc.

Incident First Responders (e.g., trustworthiness of cloud providers, response time, reconstruction, etc.) — Incident first-responder challenges in cloud forensics include confidence, competence, and trustworthiness of cloud providers to act as first responders and perform data collection; difficulty in performing initial triage; processing a large volume of forensic artifacts collected; etc.

Role Management (e.g., data owners, identity management, users, access control, etc.) — Role management challenges in cloud forensics include uniquely identifying the owner of an account; decoupling between cloud user credentials and physical users; ease of anonymity and creating fictitious identities online; determining exact ownership of data; authentication and access control; etc.



Digital & Multimedia Sciences Section - 2015

Legal (e.g., jurisdictions, laws, service level agreements, contracts, subpoenas, international cooperation, privacy, ethics, etc.) — Legal challenges in cloud forensics include identifying and addressing issues of jurisdictions for legal access to data; lack of effective channels for international communication and cooperation during investigations; data acquisition that relies on the cooperation of cloud providers, as well as their competence and trustworthiness; missing terms in contracts and service level agreements; issuing subpoenas without knowledge of the physical location of data; seizure and confiscation of cloud resources may interrupt business continuity of other tenants; etc.

Standards (e.g., Standard Operating Procedures (SOPs), inter-operability, testing, validation, etc.) — Standards challenges in cloud forensics include lack of even minimum/basic SOPs, practices, and tools; lack of inter-operability among cloud providers; lack of test and validation procedures; etc.

Training (e.g., forensic investigators, cloud providers, qualification, certification, etc.) — Training challenges in cloud forensics include misuse of digital forensic training materials that are not applicable to cloud forensics; lack of cloud forensic training and expertise for both investigators and instructors; limited knowledge by record-keeping personnel in cloud providers about evidence; etc.

Digital Forensics, Cloud Computing, Forensics Challenges



Digital & Multimedia Sciences Section - 2015

C18 Federated Testing: Shared Test Materials From the Computer Forensics Tool Testing (CFTT) Program at NIST for Digital Forensics Tool Validation and Shared Test Reports

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After attending this presentation, attendees will be familiar with the Federated Testing materials being developed by Computer Forensics Tool Testing (CFTT), understand how they can be used in their labs to timely and effectively validate tools, and learn how the Federated Testing initiative encourages shared tool validation results. Federated Testing at the National Institute of Standards and Technology (NIST) is an expansion of the CFTT Program that provides digital forensics labs and practitioners with test materials for tool validation.

This presentation will impact the forensic science community by providing information as to how the NIST Federated Testing materials can be used in digital forensics labs for tool validation at a savings of time and expense and how the Federated Testing materials support shared tool validation results.

Forensic tools need to be validated before being used in the forensic process. These tools need to be validated: (1) to ensure that digital evidence is being correctly processed; and, (2) to support the admissibility of digital evidence in court and legal proceedings. Tool validation is difficult, expensive, and time consuming.

The various federal, state, and local digital forensics laboratories use the same tools or the same types of tools. It follows that a lot of work is duplicated in tool validation. The CFTT program currently creates tool specifications, test methods, and test reports. The goal and purpose of Federated Testing is to simplify, package, and export the CFTT test methodologies and the expertise used to produce the NIST test reports to laboratories and individual examiners for use in tool validation.

The Federated Testing initiative offers several anticipated benefits to laboratories and examiners. A primary benefit is one of time and cost savings. If laboratories can use the NIST methodology and materials for tool validation, they save the time otherwise spent to develop their own. Federated Testing can also improve the quality of testing. CFTT's shared test materials additionally present a new opportunity for shared tool validation reports. A current barrier for laboratories for sharing tool validation results is that each laboratory will test a tool differently. A further barrier is dissimilar formats between laboratories for documenting and presenting tool validation results. This makes it difficult for a laboratory to understand how an externally validated tool was tested and to determine if the tests and validation results are acceptable for use in their own laboratory.

When tools are validated using the CFTT shared test materials, a common methodology is used and the results are reported in a common format. This makes it easy for a laboratory receiving a shared validation report to quickly understand how a tool was tested and to determine whether the validation results are acceptable and applicable for use in their own laboratory. Tool validation results from CFTT's Federated Testing shared test materials can be shared informally between laboratories, could be submitted to and shared via public websites such as the Department of Homeland Security Science and Technology (DHS S&T) -sponsored cyberfetch.org or CFTT's Computer Forensics Tool Catalog, or be kept private within the tester's organization.

Federated Testing is a work in progress. CFTT currently has test methodologies for disk imaging, forensic media preparation, hardware write blocking, deleted file recovery, mobile device acquisition and analysis, and file carving. CFTT is first implementing Federated Testing for disk imaging; test materials for the other functionalities will follow.

The test materials are being packaged on a live Linux® Digital Video Disc (DVD). The materials consist of the DVD, which contains video tutorials on how to use the materials, a website, and command line test support tools. Using the website, users select the type of tool they wish to test (e.g., disk imaging tool), then select the specific features they wish to validate. This results in a list of the tests to run in order to test these features, along with the steps and instructions for each test. The test instructions reference CFTT's test support tools. Run from the command line on the DVD, the support tools are used to set up each test and to analyze the results. When all the tests have been run, the website generates the tool validation results in the CFTT Federated Testing common report format.

Digital Forensics, Tool Validation, Federated Testing



Digital & Multimedia Sciences Section - 2015

C19 Implications of Valid Data Length (VDL) Slack and the Facts That It Presents

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After attending this presentation, attendees will be familiar with Valid Data Length (VDL) slack and its implications for forensic examinations. Examiners will learn what VDL slack is, how to detect it, and how to handle the two distinct hashes that these files have.

This presentation will impact the forensic science community by bringing awareness to the fact that VDL slack is not widely known by examiners, few have run into it, and it is rarely taught in examiner training. VDL slack has a number of interesting properties, one of which is that it has two distinct hashes.

Many examiners are unfamiliar with VDL slack and its potential impact on their reports or potential testimony. This presentation introduces the topics and presents some interesting facts that can be derived from files with VDL slack. After attending this presentation, attendees will understand some of the unique characteristics of files that contain VDL slack (sometimes called file tail), how to detect these files, how they are created, and what they can mean to forensic examiners. One of the unique characteristics is that files that contain VDL slack have potentially two different hashes. In addition, copies of files with VDL slack can be traced back to an original file with certainty (this could be useful in child pornography distribution cases).

VDL slack is an artifact of the Microsoft's® object reuse strategy for disk space. When Microsoft® introduced New Technology (NT) and the New Technology File System (NTFS) in the 90s, they wanted to get a C2 security rating from the National Security Agency (NSA). This required that, when disk space was reused, the new user could not be allowed to see what was there before. To allow efficient reuse, Microsoft® added the VDL value in the Master File Table (MFT) entry for each file. The VDL value is in addition to the file length in the MFT as they are separate and distinct values in the MFT. When a file is created by Windows®, if the file size is known, the file is created with the file length equal to the size provided by Windows® and the VDL is set to zero. So, the VDL is always smaller or equal to the file length. As data is written to the new file, the VDL is increased to include the new data. In practice, the VDL value in the MFT is almost always the same as the file length value. When the two values are not the same, the file contains VDL slack.

VDL slack has been around for years, but it is relatively rare and only occurs on NTFS partitions. A survey of more than 10,000 partitions found that 50% of the drives had at least one file with VDL slack and on average there were ten files with VDL slack per partitions. Most of the time, the files with VDL slack are relatively large (over 1GB).

So why should an examiner learn about VDL slack? For a forensic examiner, a working knowledge of VDL slack, at a minimum, can be useful in limited cases. In some cases, a file containing VDL slack could contain data that is crucial to the case. An examiner that is ignorant of VDL slack runs the risk of appearing to be confused on the witness stand or confusing a VDL slack for an intentional attempt at hiding data. In either case, their testimony could be lacking in true understanding.

VDL, Valid Data Length, NTFS



Digital & Multimedia Sciences Section - 2015

C20 Steganography Analysis: Efficacy and Response Time of Current Steganalysis Software

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After attending this presentation, attendees will understand the basics of steganography including methods of hiding information, applications, practical use, and the current software used to analyze hidden data.

This presentation will impact the forensic science community by educating digital forensic investigators about a form of data obfuscation that is difficult to detect in a practical setting. Awareness of its presence, especially in the realm of organized crime and terrorism, is a first step in addressing the proliferation of potentially illicit data.

Steganography, Latin for “covered writing,” is a method of hiding information within digital media. In steganography, a message is embedded into a carrier or host file through means such as least-significant bit encoding, appending, or watermarking. Many file types, including audio, video, image, and text, can be embedded into carrier files of equally diverse formats. Today, steganography grows more complex with an increase in open-source applications, which hide data. As applications become more sophisticated, the need to detect, analyze, and stop the flow of dangerous information becomes more crucial.

Due to the increasing need for steganalysis software, companies like Backbone Security have developed programs that detect and decode steganography. StegAlyzer™ is a software program that detects and analyzes suspect files in order to aid law enforcement in the discovery of evidence that may condemn criminals. While there are four programs within the StegAlyzer™ suite, this investigation dealt with its Signature Search (StegAlyzerSS™) and Artifact Scanner (StegAlyzerAS™) due to their abilities to detect steganography applications and the steganography created from these applications.

Several questions were asked in this study: (1) does analysis time change with different carrier and message sizes and formats; (2) how well does StegAlyzerAS™ detect multiple steganography applications; and, (3) can StegAlyzerSS™ detect steganography from these applications? For the first question, a free application named GhostHost was selected to create steganography of differing sizes and formats. The open-source steganography applications chosen for the latter two questions were GhostHost, ImageSpyer G2, OpenStego, Steg, Steganography Studio, Open Puff, Silent Eye, Steghide, and Secret Layer.

StegAlyzerAS was able to identify signatures from five out of nine applications investigated in this study and StegAlyzerSS had a success rate of 33% in identifying steganography created by the applications. StegAlyzerSS was also used to analyze the duration of detection for image steganography created by GhostHost, a steganography appending, open-source application. Analysis time fell within the range of 0.15 and 0.25 seconds regardless of carrier or message file size. A one-way analysis of variance showed that different carrier and message sizes and formats had no statistical effect on analysis time.

Further studies should investigate StegAlyzer’s™ abilities compared to other steganalysis software, such as WetStone’s StegoHunt™ or open-source steganalysis software such as Steganography Studio. StegAlyzer™ is an invaluable tool for investigations of digital crimes, and requires competent analysts to be effective.

Steganography, Steganalysis, Investigation



Digital & Multimedia Sciences Section - 2015

C21 Generating a Corpus of Mobile Forensic Images for Masquerading User Experimentation

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After attending this presentation, attendees will understand the research outcomes and results of a recently conducted experiment identifying masquerading users on mobile devices using traditional and mobile forensic techniques.

This presentation will impact the forensic science community by providing forensic investigators/researchers with an example of successful human subject experimentation used in support of forensic analysis.

Periodic Mobile Forensics is a research project investigating user behavioral measurement on mobile devices by applying both traditional and mobile forensics processes. Forensic techniques have been applied to an enterprise mobile infrastructure where a mobile on-device agent named TractorBeam was utilized. This agent periodically collects changed storage locations from each device to allow for later image reconstruction and analysis. TractorBeam operates silently in the background during the normal use of the device. TractorBeam provides its collected data periodically to an enterprise infrastructure, which consists of a cloud- or server-based queuing service, a relational database, an analytical framework for running forensic processes, and a Mongo database for storing the analytic output.

Collaborating with Purdue University, a three-month experiment was performed where TractorBeam's operation in a simulated operational setting to identify masquerading users (i.e., users operating the devices other than the enterprise designated mobile device user) was evaluated. It was surmised that even if a masquerading user on an enterprise mobile device lacked malicious intent; this masquerader would still be undesirable to the enterprise. A set of human-subject volunteers were provided with the following: preconfigured mobile devices with cellular voice and data plans, with the TractorBeam agent pre-installed; a simple acceptable-use policy; and deceptive project background information to stimulate normal behavior. TractorBeam transmitted encrypted incremental backups to an Amazon® Web Services cloud instance. In the relational database, redundant information within the collected data was deduplicated, resulting in a 50-times reduction in storage size of the images. Most of those images were rebuilt and a series of developed forensic processes were executed on them, resulting in a Mongo collection of extracted audit data. As a result of the experiment, enough data was collected to successfully reconstruct 821 forensic images, extract over one million audit events, and perform masquerading user analysis. This study characterizes the collected corpus, the extracted audit events, and the performance of TractorBeam throughout the protocol. For masquerading detection, a set of features from the collected audit data was produced and associated those features to user sessions, or periods of device usage. Those sessions and their associated features are being used to train and evaluate a set of classifiers. This analysis is in progress and will be documented in a future paper.

Mobile, Masquerading, Experiment



Digital & Multimedia Sciences Section - 2015

C22 The Intersection of Physical and Digital Security and Investigations

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After attending this presentation, attendees will understand some basic principles and behaviors related to physical security and cyber security and how they interact.

This presentation will impact the forensic science community by providing a basic understanding of the common ground and responsibilities in physical and cyber security.

Many organizations treat physical security and cyber security as separate and distinct responsibilities. In fact, the intersection of these security responsibilities is critical to understand. Cyber security practitioners must interact, train, develop policies, and learn with physical security practitioners in order to adequately secure the environment in which they work.

Issues such as entry and search policies and how they relate to cyber security policies regarding external media can and do create conflict between these two groups of practitioners. Indeed, many recent cases have allowed exfiltration of sensitive data aided by the ability of the perpetrator to enter the facility with writable external media.

Additionally, physical security relies heavily on technology, the security of which these practitioners may not fully understand, allowing for physical breaches caused by weak cyber security policies in their security networks. They also require interaction with cyber security, digital forensic, and multimedia practitioners to assist in investigations into physical security incidents.

How this interaction must develop will be addressed as well as suggestions for policy improvement related to these two groups working together.

Physical Security, Digital Security, Cyber Security



Digital & Multimedia Sciences Section - 2015

C23 Memory Forensics: Reliable In-Memory Code Identification Using Relocatable Pointers

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After attending this presentation, attendees will understand that memory forensics requires the exact knowledge of code that is present in a physical memory in order to apply code-specific data-structure knowledge for extraction of forensic-relevant artifacts. Attendees will learn about the concept of the relocation table and its ubiquitous use in Microsoft® (MS®) Windows® executable files and how relocations can be used to create distinct fingerprints of executable code for extracting their in-memory pages from a memory dump. Using a comprehensive test of more than 50,000 executables, a large-scale experimental validation and statistical analysis of the methodology is presented. It shows that relocation tables are closely tied to the specific version of a code executed in memory and can be used to strongly identify (a piece of) code found during the analysis.

This presentation will impact the forensic science community by discussing a code fingerprinting technique that is able to identify a specific version of code in a memory. Unlike previous techniques, which require parsing of memory contents, disassembly of code, and identification of the address space of kernel and processes, this presentation will show that the derived fingerprints from relocations works reliably on a memory capture without any prior knowledge about the content of the memory. Prior techniques also have a limited focus on identifying operating system code and its specific version. The presented technique works on any relocatable code and is effective in identifying any MS® Windows® executable code.

The relocations are pointers in the code that need to be adjusted depending on where the executable is loaded into the memory. A relocation table in an executable file contains the locations of the pointers in the code, which are the offsets to the pointers from the beginning of the file. The table is only required during the file's load time and is then discarded from the memory. The presented technique extracts the relocation table from the file. It then divides an executable file into memory-size pages and uses relocations to create separate signatures (or fingerprints) for the pages of the executable file. A signature consists of offsets to pointers from the beginning of a page and the pointer values. The signatures are then used to search physical memory page-by-page to find the executable file pages with no intrinsic knowledge about memory content.

The presentation will address a number of additional challenges including paging and Address Space Layout Randomization (ASLR). Most code is, by default, pageable which implies that parts of the executable may not be physically present in volatile memory. This study shows that a *majority-wins* approach is sufficient to overcome this obstacle. ASLR presents a different challenge in that, on every execution, it loads the code starting from a randomly chosen base address and adjusts the necessary pointers in the loaded code. As a result, the representation of the code on-disk and in-memory diverge; however, this study identifies reliable invariants in this process, which enable the correct one-to-one mapping as well as the computation the actual run-time base address.

Code Fingerprinting, Memory Forensics, Relocation Tables



Digital & Multimedia Sciences Section - 2015

C24 Graphic File Carving Tool Testing

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After attending this presentation, attendees will be aware of the importance of tool testing and will have gained an understanding of the file-carving tool-testing process conducted within the Computer Forensics Tool Testing (CFTT) project.

This presentation will impact the forensic science community by increasing awareness of the impact tool testing has on informing the forensic community of tool capabilities and limitations. Test reports provide a foundation for toolmakers to improve tools, help users make informed choices, and provide interested parties with an overview of any anomalies found. This presentation will provide an overview of tools capabilities for carving graphics files and the CFTT test results produced for various tools.

The CFTT project has been researching and testing forensic tools capable of reassembling files from fragments in the absence of file system metadata, typically accomplished by searching an input for files based on content or header/footer file signatures. This presentation discusses all aspects of the testing process that are critical for producing a test report.

A summary for the test results of the graphic file carving tools examined will be discussed for each dd image created for performing the following test cases:

Nofill: Contiguous files with no other content between files.

Simple: Contiguous and sequential fragmented files with content separating the files.

Partial: Contiguous and partial (i.e., only a portion of the file is present) files.

Disordered: contiguous and disordered fragmented files separated by other content.

Braided: Contiguous and intertwined fragmented files.

Not-Shifted: Contiguous files that are aligned on sector boundaries.

Shifted: Contiguous files that are aligned on non-sector boundaries.

Each test report contains an associated table that identifies the test, the total number of files carved, and a classification based upon the data recovered. The categories classifying the recovered data for each test follow:

Viewable — Complete/Minor Alteration: Carved data appears to be unchanged from the original or the changes are so minor that the full content and most attributes of the video are maintained.

Viewable — Incomplete/Major Alteration: Include partial recoveries (i.e., only parts of the file are viewable), scrambled files in which the fragments are assembled incorrectly (making the content of the file unrecognizable), color shifts, and similar changes.

Not Viewable: Describes carved files that are not viewable, could not be opened, or had no content when opened.

False Positive: Reports a count of files that were incorrectly identified.

The presentation gives an overview of the CFTT process as applied to graphic file carving tools while providing information on file carving and scanning unallocated space enabling the recovery of specific file types with based-upon file signatures and various carving schemes.

The test reports are available from the Department of Homeland Security Cyber FETCH web site: <https://www.cyberfetch.org/>.

File Carving, Digital, Testing



Engineering Sciences Section - 2015

D1 Vehicle Seat-Adjuster Failure in Collisions: Unreliable Safety Devices

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The goals of this presentation are to: (1) inform forensic and field investigators for police, medical, and traffic safety organizations how and why various seat adjusters fail in various collisions; (2) teach methods of recognizing, analyzing, and documenting such failures; (3) explain how such seat failures affect vehicle occupant safety; and, (4) demonstrate via static and crash testing how alternative designs prevent such predictable failures.

This presentation will affect the forensic science community by providing information on largely hidden vehicle safety defects that affect the motoring public. Many severe injuries and deaths have occurred due to such failures, which are not adequately addressed by many government or auto-industry safety standards.

Seat adjusters are not merely devices to assist motorist comfort and fit within a vehicle. Vehicle manufacturers warn motorists to never adjust seat tracks, recliners, or other adjusters while vehicles are in motion and that seatbacks must be upright. They warn that any seat movement due to release of a seat adjuster can reduce the effectiveness of seat belts and increase the likelihood of injury; however, the vast majority of seats in vehicles are subject to exactly that kind of predictable unsafe seat movement due to inadequate crashworthiness of seat structures and seat adjusters. Most seats are too weak to be capable of meeting the requirements for occupant protection set forth in the vehicle owner's manual. There are no sensors or warnings provided in vehicles to indicate that seats adjusters are not fully engaged or that seatbacks are not adequately upright while the vehicle is in motion. These seats are legal to sell due to inadequate or non-existent seat safety and occupant protection regulations and resultant lack of meaningful testing. The enhanced potential for serious and fatal injury to front and rear seat occupants, including children, is clearly demonstrated.

Decades of research into vehicle crashworthiness and occupant protection effectiveness of various seat and belt combinations have demonstrated numerous random modes of seat adjuster failure in foreseeable collisions. Over time, seats have grown more complex, often adding vertical and tilt adjusters as well as recliners and latches that allow fold-flat capability of not only rear seats in utility vehicles but also some front seats. This study involves analysis of various seat adjuster failures during static, sled, and crash tests, as well as related forensic evidence found on front and rear seats and vehicle structures during field investigations. This includes: seat track-to-floor anchors; seat track adjusters; seat cushion-to-seat track separation; seat cushion vertical and tilt adjusters; recliner gear, bolt, and frame fracture; and, adjuster friction mechanism slippage. Seat adjusters also fail due to occupant and/or seat belt contact to unshielded controls. Inertial release of seat controls which are engaged merely by spring tension is also a risk. Bending and fracture of inadequate seat frames can contribute to catastrophic failure of one or more seat adjusters, especially in offset and angular impact loading. Angular loading of seats can often reduce static strength capacity by 30%-40% compared to straight rearward loading. One type of seat has demonstrated at least seven random failure modes, often with multiple adjuster failures in one collision. Seats with multiple adjusters or multi-function recliners often show greater potential for failure at lower occupant load levels than those achieved by more basic seats. Seat adjusters have also shown significantly reduced strength, crashworthiness, and reliability if they are placed in less than optimal mechanical engagement, within their normal operating range. All of these factors contribute to the unreliability of seat adjusters as an occupant safety device.

This study shows direct comparisons between evidence found in field investigations of front and rear seat failure and laboratory studies performed to demonstrate the circumstances of the collision. This includes sled testing with adult and child dummies, vehicle-to-vehicle crash testing, and vehicle-to-fixed object crash testing. These seat adjuster failures usually involve rear impacts but have also occurred in front, side, and rollover collisions. Seat adjuster failure modes, seat belt slackening effects, resultant occupant kinematics, ejection, and injury are analyzed in detail. This includes static testing as well as side-by-side dynamic testing of various seat, seat adjuster, and seat belt designs under identical collision circumstances.

Seat-Adjuster Failure, Rear Impact, Vehicle Occupant Injury



Engineering Sciences Section - 2015

D2 Enhanced Injury From Failure of Seats, Seat Belts, and Unsafe Vehicle Interiors

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The goal of this presentation is to inform investigators for police, medical, and transportation safety organizations of the hazards presented to vehicle occupants in collisions when seats and seat belts fail. Injuries can be enhanced when such failures allow occupant contact with hazardous structures within vehicle-occupant compartments. The combination of weak seats, slackened seat belts, and unpadding, force-concentrating structures presents extreme hazards to front and rear seat vehicle occupants.

This presentation will impact the forensic science community by educating field investigators, medical personnel, and safety researchers on how to better understand the hazards created when there is a combination of weak seats, slackened seat belts, and unpadding structures within vehicles. These hazards are not permitted in the front of vehicle-occupant compartments, and vehicle seat belts, airbags, and structural padding all combine to prevent such injuries in frontal impacts. No such protection is provided for rear impacts.

Front seat and belt failure in rear impact has been known to cause severe injury and death to both front and rear seat occupants. Several cases of predictable enhanced head and spinal injury have been discovered in vehicles where front seat and seat belt failure was combined with inadequately padded force-concentrating structures in the rear of vehicle occupant compartments. Causation of these enhanced injuries was proven by a combination of accident reconstruction, static testing, and dynamic testing. Prevention of injury by lightweight, technologically and economically feasible means was also demonstrated.

It has been known for decades that head impact into narrow, force-concentrating structures can result in skull fracture, brain injury, spinal injury, and death at much lower load levels than if those same structures were adequately padded or contoured to reduce their lethality. To reduce or eliminate such injuries, FMVSS 201, Occupant Protection from Interior Impact, has been part of the Code of Federal Regulations related to vehicle crashworthiness since 1967. It has required certain interior structures to be effectively padded, flattened, rounded, and otherwise made less lethal when contacted by vehicle occupants whether belted or unbelted. This is why dash panels and other vehicle interior trim is padded and why narrow, projecting controls and other structures have been eliminated in the forward areas of passenger vehicles.

Several contradictions to the well-known foregoing occupant protection principles have been discovered in late model vehicles, in which unpadding, force-concentrating structures have been placed into the path of vehicle occupant heads that are allowed to catapult rearward as a result of predictable front seat and belt failure. These dangerous conditions have occurred in vehicles produced by multiple manufacturers. There have been enhanced brain and spinal injuries and death caused to vehicle occupants that would otherwise not have occurred if the front seat had remained reasonably upright and, therefore, the belts and head restraints would have been effective at protecting the occupant; and if there had been energy-absorbing padding or elimination of the rigid, force-concentrating structures in the rear of the vehicle interior. Some of these force-concentrating rear interior structures are hazardous to all vehicle occupants, including those in the rear seats.

The reason these dangerous conditions are occurring in late model vehicles is that there are not occupant protection standards for rear impact, no dynamic seat or belt performance standards for rear impact, and there are no requirements for equivalent padded structures in the rear of vehicle interiors as those found in the front seating areas.

This study presents the forensic field investigations that have been conducted to identify these dangerous designs, as well as static and dynamic testing that discovered both the failure mode of the seats, head restraints and belts, as well as how to prevent such injuries. Improved seat and head restraint designs were incorporated in the static and dynamic testing to prevent any occupant contact with the force-concentrating structures. Alternative designs that would eliminate the force-concentrating structures in the rear of vehicles were also determined.



Engineering Sciences Section - 2015

This presentation will benefit the forensic science community by showing that these severe hazards exist, why they exist, how they create enhanced injury, and how those injuries can be prevented using lightweight, economically and technologically feasible means. Vehicle crash investigators will learn how to identify vehicle seat and belt failure, vehicle interior occupant contact witness marks, and associated forensic evidence that will allow more accurate assessment of occupant injuries in such circumstances.

Enhanced Injury, Seat and Belt Failure, Unpadded Vehicle Structure

D3 Motorcycle Helmet Translational and Rotational Head Injury Risk Measures Using H-III Head Impact Tests

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After attending this presentation, attendees will understand an experimental pendulum impact test method, using H-III biofidelic surrogate head and neck components, for measuring impact and rotational head injury risks of motorcycle helmet safety performance.

This presentation will impact the forensic science community by illustrating how this test method provides a means for efficiently evaluating helmet safety and injury risks related to both translational and rotational head/brain injury mechanisms by utilizing a human-responding H-III head and neck with a multidimensional pendulum impact device that also allows for full-face helmet safety evaluation not tested in current European and United States helmet certification standards.

Concussion and severe head injuries sustained by helmeted motorcycle riders are often related to both direct translational contact impact and brain rotational shearing mechanisms. Research by Hodgson (1975), Gennarelli, (1982), and Ryan (1994), among others, have addressed biomechanical injury risk levels of direct impact and rotational effects on humans.¹⁻³ More recent studies by O’Riordain (2003) and Antona-Makoshi et al. (2013) have also studied brain injury risk as functions of both direct impact and rotational effects.⁴⁻⁵ Unfortunately, current helmet certification standards do not test with Human Responding (HR) head forms nor do these criteria measure multi-directional impact conditions necessary to evaluate both direct contact impact and rotational effects. Also, the current helmet certification impact test devices do not enable measures of “full-face” head impact modes.

In this study, a 3D pendulum impact device, with an HR Hybrid-III head form and neck, is used to study multi-axial direct contact impact and head rotational effects on helmet safety. The H-III head is instrumented with a tri-axial accelerometer and multi-axial angular motion recording devices that allow measures of the Head Injury Criteria (HIC) and angular velocity versus angular acceleration levels for the helmeted head form when subjected to various impact conditions including full-face frontal loads. The test impact speeds and energy levels are comparable to current helmet certification test criteria. The figure below shows a sequence of photo clips taken from one of the pendulum impact tests of this study that involved a “full-face” 110 Joule (J) impact to a helmet when impacted at 25kph. The photos show, from left to right, film clips at: 50ms before impact; 12ms into the impact; and, 100ms after impact. The peak acceleration was 145 G’s at 11.4ms, and the 15ms calculated HIC was 758. The head pitch-rotation rate was 19.8r/s at 10ms and the angular acceleration was 11,210r/s/s at 11.5ms.



In addition to the above 25kph test, another full-frontal impact test was conducted on the same model helmet but at an impact speed of 28.4kph to examine helmet safety performance at a higher 150J impact energy level. In this test, the peak resultant acceleration was 266 G’s at 4.6ms, and the 15ms calculated HIC was 2009. The test HIC levels of 758 and 2009 both violate the NHTSA recommended head injury limit of 700 and indicate danger of a severe head injury. The head pitch-rotation rate in this second impact test was 28.9r/s at 4.9ms and the angular acceleration reached 13,776r/s/s at 4.1ms. The measured acceleration-time data from the above two tests were at the upper tolerance level for Acute Sub-Dural Hematoma (ASDH) and Sub-Arachnoid Hematoma (SAH) as predicted by Auer et al. (2001) based on head injury reconstructions from 25 fatal pedestrian accidents.⁶ With regard to Diffuse Axonal Injury (DAI) effects, the rotational acceleration measures for the first test were indicative of a classical concussion level as defined by Gennarelli. The much higher rotational acceleration measures for the second test were indicative of a “mild” DAI level as defined by the Gennarelli reference.



Engineering Sciences Section - 2015

Finally, the above ASDH, SAH, and DAI injury risk findings derived from the direct contact impact and rotational measures obtained from the two full-frontal impact tests on the helmet model shown in the figure above were consistent with the medical record descriptions related to severe brain injuries sustained by a motorcycle rider wearing the same model helmet in an actual accident. Damage to the accident helmet indicated primary impact to the chin bar and forehead full-frontal region of the helmet, like the region tested on the exemplar helmets. The correlation of the tests with the actual accident injuries suggests that the pendulum impact apparatus, with the human responding H-III head form and H-III neck, provide a reasonable method for assessment of overall helmet safety performance as related to both direct contact impact and rotational acceleration forces in all modes of impact, including full-frontal, and improves on current helmet certification test limitations.

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Head Injury, Helmet Testing, Impact Analysis



Engineering Sciences Section - 2015

D4 Ejection of Belted Vehicle Occupants Due to Seat and Belt Failure in Rear Impacts

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The goal of this presentation is to inform attendees how and why restrained vehicle occupants can be partially or completely ejected from vehicles as a direct result of seat failure which can also cause failure of vehicle-anchored seat belts by causing excessive belt slack.

This presentation will impact the forensic science community by illustrating how seat belts can be defeated by weak seats in vehicles. Defeat of the seat belts can lead to the partial or complete ejection of initially restrained occupants.

Seat belts, seats, and head restraints are equally critical components of vehicle occupant protection systems. Based on human tolerance testing, Federal Motor Vehicle Safety Standard (FMVSS) 210 requires lap/torso belts to withstand 6,000 pounds of forward static load. Belt webbing and anchorages therefore are rarely overloaded in survivable front and side collisions. At 30mph ΔV , only 1" of belt slack increases vehicle occupant injury by 50%, and 4" of belt slack is equivalent to being unbelted. Pretensioners may minimize belt slack in frontal impacts where airbag deployments occur. Pretensioners rarely activate in rear impacts and have proven to be inadequate to provide belt effectiveness if significant seat deformation occurs.

Vehicle manufacturers warn against adjusting seats when vehicles are in motion because of increased injury risk and decreased effectiveness of seat belts. FMVSS 207 requires less than 300 pounds static load capacity for seats and does not consider human tolerance to impact or presence of an occupant in the seat. No occupant protection requirements exist for rear impact or rollover. Therefore, seats, head restraints, and seat belts predictably fail in moderate rear impacts and rollovers. When a seat fails rearward, head restraints cannot function and dangerous amounts of slack are created in any vehicle-anchored seat belt system, rendering the belt ineffective in exactly the manner warned about by manufacturers. Seven to 14 inches of static belt slack is created solely by recline of the front seat. Many vehicles allow ten or more inches of additional rearward belt buckle displacement. Any of these belt-slackening mechanisms created by seat failure will allow the lap belt to easily slip off the pelvis, negating any effective restraint. Mis-positioned lap belts cause serious or fatal injury from submarining as well as partial or complete ejection in subsequent collision or rollover. In 1967 FMVSS 209.4.1, it is required that lap belts remain on the pelvis at all times during collision or rollover. Despite objection from the National Transportation Safety Board (NTSB) and others, and decades of consistently poor crash test results proving this danger, in 1998 vehicle manufacturers successfully petitioned the United States Department of Transportation to rescind this critical dynamic seat belt performance requirement. No conceivable benefit to motorist safety occurred.

This study shows how and why predictable seat/head restraint failure and resultant dangerous seat belt slackening have allowed partial and complete ejection of belted occupants in crash testing and real-world collisions and rollovers. There have been numerous crashes and rollovers investigated for this study where belts were properly worn and seats were upright prior to the crash. The belts remain buckled but due to seat failure and belt slack the occupant was ejected either into the rear interior, other occupants, and/or completely out of the vehicle. In several instances, lighter-weight occupants, or similar-size occupants who loaded into stronger seat structures, were effectively restrained and protected by seats and head restraints that remained upright and belts that therefore remained effective. Published crash and sled test research since the 1960s has consistently demonstrated that when seats fail, no effective lap belt loads are generated prior to occupant contact with either the rear seat, other occupants, and/or ejection from the vehicle.

Effective countermeasures which prevent seat and belt failure are presented in this current study, utilizing recent dynamic side-by-side testing of crashworthy seats and head restraints, resulting in belts that function as intended. This is compared with **Original Equipment Manufacturer (OEM)** collapsing seats, head restraints, and resultant slackened seat belts that allow ejection. There is also comparison of biofidelic dummies having articulating pelvic and leg structures with non-biofidelic dummies that artificially snag lap belts.

The forensic community will benefit from this presentation by learning how to recognize forensic evidence associated with seat and belt failure, including deformation and witness marks on vehicle interiors, seats, and belts. Investigators will learn how and why ejection of belted occupants readily occurs in passenger cars, vans, and light trucks. Fatal injuries have occurred to such occupants while those in upright seats beside them were unhurt.

Vehicle Occupant Ejection, Seat Failure, Seat Belt Failure

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Engineering Sciences Section - 2015

D5 Vehicular Rear-Impact Accident Reconstruction: Validation by Crash, Sled, and Static Testing

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The goal of this presentation is to inform attendees of details concerning how accurate reconstruction of vehicular rear impacts is performed and how validation of calculated results has been determined via repeatable scientific testing.

This presentation will impact the forensic science community by providing knowledge about the proper manner in which to investigate, analyze, and reconstruct rear-impact collisions. These are common collisions that are inadequately represented in research efforts and safety emphasis on both governmental and automobile manufacturing levels.

Rear impacts are among the most common crash modes, yet they receive relatively little attention from government safety regulators and auto manufacturers. With relatively rare exceptions, the only common rear crash testing done is per Federal Motor Vehicle Safety Standard (FMVSS) 301-75 to evaluate fuel system integrity. Such tests typically utilize rear moving barriers with little to no instrumentation and net impact severity tends to be only about 60% or less of typical frontal impacts at the same contact velocity. Such impacts rarely are conducted at angular principle direction of force. There are no occupant protection or dynamic head restraint requirements for rear impact and static strength requirements for seats are less than 10% of those for torso belts. Rear bumpers often are made of very weak composite materials and plastics which contribute to override and intrusion of rear cargo compartments and occupant survival space. As a result, rear impacts of otherwise readily survivable severity commonly result in serious or fatal injury to front and rear occupants, including children, due to failures of front and rear seats, slackened seat belts, and occupant compartment intrusion.

Compounding the foregoing relative lack of information concerning vehicle rear crush characteristics is the fact that almost all vehicles utilize front engines with firewalls and structural bulkheads between the engine and passenger compartments, while the equivalent rear cargo areas are full of air. Utility and van-type vehicles typically have nothing between the seats and the cargo area and most passenger cars tend to have open bulkheads between the rear seat and the trunk. Therefore, the risk of rear intrusion tends to be greater than frontal intrusion in a given impact severity, particularly if there is override of the rear bumper. Approximately two-thirds or more of rear structural integrity is carried in the frame and bumper areas.

This study involves various types of vehicles and impact severities in which a combination of crash, sled, and static testing was conducted on a given vehicle design. The various tests served to validate the accident reconstruction analysis. This research showed that in many instances, "default" stiffness coefficients utilized by common accident reconstruction computer programs tend to overestimate actual rear impact severity. The similarity between predicted crash pulse amplitude and duration is also demonstrated via direct comparison between calculated values and test data. Static testing of seats can also serve to validate calculated crash severity values by determining the seat deformation caused by occupant loading under known conditions with the evidence found in crashed vehicles.

This study demonstrates the forensic science involved in determining the accurate severity of foreseeable rear collisions involving several common types of vehicles. Current methods used to determine rear crash severity may overestimate collision velocities, thus giving the incorrect impression that severe injuries or death were caused in much higher-speed crashes than actually occurred. The incorrect conclusion is that vehicle structures and seats are actually performing far better than they actually do in the real world. The safety implications for motorists in millions of reasonably similar vehicles under similar impact conditions is clear. The design and testing process (and lack thereof) used to develop these vehicles allow common failures of seats, seat belts, and rear structures. The testing shown in this study clearly demonstrates that what should be among the most readily survivable crash modes commonly results in severe or fatal injuries to vehicle occupants. These failures could be prevented if properly tested prior to initial vehicle production.

Accident Reconstruction, Rear Impact, Crash and Sled Testing

D6 Crash Test Verification of Offset Rear-Impact Accident Reconstruction and Crashworthiness-Design Concepts

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After attending this presentation, attendees will be presented with a vehicle-to-vehicle crash test methodology for validating physics-based reconstruction of offset rear-impact override crush accidents, with verifications of occupant injury analysis, a demonstration of safer available alternative vehicle designs, and the benefits of using basic, accepted crashworthiness-design concepts.

This presentation will impact the forensic science community by explaining how the crash test method provides a means for scientifically verifying accident reconstructions with correlation of injury measures, as well as demonstrating safety improvements and reduced injury risks related to following well-established, good crashworthiness-design concepts.

In this study, a physics-based accident reconstruction was performed on a rear-impacted, four-door sedan vehicle that was struck off-set to the right rear half by a Sports Utility Vehicle (SUV) that over-rode the rear bumper and trunk floor structure. This allowed the sedan's weaker sheet-metal fender material to easily crush and allow displacement of the rear shock tower structure into the right-rear occupant space. The sedan had two average-size males in the front and three smaller rear seat occupants. During the crash, the occupied front seats yielded rearward into rear occupant space. Simultaneously, intrusion of the right-rear occupant space by the shock-tower structure shoved the right rear 13-year-old forward toward the rearward collapsing front seat and occupant. As a result of the above occupant space violations, the right-rear occupant received a severe disabling head injury. In contrast, the front occupants and other two rear occupants, who were located farther from the right-rear penetration, did not receive any permanent injuries. In order to understand the engineering parameters that led to the injury of only the right-rear occupant, a reconstruction of the accident was performed by using energy and momentum balance principles. The reconstruction analysis (see Figure 1 diagram) indicated that the SUV impacted into the stationary sedan right-rear at 54kph.

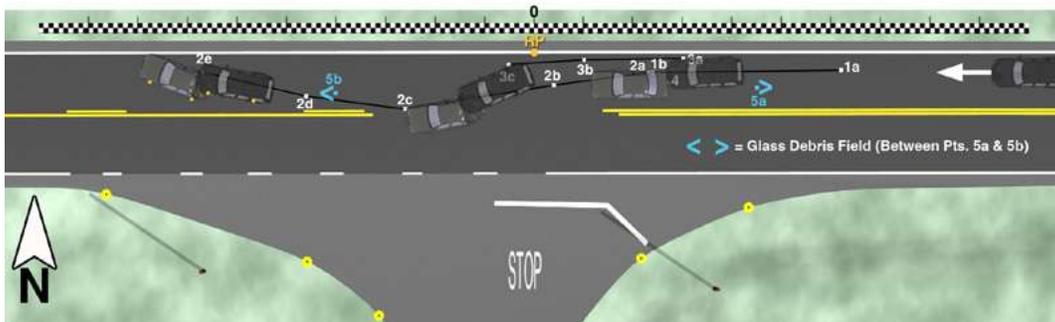


Figure 1: Reconstruction Diagram of Right Rear 50% Off-Set Overlap Rear Impact of SUV into 4-Door Sedan.

A vehicle-to-vehicle confirmation crash test, with instrumented surrogates, was run to verify the reconstruction impact speed and show consistency of surrogate injury measures with accident occupant-injury levels as further proof of the analysis. Figure 2 shows a comparison of the actual accident vehicle damage with the sedan used in this crash test. Figure 3 shows the pre-test positions of the rear seated surrogates and also a photo clip from the high-speed interior camera recorded at 173 milliseconds after impact. The 173ms clip shows the left side of the right-rear surrogate head (red chalked) turned toward the right and striking the back of the right-front surrogate head (blue chalked), and the front seat headrest, due to the inertial rearward loading of the front occupant while the right-rear surrogate was being shoved forward from the rear intrusion. The Head Injury Criteria (HIC) measures of this test, like the accident, indicated that only the right-rear surrogate received a severe HIC level of 1,217.4 (i.e., in violation of the National Highway Traffic Safety Administration (NHTSA) injury level), with a dangerously high-peak G resultant head load of 281 G's.

In addition to the above reconstruction confirmation test, two other tests were also run with the SUV impacting offset into the right-rear half of a four-door sedan at 54kph. One test was run with a crashworthy modified exemplar of the accident vehicle to demonstrate how good crashworthiness redesign of the **Original Equipment Manufacturer (OEM)** vehicle could have been utilized, with state-of-the-art concepts recommended in the 1960s and 1970s, to eliminate the right-rear occupant head injury caused by the space-intrusion dangers of the accident vehicle design.¹⁻⁴ Another repeat 54kph test was also run, but in this test the struck accident vehicle design was replaced by a different commercially available model year OEM European designed four-door sedan, which demonstrated how a vehicle with well-designed barrier resistance and stronger less-rearward yielding front seats could have also eliminated the injury sustained by the right-rear teenager. In both of these tests, the front and rear intrusion, and violation of the rear-seated occupant safety zone, was reduced such that none of the rear-seated surrogates had injurious head contact with the front seats or front occupants.



Figure 2: Comparison of Accident Vehicle Right Side Damage (Blue Vehicle) with Confirmation Test Vehicle.



Figure 3: Pre-Test Surrogate Positions (Left Side Photo) and Confirmation Test Impact Intrusion Photo at 173ms.

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Accident Reconstruction, Crash Testing, Crashworthiness Design



Engineering Sciences Section - 2015

D7 Biomechanical Evaluation of Pediatric-Inflicted Head Trauma

*John D. Lloyd, PhD**, 32824 Michigan Avenue, San Antonio, FL 33576; and *William E. Lee III, PhD*, University of South Florida, College of Engineering, Tampa, FL 33620

After attending this presentation, attendees will have an appreciation for biomechanical evaluations that can be applied in order to objectively determine the mechanical causation of inflicted vs. accidental head trauma in a pediatric population.

This presentation will impact the forensic science community by informing attendees that in cases of head trauma in infants and young children, it is often alleged that the parent or care provider struck the child victim with their hand or with or against a hard surface, causing serious and sometimes fatal bodily injury. By performing a systematic biomechanical evaluation, this presentation will quantify the risk of head and brain injury associated with such mechanisms of injury.

Methods: Two adult male investigators served as participants; neither had any physical disabilities that might affect their performance. An instrumented Child Restraint and Airbag Interaction (CRABI) -12 biofidelic mannequin, of height 0.75m and mass 10.0kg, was utilized as the infant surrogate. A 500G piezo-electric tri-axial accelerometer was installed at the Center of Mass (CoM) of the CRABI headform along with a tri-axial digital gyroscope, in accordance with the **Society of Automotive Engineers** (SAE) J211.

Eight conditions were investigated, including striking the head with both an open hand and baseball bat as well as impacting the mannequin head against interior wall structures, both on and between supporting studs. Such walls were manufactured for the purpose of this study using 2"x4" white wood with both 16" Outside Corner (OC) and 24" OC separation between vertical studs, over which 3/8" and 1/2" gypsum drywall was fastened in accordance with state building codes. Participants were instructed to impart gentle, moderate, and vigorous impacts on the infant surrogate, repeated five times for each of the eight conditions, resulting in a total 240 trials.

Results: Data from both the analog linear accelerometer and digital gyroscope was acquired at 10,000Hz, per channel, using LabVIEW™ and filtered in MATLAB® using a phase-less 4th-order Butterworth filter with a 1650Hz cutoff frequency, per SAE J211. Head linear acceleration and angular velocities were recorded, angular acceleration was derived, peak magnitude values were calculated, and Head Injury Criterion (HIC15) computed. Descriptive statistics are reported across repeated trials and participants.

Conclusions: Forces associated with linear acceleration are typically responsible for focal injuries, including subgaleal hematomas, lacerations, skull fractures, and brain contusions, whereas forces associated with rotational inertia account for diffuse injuries, including concussive brain insult, axonal injury, and bridging vein rupture, leading to space-occupying subdural hematomas. Findings from this biomechanical evaluation can be applied to objectively determine the mechanical causation of inflicted trauma from claimed accidental reasons.

Biomechanics, Head and Brain Injury, Pediatrics



Engineering Sciences Section - 2015

D8 Low-Energy Bone Fractures: Part III

David Pienkowski, PhD, University of Kentucky, AB Chandler Hospital, Rm MN 564, Lexington, KY 40536-0298; and Hartmut Malluche, MD, University of Kentucky, Chandler Medical Center, 800 Rose Street, Lexington, KY 40536*

The goals of this presentation are to raise attendees' awareness of bone microdamage, illustrate how above-normal levels of bone microdamage are linked to altered load-bearing mechanical competence, and thereby help the forensic investigator understand how excess levels of microdamage may partially explain low-energy bone fractures.

This presentation will impact the forensic science community by providing a partial explanation for seeming incongruities among low-energy events, reconstruction-derived traumatic event energy (or force) amplitudes, and bone fractures. Bone fractures accompanying low-energy trauma are perplexing to the forensic investigator. Ostensible disparities between the energy (or force) amplitude of the traumatic event and the level of injury can often lead to uncertainty regarding the facts of the event or the validity of the reconstruction.

Application of service loads to engineering structures creates stresses in the constituent materials. These stresses generate material defects which, if accumulated, result in reduced structural stiffness, diminished load-bearing capacity, and may eventually lead to catastrophic failure.

Bone is unique among engineering structures because of its capacity for self-repair of defects caused by physiologic loading. These defects are known as "microdamage" and consist of observable microcracks and not-so-readily observed diffuse damage. Microcracks are quantified by crack number, crack length, and number/length density distributions. Bone microdamage is self-repaired by bone turnover, a complimentary set of on-going cellular events consisting of bone resorption (targeted local destruction of bone with microdamage) followed by bone matrix formation (production of new organic material for subsequent mineralization and maturation into mechanically competent bone). Normal bone turnover prevents damage accumulation from reaching critical levels and confers bone with the biomechanical properties needed to resist excess deformation and avoid fracture.

Rates of bone formation and resorption must be maintained, from both relative and absolute perspectives, to sustain these biomechanical properties. Relative rates of resorption and formation must be equal to avoid losing or gaining bone mass. Absolute rates of turnover must also be controlled to avoid premature bone resorption or prevent microdamage accumulation. Specifically, if the rate of bone turnover is inadequate, then the rate of microdamage repair is less than the rate of microdamage creation and the net amount of microdamage increases. Unrepaired microcracks will increase in length while bone is physiologically stressed and these cracks will eventually coalesce to form larger cracks that could become macroscopic. Thus, bone with above-normal levels of microdamage will have a reduced load-bearing mechanical competence, a diminished injury threshold, and may catastrophically fail (fracture) when subjected to low-energy trauma.

The forensic investigator should appreciate the variety of bone-quality factors, including microdamage, which collectively govern the mechanical competence of this organ. To reconcile the seeming disparities between fracture and low-energy trauma, the forensic investigator should search for mechanisms linked to abnormal bone turnover. This search should begin with a review of the subject's medical history because low bone turnover is a "silent" phenomenon that accompanies particular diseases or therapies. Excess consumption of certain anti-osteoporosis medications, for example, is known to cause low bone turnover. Advanced age also is associated with low bone turnover. A more thorough investigation requires a surgically procured bone sample upon which histological processing and quantitative microscopic analytical measurement methods are employed to quantify the distributions of mean crack number, length, and density.

The material quality analytical approach described in this and related prior "low-energy bone fracture" presentations offer the forensic investigator a general tool for reconciling low-energy traumatic events with seemingly incongruous injuries in other non-osseous tissues or organs.

Low-Energy Bone Fracture, Human Injury Analyses, Accident Reconstruction



Engineering Sciences Section - 2015

D9 Biomechanics of Short Falls in Children

John D. Lloyd, PhD, 32824 Michigan Avenue, San Antonio, FL 33576*

After attending this presentation, attendees will comprehend the risk of traumatic head and brain injuries in a pediatric population associated with short household falls.

This presentation will impact the forensic science community by informing attendees of the mechanisms of head and brain injuries in pediatric populations and the risks associated with short falls.

Introduction: This study involved systematic assessments of falls from heights ranging from two to six feet onto varying flooring surfaces including concrete, linoleum, apartment-grade carpeting with underlay, Berber carpet with underlay, commercial carpeting without pad, and wood laminate.

Methods: A Child Restraint and Airbag Interaction (CRABI) -12 biofidelic mannequin (29.5in/22lb) and a Hybrid III three-year-old (37.2in/35.65lb) biofidelic mannequin were used during this systematic evaluation of short falls. A tri-axial piezo electric accelerometer, calibrated and certified by PCB® Piezotronics, was installed at the center of mass of the CRABI headform, in accordance with convention described in the **Society of Automotive Engineers** (SAE) J211 along with an InvenSense® tri-axial digital gyroscope. Still photography and high-speed video were used to record the fall sequences.

A height-adjustable platform was used to represent the fall surface. The platform has trap doors, which are held in place by electromagnets. Interruption of power to the electromagnets causes the sprung trapdoors to open instantaneously, thereby initiating the fall sequence. To investigate biomechanical mechanisms of injury associated with short falls in children, 175 trials were completed.

Data from the tri-axial piezo-electric accelerometer, mounted in the head of the biofidelic mannequin, were acquired at a rate of 10,000 samples per second using LabVIEW® software. Data from the Microelectromechanical System (MEMS) tri-axial gyroscope were acquired at 3,800Hz per channel. Raw data were displayed on-screen for visual verification. These data were analyzed using MATLAB®, including Fast Fourier Transform analysis to visualize the frequency spectrum of the data, followed by phase-less filtering using a 4th-order low-pass Butterworth filter with a cut-off frequency of 1650Hz. The peak magnitude value of head linear and angular acceleration components were derived and Head Injury Criterion (HIC) computed.

Results: Both the CRABI-12 infant representative and Hybrid III toddler representative exceeded injury threshold values from a fall height of only two feet (61cm), based on peak magnitude linear acceleration and HIC, which indicates that such short falls can cause substantial head/brain injuries in young children.

Conclusions: Results clearly show that household short falls events do exceed established thresholds of injury based on linear and angular accelerations. Furthermore, kinematic measures associated with household short falls are comparable to high-risk adult sporting activities, such as a typical boxing punch or a concussive tackle in college football. Based upon these evaluations and associated results presented herein, it is the expressed conclusion that household short falls present a very real and significant risk of head and brain injury among infants and toddlers.

Biomechanics, Pediatrics, Falls



D10 An Examination of Dynamic Mechanical Properties and the Strain State Evaluation Technique of Soft Tissue

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After attending this presentation, attendees will understand the evaluation method of the dynamic mechanical properties of soft tissue.

This presentation will impact the forensic science community by providing the physical model of personal injury evaluation that takes into account the dynamic mechanical properties of soft biological tissue.

To clarify the presence or absence of murderous intent in cases of murder, attempted murder, and injury, the relationship between personal injury and external forces has been extensively studied for a long time in university forensics medicine classrooms and by the police. As a result, it has been revealed that the presence of soft tissue such as skin and muscle has a great effect on the presence or absence of bone fracture resulting from a dynamic load; it is therefore important that the physical model used for personal injury considers soft tissue. Since the technique of measuring dynamic mechanical properties of soft tissue does not yet exist, the effect has not been quantified to date. In addition, data regarding the stress-strain properties and viscoelastic properties of skin are essential to evaluate skin damage caused by external forces, but it is difficult to accurately measure and evaluate the strain in the skin. Because the handling of soft biological tissues is difficult, soft tissue is usually not taken into account in the physical model of personal injury evaluation. For example, there is the Hybrid III 50th dummy that is used to evaluate personal injury in car accidents. This is used not only in automotive frontal collision tests, but also in the evaluation of human body damage caused by impact, drop, fall, vibration, etc.; however, the presence of soft tissue is not considered. The evaluation of safety standards for life-support robots conforming to the International Organization for Standardization 13482 uses a “dummy skin” that utilizes substitute materials to reproduce the mechanical properties of soft tissue. Dummy skin uses human skin gel as artificial subcutaneous tissue and is an alternative material used for considering the influence of a static external force on soft tissue. Currently, the evaluation of human skin gel remains primarily a static evaluation technique and it has been questioned whether the dynamic mechanical properties of soft tissue with respect to dynamic loads such as those that occur during personal injury can be accurately reproduced. It becomes necessary to consider alternative materials to reproduce the stress-strain properties of skin and the dynamic mechanical properties of soft tissues, and the goal is to quantify these properties for the development of alternative substitute materials.

In this study, the dynamic viscoelastic properties of soft tissue was measured using a rheometer and a durometer and the evaluation methods were studied. The rheometer has so far been used to measure the dynamic viscoelastic properties of industrial materials having fluidity, such as grease; however, improvements in recent years have resulted in its use for soft solid materials such as rubber, resulting in this study attempting to measure the viscoelastic properties of skin using a rheometer. In addition, the transformation of artificial skin was measured using a strain gauge and the evaluation technique of the stress-strain state was measured. Generally, the strain gauge, typically used to measure small deformation strain, cannot adapt itself to the large deformation field of soft tissue. In order to correctly evaluate the strain state of the skin surface, measurement was therefore attempted using a bonding agent as a material to promote reduction in the strain between the strain gauge and the artificial skin. It became clear from comparing the dynamic mechanical properties of the human arm hardness-equivalent artificial skin that its dynamic viscoelastic properties were considerably different from those of real skin. When the tension of the skin surface of humans was reproduced in the artificial skin, values equal to the result of a measurement of the human arm were obtained. Based on that result, it was suggested that the tension of the skin surface has a significant impact on the mechanical properties of human skin. It was revealed that when artificial skin is used in personal injury evaluation, not only is the material important but also the skin tension.

Soft Tissue, Dynamic Mechanical Properties, Rheometer



Engineering Sciences Section - 2015

D11 Biomechanical, Mechanical, and Epidemiologic Characteristics of Low-Speed Rear-Impact Collisions

Michael Freeman, MD, PhD*, 425 NW 10th Avenue, Ste 306, Portland, OR 97209

The goal of this presentation is to provide a reliable description of the mechanical, biomechanical, and injury-risk characteristics of minimal and no-damage rear-impact collisions based on an analysis of epidemiologic data.

This presentation will impact the forensic science community by demonstrating a validated methodology for investigating and describing the dynamic vehicle and occupant characteristics of minimal and no-damage bumper-to-bumper, rear-impact collisions as well as the injury risk associated with such collisions.

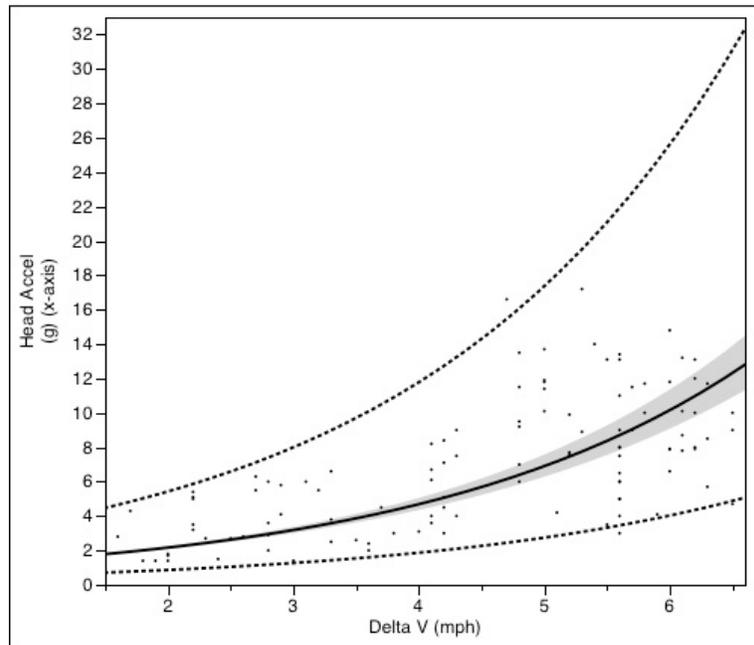
Bumper-to-bumper, rear-impact collisions are unique events that can result in substantial occupant forces while leaving little physical evidence of the collision. Highly elastic bumper components protect the vehicle from visible damage in ≤ 10 mph (16km/h) closing speed and ≤ 8 mph (13km/h) delta V collisions. Precise reconstruction of the delta V of the target vehicle is challenging, as a 3mph delta V crash can leave the same degree and pattern of physical evidence as a 7mph delta V crash (little to none); however, the energy of the latter collision is more than four times greater than the former. Absent multiple full-scale reenactments of the collision, commonly employed reconstruction methods, such as a Mass, Energy, and Restitution (MER) or conservation of momentum calculation, are error-prone because of the speculative basis of some of the critical input variables (closing speed, coefficient of restitution, etc.).

Regardless of the shortcomings, such reconstructions are common in litigation involving minimal damage collisions. This practice is in part due to the fact that it is not uncommon to see claims for injuries in such collisions that are significant, including claims of spinal disk derangement resulting in surgery. The most common purpose of the reconstruction is to estimate vehicle forces, given in terms of acceleration (g). Vehicle acceleration is often used for an inference regarding occupant forces, which are often used to estimate the injury risk of the collision. The relationship between the vehicle delta V and acceleration is based on an assumption regarding the duration of the crash pulse. The method of estimating the occupant acceleration is often unspecified, and typically estimated as equal to two or more times the average vehicle acceleration. The estimate of injury risk from the delta V is most commonly based on the lack of significant injury reported in full-scale human volunteer crash testing and the estimate of injury risk from assumed occupant acceleration is based on comparisons with reported accelerations of daily activities and other non-injurious events.

There are available data sources for estimating all of the above-mentioned parameters of rear-impact collisions, thus eliminating the speculation inherent in the approach described above. Several researchers have examined and described vehicle dynamics associated with low-speed rear-impact collisions via information collected contemporaneously by data event recorders in the vehicles and matched the vehicle information to injury outcome and duration.^{1,2} Other researchers have described the results of volunteer crash testing such that the range of occupant acceleration at a specific delta V can be estimated.³ The purpose of the present investigation is to describe the range of vehicle and occupant dynamic and injury response to a range of rear-impact-related delta Vs associated with minimal and no-damage collisions (3-7mph delta V (5-11km/h)), based on an analysis of previously published observational data. The statistical methods used for the continuous variable outcomes were regression utilizing a general linear model and for dichotomous outcomes binomial logistic regression (JMP®, Version 11).

Delta V (mph, (km/h))	Peak vehicle accel. (g)	Peak occupant head acc. (g)	Crash pulse duration (msec)	Any injury %	Injury >6 months %	Cervical disk injury %
3 (4.8)	6.1 (5.5, 6.7)	4.4 (3.5, 5.9)	55.8 (52.0, 59.6)	24.4 (14.8, 37.4)	1.9 (0.5, 6.8)	1.6 (0.3, 7.3)
4 (6.4)	6.7 (6.2, 7.2)	5.4 (4.5, 7.0)	61.3 (57.9, 64.7)	29.3 (19.6, 41.5)	2.5 (0.7, 8.2)	1.9 (0.5, 7.9)
5 (8.0)	7.2 (6.8, 7.7)	7.2 (6.0, 9.1)	66.7 (63.6, 69.8)	34.9 (25.0, 46.4)	3.5 (1.2, 9.8)	2.5 (0.7, 8.6)
6 (9.6)	7.8 (7.4, 8.2)	10.6 (7.9, 15.9)	79.2 (62.2, 75.2)	40.9 (30.4, 52.3)	4.7 (1.8, 11.8)	3.0 (0.9, 9.3)
7 (11.2)	8.3 (7.9, 8.7)	13 (10, 20)	77.6 (74.6, 80.6)	47.2 (35.5, 59.1)	6.4 (2.7, 14.2)	3.7 (1.3, 10.3)

The above table depicts the results of the analysis of event data recorder-reported findings and the associated medically observed outcomes in 114 occupants exposed to rear-impact collisions. The reported vehicle accelerations were based on a 3g recording threshold, which may have affected the speed change calculation. The risk of cervical disk injury is based on the classification of an injury as a “WAD III,” which includes findings of neurologic involvement. Peak head acceleration is based on the results of 93 volunteer crash tests. The values for occupant acceleration at 7mph delta V are estimated due to excessive variance. The values in brackets are the 95% confidence interval for the point estimate.



The above graph demonstrates the curvilinear relationship between vehicle delta V and peak head acceleration in 93 human volunteer crash tests. The shaded section is the 95% confidence interval, and the dotted line is the predictive interval depicting the likely range of accelerations in the general population.

This presentation will demonstrate the mechanical, biomechanical, and injury risk characteristics of minimal and no-damage rear-impact collisions.

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Rear-Impact Collision, Crash Reconstruction, Biomechanics



Engineering Sciences Section - 2015

D12 Biomechanics of Spinal Injury

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After attending this presentation, attendees will better understand the forces and accelerations required to produce “whiplash” cervical soft tissue injuries.

The presentation will impact the forensic science community by quantifying a method to assess the potential for spinal soft tissue injuries based on the known scientific literature on the strength of the affected biological materials.

Over the years, there has been considerable argument over the occurrence of soft tissue injuries during low-speed collisions. Although a variety of ailments have been claimed during these low-speed impacts, only cervical soft tissue injuries are addressed in this presentation. Some of the most common unrelated injuries claimed include rotator cuff injuries, carpal tunnel injuries, Temporomandibular Joint Injuries (TMJ), Anterior Cruciate Ligament (ACL) injuries, and thoracic and lumbar spine injuries. Cervical soft tissue injuries are commonly referred to as “whiplash.” The range of complaints for this type of injury includes headaches, numbing and tingling of the arms, pain, and loss of range of motion.

In this presentation, the other types of injury claims are disregarded simply because they have no merit in low-speed rear-end collisions because a biomechanical effect cannot be attributed to them. Cause and effect must be established in order to proceed with biomechanical calculations. In this context, several researchers have studied the “whiplash” phenomenon. Clemens and Burrow determined that there are three areas of the spine susceptible to injury, at the base of the cranium and C1, the region of C3 and C4, and the region of C6 and C7. Their tests in the range of 17 miles per hour and decelerations of 30gs correlated to serious injury involving 90% of ruptures and 30% of fractures. They found that the limit of vulnerability to injury to be 13mph and 13gs. Damask places the vulnerability to injury between 13mph and 16mph and 13gs to 15gs to include isolated fissures of discs without injury to the ligaments or bones. The known scientific literature indicates a high probability of cervical soft tissue injury at accelerations of 17gs or greater.

From tests performed on biological materials, Yamada determined the lowest value of the ultimate strength of discs to vary from 0.20 Kg/mm² to 0.24Kg/mm². Calculations can be performed on the number of discs, a variation in the size of the disc structures, the weight of the individual affected, and various speed changes to determine the probability of injury to the cervical spine. This type of parametric analysis lends itself well to assess and correlate potential injuries to the work of other researchers that have established well-recognized limits on vulnerability to injury. Charts and graphs will be presented to show the analysis and the underlying basic equations used to formulate the problem.

Whiplash, Biomechanics, Spinal Injuries



Engineering Sciences Section - 2015

D13 Biomechanical Evaluation of Head Kinematics During Infant Shaking vs. Pediatric Activities of Daily Living

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After attending this presentation, attendees will understand the mechanisms associated with traumatic brain injury in a pediatric population. Thresholds for injury will be discussed and compared against alleged forms of physical harm.

This presentation will impact the forensic science community by informing attendees that abusive shaking of infants has been asserted as a primary cause of subdural bleeding, cerebral edema, and retinal hemorrhages. This presentation seeks to compare linear and angular accelerations between infant shaking and pediatric activities of daily living.

Background: A biomechanical evaluation was performed to quantify kinematic variables associated with various infant shaking techniques, with comparison to a series of pediatric activities of daily living, as abusive shaking of infants has been asserted as a primary cause of subdural bleeding, cerebral edema, and retinal hemorrhages; however, manual shaking of various biofidelic mannequins has failed to generate the head kinematics believed necessary to cause these intracranial symptoms in the human infant. This study sought to compare linear and angular accelerations between infant shaking and pediatric activities of daily living. Additionally, injury risk as a function of known biomechanical thresholds of injury was determined.

Methods: Using InterSense^o sensors attached to the heads and torsos of two infant surrogates, investigators collected linear and angular motion data during resuscitative, aggressive, and gravity-assisted shaking as well as during various non-abusive activities normally experienced by infants, such as burping, rough play, etc. Tasks were performed by nine subjects, ranging in age from 20 years through 77 years and included two females and seven males. The researchers also collected data from a 7-month-old infant spontaneously at play in a commercial jumping toy. Raw data including orientation, angular velocity, and linear acceleration were acquired wirelessly. Using MATLAB[®], data was filtered by a 4th-order Butterworth low-pass filter. Angular accelerations were subsequently derived, root mean square values calculated and Head Injury Criterion (HIC15) computed. Results were compared between the experimental conditions, against other biomechanical studies of shaking, and in contrast to accepted biomechanical thresholds of injury.

Results: In these experiments, the peak rotational acceleration generated, averaged across nine adult subjects, during aggressive shaking of the Child Restraint and Airbag Interaction (CRABI) -12 biofidelic mannequin (1,068rad/s²) were both consistent with the reports of prior biomechanical studies and, most interestingly, statistically undifferentiated from angular accelerations spontaneously generated and well tolerated by a normal 7-month-old infant at play in a commercially available jumping toy (954rad/s²). Furthermore, measures of head angular acceleration are substantially below scientifically accepted biomechanical thresholds of injury. Thus, shaking produces head kinematics that are clearly benign and well tolerated by normal infants, even if repetitive. If intracranial injury (SDH, EDH, SAH, DAI, concussion) is clinically presented, this data would indicate that shaking would not be part of its etiology.

Conclusions: This is the first scientific study of the effects of shaking-related shear forces on a human infant brain and, of course, there were no ill effects experienced by the infant.

Non-contact shaking appears to result in head kinematics that are well tolerated by normal infants, even if these rotational accelerations are repetitive, as experienced by the infant at play. This data would indicate that intracranial injury in an infant is unlikely to be the direct result of the linear and/or angular accelerations generated during non-contact shaking.

Biomechanics, Pediatrics, Shaken Baby



Engineering Sciences Section - 2015

D14 Relationship of Risk Factors and Specific Tissues at Risk in Rear-Impact Collisions

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After attending this presentation, attendees will understand the most pertinent and valid risk factors associated with injury in motor vehicle occupants subjected to collinear rear-impact collisions. This presentation will address four common mechanisms which alter the biomechanical loading capacity of the cervical spine and one mechanism which is unique to rear impacts. For each mechanism, the presenters will describe what tissues or structures are placed at risk and how the pain of injury to these structures is expressed.

This presentation will impact the forensic science community by elucidating common factors which increase the risk of injury in rear-impact collisions. This will enable biomechanical experts to better understand and analyze the mechanisms of injury in these collisions and aid care providers in assigning prognoses to patients recovering from these events. This presentation will help illuminate a field which has been fraught with bias due to the lack of understanding of the true injury mechanism and interaction between human, vehicle, and collision factors which can increase injury risk and lower long-term prognosis for recovery.

The risk factors most commonly identified in motor vehicle collision testing include: (1) Gender — females are more likely to be injured in rear-end collisions than males.^{1,2} The female anatomy and physiology is less conducive to withstand impact loads, shear, compression, tension, torsion or jolt injuries as sustained in a rear-end collision; (2) Stature — persons with smaller frames and smaller neck circumferences are more likely to be injured in rear-end collisions.³ Tissues with smaller circumference or geometrical size are less able to withstand trauma; (3) Occupant Position — studies have found that when the head is rotated from ideal position, neck strain becomes more common.⁴ Additionally, multiple injuries and more severe injuries occurred with increased frequency.⁵ When an occupant is not seated in ideal position, structures are less likely to be in an optimal load tolerance state and likelihood of injury is increased; (4) Unpreparedness— it has been shown that unprepared occupants are more likely to sustain multiple injuries and more severe injuries.^{1,5} When an occupant is hit unaware, he or she is unable to brace for impact, which increases risk of deep spinal injuries and lowers prognosis for recovery; and, (5) Rear-Impact Unique Loading Event — when struck from the rear, a motor vehicle occupant experiences an S-shaped curve in the cervical spine, which produces a loading event unlike daily activities, sports, or other traumatic events.^{6,7} This event combines a pre-loading level of compression that decreases the load tolerance of the pain-sensitive structures of the upper quarter. This compression is immediately followed by a shear-torsion mechanism causing differential loading between the lower and upper cervical spine, concentrating forces and increasing the risk of injury of deep and superficial pain-sensitive spinal structures.

Forensic analysis and medical examination are further complicated by intrinsic links between these factors. For example, females are typically smaller than males and are more likely to have a smaller stature and automobile seats often do not accommodate persons of smaller stature, making it difficult or impossible for them to sit in ideal occupant position or brace effectively for collision. This presentation will discuss how the interplay of these factors affects likelihood of injury and prognosis for recovery of individuals involved in rear-impact collisions.

There is a confluence of evidence from international sources including independent and institutional researchers which consistently documents increased injury risk when these factors are present. This evidence comes from a variety of sources, including cadaver and human volunteer studies, autopsy studies, provocative injections, palliative injections, and long-term population studies. The data provided by each of these sources will be discussed as it relates to cervical injuries in rear-impact collisions.



Engineering Sciences Section - 2015

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Injury Risk, Cervical Spine, Rear Impact



Engineering Sciences Section - 2015

D15 Biomechanical Evaluation of Shaking Impact Syndrome

John D. Lloyd, PhD, 32824 Michigan Avenue, San Antonio, FL 33576*

After attending this presentation, attendees will understand the risk of traumatic head and brain injuries in a pediatric population associated with shaking impact syndrome, a method often alleged in cases of abusive head trauma in young children.

This presentation will impact the forensic science community by informing attendees as to the mechanisms of head and brain injuries in pediatric populations and the risks associated with shaking and impact.

Introduction: A biomechanical evaluation of shaken impact syndrome was performed to evaluate the risk of injury to an infant. Injury risk was measured as a function of linear and angular head kinematics of a biofidelic infant surrogate during a biomechanical recreation.

Methods: Two adult males performed the shaken impact and impact activities. A Child Restraint and Airbag Interaction (CRABI)-12 biofidelic mannequin, height 0.75m and mass 10.0kg, was utilized as the infant surrogate. A 500G piezo-electric tri-axial accelerometer was installed at the Center of Mass (CoM) of the CRABI headform, in accordance with the Society of Automotive Engineers (SAE) J211 along with a tri-axial digital gyroscope.

A number of conditions were explored using a height-adjustable test apparatus. These included: non-contact shaking; shaken impact (which implies a brief shaking episode, followed immediately by impact); and, impact only. For the shaken impact and impact-only scenarios, participants were instructed to impart gentle, moderate, and vigorous impacts on the infant surrogate. In addition, the act of dropping the mannequin onto the surfaces was explored for the impact-only technique. Surfaces impacted included a standard infant crib mattress, a standard changing table pad with cover, and a hard wooden tabletop. Mattress height was set at a standard bed height of 23 inches, whereas the changing mattress and tabletop were both studied at 35 inches, as measured from the floor. Both participants performed five repeated trials for each condition, for a total of 230 trials.

Data from the analog linear accelerometer was acquired at 10,000Hz, per channel, using LabVIEW™ and filtered in MATLAB® using a phaseless 4th-order Butterworth filter with a 1650Hz cutoff frequency, per SAE J211. Data from the gyroscope was recorded at 5,585Hz, per channel, with no filtering necessary for this digital sensor. Head linear acceleration and angular velocities were recorded, angular acceleration was derived, peak magnitude values were calculated, and Head Injury Criterion (HIC15) computed.

Results: Findings indicate that angular accelerations associated with intentional impact and shaken impact are typically below infant brain injury thresholds of 8,000-10,000rad/s². The exception is vigorous impact against the changing pad or wood tabletop. It was also noted that dropping the infant surrogate onto the test surface produced injury risk similar to the moderate impact condition.

Conclusions: Across all events where sufficient rotational brain motion was recorded to produce significant brain injury, sufficient linear acceleration to cause skull fracture in an infant was also documented. Thus, considering the shaking impact or impact-only mechanisms, findings suggest that for there to be an underlying brain injury, a skull fracture is also likely.

Biomechanics, Pediatrics, Shaking Impact



D16 Can Barefoot Slip Resistance Be Quantified Using the ASTM F2508 Standard for Tribometric Testing?

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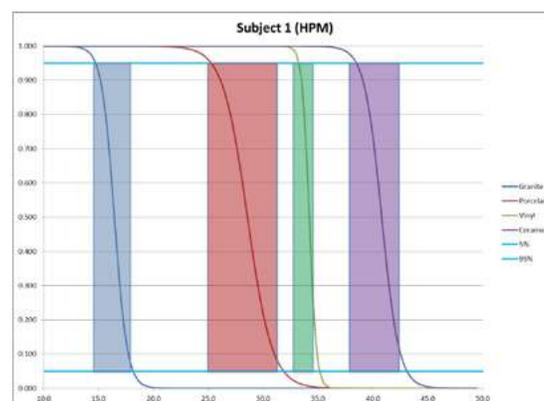
After attending this presentation, attendees will better understand the latest developments in barefoot walkway-safety tribometry.

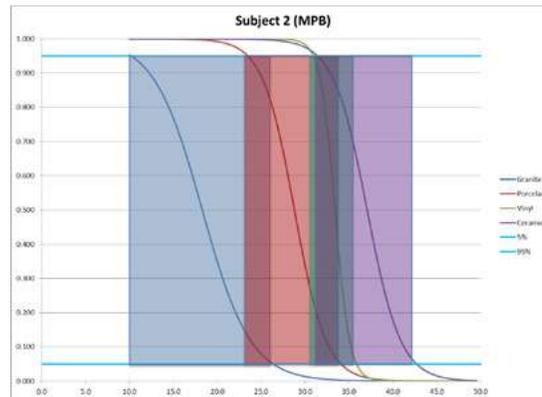
This presentation will impact the forensic science community by providing practitioners with an understanding of the problems and progress in barefoot tribometry and apprising researchers with an understanding of the state of barefoot tribometry.

Background: Walkway tribometers have been used to characterize slip resistance of walkway surfaces. Such tribometers typically use a Neolite® or leather “test foot” to assess slip resistance. This study explored barefoot slip resistance; while a leather or Neolite® Test Liner (NTL) test foot may resemble the outsole or heel of a shoe, it is a poor model for the human heel. In this experiment, a custom step meter was used to explore the behavior of the bare foot during slip, using the American Society for Testing and Materials (ASTM) F2508 *Standard Practice for Validation and Calibration of Walkway Tribometers Using Reference Surfaces* methodology.^{1,2}

The step meter is a device which can control the motion of the lower leg of an individual and allow the *in vivo* heel to be “dropped” onto a test surface in a repeatable manner. The test surface is inclined incrementally until slip occurs, similar to a “ramp test.” Previous studies have shown the step meter results to agree with those of validated tribometer tests.^{3,4} For this study, a more sophisticated statistical analysis than contained in F2508 is utilized: the logistic regression is used to find the point at which $p(\text{slip})=p(\text{no slip})=0.5$.⁵ A concurrent study validated the step meter fitted with an NTL test foot using the ASTM F2508 protocol.⁶ This study explores the behavior of the *in vivo* bare heel of two test subjects on the four reference surfaces used in the F2508 standard.

Experiment: There were two test subjects for this study. The testing procedure was as follows: prior to testing, the subject’s foot was allowed to hydrate for five minutes by submersion in a water bath. The subject’s right leg was then fixed in the step meter. The step meter allows the subject’s heel to be dropped vertically onto the test surface in a repeatable manner. The four reference surfaces described in ASTM F2508 were used in this study: granite (RS-A), porcelain (RS-B), vinyl (RS-C), and ceramic (RS-D). Surfaces were successively installed in the modified step meter and wetted with distilled water. For each surface, the instrument’s test-surface inclination angle was incrementally increased. At each angle, the heel was dropped onto the surface ten times and the number of slips occurring was recorded. A logistic regression was performed on the data to determine the $p(\text{slip})=0.5$ point and that value was recorded as the tribometer threshold for slip for that surface. The 5th and 95th percentile values were used as the lower and upper boundaries of the confidence interval for this measure.





Results: Graphs of the results of the logistic regression are shown above. For one subject, the combination of the modified step meter and the bare foot as test foot satisfied the F2508 standard. The results from the second subject, while similar to those of the first subject, did not statistically differentiate between the test surfaces.

Discussion: As the tribometer used in this study has been validated with a standard NTL Test Foot, one is left to speculate on the differences between the bare heels of the two test subjects evaluated.⁶ Why would one test foot “pass” F2508 and another not? It is suspected that there are differences between feet in a number of areas. Levels of hydration of the foot may have been different between the two subjects; no attempt was made to evaluate this hydration. There may be subject-to-subject differences in the skin ridges (heel “fingerprints”) that would affect slip resistance on a wetted surface; subject-to-subject differences may mean that some people have “slipperier feet” than others. While the reference surfaces were prepared as per F2508 (cleaning), no standard cleaning of the subjects’ feet was performed; it is possible that perspiration or other materials or factors “contaminated” the human test foot.

Further Research: The engineering solution to the problems discussed above would be to “standardize” the human foot for tribometric testing; however, if the objective is to assess the slip resistance of the barefoot pedestrian with any degree of biofidelity, such a test (or test protocol) *must* allow for subject-to-subject variations in the human heel. Such variation must be quantified to allow the application of tribometric walkway safety testing to the problem of barefoot walkway slip resistance.

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Barefoot, Tribometry, ASTM F2508



Engineering Sciences Section - 2015

D17 Additions to Accessibility Standards for Nursing Home and Assisted Living Residents in Toileting and Bathing

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After attending this presentation, attendees will: (1) understand the nature and extent of the needs of frail elderly adults in daily living; (2) be informed about the evidence base identifying gaps in toileting and bathing sections of the Americans with Disabilities Act (ADA) and American National Standards Institute (ANSI) Standards; (3) be informed of assisted-use supplements needed to meet ADA and ANSI Standards; (4) see research underway at the Georgia Institute of Technology's Center for Assistive Technology and Environmental Access and at IDEAS, Inc. where sufficient evidence does not exist regarding assisted use; and, (5) see a demonstrated example of the forensic process applied to creating new standards.

This presentation will impact the forensic science community by describing the work of a task force created to propose supplements to the 2010 ADA Standards for Accessible Design and the ANSI 2009 A117.1 Standard for Accessible and Useable Buildings and Facilities. For the purpose of this project, the Hulda B. and Maurice L. Rothschild Foundation partnered with the American Institute of Architects (AIA) Design for Aging Knowledge Community. This presentation was initiated by the Hulda B. and Maurice L. Rothschild Foundation, a national philanthropy focused on improving the quality of life for elders in nursing homes throughout the United States.

This task force is comprised of individuals committed to providing quality environments for *all* individuals, especially including frail elderly adults. Representatives include those of the foundation, architects, interior designers, researchers, providers, and others working collaboratively toward the goal of ensuring that standards which govern design of our environments provide for both safety and quality of life for *all* users. Since the ADA Accessibility Guidelines were initially issued in 1991, millions of Americans have benefited from changes to the built environment that provide greater opportunity and enhanced participation in society. Like most accessibility standards, they are intended to promote *independence*, generally based upon the stature, strength, and abilities of younger adults with a single disability, most of who transfer directly from wheelchair to toilet. In fact, much of the initial research utilized in development of the guidelines was based upon the physical challenges of returning veterans from the Vietnam War. These standards were developed around the capabilities of adults who: (1) have upper body strength; (2) can make sliding transfers; (3) have full cognition; and, (4) transfer independently.

Since the development of these early guidelines and standards, demographics of the population of people with disabilities have changed dramatically and now the limited functional abilities of the growing number of frail and elderly persons are not being adequately served by existing design standards as cited by the AIA Guidelines of 2002, which stated, "Hospital patients, and especially nursing facility residents, due to their stature, reach, and strength characteristics, typically require the assistance of caregivers during transfer maneuvers. Many prescriptive requirements of model accessibility standards place both elderly persons and caregivers at greater risk of injury than do facilities that would be considered non-compliant."

Initial phase of this project produced a white paper providing an evidence base and recommendations for *assistive* accessibility standards as supplements to the ADA and ANSI Standards to support and optimize functional abilities of frail elderly adults, reduce falls, and improve caregiver safety.

Recommendations for toilet room design are based on how frail elders transfer to the toilet with and without assistance and how space needs for staff and equipment must be accommodated. Changes in the bathing and showering area standards would provide *assistive* design options better enabling the person-centered care being mandated by the Centers for Medicare and Medicaid Services. Further, the proposed changes are consistent with the intent of 2002 AIA Guidelines that called attention to the disparity between accessibility standards and the needs of older adults and their caregivers.



Engineering Sciences Section - 2015

Forensically, adoption of supplementary guidelines and standards for residential care environments will reduce ambiguously based law suits regarding personal injury from falls for frail elders and back injuries to caregivers. Jurisdictional authorities will be able to make more informed decisions about accessible design for frail elders in assisted living and nursing home communities. Forensic expert architects, specialized in ADA and ANSI compliance will have strong bases upon which to investigate, report, and testify in court.

This presentation will conclude with a description of research progress and an example of successful forensic endeavor.

Frail Elderly, Assisted, Standards



Engineering Sciences Section - 2015

D18 Evaluating the Impact of Reroofing on Increasing the Risks of Ponding of Water

Daniel M. Honig, PE, Structures Consulting Engineer, PO Box 125, Swarthmore, PA 19081*

After attending this presentation, attendees will understand some factors that affected and contributed to a roof ponding event, as well as how the selected materials, installation procedure, and localized drainage conditions caused the collapse of a recreational facility roof.

This presentation will impact the forensic science community by using a case study to illustrate how modification work done on a building can negatively impact the structural integrity of that building. This presentation also highlights the importance of building code compliance, which often prevents building collapses as building modifications are made over time.

Ponding, the unexpected pooling of water, often occurs with catastrophic events and is a predictable condition in areas of heavy rainfall. The weight of rapidly congregating water on a rooftop can cause an existing structural roof system to deflect and progressively collapse during intense rainstorms.

In this instance, reroofing work completed on a recreational facility negatively altered the roof drainage capability of the building. The continuing adequacy of the rooftop drainage system was not properly reevaluated as part of the reroofing project, thereby leading to a significant structural roof failure incident approximately a year later during a rainstorm microburst. In this case, a localized portion of the structural steel support system of the roof collapsed due to ponding from a blockage created in the drainage system.

Prior to this ponding incident, the original roof drainage system had functioned properly for more than 20 years since the time it was constructed.

The major factor contributing to the ponding failure was the installation of two-inch-diameter drain inlets without proper debris-protection baskets during the reroofing process. The thickness of the drain inlets and the installation of an Ethylene Propylene Diene Terpolymer (EPDM) membrane within the inlets reduced the effective drainage diameter to approximately the size of a common bathtub or shower drain. This reduction in diameter significantly and proportionally reduced the effective drainage area of the roof drainage system by as much as 80%. In addition, no secondary or overflow roof drainage system was provided as per code. The new roofing material installed, including flute fillers and insulation, also created additional dead loads to be supported by the existing structural roof framing members.

While the roofing design of the original building would not have required emergency scuppers, the drainage system was altered, thereby making it inadequate to withstand even regular rainfall events. The significant drainage capacity reduction, in combination with the lack of protection baskets and the potential for blockage of the inlets, allowed the significant roof ponding and additional roof loading to occur. In this case, the reroofing contractor did not conduct a proper engineering review of the rooftop surface conditions as part of the completion of the reroofing job. While plumbing codes prohibit the reduction of drainage diameters on existing buildings, appropriate attention by installers to the impact of modification work on the structural integrity of a building could also help prevent such failures in the future.

Ponding, Roof Collapse, Reroofing



Engineering Sciences Section - 2015

D19 A Test Program to Validate a Step Meter Using Neolite® Test Liner (NTL) as a Test Foot per the ASTM F-2508 Protocol

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After attending this presentation, attendees will understand the steps needed to validate a new tribometer under the American Society for Testing and Materials (ASTM) F2508.

This presentation will impact the forensic science community by showing how tribometer validation, important under the *Daubert* and *Frye* court decisions, can be accomplished.

ASTM committee F13 (Pedestrian/Walkway Safety and Footwear and Walkway Safety) is charged with developing standards and procedures to increase pedestrian-ambulation safety. “A primary focus of the Committee is the measurement of slip resistance” (ASTM F13). To that end, over the years, this committee has approved specific tribometers (devices to measure on-site slip resistance between flooring and shoe bottoms) (e.g., ASTM F1677 — Portable Inclined Articulated Strut Slip Tester, ASTM F1678 Portable Articulated Strut Slip Tester, and ASTM F1679 — Using a Variable Incidence Tribometer). A number of years ago, each of these standards were withdrawn from active development, mainly because these standards referenced proprietary instruments. Replacing them was a non-proprietary standard (ASTM F2508 — Standard Practice for Validation, Calibration, and Certification of Walkway Tribometers Using Reference Surfaces) to verify the ability of a tribometer to measure the slip resistance (between the floor and test foot.) This standard included four reference surfaces: black granite, porcelain tile, vinyl composition tile, and ceramic tile (with an increasing slip resistance in the referenced order). Essentially, if a tribometer’s test results were ordinally correct (with the test results in same order as the reference surfaces as listed above) and statistically discriminated, the tribometer was “validated” as per ASTM F2508.

Preliminary Studies: (1) In a previous study, a step meter (variable inclinable) was developed to compare slip-resistance results of a test subject and a tribometer.¹ The device is similar to a ramp test, except the test subject stood in the device and stepped down (successively) on a test surface that had its inclination increased until a slip occurred. The test results were comparable between the step meter and a Portable Inclined Articulated-Strut Tribometer (PIAST); and, (2) In a later study, the step meter was modified to allow the test subject to remain seated.² The test subject’s leg was raised and dropped on a test surface that had its inclination increased until a slip occurred. The leg was constrained to move vertically. This seated configuration for the test subject (as compared to standing) was found to have increased reliability as shown by a steeper logistic-regression curve.



The Current Experiment: The goal is to verify that the step meter equipped with a grooved Neolite[®] Test Liner (NTL) test foot will pass validation under the ASTM F2508 protocol. This same NTL test foot, when used in a PIAST, had been found to meet the ASTM F2508 criteria. The hypothesis is that the step meter-measured available friction will result in these reference surfaces being “placed” in the rank order (section 9.2.1, ASTM F2508) and the differentiation between these surfaces will be statistically different (section 9.2.2, ASTM F2508).

In order to validate (as per ASTM F2508) the seated step meter, it was equipped with an articulated tribometer strut (hinged at the top and dropping vertically) with a grooved NTL test foot, contacting water-wet ASTM F 508 reference surfaces. The surfaces (resting on a bottom plate) could be rotated, changing the orientation between the grooved NTL test foot and these surfaces. The reference surfaces were bathed in a continuous film of water supplied by a recirculating pump. (Triton[™] X-100 was not used as a wetting agent on the black granite surface as the test surface was continuously bathed in the water.) The NTL test foot was dropped onto the reference surfaces successively (with the reference surfaces initially horizontal). The reference surface was rotated toward the vertical in small increments until slip occurred.



Figure 2: The Modified (Hybrid) Step Meter.

The articulated strut moves vertically down onto the test surface. The test foot (grooved NTL) is attached to the strut by means of a clip. Water is continuously sprayed onto the test surface by means of PVC drilled tubes (on either side of test surface). The test surface rests on a flat surface (shown in photo). The test surface is “tilted” to provide any angle between the vertical strut and the test surface. The strut “drops” onto an angled, water-wet test surface. When slip occurs, the angle of the test surface is measured.

The testing was conducted as per the ASTM F2508 protocol (10 tests in each of four perpendicular directions). The angle of the reference surface was measured and recorded when the test foot slipped. The results were statistically analyzed as per ASTM F2508.

The results show that this modified step meter, using the ASTM F2508 reference surfaces, was able to differentiate the slip resistance of these surfaces in the proper order, and met the F 2508 criteria for validation of a tribometer.

	Grooved Neolite Test Foot Step Meter Tangent of Slip Angle			
	Black Granite	Porcelanosa	OVCT	Ceramic
Mean	0.1801	0.2137	0.403925	0.756625
Std Dev	0.027192	0.02252998	0.03283321	0.053789565
LCL	0.171673	0.20671789	0.39374988	0.739955441
UCL	0.188527	0.22068211	0.41410012	0.773294559



Engineering Sciences Section - 2015

References:

1. Medoff H, Brungraber R, Hilferty C, Patel J, Mehta K. 2002. Variable inclinable step-meter: using test subjects to evaluate walkway surface/footwear combinations. In: Marpet MI, Sapienza MA, editors. Metrology of pedestrian locomotion and slip resistance, ASTM STP 1424. West Conshohocken, PA: *ASTM*:51-72.
 2. Besser, M., Marpet, M., Medoff, H. 2008 Barefoot-pedestrian tribometry: in vivo method of measurement of available friction between the human heel and the walkway. *Industrial Health National Institute of Occupational Safety and Health*, Japan 46:51-58.
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Neolite® Test Liner, Daubert, Frye



Engineering Sciences Section - 2015

D20 Anomalous Initial Readings in Tribometric Testing of Barefoot Subjects

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After attending this presentation, attendees will be familiar with certain problems that appear in walkway-safety tribometry and how to statistically test for the presence of these problems.

This presentation will impact the forensic science community by alerting practitioners to potential problems in tribometric testing, how to determine if such problems exist, and how to eliminate them.

Background: A modified Stepmeter is in the process of being validated under ASTM F2508 Standard Practice for Validation and Calibration of Walkway Tribometers Using Reference Surfaces.¹ Briefly, the Step Meter is a cyborg-like take-off of a Slip-Test Mark II Portable Inclinable Articulated Tribometer, where a human subject's leg is constrained to move only vertically and/or rotate forward from the knee joint. The subject's constrained leg is passively lowered against an inclined test surface.² More sophisticated statistical analysis than contained in F2508 is utilized, viz., logistic regression is used to find the point at which $p(\text{slip}) = p(\text{no slip}) = 0.5$.³ In the validation process, repeated tests (foot lowerings) are conducted at a given test-surface inclination angle and the sequence of slips versus no-slips is recorded. A visual inspection of the data raised the hypothesis that the initial foot lowerings in any sequence of same-angle tests appears to be somehow different from the rest of the sequence.

Experiment: To investigate this hypothesis, Official Vinyl Composition Tile (OVCT, Reference Surface C in F2508) and then Ceramic Tile (Reference Surface D) were successively installed in the modified Step Meter. The instrument's test-surface inclination angle was adjusted for each test surface so that the $p(\text{slip})$ was approximately 0.5. For each surface, repeated series of ten foot-lowerings were conducted (11 sequences for the OVCT and 10 for the Ceramic Tile). The slip/no-slip results were recorded in sequence (i.e., slip, slip, no-slip, ..., slip). The number of slips was histogrammatically tabulated. If the probability of a slip is constant from foot lowering to foot lowering, the probability of the first slip at the k th trial will follow a geometric distribution:

$g(k; p) = p(1 - p)^{k-1}$; $k = 1, 2, \dots, 10$ and $0 \leq p \leq 1$ where $p = \frac{\text{number of slips}}{\text{number of trials}}$. For example, this study observed in total 53 slips in 110 trials for the OVCT, thus $p = \frac{53}{110} = 0.482$. A χ^2 goodness-of-fit test was conducted, where the expected number of occurrences was compared with the observed number of occurrences: (1) H_0 : Distribution cannot be distinguished from

a Geometric Distribution; (2) H_1 : Distribution doesn't follow a Geometric Distribution; (3) Test Statistic: $\chi^2_{\text{test}} = \sum_{k=1}^{11} \frac{(O_k - E_k)^2}{E_k}$

with $k-2$ degrees of freedom, and, (4) Where $E_k = 11g(k; 0.482)$.

First slip=k	$g(k; 0.482)$	$E_k = \text{Expected}$	$O_k = \text{Observed}$	$(O_k - E_k)^2 / E_k$
1	0.482	5.30	1	3.4887
2	0.250	2.75	0	2.7464
3	0.129	1.42	4	4.6661
4	0.067	0.74	1	0.0935
5	0.035	0.38	5	55.8059
6	0.018	0.20	0	0.1980
7	0.009	0.10	0	0.1026
8	0.005	0.05	0	0.0532
9	0.003	0.03	0	0.0276
10	0.001	0.01	0	0.0143
11	0.001	0.01	0	0.0074
Totals->	1.0	10.98	11.00	$\chi^2 = 67.20$



Engineering Sciences Section - 2015

The p -value (i.e., $P(X \geq 67.20)) = 0.0$. That is, the probability that, under a geometric distribution, one could achieve the X^2 result found would be effectively zero: effectively an impossibility. For context, the 1% critical value is $\chi^2 = 21.7$.

The first and then the first and second foot drop results were then removed from the calculation. With the first reading in the sequence dropped, $\chi^2(8 \text{ d.f.}) = 31.4$, with corresponding p -value of 0.0001, still highly significant. With the first and second readings in the sequence dropped, $\chi^2(7 \text{ d.f.}) = 11.89$, corresponding to a p -value of about 10%, which is conventionally considered not significant. That is, with the first and second readings in each sequence ignored, the data could not be distinguished from a geometric distribution.

For the ceramic tile, $\chi^2(8 \text{ d.f.}) = 31.1$, with a corresponding p -value of 0.0001. With the first foot-dropping result eliminated, $\chi^2(7 \text{ d.f.}) = 12.17$, with a p -value of about 10%: not considered to be significant. With the first two foot-dropping results eliminated, $\chi^2(6 \text{ d.f.}) = 2.17$, with a p -value of about 90%. (If you believed the results didn't follow a geometric distribution, you would have an approximately 90% chance of being *incorrect*.)

Recommendations: On the basis of these results, it would preliminarily be recommended that, at least for barefoot tribometry, the first two readings in any set of tests be ignored. That is, one should not record the first two test-foot drops. This study suggests that at least one of those two test-leg drops should be a slip.

Further Research: This study intends to extend this experiment to the other two ASTM Reference Surfaces (Granite and Porcelanosa Ferrocker, A and B respectively). More important, perhaps, is to extend this work to testing using a Neolite® Test Liner Test Foot, a commonly used surrogate for a shoe heel. There are anecdotal reports of the same anomalous behavior in NTL tests, and thus bears investigation.

References:

1. ASTM, F2508 Standard Practice for Validation and Calibration of Walkway Tribometers Using Reference Surfaces, *ASTM International*, West Conshohocken, Pennsylvania.
2. Besser M, Medoff H, and Marpet M. "Biofidelity-based Comparison of Barefoot Slip Resistance (Laboratory) against an in vivo tribometer and a standard Tribometer," in *Proceedings of the 2010 International Conference on Fall Prevention and Protection* (NIOSH sponsored–2010)
3. Medoff H, Besser M, Marpet M. "Visual Characterization of Tribometric Reference Surfaces Using Logistic Regression" in *Proceedings of the American Academy of Forensic Sciences, 62nd Annual Scientific Meeting, Seattle, WA. 2010.*

Tribometry, Geometric Probability Dist., Barefoot Friction



Engineering Sciences Section - 2015

D21 Forensic Investigation of a Premature Mortar Explosion That Resulted in the Death and Maiming of Several United States Marines

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After attending this presentation, attendees will understand how a mortar system is designed and works and what can go wrong. Additionally, attendees will learn how defects can be identified post-accident using deductive reasoning, the importance of correct designation, and the use of fact vs. expert witnesses.

This presentation will impact the forensic science community by establishing that deductive-reasoning analytical techniques may be gainfully employed outside their typical use in medical diagnoses. This presentation will also highlight the importance of correctly designating expert and fact witnesses.

This presentation will educate attendees as to potential failure modes in medium caliber mortar systems, civil litigation fact/expert witness procedure pitfalls, and illustrate the legitimate use of deductive reasoning to form scientifically valid conclusions in instances where much of the physical evidence is lost or destroyed.

This presentation focuses on an actual forensic case study relating to a training accident involving United States marines who were conducting a training exercise using a battery of medium caliber mortars. The case is interesting for a number of reasons. First, mortar system failures are very rare. Second, the use of deductive reasoning, while widely accepted in the medical profession when making diagnoses, may be frowned upon outside of the medical profession; however, in this instance, the court ruled that the technique was appropriate to utilize in a situation where the majority of the evidence was destroyed and/or lost. Third, the case demonstrated, from a legal perspective, the importance of correct identification and use of fact versus expert witnesses and the perils of flouting relevant correct procedures.

Medium caliber mortar systems are the heaviest portable artillery that may realistically be carried by infantry troops. A mortar is the most basic form of artillery, but is nonetheless a very valuable weapon system that provides a tactical edge to infantry during forward operations. The mortar system is comprised of a muzzle-loading smooth bore gun (tube) and fin-stabilized high-explosive projectiles with an integral propelling charge system. The gun itself is comprised of a barrel, a base plate, and an aiming system. The firing pin is fixed, at the bottom of the tube, and the ammunition propelling charge is ignited by a percussion primer that impacts the firing pin when the ammunition is dropped down the tube by the loader. It is a low-pressure, low-stress system, and this enables the tube weight to be kept to a minimum, thereby ensuring that the system is man portable.

If all goes according to plan, the crew sets up the mortar and sets the tube to the correct angle of elevation to achieve the range they need using the propelling charge they have selected. The loader drops a round of ammunition, fitted with the appropriate number of propelling charge segments, into the muzzle end of the tube. Gravity takes the round of ammunition to the bottom of the tube at which point the percussion-actuated primer makes contact with the fixed firing pin and the propelling charge is initiated. The round of ammunition is accelerated up the barrel (tube) and makes its way to the target. The ammunition incorporates a safety and arming device in the nose-mounted fusing system. This safety and arming device ensures that the high-explosive charge in the warhead cannot be detonated by the fuse/booster while the round is still in the tube or while it is within an unsafe distance of the gun emplacement.

These mortar systems are comparatively simple, safe, and reliable. The gun tube is inspected regularly and the degree of wear noted. At some point, the tube will fall below the minimum safe-wall thickness, exhibit signs of cracking, and be replaced. There are several things that can potentially go wrong and result in crew injuries or fatalities. In broad terms, these fall into three main categories: (1) the tube may rupture due to excessive wear and/or metallurgical defects (tube failure); (2) the ammunition warhead may detonate prematurely due to body failure, fuse failure, or high-explosive warhead defects; or, (3) the crew may insert a live round into the tube before the previous one has left (double charge=operator error). Any of these events are bad news for the crew who are, by necessity, in very close proximity to the tube. A tube failure is less catastrophic than a premature detonation of the high-explosive warhead. A double charge may result in the two rounds of ammunition being propelled harmlessly downrange, or a very violent in-bore explosion. The case under review was further complicated due to issues relating to evidence examination (resolved pre-trial) and designation and use of expert vs. fact witnesses (resolved on appeal).

Mortar Accident, Deductive Reasoning, Expert vs. Fact Witnesses



Engineering Sciences Section - 2015

D22 Catastrophic Failures in Firearms and Ammunition Attributed to Propellant Issues, Metal Fatigue, High Pressure, Incorrect Ammunition, and Other Factors

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The goal of this presentation is to provide an understanding and appreciation of the multiple issues and conditions which can produce a dangerous potential for firearms and ammunition to become unintended explosive devices which often results in catastrophic failure and consequential degrees of property damage and serious personal injury including death.

This presentation will impact the forensic science community by informing attendees that these explosive incidents can occur with perfectly safe and fully functional weapons that have not previously shown any signs of an impending problem. Inadvertent lapses in quality control during the manufacturing processes may permit substandard products to enter the market with this fact becoming apparent only after a catastrophic incident.

Injuries from firearm malfunctions are mostly preventable through common safe practices including the use of protective equipment, routine maintenance and inspection, and being aware of the typical symptoms and signs which precede a catastrophic failure; however, even a proactive approach will not uncover the hidden dangers.

Firearms and ammunition are dangerous weapons which have been designed for a variety of purposes. The average shooter will not encounter any significant or serious problem over their lifetime while engaging in the use of firearms. Despite the warnings and practices that are learned in common safety education programs, there are circumstances which can develop through no fault of the shooter that results in the firearm becoming a pseudo pipe bomb resulting in its spontaneous disassembly and fragmentation.

The typical injuries suffered during these explosive events involve the hands and face. Forensic investigations into these incidents are not commonly conducted by personnel in crime laboratories since the personnel are usually restricted to basic firearms testing for functional design and the identification of evidence; they are most often analyzed by engineers or other specialized consultants who have backgrounds in firearms and ammunition design and who have studied the causes and effects of blast damage.

Over the last several years, the civilian shooting environment has seen an influx of very large caliber weapons that were restricted to the military. This presentation will discuss some specific areas of catastrophic failure which result from the use of incorrect ammunition, metal fatigue, plugged barrels, high- and low-pressure detonation, improper assembly, use of the wrong type of propellant including how burning rates affect the movement of projectiles in the barrel, and discuss the correspondence of peak pressures in relation to barrel length.

Firearms, Catastrophic Failure, Injuries



Engineering Sciences Section - 2015

D23 Injuries Arising From Glass Drinking Vessels Used in Stabbing and Slashing Attacks

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The goal of this presentation is to show how breakage of glass pints used in stabbing and slashing attacks can cause injury and the typical forces that are generated by an attack with a glass drinking vessel.

This presentation will impact the forensic science community by assisting forensic engineers and other professionals who need to interpret glass injuries to understand the forces generated and the way in which the glass fracture and fragmentation leads to injury.

Recent reports in the United Kingdom estimate the annual cost to the National Healthcare System as a result of alcohol harm at £2.7bn. Glassware is used as an impulsive weapon in 4% of violent incidents in the United Kingdom. The injuries that occur can be significant, leading to serious injury and death and usually fall into categories of either stabbing or slashing. Injuries can also have a component of blunt force trauma depending on the way in which the weapon is used. In order to better understand the injury potential of different types of glassware and measure the forces involved in glass-related attacks, a study has been conducted using English pint glasses, in particular “Nonic” glasses and “Tulip” glasses. Slapping attacks, where a glass is held in the hand and slapped onto the victim, are dynamic attacks and in order to determine the level of force that can be generated, a novel force-plate dynamometer was used to record the peak forces generated by a number of assailants. A typical example of the force-time data is shown in Figure 1. The peak force generated in this instance was 1,208N. The further peaks arise from the dynamic oscillations (vibrations) induced in the dynamometer by the initial impact. The force generated is considerable and easily sufficient to penetrate skin with a glass fragment from the fracture of the glass on impact.

Additionally, high-speed video was used to record the way in which the glasses fractured and any shards from the glasses penetrated a synthetic skin simulant. Tests were made onto both a flat plate and a mannequin head. A silicone rubber skin simulant was used to allow the damage created by shards to be assessed. Annealed and tempered glassware was tested and the glass fracture patterns and types of shards that are generated were compared in terms of the damage that was obtained. The average force generated during a slapping attack was found to be ~1,000N. This is a significant force and therefore it would be expected that the injuries would be a combination of blunt force injuries and sharp force injuries from the glass fragments that result on impact. The results of the engineering experiments are presented in terms of observed forces and damage patterns and compared to those found in a pathology context in order to gain an improved insight into the way in which injuries arise in assaults using glass as an impulsive weapon.

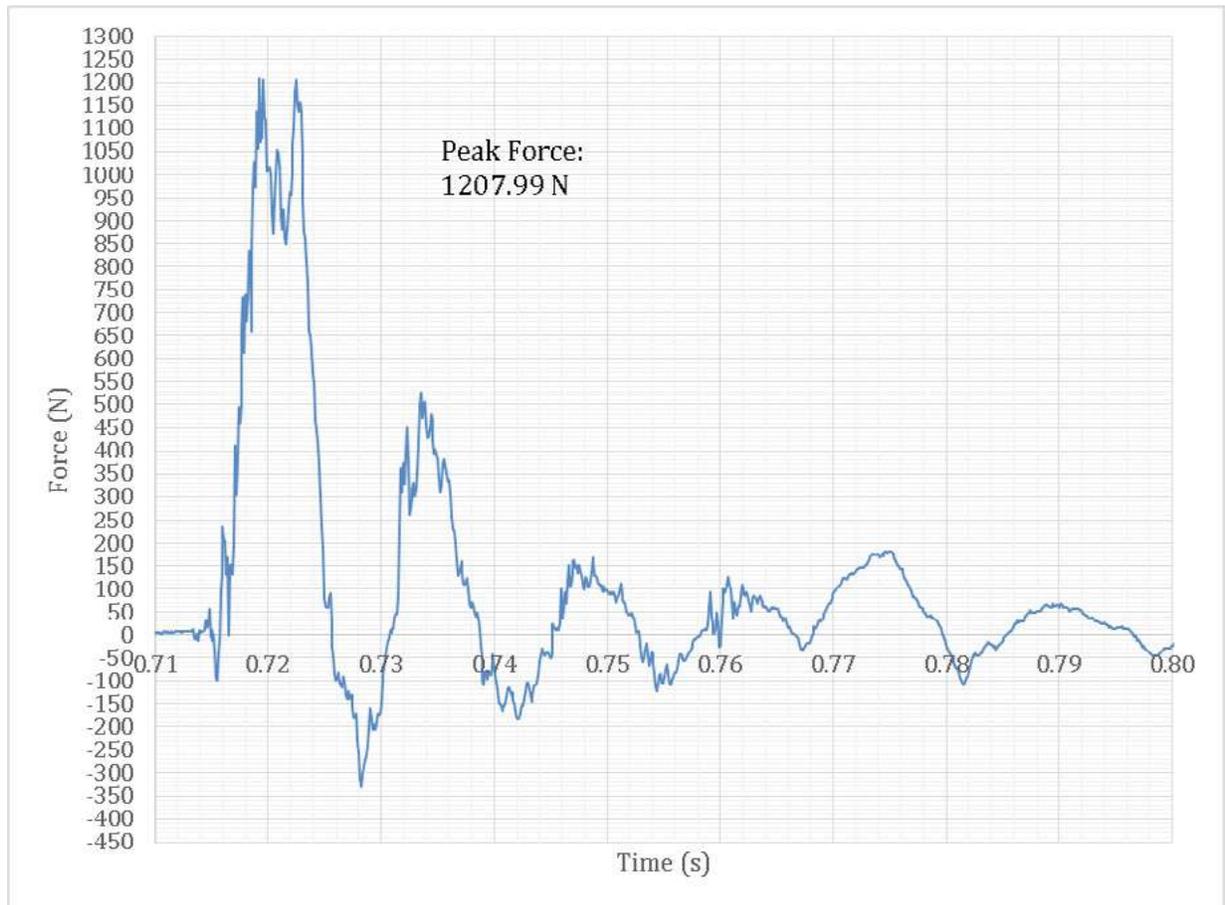


Figure 1: A force-versus-time graph for a slap with a “Tulip” geometry glass

Glass, Glassing, Fracture



Engineering Sciences Section - 2015

D24 Investigation of “Inert” Artillery Shell Explosions

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The goal of this presentation is to demonstrate the importance of ensuring that staff have a responsible disposition and that they are appropriately qualified and trained to deal with the hazardous materials likely to be encountered during munitions decommissioning operations.

The presentation will impact the forensic science community by highlighting the importance of repeated safety inspections, even when multiple levels of paperwork indicate that a proposed operation will be safe to perform.

The case involved the decommissioning and recycling of military munitions that had been in storage and had exceeded their specified service life. Decommissioning of live explosive munitions is a delicate, potentially dangerous, and expensive process. The usual procedure is to inert the munitions by removing fusing and explosive warhead materials, then ship the remaining inert components to scrap metal processors for final disassembly and metals recycling.

Military authorities typically follow one of two courses of action for inerting the munitions. They can have a government/military entity perform the inerting process or they can have an approved private contractor do it. Needless to say, it is important that each step of the process be carefully monitored and documented to ensure that no munitions, removed fusing components, or energetic warhead materials are mishandled, misplaced, or lost to potentially dangerous criminals/terrorists.

Once the residual munitions components are certified as inert, they can be sold to scrap metals processors for final disassembly, destructive breakdown, and sale to metals smelters/recyclers. In recent years, some insurance companies have insisted that recycling processors not accept decommissioned munitions for processing, presumably because of the perceived high risk.

In this case, the empty shells were sold and transported to a large scrap metals processor who deemed it necessary to cut the shells into smaller parts. This cutting was to be accomplished using dual gas cutting torch equipment. Unfortunately, one or more of the shell bodies contained a significant amount of high explosive and a young contract employee was fatally injured when he took a cutting torch to a steel shell body and the high explosive detonated. Warning signs were not recognized and/or ignored. The young worker lost multiple body parts and faculties and took several weeks to die. The subsequent litigation investigation uncovered numerous alleged errors and lack of care issues on the part of the scrap metals processing plant and the employee's family were given substantial financial damages for the death and suffering of their family member. As with many investigations, this one revealed some interesting issues with both plant procedures and supervisors.

Artillery Shell, Munitions Decommissioning, Explosives Fatalities



D25 Killing-Power Ranking of Blunt Instruments — Establishment of a Method to Evaluate Human Injury

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After attending this presentation, attendees will understand the relationship between impact velocity and maximum impact force by typical blunt instruments in order to determine a technique for quantification of impact force

This presentation will impact the forensic science community by determining the existence or non-existence of murderous intent for a criminal trial.

In a murder case and accident, evaluation of human injury was conducted at the same time as a postmortem examination and investigation. It is difficult to quantify when the size of impact force from human injury is evaluated. There are many parameters presented in this study, such as acting force, maximum force, and acting time. Then, in the case of bone fracture-risk evaluation by blunt instrument, the circumstances of a murder case and accident can be explained. According to an on-the-spot investigation report and recording of a statement, it's possible to perceive the circumstances of a murder case and accident.

This study investigates and analyzes the following: (1) estimation and selection of impact position by blunt instrument; (2) impact force that an assailant can show (physical or environmental factor); (3) the case of situation which a sufferer receives (a back or a wall); and, (4) choice of injury parts.

The results of the analysis were carefully examined. Then, the condition of impact force-measuring experiment was set and the impact experiment was conducted by the same or similar blunt instrument compared to the actual weapon. In a murder case and accident, the range $F_{min} \sim F_{max}$ of impact force F were narrowed. Furthermore, as a result of the examination, bone fracture limits force f of the sufferer was presumed. Last, impact force F and bone fracture limit force f were compared: $F_{min} > f =$ bone fracture risk low; $F_{min} < f < F_{max} =$ there is the possibility of the bone fracture; $F_{max} < f =$ bone fracture risk high. Judging from this, a test result is assumed.

Using a variety of blunt instruments, the following three experiments were performed: (1) a difference in impact force of impact position by blunt instrument; (2) measuring of down swing velocity from an individual difference; (3) an impact experiment concerning artificial human soft tissue.

As a result of the experiments, the following were suggested: (1) it was found that down velocity by blunt instrument was so fast as to be below the impact position; (2) because of material and impact velocity, it was clear that the tendency is to decrease load from a position which is measured maximum force to the tip; (3) there were good relationships between impact velocity and maximum impact force using a modeled skull made of formed styrol; and, (4) the blunt instrument confirmed that it's possible to estimate the maximum impact force the skull receives from impact velocity.

Human Injury, Human Soft Tissue, Impact Velocity



Engineering Sciences Section - 2015

D26 A Study of Batch-to-Batch Handgun Ammunition Propellant Variables and Their Influence on Muzzle-to-Target Distance Determinations

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After attending this presentation, attendees will understand ammunition construction and the variables in propellant composition and morphology that exist between different batches of externally identical ammunition. The impact of these variables on the validity of muzzle-to-target distance determinations will be discussed.

This presentation will impact the forensic science community by affecting the way in which muzzle-to-target distance determinations are performed when a sufficient quantity of identical ammunition is not recovered.

Deceased gunshot wound victims frequently have close range wounds to bare skin that exhibit sooting and/or stippling, to varying degrees. It is common practice for technicians to perform muzzle-to-target distance determinations using the same brand and, when known, loading of ammunition. This presentation illustrates and discusses the variability of propellants (especially propellant geometry/morphology) discovered within different lots of the same brand and loading of premium ammunition. The variability in combustion, stippling, and sooting characteristics were studied in the context of their impact upon the accuracy and validity of muzzle-to-target distance determinations that are based upon these visual indicators. The results and conclusions of the study will impact the way that muzzle-to-target distance determinations are performed when a sufficient quantity of identical ammunition is not recovered.

The degree of sooting, and the maximum range for sooting, vary significantly depending upon the propellant used in the subject ammunition. Typically, propellants manufactured with a greater variety and/or quantity of additives will produce more sooting. For most handgun/ammunition combinations, visible sooting will cease at a muzzle-to-target distance of approximately nine to twelve inches, but it is not uncommon for some “dirtier” ammunition types to leave visible soot deposits out to fifteen inches or more.

Additionally, old propellants or propellants that have prematurely aged due to being inappropriately stored (high temperatures and/or repeated temperature cycling — such as may be encountered in a vehicle, for example) typically produce a greater degree of both sooting and stippling than would otherwise be the case. It is generally accepted that stippling from a handgun will cease at a maximum muzzle-to-target distance of approximately three feet. Occasionally, stippling may occur beyond that three-foot limit, but it is more usual for stippling to cease at a muzzle-to-target distance of approximately two feet; however, the propellant characteristics that most influence maximum range and dispersion of stippling are propellant grain mass, size, and morphology. Propellants used in handgun ammunition typically fall into one of three morphology categories — ball, flake/disk, and cylindrical. It is not uncommon for propellants to be blended, and thereby be comprised of more than one propellant grain geometry type. Clearly, there are implications for the aerodynamic characteristics for each of the three propellant grain geometries cited — a grain of ball propellant will travel further than a grain of flake propellant, for example.

The research conducted for this presentation focused on a practical study of several batches of the same loading of premium defensive handgun ammunition that had been manufactured by a long-established and reputable manufacturer. Disassembly of samples from the different batches revealed that different propellants had been utilized — the primary concern of the manufacturer being to achieve the same batch-to-batch average muzzle velocity, without regard for propellant type. The differing propellants encountered in each batch may have had varying additive content (in terms of both composition and quantity). The propellants were primarily identified and classified merely by their size and morphology. The primary purpose of the research was to investigate the variation in sooting and stippling characteristics between different batches of ammunition of the same brand and loading, given the knowledge that the propellants used differed significantly from batch to batch. The research demonstrated that sooting and stippling characteristics varied widely between different batches of the same brand and loading of ammunition, due to the varying size and morphology of propellant grains used during manufacture. Consequently, it was concluded that a technician who tested for muzzle-to-target distance using a given brand and loading of ammunition, assuming that the results would be representative, may obtain erroneous muzzle-to-target distance results if the ammunition batch were not identical. Current laboratory protocols and generally accepted practices do not consider these ammunition variables.



Engineering Sciences Section - 2015

If a significant quantity of ammunition is recovered for testing, the previously cited problems will be of little concern, assuming a visual examination of propellant samples confirms that it is all identical; however, in cases where only one round or a limited quantity of ammunition is available, it is recommended that it be dismantled and new ammunition procured for testing. The propellant from the new batch(es) of ammunition should be compared to the case sample to ensure propellant similarity and thereby guarantee the validity of any subsequent muzzle-to-target distance determinations.

Propellant Morphology, Propellant Stippling, Distance Determinations



Engineering Sciences Section - 2015

D27 Testing of Police Radar

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The goal of this presentation is to improve attendees' understanding of the limitations of police radar, as well as to impart how it is technically implemented in both software and hardware. The outcome will show that improvements in electronics techniques have resulted in radar systems that ignore some reflected target signals by treating them as "noise," when in fact they are signals from valid vehicles. Attendees will also see that displayed speeds are not displayed in a way that correlates with an officer's discernment of traffic.

This presentation will impact the forensic science community by demonstrating that although police radar is used for enhancing traffic safety, it is also used for enhancing governmental revenue, as well as serving as the basis for traffic stops in which law enforcement then searches a vehicle for contraband. Such law enforcement techniques are appropriate when the basis for executing a traffic stop are lawful. This presentation demonstrates that traffic radar is not reliable when subjected to *Daubert* criterion in that it leaves too much discretion to the officer. In that it is not reliable, its use as a basis for searches of vehicles and resultant convictions due to the carrying of contraband (drugs, weapons) raises 4th-amendment questions. The outcome of this research is that there may be post-conviction relief for citizens who were righteously convicted for contraband offenses after being searched without the necessary probable cause.

Police radar forms the mainstay of many police department traffic enforcement programs. While enforcement of traffic laws is within the purview of a police department or state police group, anecdotal experience has shown that the issuance of citations for speeding is sometimes done to enhance governmental coffers. In that speeding tickets represent the most frequent brush with the law that many citizens have, one would think that police radar is a highly sophisticated tool that leaves the operator no discretion in determining the speed of a vehicle.

The reality of police radar is that it fails when subjected to the *Daubert* test. In this regard, police radar operation *should* be repeatable — this research demonstrates that it is not a repeatable technique and is, in fact, subject to operator interpretation when multiple targets are present.

The research utilized several brands and models of radar units, operating simultaneously. When one target was present, the radars were usually within tolerance of one another; however, when multiple targets are present, there is no guarantee as to which vehicle's speed will be displayed — the radar units can well read different speeds, leaving interpretation up to the operator. It is demonstrated with these units that the decision to issue a citation is highly dependent upon the operator, relative to the instrument.

Radar can pick out the *strongest* signal (which is a function of target gain, cross sectional area, and distance) or it can pick out the *fastest* signal. Note that these selections are relative — they are relative to what other vehicles are doing, what kind of vehicles they are, and their relative distances. Police radar cannot pick out the speed of the *nearest* vehicle. To make matters worse, one manufacturer has installed "gun sights" in an attempt to aid in target discrimination — the gun sights are useless. One manufacturer also allows the officer to "blank" out his/her own displayed speed in a moving mode, so as to not reveal to a potential violator that the officer was also violating the law.

In 1985, a paper was published in the Journal of Forensic Sciences, bemoaning the lack of *target specificity* with radar. In 30 years, little has changed. In an attempt to improve radar, the manufacturers have approached target speeds by using Fast Fourier Transform (FFT) spectral analysis, as opposed to analog Phase Lock Loop (PLL) techniques. The FFT technique, as does PLL usage, treats one target signal as "the" signal and processes the other return signals from other vehicles as if they were noise. In fact, this "noise" may represent the actual speed of the vehicle that is closest to the police car.

The attitude of some is that, "Well, it is only a traffic citation." In many cases, this is true. But some jurisdictions use strict traffic enforcement as the basis of *presumptive traffic stops* — they will issue a warning for the traffic violation and instead are looking for a reason to search a vehicle for contraband. This use of faulty radar as the basis for non-warranted traffic stops will also be explored.

Daubert, Traffic Radar, Doppler



Engineering Sciences Section - 2015

D28 Do I Really Have to Measure Everything Twice in My Forensic Investigation: Isn't Error Analysis Just Something One Does in Bench Science?

Thomas L. Bohan, PhD, JD*, MTC Forensics, 54 Pleasant Avenue, Peaks Island, ME 04108

After attending this presentation, attendees will be provided with an orientation to uncertainty estimates in the reporting of forensic conclusions and, in particular, to the talks immediately following which will explore the topic in detail.

This presentation will impact the forensic science community by providing one of the most important of the recommendations of the 2009 National Academy of Sciences (NAS) Report, *Strengthening Forensic Science in the United States: A Path Forward*, dealing with forensic practice in the United States.

This presentation leads off a half-day program examining the aspects of reliability analysis across a wide range of forensic practices. To set the stage for this program, it appears useful to lay out the reasons why such an examination is important so long after the NAS Report that sent shock waves through the public, this country's system of justice, and the forensic community. The shock came from the NAS Report's pointing to serious shortcomings in the practice of forensic science in this country. One of these shortcomings was the lack of appreciation the NAS Report's authors found of the importance of reliability analysis applied to stated forensic conclusions: "Few forensic science methods have developed adequate measures of the accuracy of inferences made by forensic scientists. All results for every forensic science method should indicate the uncertainty in the measurements that are made, and studies must be conducted that enable the estimation of those values."¹

This statement is embedded in several of the Report's thirteen recommendations, especially Recommendation 3 (Ibid, page 190.) Since the February 2009 issuance of the Report, the resolve within the forensic scientific community and within all three branches of the federal government to act has been mounting year by year. This reaction began within the American Academy of Forensic Sciences, which in August 2009, following Academy-wide consultations, endorsed all thirteen of the NAS Report's recommendations.

With all of the efforts expended, by forensic practitioners, by professional forensic organizations, and by the United States Congress and the White House, why is it still necessary to take time to address one of the most fundamental aspects of the NAS Report's observations, the need to report forensic results in such a manner that those receiving them can immediately grasp their reliability? Where the results are in numerical form (for example, the weight of a drug sample, the impact speed of a wrecked train, the relative humidity the night a crime took place), the reliability can be stated in terms of the likelihood that the number given could deviate by as much as plus or minus 10% from the true value. If that likelihood is very low, one might conclude that the result given was quite reliable, and conversely. Regardless of that likelihood, the conveyor of the number would have done his or her duty scientifically. If the numerical result is presented in naked form, with no "error bars," those receiving it have no way to weigh its importance and, in a scientifically ideal world, should not accept it.

But many forensic results (for example, the medical testimony that a deceased person died from a blow to the head) are not delivered in the form of a quantitative statement. Nevertheless, in addition to stating the basis for such qualitative conclusions, the person delivering them should be able to estimate the likelihood that he or she is incorrect. That is the way science must be done and forensic science should be no different.

Furthermore, as stressed by the NAS Report backed by the American Academy of Forensic Sciences through its 2009 Position Statement, if reliability estimates in a particular field are impossible or difficult to accomplish, the work necessary to correct this situation must be done and the results presented in the peer-reviewed literature.

The acceptance of the need to make quantitative reliability estimates varies across the forensic practice fields. For those that are specialized applications of a broader scientific tradition, the need to provide "error bars" with results has always been recognized. For those that originated with police practice, not so much. That there still exists resistance in some of the latter fields appears to result from two distinct problems. The easiest of these to deal with is actually one of the problems spotlighted by the NAS Report: the need to have a uniform nomenclature understood across all types of forensic practices. It should be added in connection with reliability estimates that there must be agreement across the practices as the words to be used with such estimates. It has been repeatedly noted that certain classes of practitioners strongly resist using such words as "error" and "uncertainty." Given the baggage that these words carry from the non-technical society, such resistance should not be criticized. Rather, there has to be a broad-based attempt to settle on words that convey what needs to be conveyed in the scientific context without at the same time smuggling in misleading meanings from the world at large.



Engineering Sciences Section - 2015

The other problem that needs to be overcome is a conceptual one. Most simply, it can be illustrated by the question: What relevance can studies of error over the last ten years across the entire field of handwriting identification have to assessing uncertainty in the work of a *specific* examiner in a *specific* case today? In order to achieve the goal of habituating all forensic practitioners to providing quantitative reliability estimates to all their conclusions, this question has to be answered in a convincing manner.

Reference:

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Reliability, Error Rate, Uncertainty



Engineering Sciences Section - 2015

D29 Error and Uncertainty in Toxicology and Drug Analysis — Terminology Matters

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After attending this presentation, attendees will understand the key differences in the terms “error” and “uncertainty” as applied in forensic science. The emphasis will be on quantitative methods used in forensic toxicology and seized drug analysis, but the concepts are generalizable to any quantitative measurement.

This presentation will impact the forensic science community by clarifying the definitions and proper use of these two terms (“error” and “uncertainty”) which are used to describe the utility and reliability of quantitative forensic data.

It is common for the term “error” to be used interchangeably with “uncertainty.” For informal conversation this is harmless; in forensic science this usage is misleading and potentially dangerous. The two terms are not synonymous and describe two fundamentally different properties associated with any quantitative measurement. While neither error nor uncertainty can be eliminated from any measurement process, it is possible to provide reasonable and defensible estimates of both quantities. Error is related to accuracy (how close is a given value to a true value?) while uncertainty is related to precision (how reproducible is a given result?). An estimate of uncertainty does not imply doubt or a poor measurement process. Rather, an uncertainty estimate improves the utility and reliability of any measurement. It is critical that forensic practitioners use these terms properly and convey the differences between these two words to stakeholders in judicial proceedings. This presentation will clarify the terminology and offer suggestions for explaining and presenting the differences to law enforcement and triers-of-fact. The emphasis will be on quantitative measurements obtained in forensic chemistry, but the concepts are generalizable to any quantitative measurement.

One of the challenges in the forensic context is defining what measurements are quantitative. This presentation will touch on this issue. For example, with searchable databases, a quantity is produced that expresses the closeness of agreement between a questioned and known exhibit. Thoughts to consider for such metrics will be introduced.

“Error” in a quantitative measurement refers to the difference between the reported measured value and the true value.¹⁻⁴ Given that the true value of any measurement is inherently unknowable, error is estimated using reference materials. Ideally, error values are obtained from a source such as the National Institute of Standards and Technology (NIST); examples are certified weights and standard reference materials such as ethanol in water. Uncertainty is defined as the range in which the true value is expected to lie. Uncertainty is reported along with a probability, usually stated as a confidence level. Both of these concepts will be defined and clarified using examples from seized drug analysis (reporting the weight of an exhibit) and forensic toxicology (blood alcohol analysis and postmortem drug concentration levels). Suggestions will be provided to illustrate how these concepts can be conveyed to judges and juries using everyday examples.

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Error, Uncertainty, Metrology



Engineering Sciences Section - 2015

D30 Limitations Associated With the Examination and Presentation of Fingerprint Evidence

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After attending this presentation, attendees will understand the critical decision-making points in the fingerprint comparison process, the types of errors that may occur, the research that tests examiner performance, and the limitations associated with reporting and testimony.

This presentation will impact the forensic science community by highlighting the limitations associated with both the examination and presentation of fingerprint evidence.

The analysis, comparison, and evaluation of latent fingerprints are an interpretive process. Examiners apply the knowledge gained through training and experience, in addition to the current research in the field, to reach a decision when conducting fingerprint examinations. As with any subjective process, human factors can influence the outcome of latent print examinations. This presentation will discuss the steps involved in the latent print examination process, identifying those that are potentially the most susceptible to cognitive biases. Quality assurance measures, such as documentation and verification, may be implemented to minimize the effects of various human factors and reduce variability in the examination process.

During the latent fingerprint comparison process, examiners are assessing the quality and quantity of information in the prints. If this information is misinterpreted, an error may result. Errors may occur during the examination process (false positive or false negative) or during the presentation of evidence in reports or testimony (overstating or understating the value of the conclusion). As such, examiners need to state their opinion and provide the scientific basis to support that decision, while clearly expressing the limitations of the science.

The literature related to error rates emphasizes the difficulty in calculating a meaningful error rate for both individual practitioners, as well as across the entire discipline; but the possibility for error will always exist. Recent studies have demonstrated that while examiners may reach accurate and reliable conclusions, the calculated error rates in those studies are specific to the testing conditions of the studies and may not include all the quality assurance measures of a laboratory or consider other variables inherent to fingerprint examinations.

Three main limitations associated with the reporting of fingerprint opinion conclusions include making identifications to the exclusion of all other sources, providing an absolute or numerical certainty, and stating that the error rate is zero. This presentation will discuss how these concepts are not supported by research and why they should not be used to express conclusions. Instead, examiners must clearly explain the scientific basis for their conclusions while remaining cognizant of the associated limitations.

Fingerprint, Error, Evidence



Engineering Sciences Section - 2015

D31 Certainties and Uncertainties in Accident Reconstruction — How Correcting the Other Side’s Misstatements Affects Jury Verdicts

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After attending this presentation, attendees will understand uncertainties in accident reconstruction.

This presentation will impact the forensic science community by improving attendees’ understanding of accident reconstruction.

Accident reconstruction involves the examination of the data from an accident (usually a multi-vehicle collision) to determine what happened. Since the state or local police usually provide reconstruction services for the prosecution in criminal cases, the independent accident reconstructionist is often engaged to support the defense. The goals are to provide the defense with an objective understanding of what actually happened and to assess whether the prosecution’s charges are supported by the evidence. The reconstruction addresses such issues as the speed and headings of the vehicles, an analysis of where each vehicle was located both before and after the impact, visibility issues, perception/reaction times, and analysis of any violations of the relevant driving statutes.

Although accident reconstruction can produce surprisingly accurate results, unless the limitations of the analytical methodology used are clearly understood, there can be significant potential errors associated with all the techniques employed. It is prudent to understand these uncertainties to ensure that the other side is not relying on a faulty premise when they claim that the accused was traveling at 100mph just before the collision. This presentation will discuss not only the uncertainties inherent in the physical procedures used to reconstruct accidents but also misinterpretations of the accident parameters to support charges which cannot be sustained.

Uncertainties relating to methods for determining a vehicle’s speed during an accident are described in the table below. Some of these uncertainties can lead to very large errors in the reconstructed speed value.

VEHICLE SPEED DERIVATION METHOD	SOURCE OF UNCERTAINTY
Speed loss from length of skid marks x drag factor	Vehicle travel before braking marks appear Uncertainties in the coefficient of friction value
Black box data	Crash event may not trigger recording Data record may be corrupted Sometimes provides wrong results
Vehicle damage Comparison to staged crash tests Using Numerical Models (CRASH III)	A crash test at the speed needed is not always available Need to convert Vehicle-To-Barrier results to Vehicle-To-Vehicle speeds Models contain many simplifying assumptions and are very inaccurate at onset of crush <u>Stiffness values are highly variable</u>
Momentum conservation calculation	Need accurate approach and departure angles, post-impact travel distances
ANCILLARY INFORMATION Distances from police scene survey Roadway friction Coefficient of restitution	Police laser-scene surveys found to have significant errors Friction values from drag sled and accelerometer measurements are not precise Coefficient of restitution can vary widely

The above discussion involved errors inherent in the physical procedures used to reconstruct accident speeds. A similar discussion could be held regarding vehicle headings and other factors relating to the reconstruction. Sometimes individuals are prosecuted or even convicted based on erroneous interpretations of the accident data.



Engineering Sciences Section - 2015

A number of vehicular homicide criminal case studies will be presented in which the accident data did not support the charges levied by the prosecution. This resulted in a substantial reduction in the charges that were sustained by the jury. Often the accident analysis presented by the prosecution found a speed for the defendant's vehicle which was far above the speed that was reconstructed by the defense. In some cases, the laws of physics were violated in the prosecution's analysis in order to arrive at the claimed vehicle speed. These questionable forensic analysis practices have a pernicious effect on a broad spectrum of the legal system since a civil action frequently follows the criminal prosecution. In that event, the questionable results of the prosecution's reconstruction often find their way into the civil litigation.

Reconstruction, Uncertainties, Verdicts



Engineering Sciences Section - 2015

D32 Forty Years of Squinting — Environmental Forensic Microscopy: 1974–2014

James Millette, PhD, MVA Scientific Consultants, 3300 Breckinridge Boulevard, Ste 400, Duluth, GA 30096*

After attending this presentation, attendees will better understand how different types of microscopes can be used in environmental/industrial investigations.

This presentation will impact the forensic science community by providing illustrations of how environmental forensic microscopy has been used to solve contamination and other questions by one investigator and his team over the past 40 years.

During the first 40 years of my career, I have had the opportunity to use various microscopes and forensic techniques to solve a number of interesting environmental/industrial questions involving product identification, indoor air quality concerns, outdoor darkening agents including various types of soot, lead particles, asbestos, and glass fibers, ceramic whiskers, corrosion debris, and particles from the World Trade Center disaster of September 11, 2001. The sampling and analysis methods I have used over the years in environmental forensic investigations were drawn primarily from the criminal forensics, industrial hygiene, and environmental monitoring areas. Combining various aspects of these disciplines allowed me to generate a procedure that fit the varied and sometimes very complex environmental/industrial situations.

Some investigations required only a light microscope but many of the microscopic analyses in environmental forensic investigations were accomplished utilizing a combination of visible light, infrared light, and electron microscopy. The light microscopy was usually performed initially with a stereo-binocular microscope followed by Polarized Light Microscopy (PLM) but may have involved Phase Contrast (PCM), darkfield, or fluorescence microscopy. Infrared microscopy was done using Fourier Transform Infrared (FTIR) microspectroscopy. FTIR is very useful when identifying organic molecules such as plastics and polymers. Scanning Electron Microscopy (SEM) was used in some studies because it allows the analyst to see particles that are smaller than can be seen with light microscopy and when equipped with an X-ray analysis unit, allows the analyst to determine the elemental composition of the particles. Transmission Electron Microscopy (TEM) also allows the analyst to see particles that are smaller than can be seen with light microscopy and when equipped with electron diffraction capabilities and an X-ray analysis unit, allows the analyst to determine the crystal structure and elemental composition of the particles.

One environmental method, American Society for Testing and Materials (ASTM) Practice D6602, provides a list of different types of microscopes used to investigate particles: a stereo-binocular microscope, capable of 1-60x magnification; a polarized light microscope, equipped with objectives in the 4-100x range of magnification (for a total magnification between 40x and 1000x); a transmission electron microscope equipped with a suitable camera; and a scanning electron microscope equipped with Energy or Wavelength Dispersive Analysis Equipment (EDS or WDS). A TEM equipped with Selected Area Electron Diffraction (SAED) and EDS is sometimes referred to as an Analytical Electron Microscope (AEM).

The capabilities of the various microscopy tools will be illustrated through short presentations of several investigations including: the source of asbestos fibers in a kitchen faucet filter; the composition of many building products studied during a case working for the Attorney General for the State of Maryland; characterization of dust that was generated by the World Trade Center tragedy of September 11, 2001; a spot called Ralph on a carpet in a South Carolina courthouse that kept growing larger; particles in home dust that were reported to be evidence of an alien visitor; and, the sources of black, white, and yellow deposits on automobiles.

Microscopy, Environmental, Historical Uses



Engineering Sciences Section - 2015

D33 Look at the Chemistry

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After attending this presentation, attendees will better understand the importance of giving even “non-detected” analytical results a closer look.

This presentation will impact the forensic science community by providing attendees with another valuable tool to use during forensic environmental investigations.

Environmental site investigations generally start with investigating the historical uses of the property. This information, along with experience with the background of the site, allows the investigator to design a sampling program to evaluate soil, surface water, ground water, and other materials and matrices as appropriate to the site. Selection of the chemical analyses to be performed on these samples also relies on the historical uses and experience with similar locations, as well as on state and federal lists of chemicals that may have been used and/or abused on the property. For organic chemicals, two primary analytical methods are commonly utilized to test for a large number of compounds. These are United States Environmental Protection Agency (USEPA) SW-846 Method 8260 for volatile organic chemicals and Method 8270 for semivolatile organic chemicals. Combined, these two methods list approximately 400 compounds that may be analyzed; typically, more than 100 target analytes are routinely reported.

After the analyses are completed, the laboratory presents the interested party with the results, which include the “hits” for the specified target analytes. These methods also provide a quantitative value for the concentration of the target analyte hit, which is a single organic chemical. If this concentration is above the applicable state or federal criteria, then there will be more investigation that may lead to site remediation.

There is a “but.” If there are no concentration values above the criteria, then the sample and/or site is determined to be clean. But... is it, really?

A new house was built on a hill. The water well was a major expense because it required drilling over 400 feet into bedrock and piping the water one-quarter of a mile to the house. The water smelled like paint thinner, caused the plastic pipe to soften, and readily passed through carbon treatment tanks. Yet, the analytical chemistry using Method 8260 did not give a single target analyte “hit;” however, once the full supporting data package was acquired, it was readily apparent from the total ion chromatogram that the groundwater sample was loaded with gasoline hydrocarbons. The groundwater had “weathered” the gasoline by removing the aromatic compounds associated with gasoline that were on the target analyte list, so the reported analysis results were all “non-detects.” BUT — plenty of gasoline leftovers were still in the groundwater entering this well.

Who would have guessed that farm land would be contaminated? Maybe the hydrocarbon transmission pipeline running in front of the house should have been a clue?

Chemistry, Target Analytes, Non-Detects



Engineering Sciences Section - 2015

D34 Let's Leave the Junk in the Junk Yard

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After attending this presentation, attendees will be able to recognize that junk science exists in environmental forensic science and where it is often found.

This presentation will impact the forensic science community by increasing awareness of the fact that age-dating contaminant releases is often misused in environmental forensic science and some of the common misunderstandings that often turn into junk science.

One of the first questions often asked in environmental litigation concerning the release of a contaminant into the environment is, When was it released? The answer to this question is important in determining responsibility and liability for the release, particularly when there was a series of owners or operators of the facility. Determining when a contaminant was released is often referred to as "age-dating." The literature contains numerous proposed procedures for estimating the timing of a release. Too often these procedures are used by so-called "experts" as a stand-alone magic formula to age-date the release and they ignore the science behind the procedure.

A case in point is estimating the age of the released gasoline product samples using the toluene-to-n-octane (T8) ratio technique by Schmidt, et al.¹ The T8 procedure is based on the increasing enrichment of aromatics in gasoline in the 1970s and the reduction of n-alkanes in gasoline beginning in the 1980s. Schmidt, et al. examined gas chromatograms of 130 regular and mid-grade gasolines produced between 1973 and 2001 and graphed the T8 ratios versus the year of production. The T8 ratios fell into three groups: T8 less than 5 was the 1973 to 1983 group, T8 between 5 and 10 was the 1984 and 1993 group, and T8 greater than 10 was the 1984 to 2001 group. In one recent case involving gasoline contamination from an underground storage tank, the consultant said the gasoline was released between 1994 and 1993 based on the T8 ratio; however, the consultant used the results from a groundwater sample collected from a monitoring well. In doing so, the consultant ignored basic chemistry, including solubilities and Raoult's Law in comparing groundwater results to that of dispensed gasoline. The equilibrium solubility of toluene in water is more than 700 times greater than that of n-octane at 20°C. This is akin to the idiom "comparing apples to oranges." In addition, the consultant also ignored the effect of biodegradation, volatilization from the groundwater, and transport (including sorption and retardation) differences that will create changes in the ratio between toluene and n-octane dissolved in groundwater, all of which will change the T8 ratio. This consultant's methodology was just junk science.

Another commonly misrepresented age-dating method is the Christensen and Larsen Age-Dating Model for diesel fuels.² The Christensen and Larsen model is based on the assumption that n-heptadecane (n-C17) aerobically biodegrades in the environment at a constant and uniform rate while pristane (2,6,10,14-tetramethylpentadecane or Pr) is resistant to biodegradation. Christensen and Larsen reported a linear relationship when the n-C17/Pr ratio is plotted against the known age of diesel spills from 12 sites in Holland and Denmark over a 20-year period. In order for this model to work, the biodegradation of n-C17 must be aerobic, uniform, and consistent over time. Christensen and Larsen placed a number of caveats on the use of the model and many aspects of the method are still raising a large caution flag over its use; however, many "experts" ignore the caution flag and just plug numbers into equations based on the Christensen and Larsen Model to age-date petroleum releases.³ This happened in a recent case where a consultant tried to age-date a release of No. 6 fuel oil. The consultant ignored the fact that the physical properties of No. 6 fuel oil are different from diesel fuel and what effect these differences have on aerobic biodegradation. Christensen's and Larsen's caveats were ignored. The consultant also used a sample containing more than 30% No. 6 fuel oil for the age-dating. At this concentration, there is free-phase oil present, which inhibits microbial action. With free-phase product present, there is less aerobic biodegradation on the interior of the product than on the surface. Thus, the biodegradation of the No. 6 fuel oil was not constant and uniform as required by the model. The age-date calculated by the consultant was nothing more than junk science.



Engineering Sciences Section - 2015

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Environmental Chemistry, Age Dating, Junk Science



Engineering Sciences Section - 2015

D35 Using SESOIL to Evaluate Contaminant-Release Time Frames in an Environmental Litigation Context

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The goal of this presentation is to demonstrate the application of the United States Environmental Protection Agency (USEPA)-developed Seasonal Soil (SESOIL) model to analyze contaminant transport through the unsaturated zone to estimate, in conjunction with other environmental data and forensic techniques, contaminant-release timeframes.

This presentation will impact the forensic science community by providing pertinent information to illustrate how a tool developed primarily for evaluation of environmental data in a regulatory context can be used to develop scientifically defensible arguments in environmental litigation matters.

SESOIL (SEasonal SOIL compartment model) is a 1D vertical fate-and-transport model for the unsaturated zone, which is designed to simultaneously simulate soil water movement and contaminant transport. The model is based on mass balance and partitioning of the contaminant between dissolved, sorbed, vapor, and pure phases. SESOIL simulates long-term pollutant fate and migration in the unsaturated soil zone, considering: hydrologic cycle of the unsaturated soil zone; pollutant concentrations and masses in water, soil, and air phases; pollutant migration to the groundwater; and, pollutant volatilization at the ground surface.¹

The SESOIL model was developed by the USEPA, and has been adopted by a number of federal entities (e.g., the United States Department of Energy) and state regulatory bodies (e.g., the New Jersey Department of Environmental Protection) for use in evaluating future impacts to groundwater from soil source zones. Based on this widespread regulatory acceptance, the SESOIL model provides a valuable, scientifically defensible tool that can be used in conjunction with other site-specific data to evaluate fate and transport of contaminant travel times in the unsaturated zone in the context of environmental litigation. Specifically, assuming that the approximate date in which groundwater beneath an overlying column of soil was first impacted can be reliably estimated, SESOIL modeling analyses can be performed to provide estimated timeframes of contaminant release at or near the land surface, which is often an important aspect in environmental litigation matters.

A case study will be presented in which the SESOIL model was used in an insurance litigation case to estimate the approximate start date of a leak from an underground petroleum pipeline. Site-specific data, including soil texture information provided by lithologic logs, soil concentration data from samples collected from vadose soils, and information obtained from other site investigation documents were used to define model inputs. The results of the SESOIL analyses were then compared with site groundwater quality data, including sample results for benzene and the gasoline additive Methyl-tertiary Butyl Ether (MtBE), to estimate the approximate date of commencement of the pipeline release. Corroboration of the SESOIL model results by other lines of evidence from the site historical record, as well as other considerations that should be taken into account when utilizing SESOIL modeling methods for forensic purposes, will also be discussed.

Reference:

1. Bonazountas, M., Hetrick, D.M., Kostecki, P.T., and Calabrese, E.J., 1997. *SESOIL in Environmental Fate and Risk Modeling*. Amherst Scientific Publishers, Amherst, MA.

Vadose Zone, Contaminant Transport, Modeling



Engineering Sciences Section - 2015

D36 Where Is New Jersey Going?

*James S. Smith, PhD**, Trillium, Inc, 28 Graces Drive, Coatesville, PA 19320-1206; and *Carol A. Erikson, MS*, 1433 Yarnell Station Boulevard, Knoxville, TN 37932

After attending this presentation, attendees will be aware of an important recent ruling in New Jersey that affects the practice of environmental forensics.

This presentation will impact the forensic science community by providing attendees with the background needed to determine how a 2013 court ruling will affect their practice of environmental forensics.

A dry cleaning site in New Jersey was found to be contaminated with the dry cleaning solvent Perchloroethylene (PCE); another name for this chemical is tetrachloroethene. Historically, this facility was used by two parties, the first of which ran the dry cleaning operation from 1957 to 1982. The second party took over in 1982 and continued the cleaning operation through 2011. The property was owned by a third party, having acquired it by deed in 1971. After soil and ground water contamination was found on the site in 2009, the court determined that the second party to operate the dry cleaning business was solely responsible for all of the PCE contamination. Although the second party alleged that the first party was also liable, the court disagreed because there was no proof (i.e., documentary evidence or eyewitness testimony) of actual discharges or releases at the dry cleaners prior to 1982. Simply stated, if it wasn't observed happening, and/or documented, then it did not (legally) occur.

This opinion renders reasonable environmental forensics a waste of time, effort, and money.

Forensic analysis is the recreation of an incident or many incidents that, most often, were not observed by anyone at the time they happened. The precedent set by this New Jersey ruling means that, from this point forward, there must be direct evidence of actual discharges — an often impossible standard.

Equally disturbing is that the court allowed an expert witness to offer testimony regarding his “age-dating” of the PCE release. This expert testified that the high ratio of PCE to its anaerobic biodegradation breakdown products (Trichloroethylene (TCE), cis-1,2-Dichloroethylene (cis-1,2-DCE), and vinyl chloride) was the result of “relatively recent” releases because the PCE had not had time to degrade in substantial amounts. This is pure junk science and totally false: it is not possible to age-date PCE in the environment based on parent-to-degradation product ratios or by any other chemical means. But, it sounds good, and it advanced their case.

The 2013 ruling that a party must be shown, by a preponderance of direct evidence, to have committed a discharge that was connected to the environmental damage will do nothing to ensure that all polluters are held accountable for their actions. It will instead promote science-based advocacy.

Dry Cleaner, Age-Dating, Junk Science



Engineering Sciences Section - 2015

D37 Federal Court Testimony — Tennessee Valley Authority (TVA) Kingston Slurry Ash Spill

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The goal of this presentation is to help attendees learn how to evaluate large volumes of information in preparation for federal court testimony on a large-scale environmental case.

This presentation will impact the forensic science community by describing how potential expert witnesses are often retained to play a key role on projects, even though the whole case involves many disciplines. There may be a large volume of historical data and many moving parts during legal discovery. The performance of an expert witness in federal court depends on the expert's ability to stay abreast of findings, and new facts, during the discovery process. This presentation will describe how the evaluation steps during this case were undertaken, as a review of data, reports and correspondence revealed that promises to monitor the water levels in a coal ash pile were made then found not to be done. This presentation will focus on the nature of specific testimony and questioning and how the court's decision(s) relied in part on this testimony.

A coal fly ash slurry spill occurred just before 1:00 a.m. on Monday, December 22, 2008, at the TVA Kingston Fossil Fuel Power Plant, in Roane County, TN. A reported 1.1 billion gallons of coal fly ash slurry was released from a large vertical wet pile which has been variously described as a solid waste containment area or a diked area with ponds, much of which was found to be saturated. "Dredge cells" were not constructed as described in a number of documents and drawings and the slurry traveled across the Emory River and the Swan Pond embayment, covering up to 300 acres of land, damaging homes, and flowing into waterways, including the Emory River and the Clinch River, which are tributaries of the Tennessee River. The coal ash release has been called one of the world's worst environmental disasters and was the largest coal ash release in United States history.

Questions arose regarding why the environmental release happened and who had responsibility, from an environmental standpoint, for the release. A professional engineer in 18 states, including Tennessee, provided expert witness testimony in federal court. A team of experts were retained by the parties whose homes and/or property were damaged by the coal ash release, a large number of documents from TVA and the Tennessee Department of Environmental Conservation were reviewed, and a large volume of documents continued to be produced during the discovery process.

Certain items presented in the engineer's federal court testimony were not answered by TVA and aspects of Subtitle D Resource Conservation and Recovery Act provisions were an issue. Ultimately, the court decided that there was liability on the part of TVA, based on a number of key technical facts and permitting items. Key to the court's decisions were responsibilities for properly monitoring and operating the disposal facility.

This presentation will describe the process of reviewing documents, coordinating with legal counsel on the key issues, and the approach for presenting testimony in an appropriate and organized manner. There was testimony focus on environmental management issues that did not receive attention but had been promised to regulatory agencies in the time frame leading up to the major coal ash release. When the release from the 84-acre unlined aboveground ash fill area occurred, which was 60 feet high, a mud flow wave reached out over more than 300 acres and contamination was experienced for many areas down the Emory and Clinch Rivers.

Potential expert witnesses can learn how to evaluate and focus on key issues related to large-scale environmental problems when faced with very large volumes of data, historical information, and reports to provide testimony in federal court, which focuses on the causes of, and the liability associated with, major environmental problems.

Federal Court, Testimony, TVA Kingston



Engineering Sciences Section - 2015

D38 Environmental Forensics Vanity Journals

James S. Smith, PhD*, Trillium, Inc, 28 Graces Drive, Coatesville, PA 19320-1206; and Carol A. Erikson, MS, 1433 Yarnell Station Boulevard, Knoxville, TN 37932

After attending this presentation, attendees will better understand the peer-review process for scientific literature.

This presentation will impact the forensic science community by providing attendees with insight into how improperly peer-reviewed publications will affect their practice of environmental forensics.

In court, the judge is the gatekeeper for the expert opinions rendered in litigation, and the United States Supreme Court has suggested certain criteria the judge can use to allow or ban an expert's testimony. One of these criteria is whether the scientific methodology used to develop the testimony and opinions is published in a peer-reviewed journal. This becomes an extremely important criterion because the peer-review process is the place where scientists have the opportunity to remove "junk science" (i.e., data produced using improper or non-scientific methodology) from their own scientific literature. It is the scientist's way of self-policing their discipline.

The expectation for scientific peer review is that it will be done with extreme rigor. No rubber stamps here. The sampling protocols, chemical analytical methods, quality control, data interpretation, and conclusions are all under the microscope. The publication must have merit for advancing the known realm of information of the paper's subject matter.

One expects the article to be critically challenged by each reviewer. Corrections, changes, and additions are normally necessary prior to approval for publication. More importantly, papers submitted to a peer-reviewed scientific journal that are below par should not appear in the publication. If the submitted paper was sent to a reviewer who does not have the required training, education, or experience to critically analyze the subject matter, then the reviewer will send it back to the editor so it can be re-assigned to another reviewer with the proper credentials.

Unfortunately, many published papers in the field of environmental forensics are not critically peer reviewed, despite being published in a self-proclaimed peer-review journal. This failing fosters the expert's sense of safety in opinions that speculate scenarios without a scientific basis and encourages opinions based on what might be possible, rather than what is more probable. The topic of age-dating a release is the elephant sitting on the head of this pin.

The following publications are the focus of this presentation: (1) Oudijk, Gil, "Age Dating Heating-Oil Releases, Part 1, Heating-Oil Composition and Subsurface Weathering," *Environmental Forensics*, Vol. 10, pp. 107-119, 2009; and, (2) Oudijk, Gil, "Age Dating Heating-Oil Releases, Part 2, Assessing Weathering and Release Time Frames Through Chemistry, Geology and Site History," *Environmental Forensics*, Vol. 10, pp. 120-131, 2009.

This presentation will point out some of the most egregious problems with this "peer-reviewed" reference. The concern is real because the information presented in these papers, which should never have been published in a peer-reviewed journal, is presently being used in litigation. The lack of rigorous scientific peer review prior to publication shows a lack of respect for the justice system and forensic science.

Peer Review, Scientific Literature, Publication



Engineering Sciences Section - 2015

D39 Sometimes Electrical Work Results in Damage, Fire, Injury, or Electrocution

Thomas P. Shefchick, BSEE, 853 Enisgrove Way, The Villages, FL 32163-2447*

The goal of this presentation is to illustrate how to use burn patterns to determine the origin and cause of an electrical problem.

This presentation will impact the forensic science community by discussing methods to determine the cause of an electrical occurrence.

The first case concerns an 8,000 horsepower synchronous motor that broke down and subsequently was repaired by an electrical mechanical apparatus repair company. Three months after the motor was repaired and reinstalled, problems were encountered. The motor's rotor had been rewound after the initial breakdown. Rewinding a rotor involves taking it completely apart and replacing the electrical components that produce its magnetic fields. Inspection of the motor installation found no evidence of damage. Therefore, the motor was removed and disassembled. The motor's stator showed no visible damage but the rotor's components had sustained heat damage. The rotor had cooling fins to ventilate its heat during operation; however, the motor repair company had installed the fins in a manner that would entrap the heat which caused damage to the rotor windings. This problem could have been prevented by marking the proper cooling fin orientation before the rotor was disassembled.

The second case discusses mufflers which had been installed on generators that supplied electrical power to a luxury residential establishment. The mufflers were installed to reduce the noise produced by the generators. Three generators were installed in a stand-alone building and controlled by a computer system which rotated operation of the generators. One evening the computer system failed to transfer from generator number 1 to generator number 2 and subsequently the roof of the generator building caught fire. Examination of the burn patterns indicated that heat leaking from a muffler connection ignited the combustible roof. The mufflers were removed from the generators and their internal metal components were found severely heat damaged which indicated that the problem originated within the mufflers. Analysis of the materials in the mufflers revealed that the wrong type of metal was used to manufacture them.

The third case involves a severe electrical injury at a luxury hotel. The hotel had an electrical contractor install equipment to improve their electrical power factor. The purpose of improving the power factor was to reduce their electrical utility bill; however, the contractor was not able to complete the installation because a door covering the control components did not fit properly. When the correct door was obtained, the contractor suggested that a hotel employee could install it. Subsequently, a maintenance man of the hotel was severely electrically burned when he attempted to install the door. The burn patterns on the door and motor control center indicated that his screwdriver slipped while attempting to attach the door. This problem would not have occurred if the motor control center was completely de-energized as required by warning labels on the motor control center.

The last case relates to electrocution at a school. The school had been experiencing a problem with a steamer oven in the kitchen of their cafeteria. A repair company was contacted and sent an electrical repairman to fix the steamer oven. The repairman removed covers from the steamer oven to examine the operation of its internal components. While examining the components, the man was electrocuted. Components on the interior of the steamer oven had burnt hair on them which indicated that the man's head had made contact with the electrically energized components. In addition, the workman was surrounded by grounded metal equipment. This problem could have been avoided by mounting a disconnect switch at the steamer oven and providing sufficient workspace around equipment as required by the national electrical codes.

Burn Patterns, Electrical, Electrocution



Engineering Sciences Section - 2015

D40 Hot Air Balloon Fires, Power Lines, Product Liability

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After attending this presentation, attendees will understand the mechanism of failure, accident prevention, and outcomes of hot air balloon power line strikes. Product liability issues relating to an actual case and potential design changes to prevent short circuits will assist the legal and forensic engineering community.

This presentation will impact the forensic science community by providing attendees with an understanding of the mechanisms, causes, and product liability issues of hot air balloon power line strikes.

Hot-air ballooning can be an enjoyable recreation. Pilots fall under Federal Aviation Administration (FAA) jurisdiction with a “lighter-than-free-air balloon” rating. Per Federal Regulation Sec 61.68, commercial balloon operators are required to maintain an FAA mandated pilot’s license, type-specified for the apparatus. Pilots are trained in collision avoidance under 14 CFR and a ground crew is required to assist with takeoff, flying, and landing. What is not often mentioned, or mediated upon, is that 34 hot air balloon accidents occur per 10,000 flight hours in the United States. Of these, 12% are due to balloon convergence with power lines/utility poles and 80% of all combined fatalities are the result of an accidental power line strike.

One of the most recent accidents occurred on May 9, 2014, in Ruther Glen, VA, with an event originating outside of Doswell, VA. There were three fatalities and a debris path 1.75 miles long. The National Transportation Safety Board (NTSB) reports that between 1983 and 2007 there were nine power line contacts involving balloons resulting in 17 fatalities.

Most power line strikes occur at takeoff and landing sites, close to populated areas with distribution power lines ranging in voltage from 4,160 to 13,200 to as high as 34,500 volts phase-to-phase. The distance between phases ranges from about two to six feet depending on the type of construction. Sub-transmission lines ranging from 44,000 to 69,000 volts, or transmission lines ranging from 115,000 to 735,000 volts with 10- to 30-foot spacing between phases, may be nearby. The National Electrical Safety Code (NEC) sets out phase-to-phase spacing and phase-to-ground spacing for power lines.

Power lines are rarely insulated but are bare copper or aluminum conductors. While contact with one phase may occur without incident to a balloon, contact between phases is much more dangerous. Incidents where a bird’s wingspan shorted two phases, electrocuting the bird, igniting its feathers, and starting a fire have been investigated. Balloons, given their much greater size and potential conductivity of metallic components, can likewise make phase-to-phase contact or get tangled up and cause a short circuit.

Given the foreseeable dangerous risk of contact, product liability can become a major issue, especially regarding the construction and design of the balloon basket. Those who design, manufacture, sell, or furnish products that are unsafely defective may be liable — even strictly liable for defects that make a product unreasonably dangerous. Yet despite the foreseeability of such accidents, basket design has not changed significantly.

The construction of a balloon assembly falls under the jurisdiction of the NTSB. The balloon envelope is made of nylon, which is an insulating material and would not short circuit a power line. The problem is the metallic parts of the balloon, which are not insulated. The wicker basket usually contains an aluminum frame and four propane fuel tanks which are either stainless steel or aluminum. Thus, when a basket with its frame and tanks comes in contact with a power line a phase-to-phase short circuit may occur. The KWh quantity of energy in the ensuing arcing is usually sufficient to melt and vaporize the aluminum or stainless steel and perforate the propane tank wall, releasing and igniting the propane and the wicker basket before any power line fuse or relay operates to clear the fault. The hot gas also rises, enters, and heats up the balloon causing an uncontrolled rate of climb. The occupants and pilot have only one choice...jump!

Similar accidents have been investigated and an insulation system has developed and tested in the laboratory to prevent the short circuits occurring in most instances. This insulation concept has been presented in successful product liability litigation involving balloons. This presentation includes the details of one such hot air balloon fire/power line strike, fatality, injury, and associated litigation outcome.

Hot Air Balloon Fires, Power Lines, Product Liability



Engineering Sciences Section - 2015

D41 Forensic Engineering Investigation of Burn Injuries in a Defective Spa Pool Light Installation

A.K. Aleksander, PhD, Aleksander & Associates, PA, PO Box 140558, Boise, ID 83714*

After attending this presentation, attendees will understand the need for a complete forensic engineering investigation and evaluation.

This presentation will impact the forensic science community by explaining the need for a thorough forensic engineering investigation, which systematically evaluates the injury evidence, the mechanism of injury, the type and properties of the light, the methods of installation, the electrical codes that are applicable, and the methodology of verifying laboratory tests in the field.

A middle-aged woman who was paralyzed below the waist enjoyed the benefits of a spa pool at a major national chain hotel. As a disabled person, she was particularly grateful that she had full access to the hotel hot tub and spa pool facility. She was assisted by her caretaker who helped her wheelchair to the pool and helped her into the water, using the handrail and steps provided.

After she left the pool, it became apparent that she had suffered third-degree burns along her back, which required hospitalization and significant treatment. She had been burned by a light that was installed in a defective manner and in fact that same light had the further potential to cause serious harm to others.

This presentation reconstructs the events and factors leading up to the injury event and concludes in the final and site inspection which corroborated the initial testing, along with some unexpected surprises. Attendees will benefit from this presentation by gaining an understanding of the need for a thorough forensic engineering investigation, which systematically evaluates the injury evidence, the mechanism of injury, the type and properties of the light, the methods of installation, the electrical codes that are applicable, and the methodology of verifying laboratory tests in the field.

The light at issue was a halogen bulb mounted in a watertight enclosure, manufactured for use in a pool; however, in this case, the light was mounted in the second step of a stair section that allowed persons to enter the spa pool. By mounting this light in the stair “kicker,” the vertical wall between adjacent stairs, this light was not submerged as would be the case in the manufacturer’s intended installation, but rather was adjacent to users as they entered the pool or sat on the steps. In this case, as the plaintiff sat on the step she was unaware that her body was in contact with an exceedingly hot surface.

A halogen light exemplar was examined in the laboratory and fitted in an underwater test fixture. A test device, an instrumented sponge, was developed to measure the temperature-over-time relationship, resulting in surface contact temperatures of 195°F in a matter of just a few minutes. Photos and graphs of this measurement process will be presented. Furthermore, a pork surrogate was developed to corroborate the burn patterns and depth of thermal penetration observed in the medical reports of the injuries to the plaintiff.

These findings were then taken to the site investigation. Actual tests in the hotel spa pool confirmed the lab results. This site testing was performed in the presence of 16 attorneys, experts, and unidentified participants in the case. Some of these individuals actually placed their hands against the hot light and subjectively confirmed the nearly instantaneous pain. This was a somewhat unusual event that seldom occurs in site inspections and will be further discussed.

As a follow-on to the inspection, the electrical circuit was inspected, which required the partial dismantling of the spa wooden deck covering. Several more surprises were discovered as it turned out that the installation was defective, the Ground Fault Interrupter (GFI) circuit was deficient, and the entire installation posed a real electrocution risk to anyone using the facility.

The case was settled, but a letter was sent to the city and the hotel chain advising them of the critical safety issues discovered during the site inspection. This was done as part of the code of responsibility of the Professional Engineering License in that state.

Spa Pool, Thermal Burn, Halogen Light



Engineering Sciences Section - 2015

D42 Sometimes Deficient Electrical Installations Result in a Fire

Thomas P. Shefchick, BSEE, 853 Enisgrove Way, The Villages, FL 32163-2447*

After attending this presentation, attendees will understand that electrical deficiencies are frequently missed by inspectors and that it sometimes takes several years for these deficiencies to cause a fire. Three fire cases will be utilized to show what basic visual inspection would have revealed. In addition, the use of burn patterns (damage caused by heat) to determine the point of fire origin and cause of the fire will be demonstrated.

This presentation will impact the forensic science community by illustrating that many electrical installations are not sufficiently inspected.

A fire occurred in a fast-food restaurant located on the 1st floor of a large building in the downtown area of a resort community. The burn patterns on the building and in the restaurant indicated that the fire originated in the basement. Minimal fire damage was found on the 1st floor of the building but the 2nd floor of the building was completely destroyed. The entire building was condemned and had to be demolished. Examination of the burn patterns in the basement indicated that the fire originated at a ceiling joist where an electrical cable was located. The cable supplied power to a toasting oven located in the fast-food restaurant. It had been installed three years prior to the fire by a contractor renovating the facility from an ice cream parlor to a fast-food restaurant. An architect had been retained for the renovation; however, the contractor had used an undersized electrical cable and did not install it in an electrical conduit (metal pipe) as specified by the architect. In addition, the local electrical code required the cable to be installed within a thermally protected wall. A visual inspection of the basement ceiling would have revealed the electrical deficiency.

Another fire occurred in a retirement condominium community. The burn patterns in the fire building indicated that the fire originated in a common wall between the kitchen of an apartment and a shower stall of another unit. The point of fire origin was an electrical junction box in the wall for the oven located in the kitchen unit which sustained minimal fire damage. Aluminum wiring from the apartment's electrical panelboard was spliced to stranded copper wiring from the oven in the electrical junction box. This is a violation of the National Electrical Code which requires that conductors of dissimilar metals shall not be intermixed in a terminal or splicing connector where physical contact occurs between the dissimilar conductors such as copper and aluminum. This condominium complex had a reputation for having problems with aluminum/copper wiring and over the years several electrical renovations had been conducted without resolving all of the problems.

The third case study is a wildfire which is a large destructive fire that spreads quickly over woodland or brush. Inspection of the burn patterns on the terrain and vegetation indicated that the fire originated near a highway advertising billboard. The billboard had been damaged by high-velocity winds in the area. The electrical conduit (metal pipe) supplying power to the billboard's lighting system was damaged and broken apart. A metal detector revealed the presence of metal on the ground beneath the billboard. The high-velocity winds had caused the electrical wiring in the conduit to short-circuit and drop molten metal onto dry brush on the ground thus initiating this fire. The billboard's conduit was Electrical Metallic Tubing (EMT) which is thinner than rigid electrical conduit. In addition, EMT is simply pushed together whereas rigid electrical conduit is threaded together and sturdily mounted. The local electrical utility requires rigid electrical conduit to be used and the billboard has been inspected on a yearly basis for more than 20 years.

Burn Patterns, Conduit, Copper/Aluminum



Engineering Sciences Section - 2015

D43 Did Radiant Heat From Electric Coils Cause a House Fire?

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After attending this presentation, attendees will better understand safety features in modern heating and cooling equipment and their applicability to a residential fire. Attendees will also gain an understanding of the importance of testing in forensic investigations.

This presentation will impact the forensic science community by highlighting the pitfalls of untested hypotheses in origin-and-cause investigations.

This case study involves a residential fire in southern West Virginia. A two-year-old heat pump serviced a double-wide manufactured home. The ducts and plenum boxes were replaced at that time in order to provide heated and cooled air to the home and one-story addition. On the date of the fire, the heat pump had stopped operating and the installer visited the home to return the unit to working order. Roughly 20 minutes after fixing the unit, a fire originated under the home, which eventually destroyed the structure.

An investigation was commenced by the homeowner's insurance company. The experts hired to perform the origin-and-cause investigation quickly determined that the fire started within the supply ducts near the heat pump. Particular attention was paid to the electric heating coils and control panel. The experts claimed that the unit was installed without thermal fuses, which are intended to shut off the second stage of heat when certain temperatures are reached. Additional installation deficiencies were identified, including insufficient duct diameter, improper filter, and inadequate fan setting. The homeowner had been notified of the fire by black smoke emanating from the floor registers. The experts demonstrated similar smoke by applying flames to the inner plastic jacket of the flexible ducts. The experts determined that the fire was caused by radiant heat from the electric heating coils. The experts hypothesized that the installation deficiencies allowed temperatures to rise to the point that the inner jacket of the duct ignited.

A separate origin-and-cause investigation was performed, which confirmed the area of origin. Initially, the experts' hypothesis seemed plausible, especially given the apparent lack of incendiary causes or other ignition sources near the area of origin; however, in order to adhere to the scientific method, all hypotheses must be tested in order to be confirmed as a theory. An equivalent heat pump was purchased and installed at this study's facilities. Additionally, the ducts and plenum boxes were reconstructed to depict airflow conditions at the subject home.

Several thermocouples were placed in critical areas in order to document the testing. Temperature readings were gathered near the electric heating coils, within the heating duct a few feet downstream of the discharge point, within the plenum box, and outside the unit to record ambient temperatures. In total, nine tests of the exemplar heat pump were performed. Normal operation of the unit resulted in peak temperatures of 220°F near the coils and 120°F in the supply duct. Subsequent tests involved progressive removal of safety components and increasingly obstructing airflow. Initial restrictions in the airflow caused temperatures to increase modestly; however, the electric heat sections cycled off, indicating proper function of the thermal switches.

As alleged by the other experts, the safety components were removed. Specifically, the high- and low-temperature switches were wired over, which caused continual operation of the electric heat section. Airflow restrictions were maintained to represent a worst-case scenario. Despite these efforts, temperatures stayed well below the ignition point for common plastics. Moreover, bypassing of the limit switches resulted in component failures on the control panel of the exemplar heat pump. Such component failures were not present during the origin-and-cause investigation of the subject heat pump. Air temperatures near the coils could have resulted in ignition of highly combustible materials, such as paper and dust. Such materials were introduced to the electric heat section. Although ignition occurred, the operation of the blower effectively snuffed out these small fires. None of the testing resulted in ignition of the duct liner.

The testing highlights the need for forensic engineers to fully test their hypotheses. While the opposing experts presented a compelling case, the application of the scientific method disproved their conclusions. Despite the fire occurring less than an hour after the heat pump was serviced, the installer was able to successfully defend his company against litigation. Although there are still uncertainties as to the exact cause of the fire, the allegations of installation and repair defects were disproved.

Radiant Heat, Electric Heating Coils, Ignition Temperature



Engineering Sciences Section - 2015

D44 Forensic Engineering Evaluations of Underground Gas Line Leaks: Residential Explosions Involving Marijuana-Growing Operations in Colorado

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The goal of this presentation is to educate attendees on the cause and origin of gas line leaks and the potential consequences of altering buried gas lines. Attendees will learn several important principles of gas migration in soil, underground utility placement, trench construction, yield stresses in pipe, and the importance of performing utility clearance prior to excavating soil.

This presentation will impact the forensic science community by serving as an example of potential consequences associated with impacting buried utilities. It will also increase an investigator's awareness of various conditions that should be assessed at the time of investigation. This presentation will present photographic documentation for two case studies and discuss the relevant findings and conclusions of each matter.

Case Study 1: A single-family residence, located a few miles from Vail, CO, was heavily damaged during a June 2010 fire that occurred as the result of a propane gas leak/explosion. The source of the propane was a 1,000-gallon tank that was buried approximately 75 feet north of the northeast corner of the residence. The source of the propane leak reportedly occurred at the connection between a buried polyethylene tube (i.e., supply line) and the gas line connection/penetration into the residence.

Some parties alleged that the connection between the underground polyethylene tubing and the adapter at the residence was problematic and/or inappropriately fastened. Because of these alleged issues, the polyethylene tubing became disconnected under loads that it was reportedly designed to withstand (assuming it was connected appropriately). Others alleged that inadequate soil compaction led to soil settlement which caused the gas line separation at the residence. Based on the available data, it is likely that a combination of issues contributed to the gas line separation. The potential issues evaluated as parts of this case study included: (1) soil settlement caused by inadequate compaction; (2) the inherent characteristics of onsite soils; (3) the introduction of water from a marijuana grow operation in the residence's basement; (4) the introduction of water from other sources; and, (5) the material properties of the polyethylene tubing.

Case Study 2: A natural gas explosion occurred in April 2011 at a single-family residence in Denver, CO. This explosion caused serious damage to the structure and bodily injury to the homeowner. According to the Denver Police Department report, the cause of this explosion was linked to a natural gas leak on the adjoining property to the west (a marijuana grow operation). The natural gas leak reportedly occurred due to a separation in a one-half-inch diameter steel gas line (near the gas meter at the adjoining property) and was ignited when the homeowner lit a cigarette.

The litigation and engineering evaluations in this matter primarily focused on two potentially responsible parties who likely impacted the gas line while performing excavation activities on the adjoining property. One party performed excavation activities approximately 14 hours before the explosion and the other party excavated approximately 36 hours before the explosion. Neither party admitted to impacting the gas line; therefore, an engineering evaluation was performed to assess whether gas was more likely to migrate from an underground leak within the shorter time frame. The potential issues evaluated as parts of this case study included: (1) the porosity of soil; (2) gas flow rates; and, (3) engineering calculations regarding migration of gas under various scenarios and assumptions.

Engineering Evaluation, Utility Lines, Fire/Explosion



Engineering Sciences Section - 2015

D45 Electrical Safety Components Sometimes Cause Damage

Thomas P. Shefchick, BSEE, 853 Enisgrove Way, The Villages, FL 32163-2447*

After attending this presentation, attendees will understand that electrical safety devices must be correctly designed and maintained to prevent damage or injury.

This presentation will impact the forensic science community by imparting the value of proper electrical design and maintenance of safety components.

A homeowner had developed the habit of soaking his children's clothes in the washing machine of a stacked washer/dryer overnight and letting the water run into the washer with its top open until the water was stopped by an overflow protective device. His intention was to let the children's clothes soak overnight in the washer before closing the lid and washing them in the morning; however, one evening the water overflow device failed to stop the water flow and his house was flooded with water. The washer/dryer was disassembled to inspect its control components. Lint from the dryer was found blocking the mechanism of the water overflow device and preventing it from stopping the water flow.

The water overflow device's mechanical components should have been enclosed to prevent lint from jamming their mechanical motion, since lint should be expected from the dryer on top of the washer.

Another case involved a computer surge arrestor. The surge arrestor had caused serious smoke damage in a residence. The surge arrestor was rated at 15 amperes and its overload device did not activate. A hole was found burned through its plastic enclosure. Inspection of its interior components found that the overload device was improperly wired to the incoming power line. An electrical power supply wire went to a printed circuit board ahead of a 25-ampere fuse which had not blown. Evidence of electrical short-circuiting was found between the power supply conductor and ground conductor on the printed circuit board. This device was rated at 15 amperes and its printed circuit board should not have been protected by a 25-ampere fuse.

The third case involved a man that was injured and died while operating a motorized scissors-lift vehicle. The gentleman had rented the vehicle to construct a storage structure on his property. While driving it down a hill away from the building, he hit a bump, was knocked off the front of the vehicle with its control box in his hands and dragged down the road underneath the vehicle. He sustained serious injuries and died. The control box was mounted on a removable gate on one end of the unit's platform. The gate had bounced off because it was not secured by a cotter pin beneath the platform that would secure the gate to the platform. The control box had an emergency stop button but no wires or components to activate an emergency stop. Furthermore, wiring diagrams of the control box indicated that it should contain a Mercury reed switch that would stop the vehicle if the control box moved out of position.

The rental company did not provide an operating manual with the unit or maintain it as required by law.

Water Damage, Smoke Damage, Emergency Stop



Engineering Sciences Section - 2015

D46 A Multidisciplinary Analysis of a Complex Motorcycle Fatality

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The goal of this presentation is to demonstrate the value of a multidisciplinary approach to the reconstruction of a fatal motorcycle crash.

This presentation will impact the forensic science community by presenting a case study where accident reconstruction, vehicle inspection, biomechanics of injury, structural and helmet testing, scene investigation, and innovative detailed analysis of the surveillance video all played an important role in resolving the cause of the crash and the timing available to the motorcycle rider.

A motorcycle rider sustained a fatal head injury when he attempted to avoid striking a semi-truck and trailer that made a U-turn in front of him. He was pronounced dead at the scene after landing in a grassy area behind a 66-inch wrought iron fence. He was wearing a non-DOT-approved novelty helmet and sustained a massive and unusual skull fracture. The accident occurred in the dark, early morning hours. There was little traffic and there were no witnesses to the event. The following questions had to be answered: Could the motorcycle rider have avoided the collision? How fast was he going? Did he or his motorcycle actually strike the semi-truck? What did his head strike and at what speed? Could his brain injury have been prevented or lessened had he been wearing a DOT-certified helmet? How did he end up on the other side of the fence? This presentation describes how these questions were addressed scientifically.

Although no one saw the accident, surveillance video was obtained from the security system at a nearby business. Because the video taken from a distance was of low resolution and at night, it was difficult to extract meaningful data from the video. A high-resolution camera was mounted in the same location and at the same angle. The images from both cameras were correlated in a photogrammetric study to identify distant landmarks and lights which were not easily discernable in the original footage. Speeds of vehicles on the adjacent roadway were also calculated. Using this data, it was possible to better approximate the speed of the motorcycle seen in the surveillance video. An accident reconstruction was developed using marks in photographs and at the scene that had not been considered in an earlier analysis. Subtle damage to the motorcycle and markings on the rider and his clothing were identified and correlated with contact to the rear of the trailer.

The motorcycle rider's novelty helmet showed that it was split in half. It was originally hypothesized that the damage was caused by striking a wrought iron fence post or the edge of the curb; however, closer examination showed contact marks from a distributed contact with dull irregular scratches, not the sharp or deep scratches one would expect in such an impact scenario. Instrumented helmet drop tests using a Hybrid III dummy head were performed using helmets similar to the subject helmet and a DOT-certified helmet of the same overall style. Helmeted heads were dropped onto curb sections, asphalt, and fence pieces from varying heights at speeds up to 21.5mph. Head accelerations and speeds were recorded. Through testing, the subject contact marks were shown to be inconsistent with a curb, roadway surface, or a fence impact.

Since the extensive fracturing on the subject helmet could not be reproduced in the testing, the actual impact was at speeds greater than the tested 21.5mph and the helmet impacted an object other than those tested. Using the information from the surveillance video, injuries to his body, the marks identified in the photographs, and damage to the motorcycle's handlebars, a final crash reconstruction was performed. Finally, a kinematic analysis revealed how, after making contact with the trailer and his motorcycle striking the curb, he was launched headfirst and face up over the fence into the trunk of a palm tree.

In conclusion, this complex analysis was effective in answering the questions posed, by considering and analyzing all the evidence available in a cooperative manner. Confluence of results from these various studies reveal what actually occurred in this massive head injury event.

Motorcycle Reconstruction, Novelty Helmets, Surveillance Video Analysis



Engineering Sciences Section - 2015

D47 Forensic Engineering Critical Site Photogrammetry and the Use of GPS in a Ski Collision Case

A.K. Aleksander, PhD, Aleksander & Associates, PA, PO Box 140558, Boise, ID 83714*

After attending this presentation, attendees will understand some of the complexities of the reconstruction of ski terrain collisions, photographic techniques, expert errors, and the use of novel technologies.

This presentation will impact the forensic science community by presenting a collision between two skiers at a western ski area which resulted in severe head and bilateral wrist injuries to the smaller, female skier.

A collision between two skiers at a western ski area resulted in severe head and bilateral wrist injuries to the smaller of the two, a female. Ensuing litigation excluded the ski area, but nonetheless, ski patrol reports on the incident proved to be critical to the accurate identification of the collision site and the trajectories and speeds of the two skiers.

This presentation outlines the need for systematic orthographic photos to locate otherwise unremarkable features in the ski terrain environment. Furthermore, it is imperative that opposing experts be sufficiently skilled in interpreting this information to reach reasoned conclusions, especially on terrain with which they have minimal familiarity.

Both of these factors will be developed in detail with site photos and case evidence.

In general, placing a known object in the scene allows a series of photos to be taken that will locate the spot at a future date. By judiciously selecting trees, poles, towers, buildings, landscape features, and features on ridgelines, a person can take a set of photos that will preserve the alignment of the known object and background features along three or more intersecting lines of sight.

As it happened, this litigation was initiated several years after the event. The collision reconstruction relied on the accurate positioning of the collision scene, which was made possible by the site documentation at the time of the collision.

These reports included witness statements, first responder ski patrol documents, and follow-on investigative interviews. The basic potential energy calculations indicated a minimum speed at the collision point of approximately 24mph. Actual site measurements indicated speeds of approximately 38mph were easily attainable in the moments before the collision. These factors will be presented.

A number of interesting techniques were developed during the course of this investigation and will be presented. Site measurements during summer months are compared to winter views. Opposing expert topographic models are presented, along with a discussion of the importance of deposition testimony and perceptions of position and speed on a hill.

Actual test runs along the trajectories of the participants were documented through use of the GPS capability of an iPhone®, an app called Ski Tracks®, and recorded video on a GoPro® camera with surprising results that were then plotted on Google® Earth®. These novel products allow investigators a degree of flexibility not available only a few years ago.

The general case outcome will be discussed as will the importance of the orthographic photo techniques that set the stage for this case resolution.

Ski Collision, Orthographic Views, Ski Patrol



Engineering Sciences Section - 2015

D48 Reconstruction of a Seven-Car Pileup: A Case Study

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After attending this presentation, attendees will understand the principles and techniques of reconstructing an accident with compound collisions and multiple vehicles, including momentum and energy analysis, restitution, and crush analysis. Other visual cues such as paint transfer and overlaid impacts were used to guide and confirm the results. These techniques were used to determine the closing speeds, time separating collisions, and change in velocity for a number of vehicles in a series of impacts.

This presentation will impact the forensic science community by showing how proper application of common techniques can yield valuable information to a reconstructionist and, when combined with readily available documents, can be used to analyze highly complex collisions. These same techniques can be used to determine vehicle-closing velocity and change in velocity, which will enable the reconstructionist to assign liability across a wide range of collision configurations.

The provided documents were typical of those in investigations with a legal component and included photographs of the vehicles and scene, accident reports for each set of collisions, and depositions of drivers and occupants. Combined with CARFAX® data, vehicle specification data, and scene and vehicle inspections, these documents provided the raw data for the reconstruction.

In this case study, a grass fire burned out of control resulting in thick smoke crossing a major highway. As a result, seven vehicles were involved in eleven discrete collisions. The involved vehicles were two tractor trailers, one pickup truck, two passenger vans, one Sport Utility Vehicle (SUV), and one car. Several vehicles were struck multiple times and moved from their resting positions in each collision. Reconstructing the event required analyzing not only the movement of each vehicle, but also the timing of the collisions relative to one another. As is often the case, many of the involved parties gave contradictory testimony, which was further complicated by the on-scene deaths of the two occupants in one of the vehicles. All but one of the vehicles was towed to the same storage lot, allowing the Highway Patrol to align the vehicles using a tow truck and compare crush profiles.

Each of the collisions was analyzed separately as a two- or three-vehicle event. After some collisions, the vehicles would come to rest before being impacted again, whereas others sustained multiple impacts without stopping. Passenger vehicles of several varieties and tractor semi-trailers were involved in these collisions. The reconstruction methodology presented can be used successfully in each of these situations.

Multi-Car, Accident Reconstruction, Passenger Vehicle



Engineering Sciences Section - 2015

D49 3D Animation Applied to the Analysis of Pre-Impact Visibility Obscurement

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The goal of this presentation is to illustrate the need for careful analysis of potential sight-line obscurements. While aerial and ground-based 2D still photographs may carefully document the available evidence used in collision analysis, 3D animation of the moving interaction fully illustrates the lack of objects that were suggested to have blocked potential advance warning of an impending collision hazard.

This presentation will impact the forensic science community by highlighting the benefits of 3D animation when used as a tool to accurately and clearly demonstrate driver visibility and sight lines. This case study evaluates a utility truck driver's sight line in the moments before a tragic collision that killed an adolescent bicyclist.

Driver detection and perception of roadside hazards and subsequent collision avoidance maneuvers are commonly evaluated in traffic accident-cause analysis. Tragedy often strikes when a traffic collision involves small children on bicycles. Collisions can occur when children suddenly ride into the roadway from between parked vehicles, giving alert drivers little if any time to avoid impending disaster. Likewise, inattentive drivers may have limited resources available to avoid similar tragedy. Collision analysis uses physical evidence to assist in the determination of cause; however, only after thorough evaluation of the available information can a correct conclusion be made.

A telephone repair technician in search of a customer's residence was driving his utility truck in an alley. The alley provides access to trash collection services and apartment building tenant parking. It is not uncommon for children to play nearby. The technician proceeded south at what he estimates was approximately 1mph-2mph, his foot resting on the brake pedal. After passing a few vehicles parked to his right, he heard a sound at the right front of his truck. He rolled forward another 33ft, stopped, and checked his mirrors. In the right side mirror, he observed a bicycle on the ground behind his truck. The fatal victim was a 6-year old girl who had just started riding the bicycle not more than 125ft away.

Law enforcement personnel secured the alleyway, marked and photographed the physical evidence, including the bicycle, utility truck, pavement, parked vehicles, a small circular gouge to the pavement, a 4.75ft-long tire friction mark, corresponding rear bicycle tire abrasion, and a rub mark to the outer sidewall of the utility truck's right front tire. The investigators determined that the child was riding the bicycle on a path between two apartment buildings toward the alley. After entering the alley and applying the coaster brake that locked the bicycle's rear tire, the child continued forward another 14ft and struck the utility truck. The rub mark was caused by the bicycle's front tire where it impacted the utility truck. The child then fell to the ground where she was subsequently run over by the right rear dually tires. The investigators concluded the utility truck speed was not excessive nor was a collision factor, but that the child failed to yield the right-of-way to alley traffic.

Careful forensic analysis of the conditions in the alley was used to determine the time during which the child was visible before impact. The alley was measured with a total station. Using photographs, the location of the parked vehicles, point of impact, and the tire friction mark, the child's trajectory on the bicycle was modeled using 3D software. The child's height (4ft, 8in) was found in the coroner's report. The child's speed between buildings (9.5mph) was approximated with surrogate testing of a similar bicycle in the alley. Her speed at impact after skidding on gravel/asphalt was 7.9mph. Truck speeds of 6mph and 10mph were used. Based on a time-position history analysis of the truck and bicyclist, two impact scenarios were animated. Two camera views were modeled, one from the perspective of the driver inside the utility truck cab and another overhead view tracking the forward progress of the truck leading toward the impact. At a truck speed of 6mph, the bicyclist was in view of the technician for 2.2-2.4sec. At 6mph and using a 1.5sec perception/reaction time, the technician had sufficient time and distance to stop his truck and avoid impact. At a truck speed of 10mph, the bicyclist was in view of the technician for 1.8-2.0sec. At 10mph and using a 1.5sec perception/reaction time, the technician had sufficient time and distance to slow his truck to avoid running over the bicyclist.

The results of the collision analysis highlighted with 3D animation show with graphic clarity the lack of timely response by the truck driver, who in one scenario could have completely avoided the impact, while in the other, after the bicyclist struck his truck, could have braked in time to avoid running over her.

Animation, Bicycle, Visibility



Engineering Sciences Section - 2015

D50 Engineering Ethics and an Aircraft Crash

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After attending this presentation, attendees will understand that an engineering code of ethics should be a way of life for those practicing the art and science of engineering design. This presentation will show that engineers require sound judgment to interpret how the code would apply to specific circumstances. An example of an injury to a helicopter pilot during a crash that was due to failure to follow through on a recognized hazard will demonstrate the consequences of that failure.

This presentation will impact the forensic science community by educating the community of the need to know that commitment to serving society and attending to the welfare and progress of the majority has the highest priority. When there is a basic ethical dilemma, an engineer has a duty to report to the appropriate authority a possible risk to others from a client or employer failing to follow the engineer's directions. The obligation to report overrides the duty to a client and or employer.

A military helicopter was hovering on station in a combat zone waiting for the rest of the force to arrive when the main coupling between the engine and transmission failed. The crew conducted an autorotation landing resulting in injuries to both crew members. The pilot's injuries required extrication from the aircraft while his copilot was able to exit the aircraft without additional help. The pilot sustained a burst fracture of the L1 vertebral body with sudden onset of paraplegia and bowel and bladder dysfunction.

This military helicopter had been recently upgraded to include a larger engine, a six-blade prop, and new landing gear. This increased both its internal and external gross weight capacity. The design modification company that supplied the upgrade kit recommended that the government include a Voice Warning System (VWS) with the new engine although no location for the VWS was included in the modification drawings and specifications.

The purpose of occupant restraint systems in helicopter crashes is the protection of occupants to increase their probability of survival during crashes and hard landings. Another critical feature in modern helicopters is that seats are designed to reduce lumbar spine loading during hard landings by energy transfer during impact as referenced in the Federal Aviation Regulations (FAR) 14 CFR Section 27.561 and 562.

Each front seat was equipped with a five-point harness and a seat structure that consisted of a crushable box structure and a seat cushion. The crush box was designed to deform and attenuate the kinetic energy of the load as it moves over the stroking distance, thereby transferring the kinetic energy away from the passenger.

Space was limited within the aircraft cockpit so the installers placed the VWS within the crush box. This effectively placed a vertical beam between the top and bottom of the box and defeated its purpose. During an inspection of a test helicopter, the supplier of the upgrade kit verbally recommended to the military that the voice warning box not be located within the crush box structure. No written record was ever made of the conversation and no follow up was done.

This Code of Ethics was not followed by the modification kit supplier engineer in charge or other personnel present who had observed the dangerous installation location of the VWS in the test helicopter and did not take any steps to remedy the defective condition.

Alerting the intended users and purchasers of the helicopter of this dangerous condition is an expected industry standard, as is also the correction of the problem.

Ethics, Helicopter, Crush Box



Engineering Sciences Section - 2015

D51 Left-of-Center Accident Reconstructions: A Case Study

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After attending this presentation, attendees will understand the standard techniques used in reconstruction of left-of-center accidents on two-lane roads.

This presentation will impact the forensic science community by demonstrating the importance of a complete reconstruction of vehicular accidents when the at-fault party is in doubt.

This case study involves a frontal offset collision of a four-door sedan and a pick-up truck. Both vehicles contained one occupant and there were no independent witnesses to the accident. The accident occurred on a curved and sloping section of a two-lane asphalt road. The driver of the sedan was traveling downhill along a left-handed curve and was nearing the left turn into her neighborhood. The driver of the truck was traveling uphill and in the opposite direction. After colliding, the sedan was forced off the right side of the roadway and through a guardrail. The truck entered a yaw, rolled onto its passenger's side, and came to rest within the center of the roadway. The initial investigation revealed a gouge mark within the truck's lane of travel. Threats of criminal action against the driver of the sedan were made. Furthermore, the truck driver's insurance company proceeded to hire attorneys and accident reconstruction experts to bolster their case. Given the mounting evidence against their driver, the insurance carrier for the sedan was not willing to pay for their own expert. As such, the driver of the sedan was tasked with financing her own investigation.

Forensic experts became involved in the case shortly after the accident. Thus, the scene and vehicle evidence was maintained. The examination of the scene revealed a large body of evidence that seemed to refute the initial determination. This evidence included an initiation gouge near the white fog line of the sedan's lane of travel. Placing the impact point at this gouge mark indicates that the truck had crossed the center line. Damage to a utility box matched scrape marks along the passenger's side of the sedan. Moreover, initial momentum calculations seemed to confirm this hypothesis.

The opposing experts identified gouges and scrapes that crossed the center line. One of these gouges, which was located within the truck's lane of travel, was cited as the point of impact by the initial investigators; however, a closer examination revealed a similar gouge a few feet away. The two gouges exhibited curving features consistent with contact with rotating wheel rims. Moreover, the distance between the gouges matched the wheelbase of the truck. The truck examination revealed evidence of contact between the passenger's side wheel rims and the pavement. While the opposing parties attempted to attribute one of the two gouges to the collision, the physical evidence revealed that both gouges were produced during the post-impact phase of the truck.

Interrogation of the event data recorder provided the speed change magnitudes for each vehicle. Damage-based estimates for speed change were found to be consistent with this data. A detailed momentum analysis was performed based on the physical evidence, which demonstrated that the truck had crossed into the sedan's lane of travel prior to impact. This analysis yielded speed change magnitudes consistent with the crush analysis and event data. The computations indicate that the truck was exceeding the speed limit and traveling at three times the speed of the sedan. Given the disparity in speed and mass, the sedan was driven backwards from the point of impact to its rest position. The truck continued in its general direction after impact and eventually rolled onto its passenger side due to its post-impact yaw.

The opposing party maintained their view that the driver of the sedan was at fault. An alternative scenario was devised in an attempt to explain their position. This scenario placed the sedan within the truck's lane of travel at the point of impact. This impact was defined as the rim gouge located left of the center line. In order to maintain consistency with the established speed changes, the impact angles had to be adjusted for this alternate scenario. The approach angle for the truck was not probable given terrain bounding its side of the road. Moreover, this scenario failed to account for all the scene evidence, namely the rollover gouges, initiation gouge, and collateral damage to objects bounding the roadway.

Upon presenting the evidence in a written report, the opposing party decided to admit fault, which removed the specter of criminal action against the driver of the sedan. The successful results also permitted full financial compensation for this driver, who sustained significant injuries as a result of the accident. These results were only attainable by performing a complete reconstruction.

Left-of-Center, Conservation of Momentum, Rim Gouges

D52 Sideswipe Evidence for Traffic Accident Reconstruction

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The goal of this presentation is to present physical evidence that can be found on vehicles involved in sideswipe accidents to assist in the reconstruction of these types of vehicle accidents.

This presentation will impact the forensic science community by presenting common physical evidence for accident reconstructionists and investigators to look for and by citing case studies showing how this evidence can be used to reconstruct sideswipe accidents.

It is commonly asked of traffic accident reconstructionists to determine how a sideswipe-type accident occurred. Although speeds of the vehicles are difficult to determine, common issues that are inquired about are which vehicle was overtaking the other and which vehicle turned into the other. In order to reconstruct a sideswipe accident, there is a plethora of common physical evidence that the investigator can look for.

Physical evidence including material flow, sheet metal deformation, pocketing, mirror rotation and damage, rubber transfer, and closing of body panel gaps are some examples of physical evidence that can show which vehicle was overtaking the other. Figure 1 depicts most of these examples from a sideswipe demonstration performed by researchers in which this vehicle was overtaking the other during the contact.



Figure 1: Typical sideswipe damage with side view mirror rotated rearward (overtaking vehicle).

Material flow can usually be found on plastic components such as door moldings, wheel opening moldings, and bumper covers. When the plastic component is contacted by the other vehicle, the plastic may be scraped and displaced toward the direction of contact. As shown in Figure 2, although located on the front bumper, the material flow from tire and rim contact was toward the driver's side, showing that the contacting vehicle was traveling from the passenger's side toward the driver's side during the contact.

Pocketing and sheet metal deformation occurs when the sheet metal is contacted with enough force to cause deformation. The force directed inward and forward by the overtaking vehicle can deform the sheet metal forward and can sometimes "pocket," indicating direction, as shown in Figure 3.



Figure 2: Material flow from vehicle passing from passenger's to driver's side.



Figure 3: Sheet metal deformation (left) and pocketing (right) showing force direction.

Mirror rotation and damage can occur when the side view mirror is contacted during the contact. Paint or plastic scuffing can usually be found on the leading edge of the mirror housing on the front or rear of the housing indicating which side of the mirror was contacted. The mirror is sometimes able to rotate, sometimes contacting the A-pillar, indicating direction. Figure 4 depicts mirror contact to an A-pillar from the mirror rotating forward from contact from an overtaking vehicle.



Figure 4: Mirror strike from forward rotating mirror housing.

Rubber Transfer can be indicative of tire contact. Figure 5 depicts examples of rubber transfer. The striations shown are evidence of contact with a spinning tire.



Figure 5: Examples of rubber transfer evidence.

Closing of body panel gaps can occur when the sheet metal is deformed and moved forward or rearward due to contact with the other vehicle. An example of a closed body panel gap is shown in Figure 6, where the left rear door panel was deformed rearward closing the gap.



Figure 6: Example of closing of body panel gaps.

Conclusions: In order to reconstruct a sideswipe accident, an accident reconstructionist can look for evidence including material flow, sheet metal deformation, pocketing, mirror rotation and damage, rubber transfer, and closing of body panel gaps.

Sideswipe Accident, Accident Evidence, Sideswipe Case Studies



Engineering Sciences Section - 2015

D53 WISER: Automatically Classifying Written Statements as True or False

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After attending this presentation, attendees will better understand current approaches and results in computational classification of written statements as true or false. Attendees will be able to compare current and traditional approaches and evaluate methods on linguistic sophistication, psycholinguistic realism, accuracy, data requirements, and implementability.

This presentation will impact the forensic science community by providing measures of reliability to automated deception detection of written statements and an overall framework for investigators to evaluate different methods.

Witness Statement Evaluation Research (WISER) is a project using automated text analysis and statistical classifiers to determine the best protocol for computational classification of true and false statements in the forensic-investigative setting. In this research project, current and traditional approaches are being compared and tested and methods are being evaluated on linguistic sophistication, psycholinguistic realism, accuracy across different datasets, data quality requirements, and implementability. The most well-known and famous method for deception detection or veracity assessment is Sapir's Scientific Content Analysis (SCAN) described at a website and methods following it are generally known as statement analysis.¹ SCAN has been automated to a great degree with reported accuracy results of 71% by Fitzpatrick and Bachenko on a dataset that has not been made available to the WISER project.² Another approach is Pennebaker's Linguistic Inquiry and Word Count (LIWC), a word count and categorization program that has been applied to authorship identification, gender and age estimation as well as deception, even though its original purpose and design was to classify writing samples into personality types for psychological assessment.³ A third approach that is popular among computer scientists working in text classification is the Bag Of Words (BOW) model, a term invented by Harris and developed by Salton and McGill in which each text is seen as a list of words and their frequencies without regard to any syntax, semantics, or grammatical relations.^{4,5} Both LIWC and BOW have been tested as deception detection methods by Almela and Mihalcea and Strapparava.^{6,7} A fourth approach is based in Text Analysis Toolkit Toward Linguistic Evidence Research (TATTLE), a text analysis tool; TATTLE combines linguistic analysis at the phonological, syntactic, and lexico-semantic levels and has been applied to deception detection classification on both experimental and high-stakes datasets.^{8,9} The TATTLE results showed a remarkable difference between the experimental data, in which students were asked to write two narratives of a traumatic experience, one truthful and the other false, and high-stakes data, actual statements from real criminal investigations with non-linguistic evidence of their veracity or falsehood. Using the same TATTLE algorithm, the texts in the experimental data were correctly classified using leave-one-out cross-validation 71% of the time, while the texts in the high stakes data were correctly classified using leave-one-out cross-validation 93% of the time.

This result demonstrates that there is a real difference between lies told in an experimental setting (i.e., in a classroom as part of a class assignment) and lies told in a police investigation. Further, this result demonstrates that linguistic sophistication may be needed to develop highly reliable linguistic variables. Moreover, this result demonstrates that different statistical approaches which may work better with different linguistic variable sets (e.g., Support Vector Machine or Discriminant Function Analysis) may fit better with TATTLE variables than with LIWC or BOW. In this presentation, the results of testing the text-analytic linguistic variables from LIWC, BOW, and TATTLE using several different statistical approaches such as Discriminant Function Analysis, Support Vector Machine, and others, on three different datasets (experimental from the classroom setting on trauma narratives; experimental from an electronic venue for opinion mining; and, high-stakes data from real case investigations) are reported. Further, the methods on linguistic sophistication, psycholinguistic realism, accuracy over different datasets, data quality and quantity requirements, and implementability within the investigative, interview and interrogation workflow are evaluated.



Engineering Sciences Section - 2015

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Deception Detection, Text Classification, Witness Statements



Engineering Sciences Section - 2015

D54 The Scientific and Legal Status of Forensic Speaker Recognition in the United States

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The goal of this presentation is to provide a tutorial on the current state of forensic speaker recognition with an update on Overseas Security Advisory Council (OSAC) activities in this area.

This presentation will impact the forensic science community by discussing ways to improve the introduction and acceptance of new technologies for speaker recognition in American courts.

The use of scientific techniques to identify the speaker in an audio recording has a long and mixed history in American courts. An early non-digital methodology from the 1940s, known as “visible speech,” created spectrographic, pictorial representations of speaking patterns, which were compared by humans to determine if a graphical pattern from an unknown source could have come from a known speaker. Re-christened as “voiceprinting” in the early 1960s, testimony based on this graphical, spectral analysis method was admitted by a number of courts under the Frye standard, which requires that the method has attained “general acceptance in the particular field in which it belongs;” however, in subsequent decades, critiques of voiceprinting (including two influential reports by the National Academy of Sciences) caused the method to fall out of favor in the general scientific community, although cases admitting such evidence continued to be cited as legal precedent. Graphical spectral analysis (voiceprinting) has now been widely discarded in favor of two alternative approaches to speaker recognition, both with a lengthy scientific history: Automated (computer-based) Speaker Recognition (ASR) and human examiner-based aural perceptual (or “structured listening”) techniques.

In *Daubert v. Merrell Dow Pharmaceuticals* (1993), the United States Supreme Court adopted a new standard for the admissibility of scientific evidence in federal courts — a standard that requires the trial judge to assess the scientific basis of the testimony. In its discussion of factors judges should consider, the Court referred to two cases involving expert testimony based on voiceprints — *United States v. Williams* (CA2 1978) (concerning the importance of standards regarding the implementation of a scientific technique) and *United States v. Smith* (CA7 1989) (on the relevance of the known or potential rate of error of a particular scientific methodology). The Court’s reference to voiceprint testimony as exemplars of desirable features of scientific evidence is ironic, given that voiceprints, even when generated by digital technology, are no longer generally accepted by the relevant scientific community.

In the high-profile 2013 case of *State of Florida v. George Zimmerman*, the trial court referenced the accepted but outdated precedent on the admissibility of graphical spectral analysis (voiceprints) in denying admittance of highly problematic expert testimony based on automated speaker recognition, stating that “aural perception and spectral analysis have been widely used for many years and are sufficiently established to have gained general acceptance within the scientific community,” but that automated speaker recognition proffered is “not as widely accepted at this time.” This case well illustrates the difficulties that arise when evidence from evolving scientific fields like speaker recognition enters the legal system.

This presentation will review the technical basis and legal status of these three techniques for speaker recognition: (1) graphical spectral analysis (voiceprints); (2) automated (computer-based) speaker recognition (ASR); and; (3) human examiner-based aural perceptual (or “structured listening”) techniques. An important continuing speaker recognition test program conducted by the National Institute of Standards and Technology (NIST) and the activities of the Scientific Working Group for Forensic and Investigatory Speaker Recognition, created in 2012 with a broad agenda to support scientific, technical, and legal advancements, including an acknowledgement of privacy concerns will be discussed. (In late 2014, that group was reorganized as a sub-committee under the new Organization of Scientific Area Committees (OSAC) structure). This presentation will conclude with a broad discussion of ways to improve the introduction and acceptance of new technologies for speaker recognition in American courts that avoid muddling 21st-century scientific methodologies for speaker recognition with the now-discredited field of voiceprinting.

Speaker Recognition, OSAC, Florida vs. Zimmerman



Engineering Sciences Section - 2015

D55 Comparing Statistical and Machine-Learning Techniques in Author Identification and Verification

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After attending this presentation, attendees will be acquainted with validation testing in forensic linguistic evidence, specifically author identification and current results of litigation-independent research. Attendees will be able to assess any author-identification methods they encounter in light of current research results and standards.

This presentation will impact the forensic science community by providing an example of validation testing that tests both statistical and machine-learning classification methods and by providing current research on the reliability of computational forensic linguistic author identification and verification. These problems are becoming more important due to the internet.

This presentation focuses on validation testing of methods for determining the author of a document in the forensic setting using a traditional forensic methodology and a novel biometric methodology. As Chaski explains, there are three current approaches to forensic author identification: forensic stylistics, computational stylometry, and forensic computational linguistics.¹ Stylistics has its roots in handwriting identification; stylometry in computer science and digital humanities; and forensic computational linguistics in linguistic theory and computational linguistics. Among the many ways that these three approaches differ, the most important is the attitude and productivity of a litigation-independent validation testing program to determine error rates and standard operating protocols with data requirements. Currently, only the forensic computational linguistics approach carries on a validation testing program on forensically feasible data (i.e., experimentally collected data or actual known data from cases and investigations, where all the data is “ground truth” for the tested method). Traditionally, computational forensic linguistics has focused on using hard-to-imitate, low-salience features that are psychologically real and theoretically valid: these features are syntactic structures categorized in a particular way.² Further, this feature set has yielded high accuracy (94%-95%) for author identification in two different datasets, one experimentally collected and the other from case investigations. Currently, this methodology has been used for both identification and verification. When used for an identification, the method requires a “line-up” of suspect authors who are each tested pair-wise against each other, with the resulting statistical model used to classify the questioned document to one or the other. If there is a large group of suspects, a binomial test can then be used on the multiple pairwise classifications, but often there are only two suspects (for reasons related to the case outside any linguistic analysis and unknown to the forensic linguist). When used for a verification, the questioned document must be long enough that it can be split into segments and tested against the known suspect documents; although the discriminant function procedure forces the documents into two groups, a single author’s documents often cannot be distinguished into two groups.

Using this same feature set on different datasets, this study is testing traditional forensic methodology using a pairwise procedure to determine levels of accuracy and data quantity with both statistical and machine learning classifiers. Thus the current experiments are testing if previous results using discriminant function analysis can be replicated at the 94%-95% accuracy with other statistical classifiers such as logistic regression. Further, the current experiments also test if machine learning classifiers like Support Vector Machine can attain even higher accuracy.

Again using the same feature set on different datasets, this study is also testing a novel biometric methodology to determine if this feature set can be used for verification. Distance and similarity matrices are being used with threshold settings to see how well this feature set performs as a verification metric. Further, a Bayesian analysis is being tested to see if both identification and verification protocols in this framework can be conducted.

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Author Identification, Author Verification, Bayesian Likelihood



Engineering Sciences Section - 2015

D56 Comparing Literary, Biometric, and Forensic Approaches to Author Identification

Carole E. Chaski, PhD, ALIAS Technology, LLC, Institute for Linguistic Evidence, 25100 Trinity Drive, Georgetown, DE 19947*

After attending this presentation, attendees will understand how to distinguish among the literary, biometric, and forensic approaches to linguistic evidence. Attendees will learn about the four corners of linguistic evidence and how the biometric approach relates to two of these four broad areas. After the presentation, attendees will be able to assess methods and their suitability for use in case investigations and trials.

This presentation will impact the forensic science community by providing a framework for assessing literary, biometric, and forensic approaches to linguistic evidence and how these lessons can be generalized to other forensic disciplines.

Linguistic evidence is evidence in a criminal or civil investigation that hinges on language itself to determine an element of the crime or civil action, such as the authorship of a threatening letter or the speaker of a bomb threat. Generally, linguistic evidence falls into one of four corners: identification, text-typing, intertextuality, and linguistic profiling. Two of these four corners illustrate the intersection of forensic and biometric approaches to linguistic evidence. Identification concerns both speaker (phonetic patterns) and author (lexico-syntactic patterns). Linguistic profiling concerns the sociolinguistic dimensions of language (e.g., age, gender, educational level, dialect, and associates an individual with a group identity).

In some recent high profile cases such as the J.K. Rowling pseudonym, literary author identification has been confused with forensic author identification. Author identification in the forensic setting is difficult and far more difficult than literary author identification for several reasons.¹⁻³ First, the language of forensic data is less sophisticated and more prone to ungrammaticality than literary language, so that automated parsers make far more mistakes on forensic data than on literary data. Second, the quantity of forensic data is usually much smaller than literary data, as forensic data might consist of tweets and other microtexts or emails while literary data can be tomes and novels. Third, in the forensic setting, the known and questioned documents come from different linguistic registers and genres, while literary data is consistently literary, all novels, all fiction, and all a literary register. These three differences mean that methods developed for the literary authorship question are not transferable to the forensic authorship question; however, the approaches can look similar to the public or to news reporters when the approaches both use computational tools.^{1,4} Hence, Chaski makes the distinction between computational stylometry and forensic computational linguistics.¹

A biometric is a measurement that uniquely identifies or individualizes a person by a biologically based characteristic or behavior. Language is biologically- and neurologically-based; the vocal tract and brain can be measured for unique characteristics. But language is typically seen as a behavioral biometric — it is the behavior of speaking or composing text that is proposed as the identifying and perhaps even individualizing characteristic. Behavioral biometrics are prone to intra-person variability, disguise, and imitation. The biometric and forensic approaches to individuality regarding face, iris, and fingerprint have been contrasted well by Geradts, Ruifrok, and Zoun; Houck and Seigal; and Vorder Bruegge, and these differences apply to author identification.⁵⁻⁷ The forensic author identification method developed by Chaski is influenced by the biometric approach because it is rooted in a biological/neurological conception of language, especially syntactic theory, and focused on behaviors that are almost impossible to disguise or imitate due to human memory constraints, but it is clearly within standard forensic methodology because it relies on a closed pool of extra-linguistically identified suspects — the line-up model for finding comparable knowns to which to match the questioned item.^{1,3,8} Further, like the biometric paradigm, the forensic author identification research conducted for this study is experimentally produced outside of any litigation in a proactive way and returns error rates for specific data quantities/qualities. But the biometric approach raises several research questions that are yet to be addressed adequately: Can author identification select an author in the wild, not in a lineup? Can a Bayesian approach solve this problem? How can error rates be calculated if results in the line-up model are 100% accurate, even under the dampening effect of leave-one-out cross-validation? While forensic author identification must be divorced from literary pursuits, the courtship with the biometric approach deserves a great deal of attention.



Engineering Sciences Section - 2015

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Author Identification, Behavioral Biometric, Linguistic Evidence



Engineering Sciences Section - 2015

D57 Pathway for Integrating All Hazard Preparedness/Risk Management With Command and Control

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The goal of this presentation is for attendees to see a visual presentation of a systems engineering approach to providing security, safety, and quality of life as related to forensic enterprise.

This presentation will impact the forensic science community by encouraging them with examples for making better strategic and tactical decisions by the use of current and projected mapping capabilities along with their own common sense. It will expand the definition of mapping to include small-scale events, such as crime scenes, interiors and exteriors of buildings, and below-ground objects as well as those visible above ground.

Hazard and forensic preparedness is a fundamental aspect of individual, team, and community safety. Mapping is an integral part of this preparedness; however, no single individual, jurisdiction, or agency is expected or capable of performing every activity. This presentation will describe various types of forensic mapping (surface, above surface, and below surface), both static and dynamic, that deal with current and potential forensic activities.

Forensic maps should have several attributes in common. An earth-centered international coordinate system that allows a dynamic 3D analysis of potential and actual crime scenes is proposed. Also the maps should have an accurate time and date stamp.

While many maps are available, some were developed decades ago and need to be updated using government and commercial off-the-shelf tools; they need updating at least once a year. The maps should be easily prepared, easily analyzed by experts and the general public, and easily integrated with local needs. This presentation illustrates how, with urban and rural examples, 3D maps can be applied to planning scenarios and real-time forensics using the federal government's universal task list.

Technology for specific protection and prevention for those going into harm's way is not limited to the defense sector, which has extensive transferable technical expertise. Acknowledged engineering and scientific experts in geomapping and the forensic enterprise want to use their expertise and experience in a proactive role; by being multijurisdictional trusted agents and expert witnesses.

Results from working with architects, lawyers, judges, and developers, and examples of integrated local maps will be presented.

Maps, Security, Safety



Engineering Sciences Section - 2015

D58 The Next Step: Creating and Using the 3D Working Model From Laser Scan Data to Better Seek and Illustrate the Truth

Craig T. Fries, BA, Precision Simulations, Inc, 115 S Church Street, Grass Valley, CA 95945*

After attending this presentation, attendees will better understand the 3D working model, 3D laser scanning, and their effective use in incident documentation, analysis, and visualization.

This presentation will impact the forensic science community by introducing a powerful and proven concept from traditional sciences to the forensic field. The 3D working model will facilitate the search for the truth by reducing conjecture and increasing fidelity to the physical evidence.

All phases of accident and crime reconstruction have been revolutionized by 3D laser scanning. The documentation of physical evidence is now so accurate and complete that experts can work directly with the 3D working model years after the fact as if they were working in the crime scene itself. Measuring millions of data points in mere minutes, a modern 3D laser scan is capable of capturing every inch of a scene with precision and detail sufficient to locate the smallest evidence. A typical scan contains approximately 10,000,000 data points — a level of detail akin to having the entire scene and all the physical evidence extracted and delivered to the expert's lab for thorough and exacting analytical forensics. Harnessing these rich datasets with the analytical power of modern computers allows unparalleled depth of analysis. From determining the velocity of vehicles involved in accidents, deriving critical values from video footage and scene photographs to exacting line-of-sight calculations and ballistic trajectory analysis, the 3D working model provides the expert with a toolset based upon physical evidence that was previously unavailable. The fastest growing areas of growth is the use of the 3D working model to analyze opposing expert's conclusions and opinions. Being able to plug the underlying assumptions back into the 3D working model allows the expert to determine how well, or not, the results match the physical evidence.

Once the dataset has been utilized to complete a thorough investigation and derive fact-based conclusions supported by the physical evidence, the final stage of the process also benefits greatly from the underlying 3D working model. The visualization of the dataset and the conclusions via 3D computer animation and simulation allows the experts to present their findings in a clear, compelling manner. Using the laser scan data directly in the visualization provides a level of realism and accuracy that far exceeds what was possible before. In addition to being visually compelling in its own right, the scan data gives the expert the opportunity to animate over the exact same dataset upon which their calculations were performed. This increases the accuracy of the final visualization, eliminating the need to resort to mere illustrations and elevating the animation to a true engineering visualization. This ability to maintain the highest level of scene fidelity increases the likelihood that the animation will be admitted into the trial setting and significantly helps combat the CSI-effect often seen in urban courtrooms. Today's juror comes to the trial with an expectation, born from television and other media, that the facts and findings will be presented in a visually compelling manner. The use of the 3D working model and 3D laser scanning in forensics in the United States will be demonstrated via compelling graphics and analyses in all three phases of the reconstruction process, pulling from 20 year's experience and more than 1,000 cases.

Laser Scanning, 3D Animation, Visualization



Engineering Sciences Section - 2015

D59 You View Your World in 3D — Shouldn't You View Your Case in the Same Manner? The Use of High-Definition Survey (HDS) Laser Scanning in Forensic Engineering

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After attending the presentation, attendees will understand how 3D data is collected, how the data is processed, and most importantly, how the data is utilized in actual case examples of all types.

This presentation will impact the forensic science community by bringing to the forefront the benefits of the most accurate and comprehensive 3D data collection available — HDS laser scanning.

A far cry from just a few years ago when measurements on all cases were collected using rulers and rolling wheels, new technology has allowed engineers and others to quickly collect accurate, comprehensive data in 3D. HDS laser scanning has opened a whole new world for any engineer, scientist, or technical person who needs to collect accurate, comprehensive 3D data. HDS laser scanning can be utilized to collect data from vehicular collisions, fires, building collapses, slip-and-fall events, and any other type of case that warrants accurate measurements.

The HDS laser scans provide invaluable data for analysis purposes (especially when the data is collected soon after the events occurred). The HDS laser scan data also provides a foundation of data (collected with unparalleled comprehension and accuracy) to allow the expert, the attorney/client, and the trier of fact to view the events in a 3D environment. Commercially available software allows for the creation of a 3D “real-world” environment that allows one to view the event or scene from almost any orientation. For example, a 3D environment allows for: an evaluation of what a driver could see through his/her windows and mirrors as he approached and executed a turn; an evaluation of what a witness could see through a window or door; a pedestrian's view of a pavement irregularity as he/she approached; and/or, 3D views of products such as vehicles or machines.

This presentation will focus on the accuracy of the data, how to get the data into evidence, and the cost of this new, must-have technology.

High-Definition Survey (HDS), 3D Data Collection, Laser Scanning



Engineering Sciences Section - 2015

D60 The Assessment of Facial Modifications Due to Mimicry: Possible Influences on Personal Identification From Video Surveillance Systems

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After attending this presentation, attendees will better understand the facial modifications due to mimicry and their impact on the reliability of 3D/2D superimposition for personal identification from video surveillance systems.

This presentation will impact the forensic science community by providing clues for facial modification concerns that are due to different facial expressions.

More and more frequently, forensic anthropologists are requested by judicial authorities to assess personal identification from video surveillance systems: 3D/2D superimposition is one of the most commonly used methods which consists of the comparison between a 3D model obtained from the suspect through advanced 3D image acquisition technologies (laser scanner and stereophotogrammetry) and 2D images taken from the video surveillance records. Faces to compare are expected to be neutral, but the culprit may have different facial expressions which may add a bias to the 3D/2D superimposition, therefore resulting in an error in the identification process.

This presentation proposes to expose two pilot studies concerning the assessment of facial modifications due to different expressions.

In the first project, ten male adults, between 30 and 40 years of age, underwent five acquisitions by stereophotogrammetry (VECTRA-3D[®]) with different expressions (neutral, happy, sad, angry, surprised). On each 3D facial model, nine landmarks (right and left endocanthion; right and left exocanthion; right and left cheilion; on the midline, selion, pronasale, subnasale) were identified using VAM[®] software; the acquisition of each individual with happy, sad, angry, and surprised expressions was then superimposed onto the neutral one in order to reach the best match between the corresponding landmarks. This procedure allowed the operator to also obtain a chromatic sheet of the face, where increasing zones are colored in blue and the decreasing zones in red. In all the cases, the Root Mean Square (RMS) value between the two models was calculated as well.

In the second project, five photographs with different expressions (neutral, happy, sad, angry, surprised) were taken from five male adults chosen from the previous group; the 3D model in neutral expression was then superimposed to each photo and the distance between 12 facial landmarks (right and left endocanthion, right and left exocanthion, right and left cheilion, right and left alare, right and left gonion; on the midline, selion, pronasale) located on the 3D scan and the 2D images was estimated by Adobe[®] Photoshop[®] software.

The first study highlighted that the highest difference in comparison with the neutral standard was shown by the happy expression (mean RMS 4.11mm, SD 1.13mm), followed by the surprised expression (mean RMS 2.74mm, SD 1.02mm), the sad (mean RMS 1.3mm, SD 0.49mm), and angry (mean RMS 1.21mm, SD 0.37mm). The happy and surprised expressions showed a wide modification of the mouth, chin, and cheek regions, whereas the upper third of the face does not show relevant modifications. The sad and angry expressions were affected by slight alterations.

The second study confirmed that the highest mean differences between facial landmarks on 3D scan and 2D image was reached by the happy expression (0.17cm, SD: 0.35cm), followed by the surprised expression (0.08cm, SD: 0.19cm). The sad and angry expressions showed the lowest modifications (respectively, 0.07cm, SD: 0.2cm and 0.05, SD: 0.13cm).

This pilot study shows that mimicry affects facial morphological characteristics and the position of facial landmarks, and therefore may influence the outcome of 3D/2D superimposition procedures for identificative purposes: in addition, the study highlighted the facial regions more stable with mimicry and therefore more usable for a possible identification procedures. Further studies on a larger population are needed in order to provide more details of the phenomenon and possibly add corrective values for assessing personal identification from video surveillance systems.

Facial Mimicry, Stereophotogrammetry, Personal Identification



D61 Image Processing Application to Anthropological Automatic Characterization in Forensics

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The goal of this presentation is to propose an automatic method of anthropometric data collection in forensics, making the process as automated as possible.

This presentation will impact the forensic science community by explaining the proposed procedure which was applied to anthropometric examinations performed on subjects whose photos were taken by security cameras in public places.

The goal of this work is the implementation of a software application that provides an automatic method of anthropometric data collection of an individual. In particular, it has created a tool that could detect human physiognomy information starting from a still image, taken from a movie by a security camera, using clear reliefs of sample objects taken at the scene of the crime.

The photo shows the objects on a flat surface and the illusion of depth is given solely from the perspective and chiaroscuro. It is therefore not immediately possible to detect the actual size of an object represented in a still image. The only method of obtaining this information is through direct confrontation with another item in the same creative whose size is known. Based on this principle, the proposed software application was produced. For this study, video recordings at the crime scene were viewed to capture an image that reflected the subject at full length, then a number of steps were performed, including the following: (1) identifying an item in the recording whose measurement is known or readily available, that can be used as a reference object; (2) carrying out a survey in order to detect the object reference measurements and any other useful elements for the same purpose; (3) highlighting inside the image points, which identifies the reference sample and the element to gain size perspective; and, (4) carrying out appropriate scaling operations, dictated by the rules of perspective, between actual measurements and those selected in the image. Based on the results, an anthropomorphic profile of the individual can be obtained.

Previously, it was already possible to perform all the above operations using manual methods to calculate all anthropometric data, minus a margin of error due to the tools and methods used for the measurement.

The proposed procedure was initially applied to an anthropometric examination performed on an armed subject, taken by security cameras in a public place. These pictures were used to finally conduct a functional testing of the software produced. The application that is described here will make the process the most automated possible, in such a way as to minimize measurement errors of an accidental and instrumental type.

Image Processing, Automatic Characterization, Software Implementation



E1 Use of Earprints as Evidence in Spain

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After attending this presentation, attendees will be familiar with the concept of earprint and earmark evidence used by the Spanish Cuerpo Nacional de Policia (CNP), with how this evidence is collected and classified by technicians, and with how it is used as evidence in criminal courts.¹

This presentation will impact the forensic science community by implementing new ways to use earprints, applying a scientific method to classify them, showing the problems associated with the use of this technique, and pointing out the advantages that have helped police department investigations.

While numerous studies have looked into the various parts of the ear to classify earprints, the CNP uses a pattern to measure different parts of an earmark found at a crime scene, then records it in the database. With this classification, earprints obtained in the laboratory or earmarks found at other crime scenes can be compared. This comparison helps investigators eliminate suspects or open new investigative paths. There are open research areas that are attempting to demonstrate this technique's validity and statistical research has been performed to evaluate its accuracy.^{2,3}

This study demonstrates the pattern and how the technician uses it to obtain different points for measuring the earmark/earprint. The formula obtained will have five numerical values and one non-numerical value. With this formula, classification will indicate if the earmark or earprint was from a right or left ear.

Judicial sentences from Spanish criminal courts from 2000 to 2013 were studied and it was determined that earprints have been admitted as circumstantial evidence. Circumstantial evidence has been delineated by Spanish Supreme and Constitutional court jurisprudence and this evidence must meet this criteria in order to be effective. The cases where earprints were involved came primarily from CNP burglary investigations. Credible, reliable testimony from a forensic expert in a criminal case is essential and in the studied cases, the CNP experts proved the suspect was in/at the crime scene and that the earmarks left there, after comparison with earprints taken at the police laboratory, were from one of his/her ears. Another goal of this research is to discover if this evidence is legitimate for the Spanish courts or deemed as unreliable evidence.

The opinions or assertions contained herein are the private view of the author and are not to be construed as official or as reflecting the views of the Spanish Ministry of the Interior or Spanish Cuerpo Nacional de Policia.

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Earprint, Evidence, Classification



E2 Differentiation of Human, Animal, and Synthetic Hair by ATR-FTIR Spectroscopy

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After attending this presentation, attendees will understand the current problems forensic hair analysis faces and how Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) spectroscopy combined with chemometrics provides a faster, quantitative, and inexpensive method for hair identification.

This presentation will impact the forensic science community by demonstrating a method that is able to quantitatively identify a sample of hair as human with no false negative human assignments, using a non-destructive and rapid approach without the need for sample preparation or specialization.

Hair fibers are ubiquitous to every environment and are a common form of trace evidence found at crime scenes. The primary difficulty forensic examiners face after retrieving a hair sample is determining its origin: if it came from a human or an animal and, if human, what is the race, gender, and type of body hair (e.g., head, pubic, underarm, etc.). Currently, the methodology of microscopic examination of potential hair evidence is absent of statistical probability and is inherently subjective.¹ In the 2009 National Academy of Sciences Report, *Strengthening Forensic Science in the United States: A Path Forward*, it was concluded that there are no accepted statistics about the frequency with which certain hair characteristics are distributed within a population and that hair comparisons for individualization have no scientific support without nuclear DNA.² In early 2013, the Federal Bureau of Investigation (FBI) began a review of more than 2,000 convictions based on hair evidence.³ Of the first 310 cases, DNA analysis revealed that 72 of the convictions were grounded on faulty hair evidence.³ Despite its increasing popularity, the process of extracting DNA is costly, destructive, time consuming, and the majority of times is unsuccessful due to its degradation and absence from the hair.⁴ A method for determining the identity of an unknown fiber quickly, with a high degree of certainty, and eliminating examiner bias would be extremely useful and cost-effective for the field of forensic science.

ATR-FTIR spectroscopy is a technique rising in popularity for analytical and biological purposes. The attributes of ATR-FTIR spectroscopy are very attractive for forensics because of its rapid and non-destructive nature, its ease-of-use, and minimal-to-no-sample preparation. An infrared spectrum displays the vibrational characteristics of a sample based on the different absorption frequencies of the individual functional groups.⁵ The advantage of combining ATR-FTIR spectroscopy with chemometrics is its ability to enhance the selectivity of the instrument and create classification models.⁶ With the availability of portable ATR-FTIR instruments, there is the potential for on-field analysis of the identification of a single hair fiber.

This study demonstrates the ability to discriminate natural hair from a synthetic fiber and differentiate human hair from animal hair (i.e., dog and cat hairs) using chemometric modeling of ATR-FTIR spectroscopic data.⁷ Two Partial Least Squares Discriminant Analysis (PLSDA) models were constructed: one to differentiate natural hair fibers from a synthetic fiber (binary) and the second discriminating human hair from animal hair (species specific). Hair samples were collected from a synthetic wig and a diverse population of humans, dogs, and cats. Of the many variables that can influence the chemical make-up of hair (bleaching, waving, straightening, and extensive sunlight exposure), only chemically treated (i.e., dye, bleaching, etc.) hairs were excluded from this study.⁸ Both models were successful in differentiating these classes; synthetic hair was completely separated from natural hair in the binary approach and all human samples were correctly predicted as human in the species-specific model. An external validation, using untrained donors, resulted in zero false positive and false negative assignments for the human class; however, one of the five external dog samples, from a breed of dog not accounted for in the training dataset, was misclassified and all spectra were incorrectly predicted as cat.

Overall, this demonstrates the significance of the model's unique ability to quantitatively identify a sample of hair as human with a high degree of confidence. Most importantly, the method can be conducted without the need of a trained expert, is non-destructive, requires no sample preparation, and results in rapid identification, making it of ample importance to the field of forensic science.



General Section - 2015

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ATR-FTIR Spectroscopy, Chemometrics, Hair



E3 From 20 to 12 in 42 Years: A Case of Inflated Age Estimation and the Role of Forensic Anthropology in Cold Case Investigations

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After attending this presentation, attendees will have a better understanding of the forensic anthropologist's ability to redirect 40 years of investigative efforts through routine analysis of human remains.

This presentation will impact the forensic science community by illustrating the utility of forensic anthropology in cold case investigations involving human remains that would not have originally fallen under the auspice of the forensic anthropologist. In particular, this presentation demonstrates the practical value of traditional forensic anthropology in scenarios where DNA analysis may be of limited investigative significance.

In May 1972, partially decomposed human remains were located in a pond near Daytona Beach, FL. An autopsy indicated that the victim was a White male, approximately 20 years old, and determined the manner of death to be homicide. In an effort to not only identify the victim but also to locate any witnesses or suspects, the Volusia County Sheriff's Office (VCSO) pursued leads generated from this autopsy report and evidence found at the scene. They sought the help of the media in publicizing descriptive information regarding the decedent and the crime, as known at the time, in order to identify the victim; however, their investigative efforts failed to yield an identification. The subject was interred as a John Doe and added to the list of unsolved cold cases. Over the next four decades, law enforcement focused all further identification efforts on a specific subset of the population — young, adult White males.

In 2013, John Doe was exhumed in order to obtain a DNA sample for analysis as part of continuing efforts to identify the decedent. The University of Florida C.A. Pound Human Identification Laboratory (CAPHIL) assisted in the exhumation and further performed an anthropological examination not previously conducted. As the casket was opened on-scene, the decedent's ankle was exposed; the distal tibial and fibular epiphyses were open. It was immediately clear that the assumptions of the investigation were false; the remains were not those of an adult. The VCSO was immediately notified that their decedent was significantly younger than reported and the remains were transported back to the CAPHIL for extensive analysis.

The precise age of John Doe was assessed using extent of epiphyseal closure, long bone length, and degree of dental development. Additionally, both a non-ossifying fibroma and an osteochondroma, two distinct pathologies associated with actively growing subadults, were found. It was determined that the decedent was 11 to 14 years old, and most likely 12 to 13 years old, at the time of death. The result of this study was the most significant change to the case since its origin in 1972. The anthropological findings changed the dynamics of the crime itself and served to all but nullify the previous 40 years of investigative efforts by law enforcement well before the DNA results could even be submitted. Indeed, given the limited number of nuclear DNA comparative samples available as of 1972 and the taphonomic condition of the remains, the DNA may yield little novel information.

While advancements in the ability to extract and analyze DNA and the development of comparative DNA databanks have revolutionized the field of human identification and provided numerous invaluable identifications of decedents, DNA's role in cold cases that predate the Combined DNA Index System (CODIS) may be more limited. An extended postmortem interval and poor preservation of the remains may limit analysts' abilities to extract usable nuclear or mitochondrial DNA. Nuclear exemplars are likely unavailable; mitochondrial exemplars may likewise be limited and typically require a prospective identification for direct comparison. Even in the absence of a database match, DNA analysis may provide information about the biological sex and ancestry of the decedent, but cannot provide information regarding age-at-death or numerous other identifying characteristics (e.g., stature, idiosyncratic morphology, life history traits, etc.).

In summary, cold cases are routinely being reopened due to advances in DNA analysis; however, the original assumptions implicit to cold case investigations are not always correct and DNA is not always capable of correcting these errors. When investigative entities reopen cold cases, DNA analysis should be conducted, but the unique value of forensic anthropology — even for cases that underwent full autopsy — cannot be overlooked.

Anthropology, Cold Case, Age Estimation



E4 A Study of Morphological and Metric Variations of the Human Ear — Applications in Personal Identification

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After attending this presentation, attendees will understand the significance of the variability of the human ear, which may help them in conducting further studies and in solving forensic cases usually encountered in airplane crashes, intentional mutilation and dismemberment, explosions, or other mass disasters.

This presentation will impact the forensic science community by presenting new information on the variability and uniqueness of the human ear in an Indian population and the usefulness of the human ear in personal identification.

In the past, many morphological and metric features of the human body have been used for personal identification in forensic examinations. Fingerprints, footprints, facial characteristics and features, iris, gait, teeth, bitemarks, gait pattern, lip prints, voice characteristics, and DNA fingerprinting from a variety of tissues of the human body have successfully been utilized in forensic situations as well as for identification of criminals. The human ear is another organ of the body which is unique to an individual. Like fingerprints and other individualistic characteristics of the human body, an ear retains certain individualistic characteristics which are unique due to variations in the anatomical structures of the external ear. In certain situations, where the dead body is recovered in dismembered or mutilated conditions, the shape, size, and individualistic features of a person's ears may be useful in identifying the deceased along with other identification characteristics of the human body. In the recent past, it has also been shown that, like the ear itself, the prints left by the human ear are also individualistic to an individual; the earprints can be left by the criminals/burglars while listening at the doors or windows of the target house. So looking at the value of the human ear in forensic identification, the objective of the present study was to study the morphological and metric characteristics of the human ear in a north Indian population. The sample for the present study is comprised of 90 males and 87 females ranging in age from 18 to 30 years of age taken from the upper reaches of Himachal Pradesh State in North India. The morphological characteristics such as overall shape of the ear, size and shape of the tragus, ear lobe, shape of helix, forms of Darwin's tubercle, and rare and special characteristics as well as congenital deformities were studied in all the subjects. To generate data for the metric characteristics, physiognomic ear length and physiognomic ear breadth were measured using standard landmarks.

The physiognomic ear length and breadth in males were found to be significantly larger than those of the females. The length and breadth measurements of the ear show bilateral variations, although they were statistically insignificant. The other findings of the study indicate that the oval-shaped ear was present among 40% of the males and 44.8% of the females in the study sample. The flat-shaped ear was the rarest variant, reported only among 1.1% of the males in the study. The other types of ear, such as the oblique, rectangular, round, and triangular, were found in both sexes. Bilateral asymmetry exists in regard to the shape of the ear. The size and shape of the tragus also vary with respect to the left and right sides as well in sexes. The ear lobe showed different characteristics in different individuals. In nearly half of the cases in both males and females, the ear lobe was found to be attached to the face; in many cases, it was free, and, in some, partially attached. The size and shape of the ear lobe also showed variations with respect to sides as well as sexes. The shape of the helix varies in individuals showing certain characteristics such as concave, rolled, flat and wide covering scapha, etc. The Darwin tubercle showed a variety of structures in both the left and right sides in both the sexes.

Personal Identification, Human Ear, Variations and Uniqueness



E5 A Pilot Study to Evaluate Baseline Quantities of Recovered Touch DNA From a Pistol and Ammunition

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After attending this presentation, attendees will be familiar with collecting touch DNA samples from a pistol and ammunition, the DNA profile results from different parts of the pistol, and interpretation of results.

This presentation will impact the forensic science community by describing a method of collecting touch DNA on a pistol and interpreting distribution of profiles on different parts of the pistol and on ten rounds of ammunition.

The recovery of DNA samples from firearms has proven invaluable in the course of forensic investigations, in large part due to improvements in DNA sample collection, preservation, processing, and result interpretation. Typically, this type of evidence yields a limited quantity of biological material containing DNA template from perspiration and epithelial cells, the source for “touch DNA” samples. The quantity of DNA recovered by swabbing can vary based on the frequency of handling and cleaning surfaces of firearms, types of cleaning oils and solvents, physiology of the handler, number of contributors, and downstream testing methods. This research was conducted to evaluate the baseline level of Short Tandem Repeat (STR) DNA that can be recovered from a pistol and cartridges.

A 9mm Smith & Wesson® Model 5906 pistol was handled by one right-handed owner/shooter. The pistol was fired and stored without cleaning for a period of two weeks before swabbing. The shooter removed ten 9mm full-metal jacketed cartridges from a new unopened box of American Eagle® ammunition, loaded a full magazine, then inserted the magazine into the pistol and ejected it. The pistol, full magazine, and the original ammunition box were collected and swabbed for DNA as follows: two swabs were collected from the ammunition box, one swab was collected from the outer cardboard package, and one swab was collected from the inner plastic case holding the cartridges. A total of five swabs were collected from the magazine’s right, back, left, front, and base sides; ten swabs were collected, one from each 9mm cartridge, ordered by sequence of insertion into the magazine. Finally, 19 swabs were collected from the 9mm pistol to cover right side surfaces, left side surfaces, top surfaces, and specific parts of the pistol such as the trigger, trigger guard, and hammer. All samples and appropriate controls were collected using the COPAN® Crime Scene 4N6 FLOQSwabs™ that were pre-wetted with sterile water. DNA samples were extracted using the COPAN® Nucleic Acids Optimizers (NAO), a semi-permeable basket which retains fluid until centrifuged with the PrepFiler® Express™ on the *AutoMate Express™* DNA Extraction System by Life Technologies™. DNA quantitation was performed using the Quantifiler® Human DNA Quantification Kit by Life Technologies™. The AmpFLSTR® Identifier® Plus PCR Amplification Kit by Life Technologies™ was used for DNA amplification, the fragments were run on the Applied Biosystems™ 3130 Genetic Analyzer by Life Technologies™, and the analysis was performed with GeneMapper® ID-X v1.4.

The results show full STR profiles from the user on the cartridge box (one of two swabs), magazine (four out of five swabs), and various areas on the pistol (12 out of 19 swabs). Even though this shooter was right handed, a complete DNA profile was obtained on the right side of the pistol safety lever. Characteristically, a right-hand shooter manipulates an ambidextrous safety on the left side of the pistol; however, possibly in this case, the DNA on the right safety lever originated from the method of retracting the slide on the pistol. The shooter retracted the pistol slide by placing the left thumb on the right side of the slide over the safety lever with the index finger contacting the safety lever on the left side of the slide. Consequently, a DNA profile on one side of an ambidextrous safety does not indicate the shooter’s handedness. All swabs from the 9mm cartridges resulted in partial STR profiles with no direct correlation to the number of markers compared to the loading order of the cartridges in the magazine. This data can aid criminal investigators in assessing the probative value of DNA evidence recovered from specific parts of a pistol.

Touch DNA, DNA on Pistol, Swab From Firearm



E6 Mummified Tattoo Rehydration, Photography, and Reconstruction in Cold Case Investigations

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After attending this presentation, attendees will be familiar with the procedures conducted by the Pasco County Sheriff's Office in Pasco County, FL, on a small amount of mummified tissue in order to rehydrate, clean, and expose the details of a colored tattoo on a skeletonized cold case from 1989. This protocol allowed for effective photography using various color filters to expose the details and color of the tattoos to aid in the process of identification.

This presentation will impact the forensic science community by offering details into the successful process used on a small amount of mummified tissue recovered with skeletal remains. The process allowed for the exposure, reconstruction, photography, and preservation of tattoos, which has the potential to significantly improve case solvability for highly decomposed or skeletonized remains.

On January 24, 1989, the skeletal remains of an individual were found in an orange grove in Dade City, FL. The individual has remained unidentified and, in 2007, new efforts were initiated to further investigate the case in an attempt to make an identification. The skeletal remains were sent to the University of South Florida Forensic Anthropology Laboratory for a skeletal analysis to estimate the biological profile, and forensic investigators at the Pasco County Sheriff's Office began working with a small piece of mummified tissue found with the remains. The initial police report in 1989 stated that the tattoo on the mummified skin was "what appeared to be a rose with green leaves, purple stripes going from the rose to an area that resembles a spider web colored in yellow and black lines;" however, no efforts had been made at the time to clean or rehydrate the tissue.

After the tissue was rehydrated with a 50/50 glycerin and water solution in 2007, Pasco County Sheriff's Office forensic investigators cleaned the still-submerged tissue with sterile swabs. Prior to and after rehydration, the exposed tattoos on the tissue were thoroughly photographed using colored light filters at different nanometers (including red, orange, yellow, green and blue light filters, each at 450, 485, 530, and 570 nanometers) to visualize the different aspects and colors of the tattoos. These photographic techniques allowed for the reconstruction of very detailed and colored tattoo images that were very different from the original police description, including a bicep tattoo containing the initials "HFD," wings, flames, a cross, a rebel flag and swastika, as well as a forearm sleeve tattoo of a green flaming dragon winding down the arm around a nude female with red high heels on a rebel flag background. These updated details and images were included in the law enforcement agency bulletins and media releases.

This case is an excellent example of the use of innovative forensic techniques employed by the Pasco County Sheriff's Office that allowed the details of the preserved tattoo to be exposed and reconstructed almost 20 years after the remains were recovered. A limited amount of research has been published regarding the use of various imaging techniques to visualize tattoos on cadavers; however, most of these publications involve fresh cadavers where rehydration and cleaning is not necessary.¹⁻³ This presentation clearly outlines the protocol performed on the mummified tissue which can aid forensic investigators with other law enforcement agencies or medical examiner's offices if presented with similar mummified tissue that may have the potential to contain tattoos. By having the opportunity to update the tattoo description with clear photographs and reconstructions, the visibility and solvability of an unidentified cold case in law enforcement investigations, as well as mass disaster situations or human rights recoveries, will be dramatically increased.

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Cold Case, Tattoos, Filter Photography



E7 Carbon Monoxide Poisoning: A Study of Five Cases and Literature Review

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The goal of this presentation is to highlight the importance of coloration of the hypostases in suspected carbon monoxide poisoning and the importance of quantitative measurement of Carboxyhemoglobin (COHb) in the diagnosis of the cause of death.

This presentation will impact the forensic science community by providing results from a study of five cases of carbon monoxide intoxication and a literature review of carbon monoxide poisoning. This presentation is also an alert to medical examiners of the importance of hypostases coloration in the diagnosis of Carbon Monoxide (CO) poisoning.

CO is the leading cause of poisoning death in the world.¹ CO has a high affinity for hemoglobin, decreasing its ability to carry oxygen, causing hypoxia tissular.² At necropsy, there is blood fluidity, clear hypostases, and the organs have a red carmine color.³

This study presents five cases of death due to CO poisoning. The diagnosis was confirmed by autopsy in the Central Forensic Institute of Sao Paulo and the quantitative laboratory measurement of COHb was performed by spectrophotometry at the Oscar Freire Institute Department of Toxicology. A review was made of the literature regarding the signs and symptoms of this type of poisoning, as well as COHb values found in other studies. The review used complete studies, using MEDLINE® data from 2009 to 2014, published in English and Portuguese.

The five autopsies were from the same family, but were not performed simultaneously. The first one happened approximately 30 days before the other four; cause of death was unknown, despite a highly suggestive hypostases of CO poisoning. Later, four more necropsies of this same family came to light. Due to the arrival of four more bodies of this same family, with the same descriptive characteristics, CO poisoning was suspected. Laboratory tests showed the qualitative dosage of COHb was positive and the quantitative dosages were 84%, 88%, 94%, 87%, and 88%. Death may occur with COHb concentrations above 30%.⁴ Carbon monoxide is colorless, odorless, tasteless, and invisible, features that make this agent imperceptible to an exposed person. The main cause of death due to CO poisoning occurs in fires where the release of gases is due to the combustion of fuel or from defective heaters. Local expertise revealed that the source of emission of carbon monoxide was a gas heater located in the service area of the residence.

In order to suspect CO poisoning, the coroner must be aware that there are cases in which the main symptom, if not the only one, is the red carmine color that the hypostases acquire. Therefore, while performing a necropsy, the hypostases should be evaluated for their existence and location but, most importantly, for their color.

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Carbon Monoxide Poisoning, Carbon Monoxide Intoxication, Death



E8 Does Probing Enhance the Decomposition Odor Released From a Gravesite?

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After attending this presentation, attendees will understand the impact of probing a gravesite with respect to the release of decomposition odor and its subsequent detection by Human Remains Detection (HRD) dogs.

This presentation will impact the forensic science community by expanding the general knowledge base relating to the decomposition odor profile of buried remains. The information will be valuable to law enforcement agencies who utilize HRD dogs to locate buried remains as it demonstrates the abundance of decomposition odor available at the grave surface and the impact of probing in releasing the odor for detection.

HRD dogs are trained to locate and respond to the odor produced by decomposed remains. The odor consists of a diverse range of Volatile Organic Compounds (VOCs) which are released into the atmosphere as the body decomposes over time. The concealment of a body in a gravesite will slow the decomposition rate and reduce the odor at the grave surface, proving a considerable challenge for HRD dogs tasked with locating buried remains. HRD handlers will often probe the ground surrounding a suspected gravesite with the intent of venting the soil to release the odor; however, due to its potential to damage remains sub-surface, the soil probe is typically used with caution. Damage to the remains can cause challenges for the medical examiner in determining whether the trauma occurred during the antemortem, peri-mortem, or postmortem period. Hence, the goal of this study was to determine whether probing offered any benefit to search investigations involving HRD dogs by releasing decomposition VOCs and enhancing their detection at the grave surface.

Pig carcasses (*Sus scrofa domestica*) were used as human decomposition analogues. Carcasses were buried in shallow graves to a depth of approximately 60cm. Control graves were established in close proximity to monitor the natural VOC profiles of the soil and vegetation without the presence of a carcass. The decomposition odor within the gravesites was monitored by placing a VOC sample collection tube permanently in the center of the graves. This provided a reference VOC profile throughout the decomposition period which could be compared to the VOCs detected at the grave surface. A stainless steel hood was placed over the surface of the graves prior to probing to allow the VOCs to accumulate. VOC samples were collected onto sorbent tubes and subsequently analyzed by thermal desorption — comprehensive Two-Dimensional-Gas Chromatography/Time-Of-Flight/Mass Spectrometry (TD-GC×GC/TOF/MS). This process was repeated after probing to determine whether the probe released additional VOCs that were not previously detected at the grave surface. Temperature data loggers were used to determine grave temperatures at the middle and base of the graves and a weather station was utilized to monitor environmental conditions at the site.

Comparison of the experimental and control sites allowed a list of VOCs to be identified within the grave and at the grave surface that were specific to the decomposition process. Over the eight-month study, more than 100 decomposition VOCs were detected within the grave but fewer were found at the grave surface. The abundance of VOCs at the grave surface peaked during early decomposition before quickly declining, suggesting that the soil texture can play a large role in the migration of VOCs to the grave surface. The profile detected at the grave surface did not always reflect the profile within the grave, supporting the ability of the soil microbial community to modify VOCs in the soil column. Some variability was seen in the grave surface samples collected before and after probing, which is likely attributed to the dynamic sampling environment, an important aspect to consider in search and recovery investigations. The benefits of probing should not be disregarded as on several of the sampling days, the air samples collected after probing displayed a greater abundance and more diverse profile of decomposition VOCs.

Decomposition Odor, Probing, Human Remains Detection Dogs



E9 Hammercide: A Bloody Hammer, a Dead Husband, a Bloody Wife, and a Bloody Live-In Friend, as Well as a False Confession

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After attending this presentation, attendees will understand the complexity of having an individual trying to claim responsibility for a homicide they did not commit. Attendees will see how the forensic evidence will help to determine the truth.

This presentation will impact the forensic science community by exemplifying the use of different forensic disciplines that were necessary to arrest and prosecute the proper individual involved in a violent homicide when two people confessed.

In the early morning hours of January 14, 2009, the Gilbert, AZ, police department responded to a call from a local residence. Upon arrival, the first officer found a female outside with blood in her hair and on her clothing. Inside, the officer found a male (the victim) on the floor lying in a pool of blood as well as another male with blood on his clothing. There were also two young children asleep in the house.

The injured male was taken to an area hospital in extremely critical condition. Chance of survival was minimal despite state-of-the-art medical intervention at a major trauma center. The female (Suspect 1) at the scene was the spouse of the injured party. The other male (Suspect 2) was a live-in “friend of the family.”

Evidence at the scene and injuries to the victim indicated blunt force trauma. A bloody hammer on a table at the scene appeared to be the weapon used on the victim. It was time to put the pieces of the crime scene together based on forensic evidence as well as suspect and/or witness statements. Expectations were that certain forensic evidence should or could yield valuable types of information.

Suspect 2 stated that he beat the victim and was the one responsible for the carnage at the crime scene. Suspect 1 told police that Suspect 2 saved her as she was being raped by her husband. As Suspect 1’s story was proven inaccurate based on evidence at the scene, she changed her story and admitted she was the one who beat her husband with the hammer. She said she beat her husband with the hammer because he was an abusive husband and he raped her earlier in the evening. Suspect 2 made numerous efforts to confess and take blame for the crime, including going to a newspaper and another police department to confess. The confession of either Subject 1 or Subject 2 was false and the evidence was what would contradict or support the confessions.

In this case, like many others, the forensic evidence should provide valuable information as to what occurred and who committed this crime. With two people confessing to the crime, which became murder three weeks later when the victim died, the evidence needed to be carefully evaluated. What was expected from the evidence and what the evidence yielded was not as simple as sending items to a lab and getting the positive results one was expecting.

This presentation will describe the forensic evidence, what was expected from the evidence, what the results were, and, more importantly, how the evidence was used to prove or disprove the confessions of the two people taking the blame.

False Confession, Bloody Hammer, Live-In Friend



E10 Development Process Validation for Kinship Analysis Algorithm

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After attending this presentation, attendees will gain an understanding of the established Life Technologies™ (LT) kinship algorithm analysis program, its features, flexible implementation, and standard calculations for trio analysis, including complex pedigree trees such as incest, motherless/fatherless cases, and inclusion/exclusion of mutations, and rare alleles based on currently used methods. The analysis program has established processes for Short Tandem Repeat (STR) analysis but a feasibility study using Single Nucleotide Polymorphism (SNP) data has also been proven.

This presentation will impact the forensic science community by not only using a standardized approach but will also show the ability to include concepts of both STRs and SNPs for better discrimination power.

Computing likelihood ratio in kinship analysis for autosomal markers is straightforward and well defined. Such calculation provides a value for evidence given the prosecution-versus-the-defense proposition. It is recommended and widely used in forensics, missing persons cases, and paternity cases. The forensics community has validated stand-alone software for calculating Likelihood Ratio (LR) using trios and many biologically related family members. Software such as Familias and MPKin™ FS Edition are used regularly for such calculations.¹⁻³ Because these implementations are stand-alone, transcription errors can occur on transferring data from data collection, table input, and result storage; in addition, it can also be time consuming.

LT has incorporated its version of a kinship algorithm, based on the Error Standard (ES) algorithm, to data collection and storage for ease of use and reliability of results, therefore avoiding human transcription errors.²⁻⁵ This presentation will encompass the steps taken by this study to validate the kinship analysis algorithm, given the available methods, data, and external collaborators. Building on previous literature, this study used the National Institute of Standards and Technology (NIST), the Council on Education for Public Health (CEPH), and real data from collaborators to compare results of the kinship algorithm to those currently used in the paternity and forensics laboratories. This study shows that the standard calculations, including complex pedigree trees, mutations, and rare alleles concur with currently used methods.

Through this work, the LT-kinship algorithm, a more flexible implementation with state-of-the-art models, has been established as accurate. The algorithm with SNP data has been further tested, showing that, for a small number of SNPs, the algorithm produces LR values, which may be an option once expert data and tables become available.

For Research, Forensic, or Paternity Use Only. Not for use in diagnostic procedures.

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Kinship Algorithm, STRs, SNPs



E11 An Assessment of the Volatile Organic Compounds (VOCs) of an Agricultural Biothreat Agent, *Raffaelea lauricola*, and Training Aids for Canine Detection

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After attending this presentation, attendees will learn about an agricultural biothreat agent known as the laurel wilt pathogen. Attendees will gain an understanding of how research is combatting the spread of this biothreat by analyzing the Volatile Organic Compounds (VOCs) present in the headspace above the fungus in order to create a mimic training aid to be used in canine detection.

This presentation will impact the forensic science community by presenting a novel method of preventing the spread of a biothreat through canine detection. It will also strengthen the validity of canine detection as it is currently used in the fields of forensics and agriculture by showing the canines' ability to detect biothreats and distinguish diseased trees from uninfected ones.

Invasive biological agents pose a huge threat to the agriculture, environment, and economy of the United States. These biothreats can enter the United States through ports-of-entry accidentally, as is the case of the invasive and phytopathogenic fungus *Raffaelea lauricola*. The current research identified the VOCs of the laurel wilt pathogen in order to create a mimic training aid. Using this aid, canines can be trained to detect infected trees before physical symptoms develop so that infected trees can be removed from groves and healthy trees protected. The vector of the *Raffaelea lauricola* fungus is the invasive redbay ambrosia beetle (*Xyleborus glabratus*), which entered the United States from Southeast Asia on infected wood. The symbiosis between the beetle and the fungus is a relationship where the beetle carries the fungus to a tree, bores into the host tree, and farms the fungus for food. The fungus colonizes the xylem of the tree, which then causes the tree to systematically shut down its transpiration mechanism in order to stem the spread of the infection. This leads to the tree's death within four to six weeks. Symptoms include wilting of the leaves and discoloration in the bark. Laurel wilt is advancing through the Lauraceae forests in the southeastern United States, and now is found in commercial avocado groves in Florida.

Because of the rate of advancement of the disease, a method of detection before the development of physical symptoms must be developed. Canines are often used in law enforcement and in ports-of-entry to prevent the entry of substances such as known biothreats or banned agricultural items; however, novel biothreats such as *Raffaelea lauricola* have not been stopped at the port-of-entry and are now spreading through the forests and agricultural environments. The current study focuses on identifying the VOCs above the laurel wilt pathogen in infected trees through Solid Phase Microextraction/Gas Chromatography/Mass Spectrometry (SPME/GC/MS). The goal is to create a biological mimic with which to train canines to locate the pathogen in avocado groves so the diseased trees can be removed and healthy trees preserved. Bipolar SPME fibers were used to sample compounds in the headspace above samples of infected wood and uninfected wood. The compounds were then desorbed and separated through GC/MS. Results show that avocado trees infected with the laurel wilt produce different VOCs than trees that are uninfected by the pathogen. Based on these differences, a training aid will be created and subsequently verified through canine trials. This mimic training aid can then be used to combat the biothreat caused by *Raffaelea lauricola* and the skills and practices developed applied to the wider effort of protecting the nation's food supply from foreign invasive pests and other biothreats.

Biothreat, Canine Detection, *Raffaelea lauricola*



E12 Breaking Glass: Case Review of an In-Hospital Suicide

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After attending this presentation, attendees will have reviewed a case of suicide by sharp force injuries in a hospital inpatient setting.

This presentation will impact the forensic science community by contributing to the understanding of common reasons a person may commit suicide, although, in some cases, why the person committed suicide may never be known.

Suicide is the act of intentionally causing one's own death. It is often carried out as a result of despair, the cause of which may be associated with a psychiatric condition such as depression, bipolar disorder, schizophrenia, or borderline personality disorder. It may also be associated with substance abuse problems, such as alcoholism or drug abuse. Stress factors such as financial difficulties, declining health, or troubles with interpersonal relationships often play a role in why a person commits suicide. Occasionally the underlying reason for the suicide is unclear at the time of death and can remain unknown forever.

Harris County has a population of over four million people with approximately 4,000 deaths being reported to the medical examiner's office each year. Roughly 450 cases each year (approximately 11-12%) are classified as suicides. In Harris County in 2009, a total number of 4,153 cases were examined at the medical examiner's office. Of those cases, 484 (approximately 12%) were classified with a manner of death of suicide. Suicides by sharp force injury (cut and/or stab wounds) are rare and in 2009 accounted for only 3% of those deaths were classified as suicide.

This presentation will be a case review of suicide by sharp force injuries that occurred in a hospital inpatient setting. The decedent had a medical history of Hypertension (HTN) and was transported to the hospital after experiencing a syncopal episode. He reportedly had a history of alcohol abuse and had ceased drinking approximately nine days prior to the syncopal episode initiating the hospitalization. He denied any pain, shaking, nausea, or agitation. The decedent was admitted to the hospital with the diagnoses of syncope, severe hypertension, hyponatremia, hypokalemia, tachycardia, and rhabdomyolysis. He was admitted to a regular room on a medical floor and other than a slightly elevated temperature and heart rate, he had no documented complaints. Over time, and for unknown reasons, he became increasingly agitated. He reportedly attempted to asphyxiate himself using the cord of the nurse call button. Multiple nurses were required to remove the cord from around the decedent's neck. The decedent then proceeded to run and crash through the glass window of the hospital room in an attempt to jump out the window. Hospital personnel were able to keep him from jumping out the window; however, he grabbed a large shard of broken glass and utilized it to stab himself in the neck. Prior to this episode, the family reported no history of mental illness and no history of suicidal ideations or attempts. The decedent had voiced no complaints prior to the incident. Toxicology screens performed on hospital blood from the time of admission as well as autopsy samples were negative for common substances of abuse, including illicit drugs.

Although it is important to note any history of mental illness, suicidal ideations, or past attempts, the lack of such does not indicate the death could not be suicide. Scene investigation and witness accounts of the circumstances surrounding the death are crucial. Though adequate information can be obtained in order to classify the cause/manner of death, families may not ever have an answer to the question of why.

Suicide, Sharp-Force Injuries, Hospital



E13 Mortality Directly Related to Abuse Against the Elderly in Brazil: A Reality That Needs to Be Reported

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After attending this presentation, attendees will better understand the reality of the causes of elderly deaths in Brazil. Attendees will learn how these deaths occur primarily due to physical violence; however, the majority of these assaults could be avoided if identified sooner.

This presentation will impact the forensic science community by communicating the aspects of mortality involved with the elderly due to recurrent physical assault in Brazil.

Purpose: Brazil is an “old country,” according to the World Health Organization (WHO), as more than 7% of the population is 60 or more years old (Census of the Brazilian Institute of Geography and Statistical, 2012).^{1,2} Along with this growth, the incidence and prevalence of deaths directly related to abuse against the elderly has also risen. The identification of violence against this vulnerable population is one way to prevent an increase of these fatal cases. The purpose of this study is to discover ways for professionals who care for the elderly to remain alert to this problem and, when discovered, to report the problem to the authorities.

Methods: This is a descriptive study based on data from the Information System for Notifiable Diseases (SINAN), a database of the Health Ministry of Brazil. The mortality rate of the elderly (more than 60 years of age) as a direct result of violence were analyzed for the period from 2009 to 2012 in relationship to the total number of deaths from violence in all ages during the same period.

Results: Men were the primary victims. The female mortality rate was high (above 10% in all years analyzed) and exceeded the male rate in the years 2009 and 2012 (10% and 13.8%, respectively). Regarding the recurrence of violence, men were the most frequent victims as compared to women; men were the most frequent victims of death as a direct result of violence, except in 2010 when female mortality exceeded that of males. In 2012, more than 15% of deaths due to recurrent violence in all age groups were elderly men. The instruments most used by offenders, in cases of physical violence, were blunt instruments, followed by knives and firearms, with the first resulting in the most fatalities. Among aggressors, sons were the major perpetrators, followed by friends and unknown people. Lower victim education was associated with higher frequency of deaths directly caused by violence. Neglect occurred predominantly in females, while economic violence was more common among men, although it has shown high rates in both genders.

Conclusions: The results shown in this study are alarming, even considering that the data most likely are underestimated. Many deaths are not associated with maltreatment. The omission of the aggressors concerning the precise reasons of deaths is a factor to be considered. As the elderly are susceptible victims of accidents, especially those accidents related to falls, many traumatic injuries caused by violence are not reported.

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Elderly, Violence, Death



E14 Forensic Physical Evidence vs. Eyewitness Evidence: A Look at Their Contributions to Wrongful Convictions and Exonerations

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The goal of this presentation is to provide forensic scientists and legal personnel with information that would allow them to develop stricter policies and practices regarding the handling of eyewitness evidence and physical evidence in order to prevent wrongful convictions in the future.

This presentation will impact the forensic science community by allowing attendees to better understand the impact each type of evidence has in criminal investigations as well as the importance that reliability and accuracy of eyewitness testimony and forensic physical evidence have as independent contributors to wrongful convictions.

This presentation is based on the growing epidemic of wrongful convictions and exonerations taking place in the United States since 1989. Two main databases were referenced in this research: The National Registry of Exonerations and The Innocence Project. This study examined misinterpreted or falsified forensic physical evidence and three types of eyewitness evidence: mistaken witness identification, perjury, and false confessions. After the late 1980s, modern DNA typing methods allowed for successful testing of older and smaller biological sample quantities, helping to secure 316 exonerations. In this study, statistics were generated by calculating the frequencies at which each type of evidence occurred in the 1,362 wrongful convictions to date as well as the 316 DNA exonerations.

This study examined whether erroneous forensic physical evidence or erroneous eyewitness evidence was responsible for the majority of wrongful convictions; however, in many cases, multiple types of falsified or misleading evidence were involved simultaneously; therefore, results do not total 100% and do not allow for a direct comparison. The first hypothesis tested was that eyewitness evidence will be more impactful and credible to legal personnel and juries than would be forensic physical evidence. The second hypothesis was that eyewitness evidence will be less accurate and reliable than forensic physical evidence. The third hypothesis was that forensic evidence will be more likely to exonerate while eyewitness evidence would be more likely to wrongfully incriminate.

Results showed that eyewitness evidence was more than twice as prevalent as forensic physical evidence in wrongful convictions. Out of 1,362 exonerations, perjury was the leading cause of wrongful convictions (55.5%), mistaken eyewitness identification was the second (36.5%), misinterpreted or falsified forensic physical evidence was the third (22.1%), and false confessions were the fourth (12.2%); however, modern DNA testing was responsible for 316 exonerations (23.2%) since 1989. Of the 316 DNA exonerations, 134 cases (42.4%) involved falsified or misinterpreted evidence in the wrongful convictions, while nearly all cases (96.5%) involved erroneous eyewitness evidence.

These results show that the policies regarding handling of eyewitness evidence needs the most modification. If new policies and procedures are established for handling eyewitness and physical evidence, then the likelihood of erroneous evidence leading to wrongful convictions will be reduced. More research must be done to conclude exactly how many wrongful convictions and exonerations were a result of one particular form of evidence over the other.

DNA, Eyewitness, Physical Evidence



E15 Effective Use of the Multidisciplinary Approach Is Critical to Solving Contemporary Violent Crime

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After attending this presentation, attendees will better appreciate the necessity of employing a multidisciplinary approach in violent crime investigation.

This presentation will impact the forensic science community by demonstrating that the complexity, motivations for, and high-level education-based execution of violent crimes necessitate effectively using a wide spectrum of disciplines within the forensic community.

The “Forensic Family” came together as part of task force and was responsible for solving several cold case murders and rapes.

The explosion of forensic crime dramas, true-life documentaries, and the parallel increase in available academic forensic education has forever impacted the art of criminal investigation. Savvy criminals are taught in a wide variety of areas how to circumvent traditional crime fighting strategies, thwart traditional crime scene processing techniques, and mask or obliterate various types of physical evidence traditionally associated with interpersonal violent crimes. The killer highlighted in this presentation owned multiple text books on trace evidence.

Over the course of more than 60 years of criminal investigative experience, a dramatic shift in the time and effort spent by perpetrators in staging or simply altering crime scenes, in an effort to avoid forensic detection, in even the most emotional and anger-fueled violent crimes has been seen. This study will present a case which illustrates how knowledge of forensic science affected the planning and execution of a murder, and how leveraging the multidisciplinary approach in processing the crime scene, incorporating the forensic pathology findings, and combining the results with traditional stalwart investigative methods resulted in identification of the perpetrator.

The illustrated case involved the murder of a single female soldier (mother of two children) in her government quarters where she was beaten, stabbed once, hit in the head with a hammer, manually strangled, and finally asphyxiated with a ligature. After seven months, a task force was formed and multiple members of the “Forensic Family” participated in the resolution of this investigation. The medical examiner’s office provided pathological assessment; the scene itself was assessed to include all blood patterns that allowed a sequencing of events; a panty hose wrapper was collected and analyzed which revealed a latent footwear impression that ultimately matched the footwear of the subject; skilled interviewing and cognitive interviewing techniques were used; and criminal analysis of method of operation resulted in the subject being identified in a triple murder, along with multiple break-ins, and rapes over a multi-year period along the east coast.

Ultimately, the subject was convicted and received a life sentence in prison. He was subsequently convicted of a triple murder and received the death sentence. The subject was executed in 2005 after all appeals were exhausted.

Multidisciplinary, Forensics, Violent Crimes



E16 Multidisciplinary Approach to a Staged Sexual Assault

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After attending this presentation, attendees will better appreciate the value of a multidisciplinary approach to investigations, including various principles of sex assault crime scene investigation, identification of key evidence, a diverse spectrum of laboratory examinations, and expert analysis of staging elements.

This presentation will impact the forensic science community by demonstrating how positive results were achieved through cooperation and teamwork among the entire forensic family involved in the case. No matter how far-flung the incidents, how remote the crime scenes, or how complex the scenario, investigators should coordinate efforts and approach the case from several angles for the highest chance of success.

This a case study demonstrates how an interdisciplinary approach to the investigation resolved a unique example of a complex staged sexual assault. Proper crime scene processing and collection of evidence allowed investigators, laboratory technicians, crime analysts, and subject matter experts to properly evaluate what happened, recognize how the subject of the investigation went about staging multiple scenes, and ultimately secure a confession and subsequent conviction of the perpetrator.

In this case, the victim, a soldier deployed to Afghanistan, reported she was assaulted by two men intent on raping her; however, she was able to fight them off. The victim was eventually returned from the combat zone to Georgia, where she then reported receiving two threatening notes, apparently from the suspects involved in the initial assault in Afghanistan. The victim was then transferred to Hawaii for her safety, but within two weeks of her arrival, she reported a blitz assault in her barracks room, where she was assaulted, tied up, and raped with a knife handle. In the last instance, the victim also claimed that the offender had written threatening comments on her body. Complicating the issue was the fact the victim positively identified a suspect during a lineup based on information from the initial incident. Investigation combined with several forensic examinations established all the reports were false. Once confronted with overwhelming forensic evidence, the victim confessed to falsifying each incident to facilitate a final transfer to a military base near her family.

This case study illustrates how a multidisciplinary approach provided investigators with the tools they needed to successfully resolve the case through proper application of sex assault crime scene investigation, analysis of the key pieces of evidence, appropriate use of a wide variety of laboratory disciplines, and incorporation of expert analysis.

Crime Scene, Sex Assault, Investigation



E17 Do We Have the Right Guy? Connecting One Suspect to Two Brutal Attempted Sexual Assaults in Neighboring Police Jurisdictions

Matthew C. Wietbrock, BS, 629 N 6th Street, Lafayette, IN 47909*

After attending this presentation, attendees will learn the details surrounding two brutal attempted rapes which occurred on a cold night in November 2005 on the campus of Purdue University. The offender of these attacks was quickly apprehended and the subsequent investigation led to a criminal conviction.

This presentation will impact the forensic science community by detailing forensic tasks which occurred as part of the criminal investigation as well as investigative strategies which were employed. The techniques which the suspect used to conceal his identity will be presented to educate the attendees as to these methods. The results of forensic laboratory examination, which helped lead to conviction, and lessons learned at trial will also be discussed.

The details of this case began just before 9:00 p.m. on November 29, 2005, when the Purdue University Police Department received an emergency 911 call which reported a possible sexual assault in the parking lot of a university sorority. A basic description of the suspect was obtained, and officers responded to the scene, looking for a “skinny-framed,” White male who had fled from the scene in an unknown direction.

Approximately 90 minutes later and 1.5 miles away, a second attack was reported, this time to the West Lafayette Police Department, as it occurred within their jurisdiction. Although the manner of the reported attack seemed similar, the description of the suspect was wildly different from the first attack. This description was that of a large, muscular Black male.

Shortly thereafter, a day-shift patrol sergeant, who had been working an overtime assignment, began patrolling the area between the attacks at his own initiative. He soon located an individual, matching the second suspect’s description, who appeared to be following a female who was walking down the sidewalk. Upon closer investigation, it was clear that the person had blood visible on his clothes and hands. He was then approached by officers and detained for further investigation. After a few initial denials, and a story about a bloody nose, he was taken into custody.

At the time of his detention, the suspect clearly resembled the description of the second attacker and immediate evidence as to his possible involvement in the first attack was yet to be provided by laboratory analysis.

The next night, another sexual assault was reported in the same area. This second night with a reported attack caused great alarm within and around the community. There was a question that had to be answered for investigators, more quickly than laboratory results could provide: Did they have the right suspect?

An unorthodox solution was thought of by a Purdue detective who made a call to a bloodhound handler with whom he had worked in the past. With the help of “Sam” the bloodhound and her trusty nose, investigators were able to connect, albeit circumstantially, the suspect of the first crime.

Later, the investigation proved that prior to the first attack, the suspect had fashioned a mask out of a tee-shirt to conceal his identity, and had even gone back to his residence to change clothes, prior to committing the second attempted rape.

This presentation will detail the forensic evidence which was collected and the eventual laboratory DNA results. Techniques utilized and lessons learned about the challenges of a complex investigation will also be discussed as well as the eventual sentence received by the suspect.

Attempted Rape, DNA, Bloodhound



E18 **Obscene Phone Calls: How to Consider This Hands-Off Offense**

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After attending this presentation, attendees will be informed about obscene phone callers and their victims, their different profiles and associated behaviors, and the risk and impact of this type of hands-off offense.

This presentation will impact the forensic science community by highlighting an offense that is always considered as a non-dangerous behavior.

Introduction: For more than two decades, sexual offenses have been extensively studied. From those studies, many categories and profiles have been developed. This study makes a notable difference between hands-off and hands-on sexual offenses, and one particular type of offense came to light during this study — obscene phone calls. Most of these calls contain sexual words or are driven by sexual fantasies. One of the characteristics of this offense is that it impacts random victims, sometimes in the offender's professional area. In addition, this type of offense contains a wide range of behavior, from silent calls to the most pornographic and vulgar speeches.

Methods and Results: A literature review was conducted to analyze existing knowledge regarding the matter, particularly in terms of understanding this behavior. Unfortunately, this topic does not seem to have been something of interest as less than 20 scientific papers, over a period of more than 40 years, were collected.

Discussion: The apparent low level of interest of this offense seems to be in relation to the fact that obscene phone call are often disregarded and not considered as psychological violence; however, these offenders truly suffer from a mental condition. This presentation will describe the offenders' and victims' profiles and the psychopathological theories underlying this behavior. Prevention of re-offense by leading the callers to a therapeutic program seems to be hard to develop, since the victims often do not report the calls to the police. Raising public awareness of the reality of this behavior could help to protect both the victims and the offenders.

Obscene Phone Call, Hands-Off Offense, Phone Scatologia



E19 A Ten-Year Study of Suicides From a Rural/Suburban County

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The goal of this presentation is to share trends in suicide data compiled over ten years in Dutchess County, NY.

This presentation will impact the forensic science community by providing suicide data compiled in a rural/suburban setting for reference and comparison by medicolegal investigators and pathologists.

This data was based on reporting and examination standards recommended by the National Association of Medical Examiners and Department of Justice death investigation standards. Specifically, this study looks at suicide data from 2003 to 2013 collected from autopsies and external examinations reported to the Dutchess County Medical Examiner's Office. Almost 300 cases were analyzed using Statistical Package for the Social Sciences (SPSS) 20.0 in terms of the deceased's gender, age, race, presence of a suicide note, method of suicide, and weapon used. More than $\frac{3}{4}$ of the cases were male and less than $\frac{1}{4}$ were female, agreeing with the 2011 reporting of the Centers for Disease Control and Prevention (CDC) which states that 78% of all suicides are male.¹ The large majority of the deceased were White, with Blacks and Hispanics at less than 6% each. There was a wide variety of ages ranging from 15-94 years of age, with an average age of 49.29, and a standard deviation of 18.11. The largest age group was 46-55 years of age, with just over $\frac{1}{4}$ falling into this age bracket. This also matched other studies that indicate the highest rate of suicide is in the 45-54 years-of-age group.^{1,2} Only four cases were juveniles. There was no significant difference in the average age for men and for women.

Research shows quite a discrepancy in the percentage of suicides in which a note is found. Eckert and Helpert state about $\frac{1}{3}$ of victims left a note; in contrast, a study by Tewksbury, Suresh, and Holmes found 26.3% of males left a note, and a study by Shaw et al. that only looked at adolescents, found that 39% left a note.³⁻⁵ Furthermore, while there is much research about the content of notes, little more has been said about the presence of notes. In this sample, just over 40% of the cases left a suicide note, but there was no significant difference in the presence of a note for men or women, for different races, or for different age groups. Most common methods of death were gunshot wounds, hanging or asphyxia, and overdose, which is consistent with CDC data and other studies.^{1,4,6,7} The remaining methods include carbon monoxide or other poisoning, blunt force trauma, sharp force, drowning, or self immolation. Numerous studies cite that men are more likely to commit suicide by violent means, such as gunshots, whereas women are more likely to commit suicide by poison.^{2,7,8} Results from this study agree with other reports showing significant differences for method of death both in terms of age and gender, with men more likely to choose gunshot and women more likely to choose overdose. Furthermore, the older the deceased was, the more likely they were to commit suicide by gunshot, and the younger they were, the more likely they were to commit suicide by overdose. Of note, all four juveniles committed suicide by hanging. Statistical analysis could not be performed for race and method of suicide due to small numbers of other races, but there were interesting trends that will be discussed.

Lastly, the weapon used for method of death will be discussed as time permits. Specifically, the type of gun used, classified as handgun, shotgun, or rifle; materials used for hanging, which include ropes, cords, belts, sheets, and shoelaces; different gases used for poisoning; and weapons used in blunt force trauma cases.



General Section - 2015

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Suicide, Suicide Note, Suicide Method



E20 Investigating Elder Deaths

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After attending this presentation, attendees will better understand the signs and symptoms of elder maltreatment and the need to perform complete and thorough medicolegal death investigations in these cases, including scene investigations, ancillary studies such as toxicology screening, review of medical records, autopsy, and imaging when warranted in order to properly determine the cause and manner of death. Elder Death Review Teams (EDRT) provide interdisciplinary review of elder deaths in an attempt to develop prevention strategies to decrease the incidence of elder abuse.

This presentation will impact the forensic science community by emphasizing the need for comprehensive medicolegal death investigations in order to differentiate elder maltreatment from deaths due to natural disease of elders aged 60 years and older in suspicious deaths and to conduct interdisciplinary EDRT in an attempt to improve outcomes for this population.

The population of elders aged 60 years and over is projected to double in size and comprise 20% of the total United States population by 2050.¹ As this population grows, the maltreatment of elders is an increasing and disturbing problem worldwide. Research suggests that at least one in ten elders experiences some type of abuse but only one in 23 cases will be reported, according to the National Center on Elder Abuse.^{2,3}

Elders are a vulnerable population due to their pathophysiology and lack of overall healthcare understanding. Conditions other than natural disease processes, such as hip fractures, subdural hematomas, sepsis, untreated decubitus ulcers, and malnourishment, may hasten their death. The medicolegal community must ensure that thorough investigations are completed to separate normal and expected findings from abuse and neglect.

Only two states, Arkansas and Missouri, have laws that mandate reporting of elder deaths that occur in a residential care facility, assisted living facility, intermediate care facility, or skilled nursing facility to the medical examiner/coroner regardless of whether or not the death was due to natural causes.^{4,5} Furthermore, only 33 states or United States territories require the medical examiner/coroner or persons who perform the duties of the medical examiner/coroner to report cases of suspected abuse, leaving 31 states or territories without mandatory reporting requirements.⁶ In order to address epidemiological studies, consistent reporting is necessary.

It is paramount for individuals who investigate and document elder deaths to be properly trained in order to recognize possible signs of maltreatment. A systematic approach, beginning with the death and scene investigation, followed by a complete autopsy, concluding with an interdisciplinary elder death review ensures proper classification of cause and manner of death. This methodical approach also allows for the development of prevention measures to decrease the incidence of future cases.

Deaths of elders should not automatically be assumed to result from natural disease. Accidents, suicides, and homicides can and do occur in the elder population. The development of a comprehensive standardized reporting form to collect appropriate information will assist in differentiating these cases and will be discussed.

The Georgia Department of Human Services Division of Aging and the Fulton County Medical Examiner's Office collaborated to develop a database documenting demographic and risk factors for elder abuse, neglect, and exploitation cases.⁷ The team reviews cases weekly, notifying the medical examiner/coroner of any suspected maltreatment, initiating further investigation which may not have been pursued otherwise. The project will be discussed in detail.

Premature deaths of elders should be investigated as seriously as those of children and younger adults. This outlook will decrease the incidence of maltreatment occurring in a population that continues to grow as individuals live longer.



General Section - 2015

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Elder Maltreatment, Geriatric, Scene Investigation



E21 **Bleeding Out: A Case of Mistaken Homicide**

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After attending this presentation, attendees will understand the importance of a complete and unbiased investigation when it comes to death investigations.

This presentation will impact the forensic science community by contributing to the understanding of the laws pertaining to assisted suicide and potential problems in determining the legal next-of-kin for disposition of remains.

Harris County has a population of more than four million people with approximately 4,000 deaths being reported to the medical examiner's office each year. In many cases, the cause of death is consistent with the witness statements and preliminary death scene investigation; however, sometimes the witness statements and preliminary law enforcement investigations are not consistent and a more in-depth death scene investigation is required by trained medicolegal death investigators. It is important for the medicolegal death investigator to approach all scene investigations without bias or predisposition as to cause of death.

With an evolving population, emerging technology, and the increase of crime scene-related television shows, death investigations are becoming more complicated. Not all crime scenes are what they appear to be. This presentation will review a case of mistaken homicide which was due to false/inconsistent witness statements. Law enforcement arrived on scene to find a large amount of blood throughout the decedent's residence. The decedent's 14-year-old grandson was reportedly in the residence with the decedent. Per the law enforcement report, the grandson reported that he stabbed the decedent two times. At first glance, the scene investigation was consistent with foul play due to the amount and location of blood and evidence including a bloody knife and a bleached footprint on the carpet. Trace evidence was collected on scene. During the medicolegal death investigation of the decedent's body, it was found that what had first appeared to be a homicide was now looking to be something very different. Photographs of the scene and autopsy will be used to show the evolution of the investigation, from the initially suspicious scene findings to the autopsy determination of the cause and manner of death.

This presentation will demonstrate the importance of a complete and accurate death scene investigation. The presentation will illustrate the importance of gathering accurate information and seeking further information when inconsistencies and questions arise during the initial scene investigation. Pathologists who perform the autopsies rely partly on a clear report from the medicolegal death investigator of the circumstances and observations of the death scene. Attendees will enhance their knowledge of death scene investigations and the need for clarity in death scene reports, despite inconsistencies that may arise.

Death Investigation, Homicide, Inconsistent Findings



E22 The “Spaghetti Bullet”: Difficulties Inherent in the Medicolegal Investigation of Deaths Caused by Non-Standard Ammunition

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After attending this presentation, attendees will understand the importance of forensic investigators and medical examiners being familiar with non-standard ammunition and firearms that they may encounter in medicolegal death investigations.

This presentation will impact the forensic science community by familiarizing forensic scientists and investigators with the importance of the multidisciplinary investigation in the medicolegal evaluation of deaths associated with firearms, particularly those involving atypical ammunition and/or weapons.

A wide variety of firearms ammunition is commercially available; however, many types of the ammunition are no longer being manufactured but can still be found in private collections and stockpiles. Knowledge of the composition and morphology of non-standard ammunition is essential for forensic investigators and pathologists, since these rounds are sometimes encountered in medicolegal death investigations. Similar to home-made ammunition, the interpretation of the morphology of the wounds caused by such rounds as well as the radiologic findings can be difficult for unfamiliar examiners. The following case illustrates these potential difficulties.

A 62-year-old man who was staying at the home of a friend’s parents telephoned his ex-wife, with whom he had a contentious relationship, and informed her that he planned to kill himself. She then heard what she thought were two gunshots and the phone line went dead. She called the local sheriff’s office and a deputy responded to the residence where the ex-spouse was staying. The residence was locked, but the deputy obtained a key from a neighbor and found the man deceased on the garage floor with a pistol in his hand and a pool of blood around his head. Three suicide notes were found in envelopes on the kitchen counter. The decedent was a former police chief in another jurisdiction and had been a police academy instructor.

Examination at the medical examiner’s office revealed an intra-oral gunshot wound involving the posterior hard palate. No exit wound was found. Radiographs of the head showed the presence of two unusual radio-opaque densities over the cranial vault, in addition to multiple skull fractures. The recovered projectile, which had produced an incomplete exit perforation in the posterior midline of the skull, consisted of a gray metal structure that resembled a tangled ball of spaghetti. The deformed, copper-colored jacket of a medium caliber bullet was also recovered. The postmortem examination was otherwise remarkable only for severe coronary arteriosclerosis and postmortem toxicological studies were negative. The cause of death was certified as “gunshot wound of head” and the manner of death was suicide. Because of the unusual nature of the projectile, consultation was sought from several firearms experts. It was eventually identified as a round introduced in the 1990s as non-toxic, lead-free ammunition for use by law enforcement. This round, which consisted of a woven, multi-stranded, jacketed, frangible zinc bullet, was subsequently found not to meet Federal Law Enforcement Training Centers (FLETC) standards and its production was discontinued.

This case demonstrates the difficulties that may ensue in the medicolegal evaluation of firearm deaths when atypical ammunition, including outdated and home-made rounds, as well as unusual firearms such as zip guns and nail guns, are used and highlights the importance of the multidisciplinary investigation.

Firearms Deaths, Atypical Ammunition, Medicolegal Investigation



E23 Blunt Force and Fire — Four Victims and the One Who Got Away: Case Study of a Serial Murderer

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After attending this presentation, attendees will better appreciate the efficacy of collaborative working relationships between the various professional disciplines involved in criminal investigations.

This presentation will impact the forensic science community by exposing participants to some of the key steps that were utilized in solving a difficult serial murder investigation.

In the early morning hours of August 1, 1995, a man walking on the railroad tracks in the Allapattah produce district of Miami discovered the body of Vida Hicks. There was trauma to the victim's head and she was partially burned. It was not readily apparent if she was the victim of an accident or homicide. Nine weeks later, the body of Diane Nelms was discovered approximately 20 feet away at the same location. She had also sustained blunt trauma to the head and was burned postmortem. The similarities between the two cases were striking. The police presence in the area was immediately intensified and the business owners reacted by removing all foliage and debris to prevent cover for further attacks. This tactic effectively worked for the produce district but moved the offender a mile away. Cheryl Ray was attacked in a cemetery across from Biscayne Bay approximately three months later. Like the first two victims, she was bludgeoned, doused with an accelerant, and wooden matches were used to start her clothing on fire. A witness came forward who reported seeing Ms. Ray sitting on a bus bench outside the cemetery. She was talking with an African American male who had his hair styled in long dreadlocks. The witness assisted a police artist with a composite of the suspect. The intensity of the investigation increased significantly along with the area of patrol. Two months later, the offender struck again. Janice Cox was attacked inside an abandoned gas station west of the previously targeted areas in a very similar manner. The indoor scene provided a distinctive boot print that had not been available in the three previous outdoor scenes.

With the attacks occurring over an eight-month period of time, law enforcement strongly suspected that the offender was local to the area. The victims were all similar in age and appearance. The perpetrator most likely brought a weapon to the scene along with an accelerant and matches. Investigators suspected that the purpose of the fire was for some reason other than to destroy evidence and the offender apparently did not remain at the scenes to watch the fire. A task force comprised of law enforcement officers from the Miami Police, Florida Department of Law Enforcement, and the Federal Bureau of Investigation flooded the area in an effort to prevent further attacks, develop new leads, and hunt for the perpetrator. The investigation seemingly turned a corner when a woman they interviewed reported that she had previously fled from a man who attacked her and struck her on the head — before the murders began.

The investigation was successfully concluded due to the synergy created by the collaboration of the law enforcement and forensic community.

Serial Murder, Case Linkage, Interrogation



E24 Case Study: How the Murder of a 4-Year-Old Girl Changed the System

Carrie Costello, BA, 6329 Munsee Drive, West Lafayette, IN 47906*

After attending this presentation, attendees will have an understanding of how, after police found a four-year-old girl dead in her home from a head injury and blunt force trauma and learned that she had been tortured at the hands of her stepmother for at least six months prior to her death while her biological father did nothing to stop the abuse, multiple changes occurred within the Child Protective Services Department, Law Enforcement response, and resulted in new legislation.

This presentation will impact the forensic science community by not only examining the crime scene itself, but by also showing how an abusive stepmother intimidated police, child case workers, court-appointed advocates, family members, and neighbors into not recognizing and/or ignoring the horrific torture her 4-year-old stepdaughter endured. In addition, this presentation will show how the traditional gender roles in domestic violence cases can change, with the female (wife) being the abuser while the male (husband) takes on a more traditional victim role, resulting in his failure to protect his biological daughter. Finally, it will discuss how a sheriff's deputy lied about a well-being check on the victim, resulting in the deputy pleading guilty to charges of perjury.

On the morning of March 16, 2005, shortly after 6:00 a.m., a 911 call came into the Tippecanoe County Sheriff's Department reporting that a child had been choking and was possibly dead. Through the sheriff's and the coroner's investigation, it was learned that the night prior to the 911 call, the victim had her hands bound and her mouth duct taped shut. The torture this child endured included, but was not limited to, being beaten with a broken wooden cutting board, zip-tied to a highchair, forced to sleep on plywood in a make-shift room in the unheated garage, tied to a plastic playpen, and forced to eat a type of gruel her stepmother concocted, all in the name of discipline to correct unwanted behavior. The victim also had to endure cold baths, as she was secured to the faucet as punishment for wetting her pants and bed.

This presentation demonstrates how a happy, healthy, go-lucky child who thrived in a good environment turned into a child who rarely laughed and played, causing a learned-helplessness behavioral response.

There were multiple contacts with various agencies and missed opportunities for the system to protect this 4-year-old murder victim. From the age of two, the child was appointed a ward of the court, assigned a Court-Appointed Child Advocate and a Child Protective Services (CPS) case worker. The victim's biological mother was addicted to prescription drugs and had five child neglect cases against her. There are over 1,000 pages of court records related to the victim's status as a child in need of services. The victim's father had a previous conviction of battering a 13-year-old boy.

In the five years prior to her death, there were more than 40 calls/complaints to police about the stepmother's abusive behavior, including her alleged attempt to run over a neighborhood child with her vehicle. The maternal grandparents, who had temporary custody of the victim, obtained a lawyer and filed for visitation rights due to the victim's stepmother's refusal to allow the victim to interact with her extended family. This case dragged on and never received a hearing in court. Finally, court records show that one complaint filed with CPS was not investigated because, a case manager, noted "inappropriate discipline does not meet legal sufficiency for CPS to investigate."

The end result was how all of these different agencies came together to support the prosecution of the stepmother for murder and neglect and the father for neglect.

Homicide, Child Abuse/Torture, Domestic Violence



E25 Homicide Investigation and Cold Cases: Why Aren't We Clearing More Cases?

James M. Adcock, PhD, 175 Braeburn Road, East Longmeadow, MA 01028*

After attending this presentation, attendees will be informed concerning the improvement of the low resolution rate (clearances) of homicides and cold cases.

This presentation will impact the forensic science community by providing suggestions on how clearances of homicides can be improved.

Over the past decades, there has been a significant change in the numbers related to homicides in the United States with what appears to be little progress over the past 20 years. In 1993, nearly 25,000 murders were recorded by the Uniform Crime Reports (UCR) program with a clearance of about 67%. In 2012, 13,092 homicides were reported with a clearance of 62.5%. In 2010, ScrippsNews reported that from 1980 to 2008 the United States had accumulated nearly 185,000 unsolved murders. Based on the present known clearances of reported homicides through 2012, coupled with the thousands of unidentified bodies where manner-of-death is still undetermined, there are probably in excess of 200,000 unresolved murders.

It is known that physical evidence, especially DNA, is helpful in identifying perpetrators and exonerating the innocent. But the process is limited as DNA only resolves about 27%-30% of all the cases, therefore begging the question, what about the other 70%? Experience has shown not all homicides are solvable and some are more difficult than others even when the right protocols are followed. In the meantime, political leaders have allocated hundreds of thousands of dollars through the National Institute of Justice (NIJ) for items like the NIJ-sponsored DNA Cold Case Grants.

While many have benefitted from these funds, this grant limited recipients to only evidence that contained DNA potential with no consideration or even the potential use of other personal identifying evidence being found, such as latent prints. If it did not have DNA potential, then one was not allowed to use these funds for their cases. Furthermore, looking at the cold case research conducted for the NIJ, it was found that most of these grant-receiving agencies probably would not have even looked at their unresolved homicides or rapes had it not been for the availability of outside funds. Then, following the grant, many of the cold case units fell apart with few remaining, most likely due to the lack of adequate funding and support from management. So, the question is: how much is someone's life worth?

Sadly, as the literature regarding the clearances of homicides is reviewed, a plethora of articles are found, from sociological journals to criminal justice journals and others such as homicide studies. While these all have value, they do very little toward providing police supervisors or detectives with information that will help them increase their homicide clearances. Do not misunderstand — some authors from these journals have actually described specific measures that police can take that will help them resolve more cases; however, after valid research was published in 1999, there has not been any improvement in the percentage of clearances, even with a 47% drop in the number of reported homicides. It is highly likely that the same holds true of the publications by the other authors as well. Examples will be provided to illustrate the issues at hand and introduce possible solutions.

Homicides, Cold Cases, Clearances

E26 **Hyperspectral Remote Sensing: Detection of an Experimental Mass Grave Over Time and at Different Scales in a Temperate Environment**

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After attending this presentation, attendees will have a better understanding of hyperspectral remote sensing applications in clandestine mass grave detection at different spatial scales and of its limitations in a temperate ecosystem.

This presentation will impact the forensic science community by offering insights on the temporal changes in spectral reflectance of an experimental mass gravesite and will illustrate the utility of these results for the detection of deep clandestine gravesites to narrow down search areas.

Worldwide events such as war crimes and human rights abuses can result in the death of people who have historically been buried in mass graves. Common methods used for gravesite location, such as witness testimony, geophysical resistivity, magnetometry, or ground penetrating radar, can be time consuming and cover small geographical areas. Novel advances using remotely sensed data and techniques to detect changes in both soils and vegetation characteristics due to cadaver decomposition provide an alternative tool to detect buried remains.¹⁻³

The goal of this study is to determine, through the integration of field data, spectrometry, and airborne hyperspectral imagery, how an experimental mass gravesite's surface reflectance changes over time. The study consisted of the establishment of three experiment study sites, at a depth of approximately one meter, in June 2013: one experimental mass gravesite containing 20 pig carcasses (*Sus scrofa*) and disturbed soil; one reference containing only disturbed soil; and one undisturbed control site. To investigate the changes in the spectral reflectance of the experimental mass gravesite and its distinction from the non-gravesites, hyperspectral data was collected at different spatial scales over two growing seasons. Airborne hyperspectral imagery covering the Visible Near Infrared (VNIR) range (376nm to 1,048nm) and the near to shortwave infrared wavelength range (870nm to 2,500nm), was collected over the research area. A full-range spectroradiometer, (350nm to 2,500nm) was used to collect vegetation spectra in the field. In addition, leaf pigmentation (chlorophyll and carotenoids), and soil chemistry (e.g., C, N, Ca, Mg, Na) are used to inform the spectral analyses to differentiate between the gravesite and non-gravesite (disturbance and control).

Preliminary results show that burial depth and site age play an important role in the detectability of a mass gravesite in terms of changes in soil and vegetation. In the early stages of the study, differences in spectral signatures between the gravesite and the non-gravesite are a result of the overall disturbance effects rather than attributed to changes in soil and vegetation characteristics due to cadaver decomposition.

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Hyperspectral Remote Sensing, Cadaver Detection, Mass Grave



E27 Debunking Three Myths About Rape Victims' Responses to Their Attacks

Mary Carr, MD*, Regions Hospital, 640 Jackson Street, MS 11102F, Saint Paul, MN 55101

After attending this presentation, attendees will learn that, contrary to endorsed beliefs, victims of rape do not necessarily immediately report the assault, suffer severe physical or anogenital injury, or forcefully resist their attacker during the assault.

This presentation will impact the forensic science community by providing supporting evidence, obtained from a retrospective study conducted at a Sexual Assault Nurse Examiner (SANE) program, that following a self-report of sexual assault, victims will seek medical care over a wide variation of time following the assault, rarely suffer moderate or severe physical or anogenital injury, and commonly do not resist their attacker throughout the assault.

Stereotypical and prejudicial misconceptions regarding sexual assault and victim responses to this type of violence continue to exist. These misconceptions are collectively referred to as rape myths. Three existing rape myths are that sexual assault victims: (1) immediately report the crime; (2) suffer severe physical and/or anogenital injuries; and, (3) forcefully resist their assailant. Failure by the lay public to understand the variability of victim response during and following a sexual assault can result in victims blaming themselves and lack of support provided to victims. In the courtroom, it can result in jurors misunderstanding that the victim did not consent and may result in not guilty verdicts for criminals. Having an expert witness provide testimony to the mythological nature of some long-held beliefs allows jurors to evaluate victim behaviors they otherwise find incomprehensible or counterintuitive.

A retrospective cohort study examining the time to seek medical care following the incident, presence of physical or anogenital injury, and level of physical resistance during the assault was conducted at Regions Hospital in St. Paul, MN, in 2011 and 2012. Study subjects were female sexual assault victims undergoing examination by a SANE.

SANE reports for 317 subjects met the inclusion criteria and were reviewed. Twelve (4%) victims experienced physical injury requiring medical intervention. A total of 34 (11%) sustained anogenital injuries requiring medical intervention. Overall, 253 (81%) victims did not actively resist at some point during the assault, with 178 (57%) victims never actively resisting. Nearly half (129; 43%) did not appear in the emergency department for 12 or more hours from the time of the assault.

It can be concluded from this study that female victims reporting a sexual assault seek emergency department assistance after the sexual assault in a variable amount of time, rarely suffer moderate or severe physical or anogenital injury, and commonly do not resist their attacker throughout the assault.

This study helps refute three rape myths which previously may have contributed to further victimizing the victim.

Rape Myth, Delayed Reporting, Injuries



E28 **Murder or Accidental Drowning in a Bathtub? Case Studies of Drowning and Non-Aquatic Homicides Staged as Bathtub Drowning Accidents and Suicides**

Andrea Zaferes, BA, PO Box 601, Shokan, NY 12481; and Mary E.S. Case, MD*, 6059 N Hanley Road, St. Louis, MO 63134*

After attending this presentation, attendees will be able to better recognize signs of foul play specific to several different types of pediatric and adult homicides committed either by drowning or by non-aquatic means that are then staged as bathtub drowning accidents or suicides.

This presentation will impact the forensic science community by providing an increased awareness of these types of Bodies-Found-In-Bathtub (BFIB) homicides with the goal of facilitating early recognition of red flags, more effective investigations, and successful, justified prosecutions.

Seven BFIB homicide cases will be reviewed. Initially, almost all of these cases were treated as drowning accidents until significant red flags of foul play were recognized, sometimes years later, causing the cases to be re-investigated with subsequent homicide conviction results. Each of these cases provides lessons learned that can be applied to all BFIB death investigations and prosecutions.

Homicides staged as drowning accidents in bathtubs present a number of challenges with the main challenge being a lack of specialty training. Law enforcement and medicolegal investigators investigating motor vehicle, fire, and airplane crash fatalities are usually assisted by crash reconstructionists, fire investigators, and Federal Aviation Administration/National Transportation Safety Board (FAA/NTSB) investigators, respectively, who typically have 40-1,000 hours of specialty training. When investigating aquatic fatalities, law enforcement and death investigators are rarely given similar assistance by aquatic death specialists and are at a further disadvantage by having little to no aquatic death and homicidal drowning investigation training.

The case studies presented represent several different types of BFIB homicides of which the forensic community needs to be made aware.

Two cases involved children. In one case, a 4-year-old male victim of premeditated homicide by his father was suffocated with Freon® with the scene staged as an accidental slip-and-fall bathtub drowning. The second case involved a 3-year-old male who was staged as a choking death on land after being drowned by his foster mother when dunking punishment went too far.

Five cases involved adult women murdered by their husbands or other family members. In the first case, a 45-year-old woman was assaulted and strangled in her bathroom, then deposited in her hot tub to stage an accidental drowning due to alcohol intoxication. This case remained diagnosed as a drowning accident until the offender confessed to family members years later.

A 46-year-old woman was drowned by her husband in a hot tub with subsequent accidental slip-and-fall staging. The first trial ended with a hung jury. A subsequent investigation was conducted, including a reconstruction that was necessary to support the statement of the neighbor who witnessed the assault but who didn't understand what she was observing. The second trial resulted in a homicide conviction.

Two cases involved elderly women murdered by suspected family members for monetary gain and staged to look like falls in bathtubs. One of the cases was an 87-year-old woman found in the bathtub filled with water in a secured condo with her face under the water. Autopsy revealed she had been strangled and beaten. The second case was a 74-year-old woman found in a bathtub with no water with extensive trauma to her posterior head and cervical spine and chest trauma. A soap dish on the wall was broken and fragments of that dish were found in her head wound. The trauma to the scalp was seven lacerations which certainly exceeded what would be expected in a simple fall. The cause of death was fracture of the C1 caused by the impacts to the head.

The remaining cases were of women drowned by their husbands with additional useful lessons learned regarding scene investigations and autopsy findings.

Drowning, Bathtub, Homicide



E29 Conservation Genetics of Bioko Monkeys

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After attending this presentation, attendees will understand how genotypic information from three endangered primate species can be used to further develop forensic wildlife molecular methods.

This presentation will impact the forensic science community by demonstrating how the use of genetic information can be used to assess the genetic health of three endangered primate species. More importantly, it will illustrate how this information can be used by forensic biologists to develop testing methods that can be used to deter crimes against wildlife.

Wildlife forensics deals with the legal and environmental aspects of crimes against animals. Newer research in this field is exploring the use of molecular approaches to forensic testing which can be used to deter crimes against wildlife. The focus of this research is three endangered primate species, *Procolobus pennantii pennantii* (red colobus), *Colobus satanas satanas* (black colobus), and *Mandrillus leucophaeus poensis* (African drill), from Biko Island. Biko Island is home to a multitude of unique species and is one of the world's top biodiversity hot spots. Many of the primate species on the island are endangered due to a thriving bushmeat market and habitat degradation. Despite legislative efforts and hunting bans making it illegal to kill these endangered species, the people on the island continue to do so with little to no consequence. While it is difficult to fine or prosecute individuals who hunt these creatures, the availability of genetic testing which can be implemented for forensic testing purposes, may deter hunters from preying on these protected species.

The goal of this research is to genotype the three endangered primate species mentioned above in an effort to determine genetic health of the populations for conservation efforts while evaluating loci for phylogeographic utility. No previous studies regarding the loss of heterozygosity of these primates have been published. Therefore, this research is desperately needed. Three classes of loci — traditional, bioinformatic and International Haplotype Map Project (HapMap) pairs — totaling 67 loci across the whole genome, have been identified as potentially valuable for genetic testing within these populations. This research focused on those loci located between chromosomes 1 and 2, of which there are 19 in total. Of the three species of interest, 49 individuals were genotyped using the three primer methods indicated above. Loci were scored as homozygous or heterozygous. Currently, four loci with heterozygosity ranging from 9% to 33% have been identified for use in determining the genetic health of these populations. Additionally, two of these loci are promising candidate markers for implementation in forensic conservation efforts.

Wildlife Forensics, Bioko Primates, Genome Sequencing



E30 Evaluating Methods for Removing Radioactive Contamination From Traditional Forensic Evidence: Moths

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After attending this presentation, attendees will understand some of the principles behind nuclear forensics and the need to decontaminate radioactively contaminated evidence.

This presentation will impact the forensic science community by demonstrating the potential of several solvents for removing radioactive contamination from traditional forensic evidence, specifically insects, without damaging the evidence.

Traditional forensic evidence, associated with interdicted nuclear material or an attack using a Radiological Dispersal Device (RDD or “dirty bomb”), may become contaminated by dispersible radioactive material. If so, such evidence must either be analyzed by a forensic laboratory capable of handling nuclear material or decontaminated prior to entering a traditional forensic science laboratory. There are few laboratories that are capable of handling dispersible radioactive material and, therefore, decontamination of the evidence with a method that does not destroy the evidentiary value would be preferred.

In 2009, Victoria, Australia, police found 300 grams of a uranium oxide compound in a storage property. After initial analysis by the Australian Science & Technology Organization (ANSTO), aliquots of the material were sent to Lawrence Livermore National Laboratory (LLNL) for further analysis. While aliquoting the sample for analysis, researchers at LLNL found the head and body of a moth. Analysis of the nuclear material indicated that it could not have originated within Australia. Entomological study of the moth could prove useful for understanding the history of the material from production to interdiction within Australia, a type of signature referred to as a “route attribution” signature in nuclear forensics; however, before the moth could be sent to an entomological laboratory, it would need to be decontaminated, a process that could well prove destructive.

To determine an effective and non-destructive method for decontamination of the evidence moth, exemplar moths were collected and contaminated with a uranium ore concentrate. Then, these contaminated moths were ultrasonicated in 11 solvent systems chosen for their potential decontamination properties. Mass difference was initially used to determine the efficacy of these solvents for decontamination, but Inductively Coupled Plasma/Mass Spectrometry (ICP/MS) will be used in future work to determine residual uranium levels.

Four of the solvents (5% Radiacwash™, 5% Decon® 90, acetone, and 1% nitric acid) provided promising results. They removed a significant mass of the uranium ore concentrate without extensive damage to the moth; however, using mass difference to determine the amount of uranium ore concentrate removed from each moth by the solvation proved to be imprecise and sometimes difficult to interpret. For example, mass loss was sometimes greater than expected because of incomplete initial desiccation of the moths followed by a more complete desiccation after decontamination. In addition, the loss of wing scales during solvation seems to be unavoidable in all solvent systems. The moths decontaminated with the promising solvents were ashed and will be analyzed for remaining uranium content by ICP/MS. Future work may include DNA analysis of the moths to determine if DNA can be cleanly extracted from radioactively contaminated evidence.

Radioactive, Decontamination, Insects



E31 Discrimination of Human and Animal Blood Traces Via Raman Spectroscopy

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After attending this presentation, attendees will become knowledgeable about how Raman spectroscopy coupled with chemometrics can be used to analyze, accurately identify, and discriminate between bloodstains from humans and 11 species of animals.

This presentation will impact the forensic science community by introducing a unique method for non-destructive bloodstain analysis and confirmatory identification, with a statistical level of confidence, which could potentially be performed at the crime scene.

The identification of a body fluid stain is an important and necessary aspect of many forensic investigations. Various presumptive tests are currently used for identifying a stain as blood; however, these tests are most commonly oxidation-reduction assays, which use hazardous chemicals and are destructive. Common confirmatory blood tests are microcrystal assays (e.g. Teichmann or Takayama assays). The Ouchterlony test, or similar immunochromatographic assays, can be employed to determine if the blood is non-human. It is ultimately preferable to confirm the presence of blood and the species of origin before forensic DNA profiling, but this can be practically problematic.

If a DNA profile is not extracted from the sample, then the suspected blood is usually presumed to be non-human and further characterization omitted. This is an erroneous testing scheme primarily because there is a lack of confidence that the sample is in fact blood, and of human origin, due to potential false positives. This approach could also be detrimental for crime labs since time and money would be wasted on non-human or non-blood samples. Since the amount of suspected blood evidence may be miniscule, it needs to be preserved and analyzed efficiently. Therefore, a non-destructive method to competently identify human blood would be highly valuable. Raman spectroscopy is a technique that has the potential as both a non-destructive confirmatory identification for blood and as a species of origin assay.

Raman spectroscopy has been proven as an effective and versatile analytical technique for a variety of forensic applications, including identification of lipsticks, drugs, explosives, paints, and fibers. Raman analysis often requires no sample preparation, is typically non-destructive, and has the ability to analyze microscopic amounts of sample. This technique is based on the detection of light that is inelastically scattered by a sample upon irradiation from a monochromatic light source. A Raman spectrum contains numerous distinctive bands which correspond to specific molecular vibrational modes. For blood in particular, Raman spectra provide rich detail and has been targeted in previous forensic studies. Popularity of Raman spectroscopy has been growing in forensic science especially due to reduced cost of instrumentation, including portable instruments, and its numerous possible applications.

This study has analyzed blood from twelve different species, including human. For forensic relevance, the animal (non-human) samples were selected to represent three groups: (1) animals that are bred for domestication (cat, dog, horse); (2) those consumed as food (chicken, cow, pig, rabbit); and, (3) those integrated with human existence (mouse, rat, opossum, raccoon). Analyzing a variety of animals' blood enhances the forensic practicality of the study and adds more certainty to model predictions. To account for the complexity of the dataset, Partial Least Squares Discriminant Analysis (PLSDA) classification models were utilized. The first PLSDA model differentiated between human and animal blood spectra in a binary fashion (human vs. animal).¹ The second PLSDA model was used to differentiate between human blood spectra and those from each species of animal surveyed.² To validate both models, classification predictions were made for a set of internal and external unknown samples. The constructed models exhibited a great ability to discriminate human from animal blood. This study demonstrated a comprehensive and robust method for analyzing a suspected bloodstain to identify human origin. The goal of the analytical approach is to be used for the rapid and non-destructive identification and characterization of a bloodstain at a crime scene.

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General Section - 2015

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Raman Spectroscopy, Blood, Human



E32 The Optimization of Spermatozoa Extraction and the Study of the Retention of Spermatozoa on Machine-Washed Clothing

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The goals of this presentation were to determine the variables/factors optimal for spermatozoa extraction and to determine if spermatozoa has the ability to retain on machine-washed clothing under various factors. The optimization of spermatozoa was performed while examining various factors including the time of extraction, the extraction solvent, and in-house vs. commercial stain. The retention of spermatozoa was examined under the variables of type of fabric, temperature of water, and detergent type.

This presentation will impact the forensic science community by discussing the preliminary results which suggest that not only is DNA able to retain on clothing through machine washing but also enough spermatozoa retains to obtain DNA profiles.

The presence of a suspect's seminal fluid on a victim's clothing often indicates the possibility of a sexual assault, particularly when the suspect and victim are not in close relation or association with each other. Many have speculated as to whether or not the detection of spermatozoa is either hindered and/or prohibited by machine washing; however, such areas are largely understudied. Previous work has failed to not only determine this possibility, but it also failed to address varying factors that may affect the retention of the spermatozoa.

The objective of this study was to determine if spermatozoa was, in fact, able to retain on clothing after machine washing under various factors such as the type of fabric and temperature of water (hot or cold). Furthermore, this study attempts to determine if the retained spermatozoa, if found, could produce a DNA profile adequate for analysis.

The seminal samples used in this study were collected from five donors and deposited via Pasteur pipets onto approximately 6"x6" swatches of five types of fabrics: cotton (underwear), denim, a sheet, a towel, and a cotton t-shirt. The transfer of spermatozoa onto the fabrics via pipet was performed as a laboratory control in order to eliminate uneven distribution as a factor of retention. Each of the five pre-stained fabrics were tested prior to machine washing for both Acid Phosphatase (AP) activity and fluorescence under the Alternative Light Source (ALS). All five fabrics tested positive for both AP and ALS. Additionally, an approximately 3x3mm cutting from each of the pre-stained samples underwent a two-hour, 1% Hydrochloric Acid (HCL) extraction, Christmas tree (Keruechtrot-Picroindigocarmine) staining, microscopy, and rating. The ratings ranged from 3+ to 4+ for all pre-stained samples. Each of the five pre-stained fabric samples were washed with a set size of pristine clothing for both the hot and cold wash. After washing, ten cuttings were taken from each fabric and viewed via microscopy. The cuttings were extracted under the same procedure as listed above, and examined via microscopy. Each of the fabrics were positive for spermatozoa in 100% of the cuttings taken. The sperm heads were counted for each fabric and ranged from 2-352 for the hot wash and 3-390 for the cold wash. During each of the washes, the washing machine, laundry detergent, cycle type, and size of load were kept constant to ensure no other factors were affecting the spermatozoa's retention. Also, a blank was run before each load to ensure no contamination from the machine itself. Finally, the cuttings were tested for DNA using a chelex extraction and Y-chromosomal Short Tandem Repeat (Y-STR) kit. The preliminary results suggest that not only is DNA able to retain on clothing through machine washing but also enough spermatozoa retains to obtain DNA profiles.

Retention, Spermatozoa, Washing



E33 Adding Value to Your Learning Event

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The goal of this presentation is to explain how to design an effective learning event through the implementation of the concepts of constructive alignment and Bloom's taxonomy.

This presentation will impact the forensic science community by creating awareness about the optimization of the process of teaching/training-learning forensic science. Although the content/example is given within the context of criminalistics at the professional level, the pedagogical and didactical concepts and methods are applicable to all levels and all fields.

Have you tried getting oranges from a fig tree? Would you use a trace elemental chemical profile to identify a substance as cocaine? Intuitively in daily work, activities are aligned with the expected products in mind. Forensic scientists constantly build and (re)validate knowledge and skills that could be applied to the respective fields in order to strengthen the justice system; however, when it comes to transferring that knowledge and skills, the goal is sometimes forgotten. It does not help the situation that the topics are incredibly interesting or that the interdisciplinary character of the profession is fascinating. As a matter of fact, the highly motivated learners and their perception of what they have actually learned may obscure the measuring of the learning results, which are used to calculate the Return On Investment (ROI). Effectively teaching forensic scientists has become a difficult task, especially when the relevancy and amount of knowledge is changing at a rapid rate. Instructors are expected to adapt their programs with a minimal time investment. In addition, they are expected to do so without sacrificing the content or the essential academic/professional skills that new practitioners need to have, such as scientific critical thinking and independent learning.

Traditional one-way (instructor-led) learning events as the only teaching modality has become ineffective with the new generations of learners, some accustomed to cutting corners and studying for the assessments. The instructor is expected to assume diverse roles such as organizer, planner, coach, and facilitator of the learning process. In other words, the role of the instructor as an effective instructional designer has become highly relevant. This is especially true when considering both the increasing scrutiny of the accreditation agencies responsible for the control of the quality of the educational curricula and the competences desired by the future employers.

Taking a closer look at how people learn and how this process can be optimized are necessary activities for all instructors, old or new. Although there are multiple teaching-learning theories available, the preferred ones agree that stimulating deep learning is an effective teaching practice. Constructive alignment is "a design for teaching calculated to encourage deep engagement."¹ As with other approaches to active learning, the essence of the constructive alignment theory is that the instructor/trainer doesn't have the central role in the knowledge transfer, but that the learner does. Integrating the Bloom's taxonomy of the cognitive domain in the design of a learning event becomes a necessity.² In other words, the cognitive skills desired as a consequence of the learning event have to be explicit. The alignment process starts by defining the desired results — they can be as high as strategic organizational results or a hypothetical assessment — and translating them to the operational learning outcomes according to Bloom's taxonomy for the cognitive domain. Next, the beginning situation is assessed and the learning activities are designed to "practice" the cognitive processes chosen for those learning outcomes.

This process will be exemplified with a curriculum for coordinators of forensic investigations within the Dutch police. This curriculum was the product of a team effort of the police academy, the Dutch police expertise center, the Netherlands Forensic Institute, and two universities. During this presentation, the process of designing, developing, implementing, and evaluating two modules of this curriculum, pertaining to criminalistics, will be discussed.

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Constructive Alignment, Bloom's Taxonomy, Education and Training



E34 Comparing Resolution of Analog vs. Digital Imaging Systems in Postmortem Applications

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After attending this presentation, attendees will have an understanding of the advantages and disadvantages of analog and digital imaging systems and the practicality of each for postmortem radiography, particularly when high-resolution images are necessary.

This presentation will impact the forensic community by identifying the imaging methods and modalities that maximize the resolution of radiographic images.

Radiography is often an integral part of the postmortem examination, and high quality radiographs are essential. Goals of postmortem radiography include screening for the presence or absence of bony trauma and foreign bodies such as bullets or knife blades, determining the presence of air emboli, identifying the decedent, and determining accidental vs. non-accidental trauma. In the past, film was the only available recording media for radiographic examinations and limitations of analog systems often made it difficult to acquire optimal images. Presently, several types of recording media are available for both medical and postmortem radiographic examinations. Analog systems, also known as film-screen radiography, consist of intensifying screens contained within a cassette and film as a recording media. A significant advantage of analog systems is their unmatched spatial resolution. Unfortunately, a number of disadvantages exist such as the inability to manipulate an analog image once it is taken and sub-optimal radiographs needing to be repeated. Analog radiography requires an automatic processor and darkroom to convert latent to manifest images and maintenance of the film processor and chemistry is essential for optimum image quality. Film cost and storage space are considerations, as are the inability to store, transmit, or view analog images electronically without first digitizing them.

In addition to analog, two types of digital imaging systems exist: Computed Radiography (CR) and Direct Digital Radiography (DR) systems. Digital recording systems, both CR and DR, provide high-quality images while overcoming many of the limitations of analog systems such as the inability to manipulate the image and the need for a darkroom, processor, and storage space. Unfortunately, many digital imaging systems are unable to achieve the resolution of their analog counterparts. This presentation compares the resolution of skeletal survey and specimen images of several infants radiographed using both analog and CR imaging systems. Each radiograph was evaluated for visibility of recorded detail by a board-certified radiologist and a pathologist. This presentation will weigh the advantages and disadvantages of analog and CR imaging systems, identify the imaging methods and modalities that optimize image resolution, and discuss the practicality of each type of imaging system for its use in postmortem radiography.

Forensic Radiography, Image Resolution, Digital Radiography



E35 Forensic Discrimination of Ballpoint Pen Inks on Documents Using LA-ICP/MS

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After attending this presentation, attendees will learn the differentiation of ballpoint pen inks (black and blue) written on documents through an Laser Ablation-Inductively Coupled Plasma/Mass Spectrometry (LA-ICP/MS) methodology.

This presentation will impact the forensic science community by describing how to achieve discrimination on a forged document.

The differentiation of ballpoint pen inks (black and blue) written on documents through an LA-ICP/MS methodology is proposed. Size A4 white office paper containing ink strokes from ballpoint pens of known origin were cut and measured without any sample preparation. In a first step, Magnesium (Mg), Calcium (Ca), and Strontium (Sr) were proposed as Internal Standards (IS) and used in order to normalize elemental intensities and subtract background signals from the paper. Then, specific criteria were designed and employed to identify target elements which resulted independent of the IS chosen in most of the cases and allowed a qualitative clustering of the samples. In a second step, a normalization data based on the targets previously identified was used to obtain mass independent intensities and perform pairwise comparisons by means of statistical analyses. This treatment improved the Discrimination Power (DP) and provided objective results, achieving a complete differentiation among different brands and a partial differentiation within pen inks from the same brands. The results show that 25 samples of black ballpoint pens and 14 samples of blue ball point pens, all available on the local market, were successfully discriminated and identified.

Ballpoint Pen Inks, LA-ICP/MS, Forensic Discrimination



E36 Comparing 6,000 Consecutively Fired .40 Smith & Wesson® Bullets and Cartridge Cases From a Sig Sauer® P320 Pistol Utilizing 3D Imaging and Objective Comparative Analysis

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After attending this presentation, attendees will be aware of the acquisition techniques used by two types of 3D instruments, the correlation procedures used to interpret the data collected from test-fired bullets and cartridge cases, the application of the results as it relates to firearms identification, variations occurring throughout the sequence, and interpretation of the variations.

This presentation will impact the forensic science community by establishing/developing an objective means to evaluate an identification and serve as a confirmation to the firearms/tool marks theory of identification that the extent of sufficient agreement of individual characteristics occurring in tool marks produced by the same tool exceeds that agreement which occurs in tool marks produced by different tools. The presentation will also inform the forensic community of applications of emerging technologies within comparative-based disciplines.

This study was conducted to determine the variation of individual characteristics on test-fired bullets and cartridge cases over the lifetime of a Sig Sauer® P320 .40 Smith & Wesson® semiautomatic pistol. The pistol was purchased new and had only been fired by the manufacturer for a standard function test prior to this study. The ammunition selected for this study contained brass-jacketed bullets and nickel cartridge cases with nickel primers.

A total of 6,000 cartridges were consecutively fired for this study over a period of two weeks. Of the 6,000 cartridges, 342 were collected for analysis. Cartridge sets of 1-10, 91-100, 491-500, and 991-1,000 were collected and inter-compared for each 1,000-cartridge interval. In addition to the multiple test fire sets, every 50th cartridge was collected. Cartridges that were collected for analysis were fired into a ballistic water tank. Prior to test firing, a screw was inserted into the hollow-point cavity to prevent bullet expansion upon entry into the water tank. Cartridges that were not collected for analysis were fired in an indoor firearms range, then the bullets and cartridge cases were disposed of. No parts of the pistol were cleaned until after cartridge 6,000 was fired.

The first test fire was used as the reference sample for comparative analysis against all of the subsequent test fires. Prior to the 3D image acquisition, the bullets and cartridge cases were laser etched with a unique identifier and cleaned with acetone. The instruments were subjected to daily performance checks. Images of land impressions on the bullets were acquired using confocal microscopy and analyzed with the application of a cross-correlation function. Images of breech face marks on the cartridge cases were acquired using photometric stereo and analyzed with the application of a bidirectional reflectance distribution function. Both the cross-correlation and bidirectional reflectance distribution functions provided objective numerical values representing the similarity between two samples topography. The numerical values were used to determine if there was a significant variation of individual characteristics over the sequence of test fires and whether or not the variations would prevent a result of identification from being rendered. The bullets and cartridge cases were also examined by several firearms/tool marks examiners to determine if they were still identifiable by traditional means. Photographs of the pistol's barrel and breech face were taken prior to firing and at every 1,000-round interval. These photographs serve as an additional indication of variation of individual characteristics over the sequence of test fires due to wear. The photographs also indicated the extent of buildup of brass, lead, and other residues over the lifetime of the pistol without cleaning.

Reproducibility of Marks, Confocal Microscopy, Photometric Stereo



E37 Accidental Trauma Mimicking Homicidal Violence

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After attending this presentation, participants will: (1) recognize that occasional deaths that initially appear to represent homicides actually represent accidental traumatic deaths; (2) understand the importance of careful scene and autopsy investigation in establishing whether or not a particular case represents a homicide or an accident; and, (3) appreciate the cooperation necessary between police agencies, death investigation agencies, and forensic pathologists in arriving at the correct conclusions regarding deaths due to trauma.

This presentation will impact the forensic science community by highlighting inter-agency cooperation in the investigation of cases in which initial police investigations are suspicious for homicide, yet, following careful scene and death investigation, it is determined that the cases actually represent accidental traumatic deaths.

One of the most important and publicly recognized responsibilities of death investigators involves the investigation of homicides. The identification and documentation of injuries, the appropriate collection and preservation of evidence, and the proper communication of findings can represent invaluable components to the overall crime investigation and eventual adjudication of a case. Despite the importance of excellent death investigation in the overall realm of homicide investigations, it is at least equally important, if not more important, for forensic pathologists and other investigators to work together to identify the occasional case where initial presentation is suspicious for homicide, but upon closer investigation, it is determined that death is due to accidental trauma. This study covers three cases, each of which had an initial investigation indicating a possible homicide. In each, only after a thorough scene and postmortem examination did it become evident that each decedent was the victim of an unfortunate accident.

Case 1: Police responded to a call in which an unknown male was found dead, lying in a bathtub that contained a pool of blood. An extensive hemorrhage appeared to be emanating from his axilla/chest. A blood-covered, broken ceramic pedestal sink stand was adjacent to the bathtub. Police considered the case a probable homicide. Autopsy revealed a deep sharp force injury of the axilla, with transection of the right axillary artery and right axillary vein. Toxicology testing revealed acute ethanol intoxication. Subsequent investigation revealed that the pedestal had been broken for many weeks. Based on these findings, it was determined that the drunken man apparently fell into the already-broken pedestal stand, causing the severe injury.

Case 2: Police responded to a call in which a 49-year-old male worker was found dead, lying face up at his workplace, a recycling center, with apparent head injuries. Two distinct lacerations were evident on the left side of his scalp and the initial police impression was that the case represented a probable homicide due to blunt head injuries. Autopsy revealed severe craniocerebral trauma, confirming the initial police suspicion of lethal head injuries. Subsequent investigation revealed that the man had been using a crane-operated heavy weight to break apart metal scrap for recycling. He had repeatedly been reprimanded for not staying in a designated protective work booth during this activity. He was found outside of the booth, with a piece of bloodstained shrapnel near his body. Based on these findings, it was determined that the man died as a result of an industrial accident.

Case 3: An elderly man living in a retirement home was found dead next to his bed, with his head resting against a blood-soaked mattress. His scalp had a large laceration, there was extensive blood spatter on the walls and elsewhere at the scene, and there were also items of overturned and broken furniture. The initial scene findings suggested a possible homicidal attack. A postmortem examination revealed a scalp laceration with underlying arterial injury. Subsequent investigation revealed that the intoxicated man had likely fallen onto a lamp, striking his head on a metal portion of the lamp, thus explaining the head wound. Toxicology testing revealed an elevated ethanol level, providing an explanation for the fall. His past medical history was significant for chronic alcoholism.

As exemplified by the three cases presented, correct cause and manner of death determinations are the result of cooperation between police, death investigation agencies, and forensic pathologists, as well as careful, detailed scene investigation, postmortem examination, and toxicology testing. Although each of the cases presented was initially considered an apparent homicide, detailed investigation allowed for appropriate death certification. The importance of careful observation, with correlation of scene, historical, toxicological, and postmortem examination findings cannot be overemphasized.

Homicide-Mimic, Accidental Death, Death Investigation



E38 Theoretical Evaluation of the Use of the Bloodstain Pooling Method as a Screening Technique

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After attending this presentation, attendees will understand the theory of the method which is used in sampling large numbers of bloodstains following blood-pattern analysis through pooling techniques as a screening method. The possible obstacles of this technique and its positive side will be discussed.

This presentation will impact the forensic science community by reviewing the method of using blood-pattern analysis in sampling bloodstains and the use of pooling techniques to check for the presence of stains from different contributors.

There is a continuous effort to make forensic testing more efficient and economical and to lower turn-around time and cost. This is especially important in cases that include large numbers of bloodstains being submitted for testing. Differentiating, sampling, and identification of different contributors of bloodstains are important for more accurate crime scene reconstruction and to identify the suspect or other persons related to the case.

Large numbers of bloodstains can be encountered in a multitude of crime scene-related areas such as clothes, different surfaces, tools, and the human body. The Scientific Working Group on Bloodstain Pattern Analysis (SWGSTAIN) identified multiple types of stain patterns which can be detected including altered drop, projected pattern, satellite stain, splash stain, swipe stain, transfer stain, swipe pattern, bubble ring, cast-off pattern, drip (stain, pattern, and trail), flow pattern, impact pattern, insect stain, and cessation cast-off patterns. Similar and different blood patterns from the same or different persons can be found either at the crime scene or on the collected evidence. Blood patterns can be used to differentiate between possible sources, but may be less helpful when the information about the case circumstances are limited or incorrect, making sampling based on Bloodstain Pattern Analysis (BPA) less effective.

Reliability of BPA was examined by a United States Department of Justice report which indicated that contextual information can affect the analysts' conclusions, which is a confirmatory bias.¹ This could show the need for a more quantitative method to be followed in the sampling decision.

The challenges reported by different experts in examining large numbers of bloodstains include the time-consuming nature of such sampling for DNA, when there is less reliability on BPA. To solve this issue, there is a need for a stain-screening method to check for the presence of a mixture or a single profile.

The pooling samples technique, suggested by Dorfman in 1943, is a known method in genetics and chemistry which examines members of a large population based on group testing theory.² It was Dorfman's proposed pooling for blood samples of groups of men inducted into the military service that tested the combined blood samples for antigens to identify the presence of syphilis.³ Currently, this method is used in biological testing, such as mutation detection.⁴ Forensically, based on this method, groups of bloodstain are pooled and tested for mixture profile.

When required, stains are classified into groups, either on the basis of the blood pattern(s) or of their location. Each group of samples is taken using either a wet swab or by excising a small piece of the material. Each group of samples is then pooled together for DNA analysis. In the case of the pool showing a single profile, there will be no future testing. The samples number in one pool will increase if there is a higher possibility of a single contributor, but the samples number will decrease in cases where there is a higher possibility of more than one contributor. If the pool of a group indicates a mixed profile, each stain in the group will be further assisted and resampled individually to locate the bloodstain which may have caused the mixture.

The advantages of this method include less testing required, the ability to screen large numbers of bloodstains, quicker results, less manual effort, reduced cost, and quicker location of different contributor's bloodstains. The use of the pooling method has several challenges including pool group size, equivalent quantity of DNA from each bloodstain, degradation of some mixed bloodstains (missing profile), and masked allele by major profile. The conditions which can affect using the pooling technique include the sample quantity, contamination issues, stain size, stain location, case requirements, number of contributors, unknown suspects, laboratory budget, laboratory quality procedures, case strategy, and results retention time. More research is needed to evaluate this method forensically.



General Section - 2015

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Bloodstains, Blood Pattern Analysis, Pooling Technique



E39 A Bloodstain Pattern Analysis (BPA) Approach to the Shroud of Turin: A Step Forward

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After attending this presentation, attendees will understand how a Bloodstain Pattern Analysis (BPA) could be performed on the Shroud of Turin to reconstruct the original position of the man impressed on the linen.

This presentation will impact the forensic science community by demonstrating the new developments and potential of the BPA approach on uncommon pieces of evidence, suggesting further evaluation of one of the most controversial and valuable Christian relics.

Some of the presumed blood stains from the crucifixion wounds on the Shroud were approached forensically to reconstruct the body position during the blood flow, the crucifixion, and the ancient death penalty practice. A previous analysis presented the reconstruction of the position of the condemned with the hands above the head (arms/body angle near 80°).¹

Part of this new study focuses on the possibility of being nailed to a simple pole and not to the horizontal branch of a cross (patibulum), as proposed by biblical interpretation. The end of a transfusion cannula, connected to a drip chamber, was fixed at the wrist at Destot's space to simulate the bleeding from a puncture-type injury where it is usually believed that the nail for the crucifixion was positioned. Real human blood and synthetic blood for BPA were used with comparable results.

A ballistic angle finder was used, measuring the forearm-body angles when the hands are directly above the head (110° and 130°). In these cases, the blood trickled down on the radial part of the forearm, opposite to what is observed on the Shroud.

To verify whether the rivulets could be generated by a postmortem bleeding, the subject was laying with his hands crossed on the pubis, therefore the blood flow was in the same pattern as in the Shroud. Tests with different angle of the support surface (-5°, 0°, +5°) have been performed.

In none of these positions did the rivulets run as on the Shroud, but flowed parallel to the forearm for a few centimeters, then dripped toward the lateral or medial side of the forearm. The rivulets also never ran at an angle comparable to the two shorter stains located on the back of the left hand of the Shroud.

To investigate the shape of the nail wound on the hand and the two short rivulets, a preliminary test was set up to simulate the bleeding on contact with a wood surface, like the patibulum. Synthetic blood (0.3mL) was applied onto the back of the hand of a living volunteer; pieces of wood with different textures (from bark to smooth finish) were pressed on the hand for ten seconds and the resulting pattern observed. The results were not conclusive, since the wound is not clearly decipherable and in some cases the texture of the wood left its own imprint. This result underlines how difficult it is to speculate on the actual location of the nail's exit wound based on the imprint on the Turin Shroud.

In addition, this study set up a first BPA for the spear wound on the chest. On a mannequin's torso, a sponge with the same dimension of the wound was soaked in synthetic blood and then pressed at the corresponding area. In a standing position, according to the theory of the bleeding on the cross, vertical rivulets flow only on the front of the torso, with a shape congruent with the Shroud image.

Other theories suggest that the "blood belt" on the back of the image was the result of a postmortem bleeding of the subject after the removal from the cross. On a horizontal torso (support surface angle -5°, 0°, +5°), the rivulets flow sideways and posteriorly to the scapular region, where they form a large pool absorbed by the fabric and create a corresponding imprint on the body. All of this evidence is completely non-consistent with the features on the Shroud. Further studies should be performed on this topic.

New in-depth analyses on the presumed bloodstains on the Shroud of Turin will be presented, corroborating the previous results regarding the arm positions and illustrating some inconsistencies regarding the other red stains. Further BPA evaluation is highly recommended to better understand this controversial relic and the crucifixion as a death penalty.

Reference:

1. Borrini & Garlaschelli. A BPA Approach to the Shroud of Turin: A Preliminary Examination of the Left Forearm to Reconstruct the Crucifixion Practice. Proceedings of the American Academy of Forensic Sciences, 66th Annual Scientific Meeting, Seattle, WA. 2014.

BPA, Shroud of Turin, Crucifixion

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E40 The Flipped Classroom — Turning Your Forensic Education Program Upside-Down

Thomas P. Mauriello, MFS, 8775 Teresa Lane, Laurel, MD 20723*

After attending this presentation, attendees will be acquainted with the new blended-learning pedagogy movement, which is the art and science of teaching and learning, and learn how this pertains to the forensic sciences education community. Blended learning is a formal education program in which a student learns, at least in part, through online delivery of content and instruction. The Flipped Classroom format, a form of blended learning, demonstrates how students learn new content online by watching video lectures at home or in the office, and what used to be homework is now done in the classroom.

This presentation will impact the forensic science community by providing attendees with the knowledge and technical skills used to present academic content in a manner that best meets the needs and expectations of today's students. The Flipped Classroom blended-learning format provides flexible time and delivery for both the student and teacher; manages content quality control; engages students in the learning process; and satisfies the new teaching and learning pedagogy movement being experienced in our universities today.

The term "Flipped Classroom" has become something of a buzzword in the last several years, driven in part by high-profile publications in *The New York Times*, *The Chronicle of Higher Education*, and *Science*.¹ In essence, "flipping the classroom" means that students gain first exposure to new material outside of the classroom. This is usually accomplished via readings, lecture videos or YouTube® clips, and extensive online activities; students then use class time (live events) to do the harder work of assimilating that knowledge through laboratory experiences, problem-solving, discussion, case studies, and debates.

The advantages of the Flipped Classroom format is that it: (1) meets the needs and expectations of students today; (2) allows for maximum flexibility of time and delivery for both the instructor and students; (3) allows for the management of content quality control; (4) engages students in the learning process; and, (5) satisfies the new teaching and learning pedagogy movement that is sweeping the globe. For example, scheduled video lectures are not hampered by inclement weather, outside commitments, holidays, etc., so you are always on track. Video lectures are viewed on demand by the student 24/7 and therefore, reduce the in-class time requirements.

This presentation will highlight the positive outcomes achieved by "flipping" the forensic classroom and strategies used to rebuild forensic coursework derived from 36 years of teaching forensic sciences. In addition, the dynamics of rebuilding forensic coursework and a demonstration of the power of today's technologies to "make a difference" by "making it different" will be described.

Reference:

1. *The New York Times* (Fitzpatrick, 2012); *The Chronicle of Higher Education* (Berrett, 2012); and *Science* (Mazur, 2009).
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Pedagogy, Flipped Classroom, Blended Learning



E41 Asylum Seekers Alleging Torture in Their Countries: Evaluation of a French Center

Renaud Clément, MD, 1 Rue Gaston Veil, Nantes 44093, FRANCE*

After attending this presentation, attendees will better understand methods and circumstances of assault which are crucial for performing efficient forensic examination of asylum seekers.

This presentation will impact the forensic science community by presenting the clear clinical characteristics, the methods of torture used, and the evidence of corroborating wounds that permit us to build a body of knowledge of torture's physical aspects for forensic medical examiners.

This study will characterize the physical evidence of torture experienced by asylum seekers requesting treatment at an academic hospital in western France.

Materials and Methods: Over a six-year period, all refugees examined by forensic physicians were evaluated with the goal of cataloging the physical evidence of torture. Sociological data, declared violence (single physical altercation, repeated physical violence less than one year or more than one year, incarceration not more than one week, or incarceration more than one week), and method of violence (blows by blunt object, crushing, burns, electrical shocks, attempted drowning, smothering, incision, or gunshot) were studied. An association between victims' statements and physical evidence of torture was determined.

Results: Of the 570 survivors assessed, 70% were male with an average age between 31 and 93 years. Dagestan was the country most represented among asylum seekers, followed by Guinea-Bissau and Guinea-Conakry. These countries were dominated by political conflict. Beatings were reported by 27.89%, confinement was reported by 40.22%, and repeated violence was reported by 30.16% of refugees. The average time interval between the first assault and evaluation was 53 months. Forms of torture reported included blunt force trauma (82.51%) including truncheon blows (27.50%), arm incision (30%), and burns (16.3%). Statistically, truncheon blows were experienced more often by males in confinement due to political conflict. The use of crushing methods and electrical shocks also were experienced more often by males during confinement. Victims who had received incision wounds were significantly younger. Gunshots were statistically associated with male survivors of political conflict. Men experienced drowning and electrical shocks while in confinement in the Balkans, Asia, and Russia. Electrical shocks were reported by males during confinement and in northern Caucasus countries. The association between assertions of burns and the presence of cutaneous scars was significant ($p=0.0105$); similarly, assertions of incision wounds were significantly corroborated by evidence of scars ($p=0.0009$). Of note, the association between victims' claims of gunshot and the presence of physical evidence was not significant.

Discussion: Asylum seekers who requested forensic medical examinations were usually young men. The geographic distribution and proportion of asylum seeker countries of origin were different between this single site in France when compared to French national statistics and those of the European Union. Beatings with blunt objects were the most often-reported form of torture used during episodes of repeated violence and during confinement. Assertions of burns were not associated with any particular circumstances. Electrical shocks were reported during confinement and most often in countries of the northern Caucasus. Attempted drowning, smothering, and shocking were noted, but these methods typically do not leave physical evidence or have health sequelae. Wounds resulting from burns and incisions usually leave scars that corroborate refugee statements. Torture by crushing and gunshot were reported by asylum seekers for the first time.

Conclusion: Investigation of the types of torture and circumstances under which torture occurs is critical for efficient forensic evaluation of claims of torture experienced by asylum seekers.

Asylum Seekers, Physical Assessment, Torture/Scars



E42 Forensic Podiatry — Pedal Evidence in Forensic Casework

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After attending this presentation, attendees will understand the value of an important and emerging sub-discipline of forensic science, i.e., forensic podiatry, which deals with the examination of pedal evidence usually encountered at crime scenes.

This presentation will impact the forensic science community by presenting the utility of a comparatively new sub-section of forensic science which is concerned with the inspection of pedal evidence at crime scenes; this may motivate young forensic scientists to take up this discipline for research and practice.

Forensic podiatry is a comparatively new scientific sub-discipline of forensic science which deals with the examination of pedal evidence generally encountered at crime scenes. According to DiMaggio and Vernon, it is defined as the application of sound and researched podiatric knowledge and experience in forensic investigations, to show the association of an individual with a scene of crime, or to answer any other legal question concerned with the foot or footwear that requires knowledge of the functioning foot.¹ Forensic podiatrists contribute to the personal identification in forensic investigations whenever foot-related evidence is recovered from a crime scene. The need to establish the identity of dismembered remains may arise in cases of mass fatality incidents such as terrorist attacks, mass murders, transport accidents, tsunamis, floods, and earthquakes. Dismembered and mutilated remains are usually encountered in these mass fatality incidents. There is an increased likelihood of the recovery of feet (often enclosed in shoes), separated from the body in mass disasters such as high-power explosions and bomb blasts, airplane crashes, and other high-impact transportation accidents. In this regard, forensic podiatrists can collect the evidence related to the foot and assist in the identification of the individual from the foot and its parts. Furthermore, forensic podiatrists conduct the examination of footprints generally recovered at the crime scene. Footprints are commonly recovered at every crime scene in the form of bare footprints, socked footprints, or shoe prints.

There are many ways in which footprints can be used in establishing personal identification in forensic podiatry. The analysis of bare footprints involves identification based upon the individualistic characteristics of the footprints. Features such as corns, pits, ridges, humps, creases, deformity, an extra toe, riding toes, missing toe in the foot impression, and flat footedness are considered as individualistic characteristics of the footprints which can be utilized as forensic evidence in establishing personal identification. This kind of physical evidence can positively link a suspect to a crime or it can prove one's innocence. By using different anthropometric methods, the stature, sex, and body weight can also be estimated from the footprints recovered at the crime scene. As stature and body weight can provide an idea about the size of the individual, they can also provide useful clues to a forensic scientist in criminal investigation. Apart from these evidences in forensic podiatry, gait analysis and step/stride length analysis can also furnish some indication about the criminals involved in a particular case.

This presentation will discuss various methods of personal identification related to the pedal evidence which is usually recovered from the crime scene or scene of occurrence in the form of mutilated/dismembered remains, footprints, or questioned footwear.

Reference:

1. DiMaggio JA, Vernon W. *Forensic Podiatry-Principles and Methods*, Springer, New York, Dordrecht Heidelberg, London: Humana Press, 2011.

Forensic Podiatry, Pedal Evidence, Foot and Footprint Characters



E43 Analyzing Law Enforcement Officer Reaction Time in Shooting Events Using 3D Computer Animation

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After attending this presentation, attendees will understand how 3D computer animation can be used to demonstrate officer reaction time in lethal force situations.

This presentation will impact the forensic science community by demonstrating techniques for illustrating, and thus better understanding, how reaction time affects law enforcement officers' performances in shooting events.

Bullet paths in shooting events are routinely analyzed by 3D computer modeling. Computer-Aided Design (CAD) and 3D-animation software are excellent tools for the analysis of such events because of the software's ability to demonstrate spatial relationships between objects and people. When a model of a scene is created, bullet paths can be depicted as lines through space, from their point of origin to the objects they strike. When reviewing dynamic shooting events, animation software can be used to analyze the event in terms of motion and timing, which can be helpful in explaining why an officer's recollection of the event is not consistent with the forensic evidence.

A typical example of such an event is when a suspect in a motor vehicle attempts to run over a police officer. The officer, in fear for his or her life, remembers shooting at the driver through the windshield of the vehicle as it approached; however, examination of the physical evidence shows that the bullet entered through the side of the vehicle and not the windshield. How can this discrepancy be explained? If one were to diagram just that instant in time when the bullet left the gun, one might draw the conclusion that the officer was no longer in mortal danger since the vehicle had already passed by. And, that being the case, was the use of such lethal force still justified?

Law enforcement officers often have to make split-second decisions on when to use lethal force. A failure to take appropriate action could cost them their lives or the lives of others. When confronted with a threat, the officer must mentally perceive, process, and react to that threat, which requires a certain amount of time. Thus, there is a reaction-time delay between when the threat occurs and when the officer pulls the trigger on a gun. The same is true when a threat has abated. Once an officer starts shooting, it takes a certain amount of time for an officer to realize that he or she is no longer in danger and to stop firing.

This presentation will look at three shooting incidents where officers fired at moving vehicles and provided statements that were in some ways inconsistent with the physical evidence. In each case, the speed and path of the vehicle, as well as the location of the officer relative to the vehicle, could be accurately defined, and thus could be modeled and animated with a high degree of precision. Once the animated model was created, it was then possible to go back and look at what the officer saw at various points in time, prior to actually pulling the trigger. Correlating that information with data from reaction-time studies helped to explain why the officers fired when they did.

Computer Animation, Shootings, Reaction Time



E44 Death During Restraint: Excited Delirium vs. Positional Asphyxia

Werner U. Spitz, MD*, *Forensic Pathology/Toxicology, 23001 Greater Mack Avenue, Saint Clair Shores, MI 48080*

After attending this presentation, attendees will understand the mechanism of death during restraint.

This presentation will impact the forensic science community by discussing how to evaluate, certify, and testify in cases of deaths during restraint.

Rarely in the history of medicine has there been so much controversy regarding the mechanism of sudden collapse and death during restraint. Then, many years of speculation came to a sudden halt when Dr. Theodore Chan, an Emergency Room (ER) physician in San Diego who headed a number of studies on this topic, laid to rest previously held opinions that compression during restraint is unrelated to asphyxia, but due instead to Excited Delirium (ED).

On March 5, 2010, in Federal Court in Laramie, WY, Dr. Chan testified that his experiments with volunteers in the prone position, with up to 225 lbs. on their backs, were not meant to replicate conditions in the street. Indeed, Judge Johnson in his ruling states that Dr. Chan, in his testimony and in his published research, explicitly acknowledges that the two situations are not comparable (i.e., testing in the laboratory and restraint in the street).

Considering these recent developments, it is clear that ED is simply a long-known reaction to catecholamines. If ED were a cause of death, why then does it occur exclusively during restraint in prone position with compression causing immobilization of the chest and abdomen, when the abdominal organs are pushed against the diaphragm? Ronald O'Halloran, MD, past medical examiner of Ventura County, CA, asked why is it that the police are always present when someone dies of ED.

Consider the case of an autistic teenager who was in a state of acute psychosis. His parents called 911 to transport their son to the hospital. Police accompanied an ambulance to the scene.

A scuffle developed with several of the officers, during which the boy was placed in prone position and forcibly held to the ground. He suddenly calmed down and stopped fighting and screaming. His mother thought he was dead. When fire rescue personnel arrived, he was still face down, handcuffed, hobbled, unresponsive, and in cardiorespiratory arrest. Cardiopulmonary Resuscitation (CPR) was performed en route to the hospital. On arrival at the ER, he was diagnosed with anoxic encephalopathy and put on life support. Three weeks later, life support was withdrawn and death was pronounced.

The medical examiner handling the case had the event re-enacted using the two original officers to perform the restraint on one of their own officers in the same way as they had previously restrained the boy.

Within <1 minute, the officer being restrained indicated he could not breathe. The restraining officers did not believe him and continued the restraint. The restrained officer became panicked and violent because he was experiencing air hunger. When the officers finally realized he was in trouble, they released him.

Dr. Vincent DiMaio testified in this case. He opined that the cause of death was ED syndrome. Further, he stated that 225 lbs. doesn't impair the ability to breathe and it would probably take approximately 800-900 lbs. to arrive at that point.

One can only look forward to a time when compression of an individual in an acute psychotic state is replaced by treatment in a medical facility.

Positional Asphyxia, Excited Delirium, Restraint



E45 **Spatters Matter: How Bloodstain Evidence Influenced the Police at the Scene and the Prosecutor and Jury in the Courtroom**

Daniel V. Christman, MS, Snohomish County Medical Examiner's Office, PO Box 823, Bothell, WA 98041*

The goals of this presentation are to analyze the relationship of science and the law and apply the principles of forensic science with crime scene investigation techniques. This presentation will also discuss scientific methods and examine the evidentiary value of bloodstain patterns within a homicide scene, on the victim, and on the suspect and will illustrate that an investigator's opinions must be grounded in the analysis of the physical evidence and all known facts in the case.

This presentation will impact the forensic science community by helping attendees understand some basic principles associated with bloodstain pattern analysis, crime scene investigation, and scientific correlations of fluid dynamics to the resulting evidence within the featured homicide case. Attendees will also be challenged to give equal emphasis to all feasible and reasonable possibilities when considering answers to unknowns by implementing scientific methods to problem-solve the crime scene and develop a hypothesis.

This study uses a homicide case which includes bloodstain pattern analysis, blunt force trauma, and the correlation of injuries as well as a series of misdirected efforts to implicate and prosecute a husband in his wife's murder. On May 18, 1992, Gerald L. O'Grady relaxed in his West Vancouver, Canada, apartment and watched television while his wife worked in the kitchen. He wanted his wife to join him so Mr. O'Grady left the television room and walked toward the kitchen. There he found the room painted with blood and his wife's lifeless body on the floor — a victim of a brutal attack. The 70-year-old O'Grady started Cardiopulmonary Resuscitation (CPR) but, when fatigue set in, he stopped and called 911 for help.

Police and Emergency Medical Services (EMS) responded to the apartment where they found the front door dead-bolted. O'Grady opened the door from the inside and, standing before them, the first responders saw an elderly man with bloodstains covering his face, arms, clothing, and shoes. While EMS tended to his mortally wounded wife, the police became increasingly suspicious of Mr. O'Grady, questioning him at length about his alleged discovery of his injured wife as well as the exceptional bloodstains covering him. Mrs. O'Grady was taken to the hospital and Mr. O'Grady was taken to the police station for further questioning. Mrs. O'Grady died a short time later and Gerald O'Grady was ultimately charged with murdering his wife and was booked into jail.

Mr. O'Grady's defense counsel alleged that someone else entered the apartment that fateful night and savagely attacked Mrs. O'Grady in the kitchen while Mr. O'Grady watched television in another room in their residence. The prosecution countered that Mr. O'Grady murdered his wife. They believed the bloodstain evidence found at the crime scene, and most especially on Mr. O'Grady, would serve to convict him of his wife's murder.

During the first trial in 1993, the primary crime scene investigator gave conflicting, but honest, testimony. The jury found Mr. O'Grady guilty of second-degree murder. That conviction was successfully appealed in 1995, and a new trial was ordered on the grounds that "fresh evidence regarding blood spatter could have affected the result." New evidence was presented in the second trial, which ended in a mistrial in 1996 because the jury was unable to reach a unanimous verdict. The Crown Counsel (prosecution) then tried Mr. O'Grady for a third time. In the final trial, Gerald O'Grady was ultimately convicted of the second-degree murder of his wife and sentenced to life in prison; however, the physical evidence collected at the crime scene tells its own compelling story and, presented objectively, it supports Mr. O'Grady's account of the actions he took on that fateful night. In the end and in spite of the verdict, the physical evidence proves beyond a reasonable doubt that Mr. O'Grady did not commit his wife's murder.

This presentation examines the role physical evidence played in the trial process and how it was, or wasn't, used in Mr. O'Grady's original trial, in the second direct appeal trial, and in his third and last trial wherein Mr. O'Grady was finally convicted of murder. Mr. O'Grady died of natural causes while serving his sentence at the Ferndale Institution in Mission, British Columbia, Canada in 2004. He was 81 years of age.

Bloodstain, Evidence, Fluid-Dynamics



E46 Are We There Yet? The Testing of Unsubmitted Sexual Assault Kits and the Development of New Research Partnerships

Heather E. Waltke, MS, 3601 Connecticut Avenue, NW, Unit 120, Washington, DC 20008; and Gerald M. LaPorte, MSFS, National Institute of Justice, Office of Inv & Forensic Science, 810 Seventh Street, NW, Washington, DC 20531*

After attending this presentation, attendees will be more knowledgeable about federal support to state and local law enforcement agencies in their efforts to reduce the number of unsubmitted Sexual Assault Kits (SAKs).

This presentation will impact the forensic science community by demonstrating what research reveals regarding the nature of unsubmitted SAKs.

The National Institute of Justice (NIJ), the research, development, and evaluation agency of the United States Department of Justice, leads the nation in supporting the forensic sciences through research and by providing state and local crime labs with funding to help process and test evidence more efficiently in an effort to reduce backlogs. NIJ continually draws on the needs of practitioners to inform its research and programmatic agenda. This presentation will focus on a research initiative that is designed to help provide information concerning evidence collection, processing practices, and testing protocols for SAKs. Specifically, this research initiative is an endeavor to help address a major need in our nation's forensic science and criminal justice communities: to support state and local law enforcement agencies in their efforts to reduce the number of unsubmitted SAKs.

The focus of this effort is in the collection and analysis of valuable data concerning the nature of sexual assault evidence contained in previously unsubmitted SAKs. Further, obtaining an understanding of the various processes associated with analysis such as screening and testing are fundamental in the effort to inform best practices for collecting, analyzing, and testing evidence from sexual assault cases specific to the processing of large quantities of unsubmitted SAKs. The knowledge garnered as a result of this effort will be used to fulfill the long-term goal of improving current and future best practices for collecting quality evidence and processing SAKs in a more timely and efficient manner.

Over the years, forensic DNA analysis has proven to be invaluable to the law enforcement community, the victims of violent crimes, and their families. This initiative also directly supports the goal of carrying out analysis of samples from untested, unsubmitted SAKs so DNA profiles can be developed and placed in the National DNA Index System (NDIS). More violent crimes are solved as more DNA profiles are placed in NDIS, which is considered one part of the Combined DNA Index System (CODIS), the Federal Bureau of Investigation's (FBI) program of support for criminal justice DNA databases, as well as the software used to run these databases. NDIS has been particularly helpful to investigations that are very old and no longer producing new leads.

Decades ago, crimes from cold cases would have remained unsolved. The national database has been instrumental in solving violent crimes and providing valuable investigative leads to law enforcement. The upload of eligible profiles generated from DNA analysis as a result of this research initiative into NDIS provides direct aid in the investigation of violent crimes involving sexual assault and in the capability to solve more crimes to ultimately hold more criminals accountable. After attending this presentation, attendees will learn about NIJ's efforts to support state and local law enforcement agencies in their efforts to reduce the number of unsubmitted SAKs. In addition, attendees will learn about what research reveals regarding the nature of unsubmitted SAKs.

Sexual Assault Kits, Rape Kits, Untested Rape Kits



E47 Estimation of Human Age Using N-Glycan Profiles From Bloodstains

Dragan Primorac, MD, PhD, 471 Wolcott Lane, Orange, CT 06477*

After attending this presentation, attendees will learn the importance of glycans in human age estimation and that a bloodstain could be used to predict the age of an offender who committed a crime even few years ago, which represents valuable progress in the field of forensic science.

This presentation will impact the forensic science community by providing findings demonstrating that the analysis of the N-glycan profile could be a useful tool in forensics for determining human age estimation from dried bloodstains found at the crime scene.

Protein glycosylation is the most common epiproteomic modification that is involved in numerous physiological and pathological processes. Previous studies reported strong associations between human plasma N-glycans and age, prompting this study to evaluate the potential application of this biological phenomenon in the field of forensics.

Blood from 526 blood donors from different parts of Croatia was collected on bloodstain cards during the period of 2004-2007 and stored at +4°C for 6-9 years. Glycosylation profiles of the bloodstains were analyzed using Hydrophilic Interaction Liquid Chromatography/Ultra Performance Liquid Chromatography (HILIC/UPLC). The statistically significant correlation between N-glycan profiles of bloodstains and chronological age was found and the statistical model that can be used for the age prediction was designed ($Age = 75.59 - 5.15 \times (GP4)^2 + 17.07 \times GP6 - 5.30 \times (GP10)^2 - 16.56 \times GP16 + 20.07 \times GP20 - 7.54(GP20)^2 + 16.47 \times GP22$).

$$Age = 75.59 - 5.15 \times (GP4)^2 + 17.07 \times GP6 - 5.30 \times (GP10)^2 - 16.56 \times GP16 + 20.07 \times GP20 - 7.54(GP20)^2 + 16.47 \times GP22$$

This model explains 47.78% of variation in age, with the prediction error of 9.07 years. This study's findings demonstrate that the analysis of the N-glycan profile could be a useful tool in forensics, providing human age estimation from dried bloodstains found at the crime scene.

Glycans, Age, Bloodstain



E48 Ambient Ionization Mass Spectrometric Detection of Homemade Explosives in the Presence of Precursors

*Edward Sisco, MS**, National Institute of Standards and Technology, 100 Bureau Drive, Gaithersburg, MD 20899; and *Thomas P. Forbes, PhD*, National Institute of Standards and Technology, 100 Bureau Drive, Gaithersburg, MD 21075

After attending this presentation, attendees will have a better understanding of how the concurrent mass spectrometric detection of homemade explosives and their precursors can be affected by factors such as competitive ionization and differences in chemical properties.

This presentation will impact the forensic science community by providing methods for the detection of homemade explosives in the presence of their precursor chemicals and other complex matrices. The presentation will also present methods to mitigate deleterious effects caused by the presence of precursor molecules.

Chemical analysis and detection of explosives is a well-explored area of forensic science in which numerous methods have been investigated. Techniques such as Gas Chromatography/Mass Spectrometry (GC/MS), infrared spectroscopy, and colorimetric tests can all readily detect a wide range of explosives in both pure form and in complex matrices; however, the capabilities of one of the emerging techniques in forensic science, Ambient Ionization/Mass Spectrometry (AI/MS), has been significantly less researched due to the recent advent of the platform. Most work completed thus far, regarding AI/MS capabilities, has focused on the detection of pure compounds or common military-grade explosives in known matrices. While it is essential to evaluate the performance characteristics of these techniques under controlled conditions, it is equally as important to investigate common homemade and improvised explosives. Two common classes of homemade explosives are sugar alcohol-based nitrate ester explosives, such as Erythritol Tetranitrate (ETN) and Xylitol Pentanitrate (XPN), and peroxide-based explosives, such as Triacetone Triperoxide (TATP) and Hexamethylene Triperoxide Diamine (HMTD). Since these compounds are typically homemade, it is crucial to understand their performance characteristics and potential detection issues both in pure form and in mixtures with their precursors.

This work focuses on identifying potential advantages and issues in the detection of homemade explosives when found in the presence of their common precursor chemicals using AI/MS platforms. The main platform investigated is Direct Analysis in Real Time Mass Spectrometry (DART[®]-MS). DART[®]-MS has been shown to rapidly and sensitively detect a number of explosive compounds from various classes with minimal-to-no-sample preparation. Utilizing a heated stream of helium metastable atoms, ionization of explosives rapidly occurs through either adduct formation, deprotonation, or protonation. One of the major benefits and drawbacks of the DART[®]-MS and AI techniques in general is the lack of a chromatographic separation prior to ionization and mass spectral detection. While this lends itself to rapid sampling times, it also may prove deleterious when complex mixtures are analyzed. Competitive ionization and differences in chemical properties can favor ionization and detection of one compound over another.

A number of sugar alcohol-based explosives were analyzed by DART[®]-MS as neat compounds and in the presence of their common precursors, sugar alcohols and nitric acid. Through analysis of these mixtures, it was found that competitive ionization does occur when these compounds are concurrently sampled. Relative affinity for free nitrate ions and differences in vapor pressures of the explosives and related sugar alcohols components can lead to diminished detection limits of the explosive component. Furthermore, extracted ion chromatographs of the mixtures highlight how different ionization pathways are preferred in the presence of different combinations of precursors, with adduct formation with free nitrates being the dominant pathway for both components. Studies have also been completed which focused on maintaining the sensitivity of the explosive compound by altering the chemical properties of the atmosphere near the sampling area, through dopant introduction. This work has shown that different ionization pathways can be emphasized depending upon the dopant species present. While current work has focused on sugar alcohol-based explosives, work is now continuing and will be presented on understanding peroxide-based explosives and their precursors as well as investigating additional AI/MS-based techniques such as Desorption Electro-Flow Focusing Ionization/Mass Spectrometry (DEFFI/MS).

Homemade Explosives, DART[®]-MS, Competitive Ionization



E49 Casework Analysis When Reference Data Aren't Available for the Observed Insect Species

Neal H. Haskell, PhD*, 425 Kannal Avenue, Rensselaer, IN 47978

After attending this presentation, attendees will better understand how to use closely related blow fly species growth data to determine Postmortem Interval (PMI) for cases where species growth and development data is not available.

This presentation will impact the forensic science community by enabling estimation of the PMI when growth and developmental data are not available for the blow fly species which are found on decomposing remains. This is accomplished by using known developmental data on closely related species which are available.

The use of insect evidence to determine the Postmortem Interval (PMI) and to answer other questions surrounding a death scene has been in common use in hundreds of case investigations for more than two decades around the globe. In most cases, blow flies (*Diptera: Calliphoridae*) are used singly or in combination as the Primary Indicator Species for the known time of their growth and development which can then be used to calculate an estimated range of time (minimum to maximum) of when the death of the individual occurred. In this calculation, there is usually an additional estimated time for various delays which may occur prior to initial colonization (egg laying). Thus, after the blow fly species is identified, the developmental table for that specific species is then consulted. By factoring in the known environmental temperatures coupled with the growth and developmental data, the estimated range of when death most likely occurred is determined.

However, there are times when a species of blow fly is recovered from remains and the developmental data has not been determined for that species. This is usually due to the enormous effort required in resources, time, and funding to generate reliable growth data for a species. In addition, the species may be limited to a geographic area, but very commonly, the area has not been well surveyed as to what species are present, and thus the species has not been studied to any extent. The forensic entomologist is faced with the dilemma of which data set to select for that unknown (growth-wise) species. Usually, these understudied species will have a closely related, sister species which has been studied extensively. Therefore, it is possible to apply the known rearing data of extensively studied species to the species for which there is little or no data available.

This forensic entomologist has used the data set for blow fly and flesh fly species of Adel Kamal, which have been available for more than 50 years. With a few exceptions for tropical species data sets which have been found to be reliable, these growth and development tables of Kamal have proven reliable, accurate, and verified by information of the known time-of-death for a particular case. With this established, the Kamal data can then be used as the base data for sister species estimations for which there is no established growth and development data.

The most commonly used species in North America is *Phormia regina*, a warmer weather blow fly, and in the same tribe as the screw worm species. Through case work and research, it was seen that *Cochliomyia macellaria* was very near the development range of *P. regina*. Thus, before data were available for *C. macellaria*, data for *P. regina* was applied to *C. macellaria*. Once publication of the developmental data was compiled for *C. macellaria*, it was seen that *C. macellaria* was approximately 12 to 24 hours quicker for the completed total life cycle than *P. regina*. When larval stages were present and the time of development for those larvae was half the total time or less, the difference was negligible. This rationale also followed for the *Lucilia* species complex consisting of the most common green bottle flies, those being *Lucilia sericata*, *Lucilia illustris*, and *Lucilia coeruleiviridis*. There was ample data for *L. sericata*, but nothing for the other two species which were commonly encountered in hundreds of cases across North America. Therefore, the data for *L. sericata* was used as the data for these other two species. Again, once data had been accumulated for growth and development of *L. illustris* and *L. coeruleiviridis*, it was found that these two species were slightly slower than *L. sericata* by 24 to 48 hours for the total life cycle duration. As in the case with *P. regina* and *C. macellaria* where only a portion of the total life cycle was involved, the differences were greatly diminished. Since *L. sericata* is the fastest of the three species studied, when *L. sericata* was used it would establish the absolute minimum time for the time-since-death for that case. Also, the inclusion of variability into the estimated range far exceeded the small difference in the specific calculation.

Forensic Entomology, Blow Fly, PMI



E50 The Impact of 3D Digitizations and Printed Models of Osteological Trauma When Presented to the Jury as Demonstrative Evidence

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After attending this presentation, attendees will gain better understanding of the importance and the need for 3D digitizations within the courtroom. Furthermore, attendees will recognize the positive impact these digitizations have on jurors.

This presentation will impact the forensic science community by demonstrating the potential of 3D digitizations within forensic anthropology and the courtroom. Additionally, the forensic science community will see the advantages of these techniques as an explanatory tool for the jury.

Virtual environments, simulations, and 3D images are becoming commonplace within some jurisdictions, specifically in the United States; however, there has been a slower adoption of these techniques in the United Kingdom. As osteological trauma cannot be taken into the courtroom due to its sensitive nature, photography and, more recently, 3D digitization through the use of computed tomography, magnetic resonance imaging, and surface scanning has been utilized for visual representation of demonstrative evidence. Although there are a number of advantages discussed within the literature, including its visual and illustrative prowess, some of the negativities have yet to be debated or investigated.

One of the biggest limitations for using this technology within the courtroom is whether these techniques have any influence over the decision-making process or will bias the evidence visualized within the courtroom. Also, it is important to understand whether these displays deflect the viewer's attention away from the key issues and whether the jury is willing to accept the scenario into which they are placed. As a result, experiments must be designed to determine the effects of newer visualization methods so that any influence that could be created can be avoided.

The present study used a transcript to mimic an actual court case. As a control, each of the juries listened to the same case under the same conditions within an actual courtroom; however, for the demonstrational evidence presented by the expert witness, different displays for the same exhibit (osteological trauma to a cranium) were shown. This included verbal description, photographic images, 3D digitizations, and 3D printed models. Following the case, each member of the jury had to answer a questionnaire relating to the case and the particular technique they saw. The questionnaire was designed to determine each individual opinion on the outcome of the case and the demonstrative techniques that were used.

This research showed that the jurors for each of the demonstrative techniques gave the same verdicts in their case. This meant that when using 3D techniques, no influence was created over the jurors. The results showed that the jurors felt that the 3D digitizations and 3D models were more appropriate for demonstrating complicated information and helped clarify technical jargon over standard demonstrative techniques. Furthermore, these techniques do not bias the outcome of the results, demonstrated spatially what was being discussed by the expert witness, and therefore could be implemented within the courtroom to relay technical information to non-technically minded people.

Surface Scanning, Demonstrative Evidence, Jurors



E51 Multidimensional Model for Assessing Student Achievement

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After attending this presentation, attendees will be able to adapt a proposed multidimensional model for evaluating student achievement in forensic science higher education.

This presentation will impact the forensic science community by defining what successful student achievement encompasses. The assessment of student achievement demonstrates accountability and academic quality of a forensic science academic program to the forensic science community and public.

Complex tasks engage students in active learning; however, it is difficult to measure student progress with an objective exam. Students demonstrate their knowledge not as discreet items but rather across a continuum. Assessment strategies should incorporate situated judgments and contextual information to adequately evaluate the decisions made by students while performing complex tasks.

Currently, the primary tool utilized to assess student achievement in higher education forensic science programs is the Forensic Science Aptitude Test (FSAT) or an in-house comprehensive exam. A single standardized test does not accurately assess a student's ability to perform efficiently or effectively in a laboratory. This presentation will present the preliminary findings from a study directed at creating a multidimensional framework by which institutions can assess student achievement in forensic science education. Such a framework would provide a structure for forensic science programs to document student performance and demonstrate program quality and accountability to crime laboratories and the public.

Specific questions addressed in this study include: (1) How do forensic science education programs evaluate student achievement?; (2) How do disciplines other than forensic science evaluate student achievement?; (3) What knowledge, skills, and abilities are forensic science laboratory personnel expected to possess in an accredited laboratory?; (4) What standards/policies/best practices would best define successful student achievement in a forensic science undergraduate program?; and, (5) What standards/policies/best practices would best define successful student achievement in a forensic science graduate program?

The proposed framework was derived from literature assessing student achievement in higher education as well as from accreditation standards for forensic science and other academic disciplines such as nursing, medicine, and education. Accreditation standards for higher education institutions such as Council for Higher Education Accreditation (CHEA) and Middle States Commission on Higher Education (MSHEA) were also reviewed. Additionally, current standards utilized to assess student achievement by forensic science academic programs were reviewed to identify promising models. The practices identified in the various accreditation standards and promising practices were aligned with the International Organization for Standardization and the International Electrotechnical Commission (ISO/IEC) 17025 general requirements for the competence of testing and calibration laboratory standards.

Institution and discipline-specific accreditation standards incorporate several strategies for demonstrating student achievement including: (1) completion of degree coursework; (2) capstone experience or independent research projects, both with a written report; (3) completion of a comprehensive examination (in-house, FSAT, certification, licensure, or boards); and, (4) post-graduation employment or graduate school.

Literature on assessing student learning stresses that evaluation of student learning should occur at all levels and stages of the forensic science education process. Summative assessments, like the FSAT and comprehensive exams, provide one measure of a student's knowledge; however, formative assessments tend to have a greater impact on student knowledge and skills because of the reduced risk. Formative assessments, such as reflection and self-evaluation, allow students to explore both strengths and weaknesses. Most university faculty members have been given little formal training in how to develop formative assessments or measurable outcomes that can be assessed by multiple strategies.

Higher Education Policy, Evaluation, Student Achievement



E52 Creating a Positive Connection for Your Forensic Science Discipline

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After attending this presentation, attendees will better understand a solution for connecting disparate forensic practitioners within a specific field such as forensic art.

This presentation will impact the forensic science community by describing the process whereby social media has been used as a platform for professional communication.

Using the LinkedIn[®] social networking service, the Forensic Artists Discussion Group was established in 2009. It has more than 300 members globally connecting approximately 275 working forensic art professionals as well as representing affiliated disciplines including anthropology, odontology, pathology, and anatomy. Nurturing the development of this group and encouraging cross-discipline discussion is the primary purpose. Finding common ground in a forensic field where most practitioners are widely distributed and singular in their organization is an issue that has long needed to be addressed. Creating a platform for input, discussion, and exchange of ideas is the first step in developing cohesion. This development included identifying and personally contacting unattached practitioners and inviting them to the group. This is an example of a discipline taking the onus upon itself to create an outlet to advance professionalism, develop goals for advancement, and generate consensus.

The Forensic Artists Discussion Group focuses on professional concerns of the field including updates on new techniques, sponsored training, national events that affect the discipline, and employment opportunities. The group is a moderated, unbiased venue for information and materials. Discussion is monitored and guidelines have been established for professional conduct from participants. It serves as a communication conduit for all professional groups including the International Association for Identification (IAI), American Academy of Forensic Sciences (AAFS), British Association for Human Identification (BAHID), and National Institute of Standards and Technology (NIST).

There are currently 30 forensic art units in law enforcement agencies, universities, and non-profit agencies that employ 55 full-time artists in the United States. There are approximately 20 forensic art units internationally which are similarly distributed among law enforcement, educational institutions, and non-profits or Non-Governmental Organizations (NGOs).

The Forensic Art Discussion Group has developed into an invaluable resource that allows participants to learn from one another, improve their craft, and control the future of their discipline. Positive feedback is continually received from members about the group, which demonstrates the need for such a professional network. The next step is to energize members to improve the profession and increase the perceived and actual value of forensic artists. This is a means of attracting attention to the need for creating and implementing best practices across the discipline and is essential in demonstrating to NIST the need and demand for attention to the forensic art field.

Forensic Art, Communication, Forensic Team Building



E53 National Institute of Justice (NIJ) Programs to Support the Forensic Community: Strategies for Stronger Proposals in a Competitive Environment

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The goals of this presentation are to: (1) learn about National Institute of Justice (NIJ) programs that support the forensic science community; (2) learn how to read an NIJ solicitation and what key areas to focus on; and, (3) learn how to write a stronger competitive proposal.

This presentation will impact the forensic science community by providing information about funding opportunities, both competitive and non-competitive, through the NIJ.

There are various types of federal funding opportunities and, generally speaking, they can be broken down into two types: competitive and non-competitive. Non-competitive funding can come in the form of formula grants or sole-source grants/contracts. Competitive funding is just that: competitive. This presentation will focus on competitive funding opportunities issued by the NIJ for research or direct-service assistance related to forensic pathology and medical examiner/coroner services.

This presentation will discuss four programs issued by the NIJ that support the forensic community: (1) Paul Coverdell Forensic Science Improvement Grants Program; (2) Using DNA to Identify the Missing Program; (3) National Missing and Unidentified Persons System (NamUs); and, (4) Research and Development in Forensic Science for Criminal Justice Purposes Program. The NIJ program managers for each of these programs will discuss the specifics of each, including purpose, eligibility, and emphasis on how to write to the selection criteria. Common mistakes and best practices will be reviewed. Requirements including, but not limited to, the program narrative and budget documents will be discussed. A brief overview of NIJ's current forensic pathology research and development and assistance portfolio will also be provided. The goal of this presentation is to provide attendees with an overview of the current NIJ programs that support the forensic science community and to equip attendees with the knowledge and skills to write stronger grant proposals responsive to these programs.

Funding Opportunities, Eligibility, Best Practices



E54 Collaborative Retrospective Research Study Exploring STR and Y-STR DNA on 1,000 Rape Victims: Implications on Practice

Julie L. Valentine, MS, Brigham Young University, 532 SWKT, Provo, UT 84062*

After attending this presentation, attendees will understand the findings from a collaborative retrospective research study on 1,000 rape victims linking data from the sexual assault nurse examiners' forms with data from the state crime laboratory, specifically exploring the findings related to Short Tandem Repeat (STR) and Y-chromosomal Short Tandem Repeat (Y-STR) DNA analysis results.

This presentation will impact the forensic science community by increasing competency for both forensic nurses and scientists on DNA evidence collection and analyses guidelines by presenting the findings from a collaborative, retrospective research study of 1,000 rape victims. It is imperative that these professions work together to establish best practices guidelines for evidence collection in sexual assault cases. The importance of collaboration is reinforced through this retrospective study examining variables affecting positive STR and Y-STR DNA analyses.

Study Proposition: Improvements in DNA analysis methods in sexual assault cases, including Y-STR DNA analysis, have practice implications for forensic nurses and scientists that may be gleaned through a collaborative retrospective study.

Description of Study and Statement of the Methods: The impetus for this research was to explore the impact of enhanced DNA analysis methods, including Y-STR analysis, on crime laboratory findings in sexual assault cases. Implications on evidence collection guidelines in sexual assault cases are also examined in relation to the findings from this study. Practice changes for both forensic nurses and scientists that have occurred due to the findings from this study will be explained.

The setting for the study is a Mountain West urban community in the United States. For each case in the study, 203 variables from the **Sexual Assault Nurse Examiner's** (SANE's) sexual assault examination forms and crime laboratory analysis findings were identified and coded in **Statistical Package for the Social Sciences** (SPSS) statistical software program: 149 variables from the sexual assault examination form and 54 variables from the crime laboratory data on sexual assault kits were returned for analysis.

Summary of the Results: Data analysis is not fully completed, but includes the following points: (1) the percentage of collected sexual assault kits returned by law enforcement to the state crime laboratory for analysis. Preliminary data analysis has found a sexual assault kit return rate of 29.2%; (2) the percentage of returned sexual assault kits to state crime laboratory that underwent DNA analysis. Preliminary data analysis has found 44% of returned sexual assault kits had DNA analysis, with the remaining analyses stopping after serology; (3) descriptive statistics on patients' demographics, suspects' demographics, and relationship of patient to suspect; (4) descriptive statistics on STR and Y-STR DNA analysis findings; and, (5) logistic regression on the following variables — length of time between rape and evidence collection and if patient bathed or showered — on positive DNA profile identification.

Additionally, unusual cases with positive DNA profile identification will be reported as these cases reinforce the importance of expanding DNA evidence collection in sexual assault cases outside of 72 hours between rape and evidence collection.

Implications on Practice/Conclusion: Results from this collaborative study have changed practice guidelines for both forensic nurses and scientists. Preliminary findings from this study have led to the following practice changes: (1) recommended time frame between rape and evidence collection has expanded to five days; (2) biological evidence swabs from body sites other than genital swabs are recommended for collection regardless of whether the patient has bathed or showered; (3) biological evidence swabs from body sites touched by the suspect, in cases of an unknown assailant, are recommended for collection; (4) DNA analysis to be completed on most submitted sexual assault kits prior to serology testing; and, (5) state crime laboratory tracking of all sexual assault kits to determine percentage of kits returned by law enforcement for analysis.

Collaboration, Rape, DNA



E55 Analysis of Suicide Locations in Harris County, Texas

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After attending this presentation, attendees will be aware of the significance of the location chosen by some individuals when they commit suicide through careful examination of the scene and investigation of factors leading up to their death.

This presentation will impact the forensic science community by highlighting the importance of understanding the context and circumstances under which someone takes their own life. By appreciating the significance of the location, forensic practitioners will develop an awareness of how statements and behavior immediately prior to death may relate to the location chosen by the deceased.

While individuals who commit suicide do not always leave notes or other written communication stating their intent, the locations chosen for their suicidal act can often be just as significant. Factors such as religion, financial status, relationship conflicts, and mental illness are common reasons for individuals to commit suicide. Although these factors are often the driving force behind the decision to kill themselves, the location chosen often reflects the significance of those factors.

The jurisdiction of the Harris County Institute of Forensic Sciences (HCIFS) includes the city of Houston and 31 additional law enforcement agencies. According to the 2010 United States Census, the population of Harris County is 4,092,459. Each year, the HCIFS assumes jurisdiction of approximately 4,000 deaths, of which 11%-12% are classified as suicides. The demographic breakdown of individuals who kill themselves has remained constant over the past eight years. The male-to-female ratio is 3-3.5:1 and individuals of White ethnicity are encountered 2.5-3 times more than all other ethnicities combined. The most prevalent method of suicide remains the use of a firearm, followed by hanging and intentional drug toxicities. The largest age demographic is 18-39 years old. These individuals have reached a point in their lives where they are unable to use their present coping skills to address a variety of problems. In cases of spontaneous suicide, the location of their demise typically is opportunistic. In planned suicides, the location of their deaths is specifically chosen, providing clues not only to their thought processes immediately prior to death, but also to the perceived magnitude of their problems.

This presentation will review several suicides in unusual locations in Harris County, TX. An analysis of the location, previous statements made by the individual, and investigative findings will shed light on the significance of the chosen suicide location.

Suicide, Context, Location



E56 Multiple Self-Inflicted Gunshot Wounds: Case Series With a Review of Literature

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After attending this presentation, attendees will understand that a small minority of suicides — less than 1% of all suicides in Wayne and Monroe counties in the state of Michigan — do involve more than one gunshot wound, highlighting that, although rare, the presence of more than one gunshot wound is not a sole hallmark of homicide and requires an open mind when encountering such cases.

This presentation will impact the forensic science community by presenting the largest case series of multiple self-inflicted gunshot wounds. It will specifically impact forensic professionals tasked with determining or assisting in the determination of manner-of-death by reminding them not to immediately rule out suicide in deaths caused by multiple gunshot wounds, whether or not notes are left, as this can occur, evidenced by more than a decade of suicide deaths from a major American metropolitan area.

Suicide by firearm is common and usually ends in a single fatal shot. When two or more gunshots are present, it is frequently viewed as homicide by investigators, police, and the public. This study investigates the incidence, patterns, frequency, and different characteristics of suicide by two or more gunshot wounds, during the period of 2000-2011 in Wayne and Monroe counties. A total of 2,212 cases of suicide were reported and the leading method was by gunshot wound (1,066). Out of those 1,066 cases, there were 17 (1.5%) with multiple gunshot wounds. Nine of these multiple cases had two gunshot wounds and eight cases had more than two gunshot wounds. Fifteen cases involved rifled weapons and two cases involved shotguns. There were two female and 15 male decedents. People 41-50 years of age represented the most suicides involving multiple gunshots. A suicide note was left behind by three (18%) of the decedents, and 14 (82%) did not leave a note. The most common site of entrance was the chest, followed by gunshot wound to the head. In the context of manner-of-death determinations, the characteristics of fatal gunshot wounds along with the importance of keeping an open mind by exploring all physical possibilities during forensic autopsy, are emphasized.

Since multiple gunshots are such a hallmark of homicide, establishing that they are also a fact of suicides is essential, given that Wayne and Monroe counties have a slight rate of occurrence. Of all suicides between 2000 and 2011, less than 1% (17/2,212) had multiple shots. This concurs with Mernerides et al. in noting post-traumatic injury can occur and when it does, may be crucial in differentiating homicide from suicide: *Immediate incapacitation does not occur in every fatal gunshot wound that penetrates the head or perforates the heart.*¹ A study from Lorin on gunshot wounds emphasized the need for careful examination of multiple gunshot wounds for the entrance and exit wounds, estimation of close range, and vital reactions to provide arguments for or against suicidal intent.² A study from Hejna et al. discussed the ability to act in cases of multiple gunshot wounds due to suicide — a frequently asked question during the due course of an investigation and in the courts; the study notes that the character and localization of wounds determine one's ability to act following the gunshot wound.³ Racette et al. reported a rare case of suicide with drowning following two self-inflicted, non-lethal gunshot wounds to the head — this study notes a need for complete and thorough investigation when complex methods for suicide are used.⁴

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Suicide, Gunshot, Multiple



E57 Determining the Flow Rate Required to Move Submerged Human Remains

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After attending this presentation, attendees will understand some principles of how and under what conditions a deceased human body moves when submerged in a fluvial environment.

This presentation will impact the forensic science community by providing refined search and location strategies for investigations of bodies found in water. The results of this project can augment traditional means of investigation by improving references related to the orientation and velocity of a body on the bottom of a river. Understanding the movement of a body submerged in water can be useful for supporting or disputing witness statements and establishing a timeline of events in an investigation. Models and established patterns of the movement of human remains in water can be used to help determine either information on where a body entered the water once it has been recovered or to aid in searches for missing persons believed to be in the water. This will also be beneficial in stressing the importance of recording the position and orientation of a body found in a river as well as recording the river flow, which is often overlooked by investigators.

This research used two models with densities within the range of that of a healthy adult male: a 12" muslin figurine with water balloons and ¼" zinc nuts, and a 12" muslin figurine filled with silicone and copper rods. The models were submerged alternatively in a racetrack flume that had a one-centimeter layer of fine sand on the bottom and was filled with domestic tap water. Using a pygmy flow meter, the flume was set at a known speed and the model was added. Each model was submerged in four different orientations parallel to the flow of the water: face up with head upstream, face down with head upstream, face up with head downstream, and face down with head downstream. The time it took for the model to travel 100cm was recorded. Additional flow rates were used. The relationship between orientation, flow rate, density, and model velocity was analyzed. For the water balloon model, at the slowest flow rate, the face-up-with-head-downstream orientation moved almost four times faster than any other orientation. At the faster flow, the face-down-with-head-upstream orientation moved half as fast as the other three orientations. Faster flows decreased the significance of the orientation of the model and decreased the flume/model velocity ratio. The silicone model, which had a higher density, required faster flow to reach the same velocity as the water balloon model.

The controlled model was assessed using living human participants who are certified scuba divers. Each participant's height and weight were measured and their density calculated. Each participant lay at the bottom of a river with a pony scuba tank and a dive belt for weight for 15 minutes. Involuntary forward movement and positional changes were observed and the flow of the river was measured with a Price AA flow meter. This was done in three rivers with various sediments on the riverbed.

Drowning, Investigation, Water



E58 A Model for Recovery: Predicting the Location of Human Remains on WWII Bombardment Aircraft Crash Sites

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After attending this presentation, attendees will understand the spatial relationships between human remains and their assigned duty station wreckage within WWII bombardment aircraft crash sites.

This presentation will impact the forensic science community by demonstrating the value of building predictive models from previously examined cases and how that contributes to investigators' abilities to efficiently and effectively recover the remains of individuals in aircraft crashes.

The United States government makes a solemn promise to the men and women of the armed forces that if they fall on the field of battle, their remains will be returned home. In general, Americans demand that this occur in order for the individual to be properly honored and that their remaining family members can find closure. This commitment, and corresponding expectation, applies to both current and past conflicts. The Joint Prisoner of War/Missing in Action Accounting Command (JPAC) is responsible for locating, recovering, and identifying the approximately 90,000 American military personnel who remain missing from the beginning of World War II through the end of the Vietnam War. This presentation details a model based upon JPAC's previously completed casework that predicts where human remains will be found within WWII bombardment crash sites based upon each crew member's individual duty station.

Sixteen WWII bombardment aircraft loss incidents that have been resolved by JPAC since 2000 were assessed for inclusion in the model. Two primary criteria had to be met for a case to be included: (1) the archaeologist who conducted the field recovery had to have recorded the location of the crew duty station wreckage within the recovery scene; and, (2) the field provenience of the remains had to have been maintained through the mitochondrial DNA testing process. A total of eight cases met both of these criteria. Next, the Central Identification Laboratory's (CIL) identification process was run backward through a detailed examination of the skeletal analysis, odontological analysis, mitochondrial DNA, and archaeological recovery reports. Field and laboratory bench notes were also reviewed. This allowed for the determination of where the remains of each crew member were recovered within their respective crash sites in relation to the appropriate corresponding wreckage.

Based upon this, hypotheses were developed for each crew position within the aircraft based upon the patterns observed. The maximum distance that remains can be expected to be found from duty station wreckage varied from 0.8m to 9.4m, depending upon the crew position. Likewise, the maximum distance that remains of a single individual may be spread across a site ranged from 2.7m to 9.0m. The validity of these predictions was then tested against an additional bombardment aircraft loss incident. Overall, results of the testing showed that the hypotheses for each of the duty stations were confirmed and that bombardment aircraft crew members will be found no farther than approximately 8m from their assigned duty station. Additionally, for the cases included in this study, it is determined that the physics of the crash, not the actions of the crew or subsequent erosion, primarily dictates where individuals will be found within a crash site. Beyond the immediate benefits to the effectiveness and efficiency of future JPAC excavations, this study also demonstrates how careful examination of similar types of incidents can result in the ability to build accurate predictive models.

WWII, Bombardment Aircraft, Remains Recovery



E59 From the RFK Assassination in Los Angeles to the Wuornos Serial Killings in Florida: A Preliminary Examination of .22 Fired Casings

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After attending this presentation, attendees will be aware of the following information: (1) why .22 fired casings are often found at crime scenes, including the assassination of Robert F. Kennedy (RFK) and the Wuornos serial killings in Florida; (2) some unique features of .22 cartridges; and, (3) how to tell the three types of .22 firearms involved from the .22 fired casing at crime scenes.

This presentation will impact the forensic science community by reporting the results from an empirical study of .22 fired casings (N=100). The results provide a nine-category classification of the .22 fired casings by a pistol, a rifle, or a revolver. The study addresses the challenges from the 2009 National Academy of Sciences (NAS) Report, which states that firearms' examination is "not scientific." It is argued that the study can provide crime scene technicians, police officers, school teachers, and students with tools on how to determine the types of .22 weapons used based on the .22 fired casings recovered at the scene.

Shooting incidents are commonly reported by the news media and often the type of fired casing at the scene is the .22 round. This includes the historical assassination of RFK as well as the female serial killer Aileen Wuornos; however, several questions remain unanswered: (1) Why do so many criminals choose the .22 caliber as a murder weapon?; (2) Why does each shooting incident take so long to get solved?; and, (3) Was the criminal considered "smart" or "stupid" for using a .22 firearm? This empirical study involves the analysis of fired .22 casings (N=100) collected from several indoor shooting ranges with marks indicating weapon type as pistol, rifle, or revolver. Under a stereo microscope, each casing was examined and classified into a nine-category reference in terms of the firing pin shape (2D), the depth (3D), and the sloppiness (3D). From the nine-category reference, a practical table is provided illustrating how to differentiate the types of weapons (.22) involved from the types of firing pin shapes observed. Finally, the table is translated into a forensic reference for crime scene technicians, police officers, school teachers, and students.

The .22 ammunition possesses some unique challenges for firearms examinations. First, the .22 casing is the smallest caliber and the firing pin mark is smaller even under a stereo microscopic examination. Second, the .22 cartridge is the only ammunition using the rim firing mechanism with more irregular patterns as firing pin impressions. Finally, the .22 casing is the only cartridge that can be fired by three possible types of weapons, making the caliber-specific rule useless.

Preliminary results can be summarized as follows: (1) Category A were all fired from a revolver since it has a circular impression without any extractor and ejector marks on the casing; (2) Category B were all fired by a pistol. Most firing pins in pistols have a square tip that leaves a square impression on the edge with extractor and ejector marks on the casing; and, (3) Category C were all fired by a rifle, which has a rectangular firing pin tip and uses a much more powerful gas system, thus producing a firing pin impression that is deeper and much clearer than a pistol with the extractor and ejector marks on the wall of the casing.

A modus operandi analysis becomes the final part of the study to answer the question of why the .22 is one of the most popular weapons used by criminals. First, .22 firearms are less expensive weapons than other firearms. Many gang members choose .22 firearms for drive-by shootings. Therefore, the .22 ammunition is widely used by gang members and criminals due to its low cost. Second, .22 firearms (particularly pistols and revolvers) are small in size and thus easier to conceal, which provides necessary convenience in hiding or so-called "ease of concealment." There are more .22s of various types in circulation than any other caliber of firearm. Finally, the .22 casings are a problem in many cases for the crime scene technicians simply because the casing can be fired by three possible weapons, using more time for examination, exclusion/inclusion, and decision. If investigators are unable to identify the type of weapon used in a timely manner at the crime scene, it may hamper the criminal investigation.

The study of the .22 fired casing has several practical implications: (1) an empirical classification can provide a practical protocol for quick determinations at the scene; (2) a valuable tip in differentiating the three types of casings with three unique characteristics from the three .22 firearms (revolvers, pistols, and rifles) can provide school teachers and students with quick determinations for reporting to the police when .22 weapons are involved; and, (3) by employing a new digital device, a quantifiable measurement is developed, supporting the statistical requirement proposed in the 2009 National Academy of Sciences Report entitled, *Strengthening Forensic Science in the United States: A Path Forward*.

Firearms Examination, .22 Fired Casings, Digital Measurement



E60 A Prospective Analysis of the Outcomes of Violent Prone Restraint Incidents in Policing

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The goals of this presentation are to: assess the outcomes of prone positioning of violent subjects by the police, assess the relationship of using restraint techniques and prone positioning of violent subjects, examine the relationship between using electronic control devices and weight applied on the back of a subject by police during restraint and the outcome of the incident, describe the implications of study findings as they impact sudden deaths in custody, and describe recommendations based on the study findings.

This presentation will impact the forensic science community by serving to demonstrate the dynamics of safely using the prone restraint position and force devices used by the police in violent use-of-force confrontations.

Placing a violent subject in the prone position by police officers has been debated since the 1980s. It has been postulated that prone positioning may be hazardous when applied with various populations and may contribute to a sudden death-in-custody based on a theory of restraint asphyxia. Although scientific research contradicts the theory, the debate continues over the use of the prone restraint position. What is not known is how many combative persons are placed in the prone restraint position after the use of varying force modalities used by the police and the outcomes of these violent confrontations. The purpose of this study was to examine the outcomes of the police use of force and the use of the prone restraint position with violent subjects.

Using a prospective research design, officers in 17 police agencies in six states documented the use of the prone position, the use of varying force modalities with combative subjects, and the outcomes of these incidents for one year. Documentation of 1,085 prone incidents was analyzed and the majority of the confrontations involved a male subject. Subjects displayed behaviors consistent with alcohol impairment, mental distress, illicit drug use, or a combination of mental distress/illicit drugs in 85% of the incidents. Over 70% of the subjects were placed and restrained in the prone position from one to five minutes. Seven force modalities were commonly used including handcuffs and hobbles, electronic control devices, subject control techniques, an aerosol, and weight applied on the back of the subject during the restraint incident. No subject died and 80% of the subjects did not sustain an injury.

The findings of this field study confirm previous laboratory research performed on this subject which has indicated the safe use of the prone position. The findings showed that the use of the prone position provides a safe method for controlling, restraining, and securing combative individuals. Recommendations for police officers, police administrators, emergency medical personnel, and forensic investigators are presented.

Prone, Police, Restraints



E61 Biometric Research Database Catalog: Improving Access to Publicly Available Biometric Data Sets

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The goal of this presentation is to discuss the existing challenges of making existing databases open to researchers, the development of a centralized catalog to improve access to publicly available databases, and plans to develop a path forward on expanding existing publicly available databases.

This presentation will impact the forensic science community by providing researchers with information about current efforts to expand access to biometric datasets. The Biometric Research Database Catalog will provide the community with a central location to obtain information about publicly available biometric data sets to assist technology development efforts.

Biometrics refers to technologies used for the automated recognition of individuals based on their behavioral and biological characteristics.¹ The most commonly used biometric modalities include fingerprint, palm print, iris, face, voice, and handwriting. Today, biometrics are increasingly used to recognize individuals and regulate access to information, physical space, services, and to cross international borders.² In forensics, biometrics serve a critical function in helping to identify or verify the identity of an individual for crime-solving purposes. The National Institute of Standards and Technology (NIST) has been actively involved in the testing and evaluation of biometrics technologies, starting with fingerprints in the 1960s. This involvement has expanded over the decades to include efforts in various biometric modalities such as fingerprints, palm prints, faces, irises, voices, and handwriting, including the development of a comprehensive biometric data transmission standard.

One challenge to the advancement of biometric technology is the development of test data sets. A number of factors affect the appropriateness of a test data set in evaluating a biometric technology, including whether the dataset is representative of: (1) the subject population; (2) the collection environment; and, (3) the system hardware expected. Researchers need greater access to databases to further studies on distinctiveness and to advance technologies to improve the accuracy and precision of biometric systems.

The purpose of this presentation is inform attendees of the development of a catalog of publicly available datasets by the NIST in coordination with the National Institute of Justice (NIJ). Currently, the catalog contains approximately 200 existing publicly available datasets of various modalities, includes information about the samples via a detailed taxonomy, and serves as a pointer for researchers to obtain access to data supplied by host agencies. The catalog will be used as a basis for discussion among the biometric community to develop a path forward on expanding existing publicly available databases in early 2015.

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Biometrics, Fingerprints, Databases



E62 Increasing the Precision of Human Geolocation: A City Scale Investigation of Stable Isotopes in Tap Water

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After attending this presentation, attendees will understand the forensic importance of the isotopic compositions of tap water on a citywide geographical scale for the utilization of stable isotope analysis on human tissues for human provenancing.

This presentation will impact the forensic science community by demonstrating the necessity of understanding the isotopic values of drinking water sources and water distribution systems, at a high geographical resolution, in preference to estimating drinking water values derived from local precipitation water.

The geographical and temporal life history of an individual may be determined by the stable isotope analysis of human tissues. The isotopic compositions of tissues are reflective of the environment in which tissue formation took place, with drinking water being the major contributor for hydrogen and oxygen stable isotopes. Human provenancing through the use of stable isotope analysis becomes possible with the understanding of the isotopic patterns of drinking water across a geographical area, as an isotopic gradient exists for precipitation water across the globe. The term “drinking water” is commonly used interchangeably with both “precipitation” and “tap water” and while the isotopic compositions of tap water is influenced by local precipitation water, the compositions are not necessarily identical. The isotopic distribution of tap water has been previously investigated for the United States, on a large geographical scale, showing the isotopic gradient across the country; however, no previous studies have investigated the isotopic distribution of tap waters across a single city at high resolution.

This study investigated the isotopic distribution of hydrogen and oxygen stable isotopes in tap waters across Metro Vancouver (MV), at a high geographical resolution, to determine whether the isotopic compositions of tap water can vary across a city and, secondarily, can be sourced. MV tap water is supplied by multiple sources with three major watersheds supplying the majority of MV and a complex system of 18 groundwater aquifers providing the remaining areas. Tap water samples were collected from across MV with the help of volunteers during a one-week period in the summer of 2013. Samples were collected in a 30mL airtight glass vial and were stored in a refrigerator until analysis. Triple Liquid Water Isotope Analyzer (TLWIA) was utilized for the isotopic analysis of tap water samples with an instrumental precision of $\pm 0.8\%$ for d^2H and $\pm 0.2\%$ for $d^{18}O$. The Online Isotopes in Precipitation Calculator was utilized for the estimation of isotopic values of local precipitation for MV.

The results revealed that $d^{18}O$ and d^2H in tap water varied across MV with a variation of 5.3‰ for $d^{18}O_{tap}$ and 29.3‰ for d^2H_{tap} . The isotopic compositions of MV tap water were unique to its water source, depending on the water distribution system supplied by multiple water sources, and differed from estimated local precipitation values. This finding has significant forensic importance as MV tap water can be sourced solely by its isotopic compositions and suggests the possibility of ultimately sourcing human values to specific areas within MV, which could aid in forensic investigations by increasing the precision of geolocating human remains. Thus, understanding the isotopic distribution of tap water across a geographical area with high geographical resolution is considered essential for the utilization of stable isotope analysis for human provenancing purposes. It also demonstrates the power of this method and similar studies for all North American cities are recommended.

Stable Isotopes, Tap Water, Human Geolocation



E63 It's Not Over at the Death

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After attending this presentation, attendees will better understand how the United States Army Criminal Investigation Command (CID) Special Agents-in-Charge (SACs) brief family members and the challenges associated with conducting the briefings. This presentation will help make attendees aware of the policies and procedures of conducting casualty liaison briefings based on three case studies which identify the results of casualty briefings to the primary and secondary Next of Kin (NOK).

This presentation will impact the forensic science community by presenting an understanding that once an individual has died, it does not mean the death is over for the family. Understanding and the time it takes to understand the death of the loved one may take years.

In every Army CID office there is a SAC. The SAC is identified as the Casualty Liaison Officer and responsible for contacting and briefing the primary and/or secondary NOK five working days after the official initial notification. After the initial contact is made, the SAC will then contact the NOK every 30 calendar days. The SAC will brief the NOK on the status, any significant changes, or developments in the investigations.

Case Study 1: In 2003, a soldier completed a patrol in Iraq and upon returning to base became agitated with members of his unit. The soldier went to his sleeping area; later the supervisor and others heard a single shot. A thorough investigation, a full autopsy, and a toxicology examination were completed. The death was listed as a suicide by a single gunshot wound to the head. Policies for the CID were different in 2003. The soldier's mother requested information through her congressman, questioning the death investigation. This situation continued for several years as the mother did not accept how her son had died. In 2010, an agent met face-to-face with the mother. At the end of the briefing, the mother indicated she believed her son had committed suicide.

Case Study 2: In 2007, a female soldier with an intra-oral gunshot wound to the head was discovered deceased in an abandoned tent. Days before her death, she was diagnosed with a sexually transmitted disease and discovered her boyfriend no longer wanted to be with her. She gave away several personal items and destroyed others. A thorough investigation, a full autopsy, and a toxicology examination were completed. There were multiple reviews of the investigation by outside agencies and it was determined that the case had been thoroughly investigated. Multiple briefings were given to the family and to congressmen. To this day, her father does not believe his daughter committed suicide and posts on the internet how he believes she was murdered.

Case Study 3: In 2011, a soldier was dating a married woman. When the married woman decided to reconcile with her husband and work on her marriage, the soldier, prior to his death, sent her a text message with a photograph depicting all the medications he was prescribed. A thorough investigation, a full autopsy, and a toxicology examination were completed. The death was listed as suicide by multiple drug toxicity. The investigation revealed multiple searches on his computer regarding how to commit suicide with the drugs he was prescribed and with various over-the-counter drugs. He left a note with suicidal ideations; the laboratory examinations determined the note was in his handwriting and his fingerprints were discovered on the note. He also left a message on his married girlfriend's cell phone. His parents were briefed every 30 days. Approximately nine months after their son's death, the mother still had questions and contacted the CID. The investigation was re-opened to clarify the questions and concerns the mother had. A psychological autopsy was conducted and multiple coordinations were conducted. The investigation was still determined to be a suicide and the mother still questions the results.

As documented in the first case study, the mother indicated that she was not ready for the results of the investigations. As most people realize, there are different stages of grief and everyone processes their grief in a different manner. The CID will answer the family members' questions for as long as they have questions and will use every resource available to assist family members. As long as human nature is involved, there will be questions.

Investigation, Death, Briefing



E64 Detection of Residual Metal on Bone From Bullet Hole Periphery Using Digital Radiography

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After attending this presentation, attendees will be familiar with: (1) examining bullet holes in sections of bone using a NOMAD™ X-ray device; (2) reviewing radiographs for the presence of residual metal on bone; and, (3) some of the factors that affect the transfer of metal from the bullet to the periphery of the bullet hole.

This presentation will impact the forensic science community by describing a non-destructive method for identifying possible bullet holes in bone by detecting residual metal around the bullet hole periphery.

Portable X-ray equipment may be used at the scene to examine suspected bullet holes on skeletal remains or fragmented skeletal elements for the presence of residual metal. The examination is not destructive and may provide investigators with preliminary information while the skeletal remains are more thoroughly examined.¹

In this study, sections of bovine bone were cut to 10cm to 18cm in length, then cut longitudinally and used as a substrate for producing bullet holes. The sections of bone were taken to the firing range and shot at a muzzle-to-target distance of approximately one meter with a variety of firearms and ammunition. Four types of firearms were used to produce the bullet holes including: a Smith & Wesson® model 686, a Smith & Wesson® model 5906, a Smith & Wesson® model 617, and a Beretta® model 950. Fifteen brands of ammunition were used which consisted of: eight .22 caliber brands, one .25 caliber brand, three .38 caliber brands, and three 9mm brands. The bullet styles from these brands included: Lead Round Nose (LRN), Hollow Point (HP), Full Metal Jacket (FMJ), and Semi-Jacketed Hollow Point (SJHP).

Eighteen gunshots produced bullet holes. One .22 caliber bullet grazed the bone without producing a bullet hole and the round lead shot from a .38 caliber shot shell produced indentions in the bone without producing a bullet hole. The grazed bullet and the shot from the shot shell both left contact marks on the bone that were gray in color. Some bone samples shattered into multiple pieces and were assembled with the use of shipping tape before making radiographs.

After assembling the bone fragments, radiographs of the bone sections were obtained with the use of a NOMAD™ X-ray device and digital sensor. The NOMAD™ is a hand-held X-ray device powered by a 14.4-volt battery. It has been tested for safety and is approved for use in North Carolina.^{2,3} When the device is energized, the output power for generating X-rays is constant at 2.3mA at 60kVp±10%. The exposure is controlled by adjusting the time on the device. The NOMAD™ has an exposure range of 0.01–0.99sec.⁴ Average exposure time used to obtain radiographs of the bone with bullet holes was 100 milliseconds.

In conclusion, 18 of the 20 bone samples had bullet holes. One bone sample had shot impressions .140 inches in diameter from the .38 caliber shot shell and one sample was grazed with a .22 caliber bullet, both leaving gray marks on the bone. From the 20 bone radiographs examined, 16 (80%) had residual metal on the bone. Of the 18 bullet holes, 15 had metal in the bullet hole periphery. Metal was detected in only one out of five bullet holes when full metal jacketed bullets were fired. Not all bullets transfer metal to bone; however, when metal is transferred, digital radiography is useful in detecting the transfer.

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Forensic Science, Bullet Hole, Digital Radiography

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E65 Natural Causes of Death in Young Adults in an Urban Medical Examiner's Office

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After attending this presentation, attendees will better understand the incidence and causes of natural deaths in young adults (18-40 years old).

This presentation will impact the forensic science community by providing an overview of the causes of natural deaths in young adults (18-40 years old) in an urban medical examiner's office in Cook County, IL, providing insight into the causes of sudden, unexpected death in this population so risk factors and prevention strategies can be assessed.

In young adults (18-40 years old), natural deaths are less common than accidents, homicide, and suicide. Natural deaths follow a bimodal distribution across the lifespan, peaking in infancy and in the elderly. Sudden, unexpected death in young adults is a significant problem and determining the cause of death in such cases can be challenging. This study was conducted to identify causes of sudden, unexpected death in young adults.

A search of the database of the Cook County Medical Examiner's Office was performed to identify all deaths in people between the ages of 18 and 40 years of age during the years 2011-2013. Within this group, all cases in which the manner of death was listed as "natural" were identified. To focus on cases of sudden, unexpected death in young adults, this study excluded cases with known anatomic causes of death, such as cancer, infections, and asthma that were not due to cardiovascular disease.

Natural deaths accounted for 20% of the cases (775 out of a total of 3,957) in this age range. Of the 775 natural deaths in young adults, 453 (58%) were either cardiovascular-related or had no anatomic cause of death. In the group of 453 decedents, the average age was 31 years, with approximately twice as many males as females. Causes of death were divided into general categories. The most common causes of death were cardiovascular (64.7%), neurological (9.7%), chronic complications of drug or alcohol use (8.2%), metabolic (2.7%), autoimmune (1.1%), genetic (0.4%), and psychiatric (0.2%). Suspected cardiac arrhythmia or unknown natural causes accounted for 13.0% of the cases. Among cardiovascular diseases (n=308), the most common causes of death were hypertensive cardiovascular disease (30%), cardiomyopathy (28%), and atherosclerotic heart disease (17%). Obesity was listed as a contributing cause of death in 24% of the cardiovascular-related deaths. Among neurological diseases (n=48), the most common cause of death was seizure disorder (83%).

Combined cardiovascular and neurological causes of death accounted for 74% of the total natural deaths in young adults. Aside from seizure disorders, cardiovascular-related deaths constituted the majority of cases of sudden death in young adults (18-40 years). This study illustrates that chronic conditions, such as hypertension, coronary artery disease, and obesity, are significant causes of sudden, unexpected deaths in young adults and should serve as targets of prevention and risk reduction strategies in this population.

Sudden Death, Unexpected Death, Young Adults



E66 Body-Found-in-Bathtub Death Investigation

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After attending this presentation, attendees will be able to: (1) explain the investigative challenges that are specific to Body-Found-in-Bathtub (BFIB) cases; (2) offer solutions to overcome them; (3) ask critical questions that are frequently overlooked; (4) document and collect evidence that is often unrecognized in BFIB cases; and, (5) use field-tested, investigative protocols and forms which have proven to be effective during a number of cases, including eight BFIB homicide investigations.

This presentation will impact the forensic science community by demonstrating the importance of applying a standardized scientific approach to the investigation of BFIB deaths and by providing law enforcement, death investigators, forensic pathologists, and prosecutors with proven, field-tested protocols to investigate, diagnose, determine, and, if necessary, prosecute BFIB cases.

BFIB cases, which can involve bathtubs, whirlpools, spas, hot tubs, and similar bathing arenas, pose challenges when it comes to recognizing signs of foul play; this is evidenced by the large percentage of BFIB cases in which the manner of death is initially ruled as an accident but, after subsequent investigation, is changed to homicide. These subsequent investigations may not occur until months or years later, when another similar death occurs, life insurance companies raise concerns, or other indices of suspicion emerge.

Initial misdiagnosis in the majority of cases occurs as a result of faulty assumptions made at the scene. Examples include: “cause of death is drowning;” “manner of death is accidental;” “empty pill bottles, so it must be a suicide;” “it makes sense for the decedent to be in a bathtub;” “the toddler drowned due to lack of supervision;” and “a head laceration indicates a slip-and-fall death.”

These assumptions, coupled with the absence of specific BFIB training, can result in an inadequate investigation that misses red flags.

When only benign circumstances are reported in the primary investigation, forensic pathologists may perform a standard autopsy or only an external examination as they lack justification to perform an autopsy more appropriate for a suspicious death. Routine examination may overlook crucial signs of injury, such as posterior neck and back subcutaneous contusions, and prevent investigators and pathologists from spotting incongruities between documented injuries and scene and witness statements.

When the first signs of foul play are recognized at autopsy, such as signs of strangulation, critical scene and witness information may be lost. Sometimes insufficient investigations clear the way to cremations with an irrevocable loss of information.

Drowning as cause-of-death is a diagnosis of exclusion. Preliminary assumptions that a drowning occurred due to intoxication may be disproved days or weeks later, when toxicology results show levels too low for incapacitation. Conversely, positive toxicology findings may erroneously support assumptions of an accidental or suicidal manner-of-death made by investigators who fail to consider that perpetrators may use substances to incapacitate their victims and conceal a homicide. In these instances as well, too much time may elapse before a subsequent investigation, making it difficult to secure scene and witness evidence.

This presentation will include an informative study of more than 300 BFIB cases, including 27 BFIB homicides in at least six states. It will give examples of critical documentation often omitted during scene investigation including: the decedent’s airway position in relation to the water; the state of hand and foot wrinkling; eye-drying artifacts; the degree of witness and bathroom wetness; all evidence of trauma; any evidence of trauma suffered by witnesses; the decedent’s normal bathing habits or lack thereof; water depth and temperature; detailed position and posture information of the decedent when first discovered; and a detailed description of how the decedent was removed from the bathtub.

The presentation will also discuss the importance of performing reconstructions for red-flagged cases to help determine the legitimacy of witness statements; reconstructions have been used in several cases to disprove deceptive witness statements. Lastly, protocols for evidence collection and documentation specific to BFIB cases will be addressed.

Drowning, Bathtub, Homicide



E67 Organization of Scientific Area Committees (OSAC) Forensic Science Standards Activities: Helping Each Other and Stimulating the Future

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After attending this presentation, attendees will understand many of the essential requirements for developing consensus-based forensic science standards and how the Organization of Scientific Area Committees (OSAC), made up of approximately 600 subject-matter experts, addresses these requirements to support collaboration and stimulate the future.

This presentation will impact the forensic science community by educating attendees on the standards development process, the current status of the OSAC, and instructions on how individuals can become involved with the OSAC and other standards-development efforts.

The development of a quality infrastructure for forensic science was a key component of some of the reforms anticipated in the 2009 National Academy of Sciences (NAS) Report, *Strengthening Forensic Science in the United States: A Path Forward*. In response to the Report, the National Institute of Standards and Technology (NIST) and the United States Department of Justice signed a bilateral agency Memorandum of Understanding (MOU) in March 2013 which specified the establishment of “Guidance Groups” now termed Scientific Area Committees (SACs). NIST created the OSAC model to promulgate NIST’s responsibility to administer and coordinate support for the SACs and subcommittees that represent specific forensic science disciplines.

NIST envisions uniform administration of the identification, development, promulgation, and adoption of standards through the OSAC as well as supporting communication flow between the SACs and the forensic science community. The design employs the essential requirements of developing consensus-based standards which include openness, transparency, balance of interest, due process, and an appeals process that ensures each stakeholder’s viewpoints are properly considered. In addition, the OSAC infrastructure will bring a uniform standards recognition platform to the community, enhance scientific rigor, and increase communication among forensic scientists, research scientists, academicians, statisticians, attorneys, managers, and quality assurance specialists. The OSAC structure currently consists of a Forensic Science Standards Board, three resource committees, five scientific area committees, and 23 subcommittees.

Standards, OSAC, Consensus



E68 A Step Toward Likelihood Ratios in Pattern Recognition Disciplines

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After attending this presentation, attendees will understand how to represent the value of evidence in the form of a likelihood ratio addressing the issue of interest to the court. Attendees will also recognize the important role of probabilities for representing and combining information and uncertainty.

This presentation will impact the forensic science community by providing the attendees with the fundamental principles for interpreting pattern recognition evidence in a logical framework. It will highlight the importance of probabilities in this endeavor. Application of these principles allow the forensic scientist to assign the value of evidence in a transparent and scientific manner.

The role of a forensic scientist is to present the value of scientific observations and analytical results to the court. For this value to be scientifically sound, it should not only be based on scientific knowledge, but also be transparent in how it depends on this knowledge. Hence, subjective judgments and opinions may fulfill the first requirement, but it is difficult for them to fulfill the second. On this second aspect, probabilities provide a framework that allows the scientist to combine different pieces of information in a transparent way.

This presentation explains the fundamental principles of the logical approach to assigning a value to forensic findings in the context of a case. The focus is on pattern-recognition disciplines, yet the basic principles apply to all types of evidence. From a logical perspective, the value of the observed scientific results is determined by the ratio of two probabilities: (1) the probability of the observed results given the prosecution's (or the plaintiff's) position; and, (2) the probability of the observed results given the defense's (or the defendant's) position. This ratio forms what is commonly known as the likelihood ratio. In pattern recognition disciplines, the first probability (i.e., the numerator in the ratio) represents the intra-variability; that is, how the observed features vary if they were produced by the same source, and the second (i.e., the denominator in the ratio) the inter-variability; that is, how the observed features vary if they were produced by different sources in the relevant population of potential sources.

In this presentation, examples will illustrate the basic principles of how to combine numerous pieces of information to assign these probabilities and thus measure the weight of the forensic scientist's findings with regard to the issue of interest to the court. These examples will cover how to combine the value of general characteristics of the observations (i.e., the general pattern of a shoemark or the overall ridge flow of a fingerprint) with the value of specific features (i.e., observations indicating cuts or wear on a shoemark or observed minutiae and pores on a fingerprint), how to choose the most appropriate database to help inform what numerical values to assign to the required probabilities, and how to recognize what additional information is required to assign a probability, and therefore a likelihood ratio, in a particular case. These examples illustrate how a probabilistic framework helps the forensic scientist gather, organize, and present the relevant scientific knowledge in a likelihood ratio addressing the question of interest to the court.

Probability, Likelihood Ratio, Weight of Evidence



E69 Reliability, Validity, Accuracy, and Bias in Forensic Document Examination: An Interdisciplinary Approach to Understanding Forensic Decision-Making Processes and Outcomes

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After attending this presentation, attendees will understand the application of some principles of psychology, the use of eye-tracking technology to study decision-making processes in Forensic Document Examination (FDE), and the potential for this research paradigm to inform other forensic fields.

This presentation will impact the forensic science community by demonstrating the importance of engaging in theoretically based, multidisciplinary research to achieve an understanding of the nature of the methodology and expertise in forensic examinations.

A substantial portion of FDE training is devoted to handwriting and hand printing comparisons. During these comparisons, examiners seek to identify features and characteristics which may be characterized as identifying attributes. The identification — comparison — decision process is common across many forensic areas; examiners first determine the presence or absence of features, then qualitatively assign these features some degree of evidentiary weight to reach their decisions. Examiners are trained to look for both substantial similarities and differences among specimens. The number and quality of these features allow examiners to make assertions about the source of the specimens and the extent of their confidence in their decisions.¹

A substantial body of research addresses the cognitive mechanisms involved in attention and visual search. Many current theories of attention propose that attention is based on the relationship between a bottom-up, saliency-based attentional system and a top-down, feature-specific selection mechanism. Attention is guided by relational information about the target or information about how the irrelevant information of a non-target differs from the features of the target. Relational models of visual search demonstrate that visual attention can be guided by: attending to specific feature values such as color, size, or intensity; inhibiting attention to irrelevant features; or directing attention to how stimuli differ. Relational models place the target in relationship to its context, offering more specific (e.g., directional) information about differences. This relational aspect of attention may be influenced by the presentation formats of stimuli.²

Tversky pointed out that most stimuli seem to be effectively described by the presence or absence of qualitative features.³ He and others argued that an object is represented by a set of features or attributes, and that judgments of similarity are achieved through a process of feature matching. Tversky's "Contrast Model" systematizes this "feature" approach and proposes that similarity depends on the proportion of features common to the two objects and also on their unique features. Feature matching occurs by establishing differences in quality or quantity, such as differences in color or size, or the presence or absence of the features upon which the judgment is based, usually in terms of binary variables. This feature-matching process, along with the deployment of attentional resources, is a core process of forensic document examination.

This presentation discusses the application of psychological theories and methods to understanding the nature of attention, feature extraction and weighting, and decision-making in forensic document examination, and discusses the application of this research paradigm to other forensic areas.

Data from a national study of forensic document examiners (supported by Award No. 2010-DN-BX-K271, awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice) will be used to illustrate the ways in which cognitive psychology contributed to an understanding of the decision-making processes of experts in the field compared to those of lay people.



General Section - 2015

References:

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 2. Becker, S. I. (2008a). Can intertrial effects of features and dimensions be explained by a single theory? *Journal of Experimental Psychology: Human Perception and Performance*, 34, 1417–1440.
 3. Tversky, A. (1977). Features of similarity. *Psychological Review*, 84, 327-352.
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Feature Matching, Attention, Interdisciplinary Research



E70 Scientific Area Committee for Physics and Pattern Evidence

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After attending this presentation, attendees will have gained an understanding of the Scientific Area Committee (SAC) for Physics and Pattern Evidence, which includes the disciplines of friction ridge, firearms and tool marks, questioned documents, footwear and tire tread, and blood stain pattern analysis.

This presentation will impact the forensic science community by educating attendees on the standards development process and the role of the SAC for Physics and Pattern Evidence within that process.

The Physics/Pattern SAC will provide direction and oversight for the five subcommittees; interface with the resource committees (Human Factors, Legal Resource, and Quality Infrastructure); communicate activities, progress, and recommendations; review, facilitate public comment, and approve standards and guidelines; and coordinate research priorities.

The subcommittees that address each of these five disciplines will include subject matter experts who will develop and vet standards and guidelines regarding that discipline's techniques, protocols, validation of new techniques, test methods and materials, terminology, and training; will define requirements for accreditation and certification; will develop research priorities; and will coordinate the transition of existing Scientific Working Group (SWG) documents into approved standards or guidelines. These subcommittees nominally correspond to the current scientific working groups Scientific Working Group on Friction Ridge Analysis, Study, and Technology (SWGFAST), Scientific Working Group for Firearms and Tool Marks (SWGGUN), Scientific Working Group for Forensic Document Examination (SWGDOC), Scientific Working Group for Shoeprint and Tire Tread Evidence (SWGTREAD), and Scientific Working Group on Bloodstain Pattern Analysis (SWGSTAIN).

The intent of the Physics/Pattern SAC and subcommittees is to foster the development of rigorous standards and guidelines within and across these disciplines, to assist in the adoption and enforcement of these standards and guidelines, and to encourage research and evaluation to test and validate these methods. The ultimate purpose is to enhance the actual and perceived rigor of these disciplines through transparent, accurate, and reliable processes.

Standards, OSAC, SAC



E71 Objective and Quantifiable Metrics for the Determination of Latent Print “Suitability”

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After attending this presentation, attendees will: (1) be introduced to a novel technology, developed by the Defense Forensic Science Center, capable of measuring relevant image quality metrics of fingerprints; (2) understand the association of fingerprint image quality metrics and examiner performance during comparison exercises; and, (3) understand how this approach can be utilized to standardize “suitability” decisions and enhance quality assurance systems in fingerprint units nationwide.

This presentation will impact the forensic science community by introducing a more objective approach for determining “value” or “suitability” of latent fingerprint images.

Latent print examiners are routinely faced with the challenge of making determinations of “value” or “suitability” during the analysis phase of latent print examination. In the United States, there is no formal criterion for basing “suitability” determinations other than the subjective opinion of the examiner. Analyses of latent print images and the determinations of “suitability” consist of two major process steps, both of which are undertaken by the analyst without advanced instrumentation: (1) the visual analysis of the fingerprint image, assessing clarity, contrast, acutance, and other relevant image quality metrics associated with friction skin impressions in an effort to visually detect relevant features which may be used for comparison; and, (2) the consideration of the significance and reliability of that data detected for subsequent comparison and determination of “identification” or “exclusion” to a particular source.

Being a subjective process, determinations of “suitability” are susceptible to intra- and inter-analyst variations — especially for those “borderline” impressions having limited or lower quality detail. In an effort to minimize such variations and provide objective, quantifiable criterion upon which to base suitability determinations, novel fingerprint image quality assessment software has been developed to analyze the quality of fingerprint images and associate quality scores to analyst performance during comparison exercises. Such information will provide a more robust and standardized framework for dealing with “suitability” determinations, which is rooted in empirically derived data versus the vaguely defined and subjective approach of examiner opinion. Having such capability not only provides objective metrics to support analysts’ “suitability” decisions, but also provides a means by which laboratories may monitor training progress and assess performance of latent print personnel. The results of preliminary evaluations and policy guidelines which may be developed from these data will be presented along with the potential for transferring this type of technology approach to other pattern evidence disciplines.

The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the United States Department of the Army, United States Department of Defense, or United States Department of Justice.

Latent Prints, Suitability, Sufficiency



E72 Cognitive Profiling of Latent Fingerprint Examiners

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After attending this presentation, attendees will understand the background research that underpins the development of this cognitive profile for fingerprint examiners.

This presentation will impact the forensic science community by showing the forensic community how to identify applicants that have the aptitudes, experience, and skills needed to succeed in latent print examination.

Experts are characterized by specific and special abilities. Cognitive abilities are the mental skills necessary to accomplish a particular task. The accumulation of these specific cognitive abilities represents the general cognitive profile or “skill toolbox” which is needed to successfully perform the tasks required in a specific expert domain. In some cases, determining which cognitive abilities are important for a profession appears to be common sense. Forensic laboratory managers need to know whether applicants have the aptitudes, experience, and skills needed to succeed in latent print examination. For example, the ability to accurately detect sometimes small similarities or differences between two visual images appears to be important to the task of latent fingerprint examination. Therefore, examiners who possess the ability to make visual comparisons consistently and efficiently will be able to perform their job; however, “visual comparison abilities” is very broad and general, and vague from a cognitive perspective. Furthermore, as with all expertise, not all cognitive abilities are as obvious and even the expert may not be able to articulate all the mental skills that are important for the role. For example, professional racing drivers may not be able to articulate precisely how they know when to apply the brakes when approaching a corner; it just “feels right.” This, sometimes, is the nature of expertise. Correct characterization and quantification of these cognitive profiles enable better selection and screening at recruitment, and provide a very cost-effective tool for management. They can also be used as benchmark indicators and to test for job performance potential and effectiveness. In addition, such cognitive profiles provide clear targets for skill development.

Cognitive profiling has been used in several domains including military pilots and security X-ray screeners. To help in the selection of latent print examiners, the National Institute of Standards and Technology (NIST) in collaboration with the Federal Bureau of Investigation (FBI) supported the development of a test that quantifies the cognitive processes that underpin fingerprint examinations. The details of the abilities needed for forensic latent print examination is described at www.testcogpro.com. In order to develop suitable tools to measure cognitive abilities, professionals must better understand and define the nature and critical elements of expertise. This can be done through interviews, detailed task analysis, and scientific experimentation. This presentation will provide an overview of the background research that underpins the development of this cognitive profile for fingerprint examiners. The presentation will also provide a demonstration of the test instruments constructed to measure an individual’s level of aptitude for these skills.

Cognitive Profile, Special Abilities, Latent Prints



E73 Tips for the Courtroom: How to Get the Most Out of the Expert Witness

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After attending this presentation, attendees will be better prepared to select the expert witness to assist and advise them as legal counsel for their clients' cases. Attendees will learn from an experienced judge and practicing forensic pathologist, the value of the expert witness to provide technical advice to legal counsel concerning the facts and theories to be anticipated from the opposing counsel. After discussing the case with an expert, counsel will be better able to construct effective pleadings and focus counsel's attention on the relevant issues in the case. Attendees will also learn ways to locate and select the most qualified experts available. The presenters will also discuss the value of preparing the expert rather than coaching the expert so that the expert is testifying objectively and truthfully.

This presentation will impact the forensic science community by equipping lawyers to work with the expert witnesses. Lawyers will become aware of the value of the multifaceted role that an expert plays by contributing vital input in the initial stages of their cases and the importance of selecting the best expert to address the main issues in the case. Lawyers will learn of the ethical responsibilities to provide objective and unbiased testimony to the court regardless of affiliation or remuneration.

Attorneys should select and obtain experts in the early stages of the litigation process in order to reserve the best expert for the specific case and so that the expert can assist and advise counsel as to major issues of the case as soon as possible. Professional organizations may be excellent sources for obtaining qualified experts; however, counsel must proceed with caution and evaluate the requirements for membership in those organizations. Attorneys should carefully evaluate each expert's credentials such as education, professional licenses, academic and teaching appointments, publications, experience in their field of study, previous expert testimony, professional disciplinary actions, criminal record, any relationship to the parties, and any financial interest in the outcome of the case.

The scientific expert witness has a role as an educator both for the attorney's education as well as to assist the jury in understanding the scientific issues of the case. To be effective, this should occur during preparation for trial and not in the courtroom. It is difficult to test expert testimony at trial without advance notice and preparation. The courtroom is where the attorney, with the expert's help, educates the jury, not where the attorney encounters difficult scientific questions for the first time. The experts should explain facts based on information and knowledge available without entering into an advocacy role. Experts educate attorneys about their field of technical or professional expertise involved in the case.

In this presentation, forensic pathology is the scientific field used to illustrate best practices in preparing for the courtroom and selecting and effectively utilizing the expert. Experts may have limitations due to their field of expertise. Forensic pathologists, for example, may have possible limitations on their ability to be experts such as when they are estimating time-of-death, questions related to toxicology, or other related scientific fields. Experts should not be encouraged to stray outside their field of expertise.

Attorneys should also consider the importance of the demeanor of the expert, such as the expert's professional image, ability to communicate effectively to the jurors, skill and effectiveness with demonstrative evidence, ease in a courtroom environment, and the ability to not appear too relaxed so as not to project disrespect or a "hired gun" image. The expert should be polite and candid as well as appearing confident in responses and displaying patience and competence. The expert cannot speculate. Both attorneys and experts should avoid mispronouncing medical and legal terms. Attorneys should discuss with their experts the legal meanings of such terms as *reasonable medical certainty*, *beyond a reasonable doubt*, and *preponderance of the evidence*.

Attorneys should provide all facts of the case to their expert to avoid "ambush" by the opposing counsel. Experts enable counsel to be able to pose the critical questions to opposing experts in confronting contested issues of a particular case. A well-prepared expert is also better able to perform in the courtroom since he/she can analyze and organize the facts in a way that jurors can comprehend. Counsel should conduct a simulated cross-examination to optimally prepare the expert witness. Counsel should explain anticipated trial procedures, schedules, as well as trial technique and strategies to get the most out of the expert.

Expert Witness, Courtroom Preparation, Medical Testimony



F1 A Study of More Than 400 Case Reports Between 2009 and 2014, Completed in the Venice Surveillance Court to Understand, Evaluate, and Investigate the Social Hazards on the Judicial Level of Subjects Convicted of a Crime

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After attending this presentation, attendees will be able to distinguish what legal and scientific bases in Italy form the concept of social danger from a criminal (Article 203 of the Italian Penal Code: a dangerous person is one who, even if healthy or incompetent, has committed crimes and is likely to commit new crimes) by citing an unpublished study of 400 case-specific reports from 2009 through 2014 belonging to the largest surveillance court for social dangers in northern Italy: the Court of Venice.

This presentation will impact the forensic science community by informing attendees, through the cases studied, which scientific parameters (neuroscience and behavior) and jurisprudence present the criminal's personality and how the Tribunal of Venice works to understand, assess, and contain the danger presented by the offender.

From this study arises a new vision of social danger determined by a new protocol on the individual criminal by combining the results obtained on the progress of neurosciences with the personalities, the environment, and the history of the offender through the cases studied. In Italy, it is the Surveillance Court judge who acts on those convicted after the sentence is finalized and who decides on requests for alternative punishments to prison such as home detention, early release, and provisional custody to social services. It includes statistics on how a judge in Italy makes a prognosis as to the danger to society, both in terms of the risk of re-offending as well as the personality of the suspect. In this study, the alternative punishment requests have been correlated (probation with the social service, home detention, and parole) and the concept of "social danger" by an analysis covering a sample of people, mostly men of Italian and foreign nationalities. The average age is between 30 and 65 years old.

From the Court of Venice results, it is possible to say that the social danger, in cases of rejected requests for alternative measures to prison, is manifested by a complete absence of reflection on the part of the convicted person in regard to their crimes and behavior prior to the offense as well as lack of participation in social activities, violent conduct committed in prison demonstrating the presence of violent and aggressive personalities in some prisoners. The case studies seem to confirm the importance that the environment exerts on the criminal and this is also demonstrated in both neuroscientific studies and criminogenesis made on humans from a criminal anthropological perspective. In fact, the gene pool can be modified for "random variations" from which mutations can derive, "random changes" (genetic drift), and migration influx; therefore, mutations can be beneficial or detrimental and selection will favor the human ability to adapt and as such has influenced evolution of the adaptive formation of new alleles. In genetics, the study of some alleles has led to the understanding that there is a relationship between genetics and crimes through the examination of some neurotransmitters such as monoamines, including serotonin and dopamine, whose biological effects are regulated by enzymes such as Monoamine Oxidase A (MAOA), (MAOA-L allele), and Catechol-O-Methyl Transferase (COMT). These two alleles have determined a reduction of the penalty in two trials in Italy.¹ Serotonin regulates impulsive-aggressive behavior and its alterations are at the base of the aggressiveness; these alleles (including allele-s) determine a lower capacity for adaptation and therefore generate aggressive behavior. In addition to these concepts, the personality of the offender must be taken into consideration. Indeed, the environment originates the character of the offender as a result of temperament (hereditary biological matrix) and the environment where the criminal lives or has lived (including social and family environments) and this is judged by an Italian judge. Another criteria concerns the biology and structure of the brain influenced by environment and evolution. Granting house arrest (in other words, semi-liberty) to criminals has proven to the Surveillance Court of Venice to be the best method to limit social danger.

Reference:

1. Trial of Corte d'assise di appello di Trieste n. 5/2009 and Trial of Como, GIP, date 05.20. 2011.

Social Dangerousness, Criminal Law, Neuroscience



F2 An Evaluation of a Crime During a Trial in View of Cesare Lombroso's Scientific Insights on the 180th Anniversary of His Birth: This Scientist's Ideas Representing the Scientific Roots of the Evaluation of a Criminal's Personality Contained in the Italian Penal Code

Vincenzo Lusa, JD, Via Ferdinando, Palasciano #72, Rome 00151, ITALY; and Annarita Franza, PhD, Via delle Oche 15, Florence, ITALY*

After attending this presentation, attendees will learn the scientific basis of articles in the Italian Criminal Code (Rocco Code, 1930) related to a criminal's personality, which originated from the Italian Positivist School (19th century) and from the ideas of Cesare Lombroso (1835-1909).

This presentation will impact the forensic science community by acquainting attendees with the introduction of Lombroso's insights into modern Italian criminal trials, which have led to the development of criminal jurisprudence related to neuroscience (e.g., the Bayout trial in Italy, 2010).

Lombroso's book *The Criminal Man* (1876) contains his insights, which are limited to the science of his times. A composite work, *The Criminal Man* collects nearly two decades of studies on criminals. These observations led Lombroso to the differential diagnosis among normal, insane, and criminal subjects (*Introduction to the Clinical Course on Mental Diseases*, 1863; *The Man of Genius*, 1864) and the formulation of an experimental research method in which criminal cases were included. For many years, Lombroso conducted research on the existence of somatic and cerebral brain abnormalities among criminals and the insane (*Some Cases of Lesions of the Central Nervous System*, 1861; *Supernumerary Cerebral Convolutions in a Murder and Satyrist*, 1871). After other contributions on such important cases of forensic psychiatry, like the Verzeni and Agnoletti murder trials (*Verzeni e Agnoletti studiati dal prof. Cesare Lombroso*, 1873) and the publication of *Anthropometry of 400 Venetian Criminals* (1872) and *Emotions and Passions of Criminals* (1874), Lombroso illustrated the correlation between somatic stigmata and mental deformities in reference to specific factors (atavism, epileptoidism). Lombroso saw illicit criminal behavior as an "organic fatality" that drew certain people to commit crimes. Following these ideas in the Italian penal code, articles were created to prevent criminal behavior through neutralization and control structures and by combining therapy with penal sanctions (e.g., Article 203, Italian Criminal Code).

Current research into the biological causes of crime has revealed such important legal and criminological aspects as genetics during trials and what is intended as a neurobiological explanation of criminal behavior. In fact, neuroimaging and brain imaging analyses (Computed Tomography (CT), Magnetic Resonance Imaging (MRI), **Positron Emission Tomography** (PET), and functional Magnetic Resonance Imaging (fMRI)) have shown reductions in the brain's structural and functional parts among subjects prone to violent behavior, leaving them unable to feel emotions and empathy, calling to mind Lombroso's morally insane. In fact, the necessity of assessing whether the criminal impulse is a possible adaptive response to the environment has been noted. The gene pool can be modified for "random variations" from which mutations can drift and "random changes" (genetic drift) migration influx. Indeed, mutations can be beneficial or detrimental with selection favoring the human ability to adapt and this has influenced evolution in the adaptive formation of new alleles. The offender's personality should also be added to these concepts. In fact, the offender's environment gives rise to his character as a result of the union between temperament (defined as the hereditary biological matrix), and the social/family context. As regards the environmental impact on one's body, epigenetics, which associates genes and environment, also regulates gene expression rather than the same gene's mutation. Another criterion concerns evolutionary influence on the brain's biology and structure. In genetics, the study of some alleles has enabled an understanding of the relationship between genetics and crime through the examination of such neurotransmitters as monoamines, including serotonin and dopamine, whose biological effects are regulated by enzymes: (e.g., Monoamine Oxidase (MAO) (MOA-L allele) and Catechol-O-Methyl Transferase (COMT)). Serotonin regulates impulsive-aggressive behavior and its alterations are the base of the aggressiveness; its alleles (including allele-s) determine a lower capacity for adaptation therefore generating aggressive behavior.

Because of Lombroso, the current Italian Criminal Code contains articles defining dangers to society (art. 203), attribution (art. 85), tendencies to commit a crime (art. 108), criminal recidivism (art. 99), and the habitual criminal offender (art. 102). Italian trial case reports related to Lombroso's observations have been used to illustrate all of this (Bayout trial in 2009, Albertani trial in 2011, and the Cremona trial in 2012).

Cesare Lombroso, Neurosciences, Criminal Law

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F3 **Can Parents Inherit the Sperm of the Deceased Son? Presentation of an Italian Case and Review of the European Legislation in the Field of Postmortem Fecundation**

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After attending this presentation, attendees will better understand European legislation in the field of postmortem fertilization and how the legal framework regarding the use of sperm after a donor's death is still controversial.

This presentation will impact the forensic science community by providing the current legal status of sperm and its right to be inherited by relatives in the case of a donor's death.

Introduction: A recent Italian case provided the opportunity for many considerations regarding postmortem fertilization and the use of sperm after the donor's death. Numerous questions should be answered; in particular, the legal concept of sperm, if it can be inherited, and who has the right to determine the semen's destination. May the donor, by written consent, relatives of the deceased, and/or other institutions, such as the sperm bank, be involved?

The Case: Prior to undergoing chemotherapy, a 20-year-old man affected by a lymphoma decided to preserve his gametes for future fatherhood in the sperm bank of a Roman hospital. Unfortunately, the man died shortly after and his parents asked for the return of his seminal fluid from the sperm bank where it was stored. The request was denied because the hospital stated that only the interested party may have his gametes returned and the right to restitution is not transmissible to heirs because the ownership of gametes is a personal non-transferable right. The parents therefore decided to pursue litigation. In January of 2012, the Ordinance of the Court of Rome decreed that the only limitation, which may arise in the recognition of the applicant's right to succeed their son in collecting the sample, is that the parents cannot use the seminal fluid for procreative purposes. This limit is not an impediment as long as it is possible, before delivery, to make the semen unsuitable for fertilization. The Court then gave an injunction ordering the hospital to prolong the cryopreservation. The final judgment of the Court of Rome on June 2013 did not agree with the motivations reported in the previous ordinance, stating that the right of restitution has to be independent from a possible illegal use, which will be banned only if it will occur. Finally, the Court concluded affirming that the most important right, which has to be protected, is the interest of the parents to have a relic of their son and therefore it ordered the restitution of the seminal fluid to the family.

Discussion and Conclusions: This case is an example of how, in Italy, the use of biological material intended for the use of procreation is a problem which is still far from being solved. Even in countries where postmortem fertilization is strictly prohibited, such as Italy, the legislative vacuum often leads to different, very questionable interpretations that sometimes can be in conflict with current legislation. The use of sperm after a donor's death remains a very controversial issue; among European Union states, only a few countries allow this practice for purposes of procreation, with different degrees of freedom (Belgium, Greece, Spain, and United Kingdom), whereas in the majority of the remaining European Union members, this practice is either strictly prohibited or, alternatively, there is no specific regulation in this field.

Postmortem Fecundation, Donor's Death, European Legislation



F4 Moderate Force Blunt Trauma to the Head Leading to Coma: The Role of the Medicolegal Expert in the Assessment of Attempted Murder Charges

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After attending this presentation, attendees will understand the numerous difficulties in establishing a causal link between moderate use of force by the accused and serious life-threatening consequences for the victim.

This presentation will impact the forensic science community by explaining the difficulty of some rare cases wherein the role of the forensic expert is crucial in establishing the nature of the prosecution.

In both civil and common law, a profound difference can be found between the accusation of personal injuries and attempted murder in terms of the significance of accusation and consequent punishment. In most jurisdictions, the sentence may vary from a few months to several years in prison. One of the various cases in which the forensic examiner plays a key role in law proceedings is when, on the basis of the fact analysis and the injuries sustained by the victim, he is called to express an opinion about the exact charge, of the accused on behalf of the court.

The case reported here occurred just outside a nightclub in the historic center of Rome, where a young man was attacked for petty reasons by four men of the same age. The victim received several blows to the face with a scooter helmet. When the man reached the hospital, the physicians diagnosed three compound fractures, one each to the left eye socket, the nasal bones, and the left jaw. The man also reported that he had experienced several episodes of headache and vertigo in the days prior to the assault. A magnetic resonance imaging of the brain was performed, revealing the presence of a tumor-like formation surrounded by a vast area of hemorrhage. Histological exams were performed and revealed that the aforesaid formation was actually a World Health Organization Class II oligodendroglioma. Guidelines indicate that surgery resection should be performed on such neo-formation, yet the rupture of the capillary vessels of the tumor itself constitute an impassable contraindication. The man was treated with a decompressing craniotomy as a consequence of the massive bleeding and compression. The patient fell into a coma and six months later was declared to be in a persistent vegetative state. As shown by the facts, a modest series of blows (although delivered with an improvised weapon) caused the victim to fall in a serious near-dead state. The main matter of inquiry for the medicolegal experts was the assessment of the causal link between the blow dealt by the assailants and the consequences suffered by the victim. As “per se,” the strike with an improvised weapon had only provoked some marginally important fractures, yet the hemorrhage caused by the rupture of the oligodendroglioma capillary vessels led to the coma. In this case, the medicolegal experts informed the judge that the assailants were to be charged with attempted murder. Even though the victim was already suffering from the brain tumor, his condition had deteriorated due to the criminal act and there had been a significant decline in his chances of healing.

Attempted Murder, Oligodendroglioma, Legal Medicine



Jurisprudence Section - 2015

F5 The Long Road to Exoneration for Han Tak Lee

John J. Lentini, BA, Scientific Fire Analysis, LLC, 88005 Overseas Highway, #10-134, Islamorada, FL 33036*

After attending this presentation, attendees will understand some of the major changes that have occurred in fire investigation over the last 30 years and will see how, in addition to the science of fire investigation having shifted, the definition of “new evidence” also appears to have shifted.

This presentation will impact the forensic science community by making known the significant scientific and legal changes that are sweeping through the fire investigation profession.

Han Tak Lee was convicted in 1990 of setting a fire that killed his 21-year-old daughter, Ji Yun Lee. Mr. Lee had taken his daughter to a Korean Baptist camp in Hebron, PA, because she was having serious mental health issues. He hoped that the demons could be “prayed away” from his daughter and an actual exorcism was performed.

On the evening of their arrival, after Ms. Lee stated, “This place is going to be my tomb,” a fire erupted in the cabin they were sharing. Mr. Lee was able to escape, but his daughter was not. She had only 9% carboxyhemoglobin in her blood at the time the reading was taken, erroneously as it turns out, as a sign that she had been murdered or placed “at death’s door,” rather than the now-accepted explanation that she was close to the origin of the fire. Investigators from the Pennsylvania State Police and a private consultant came to the scene and declared the cause of the fire to be arson. The private consultant estimated that there had been 662+ gallons of fuel oil and 12.2 pounds of gasoline, even though none of the debris samples were positive for any ignitable liquid residue.

Some ignitable liquid was found on Mr. Lee’s shirt and next to the filter for the kerosene-fired furnace. The state’s chemist, when asked if the ignitable liquid residues he detected on three samples were similar, evaded the question and stated, “They covered the same carbon number range.” The prosecutor then argued that the three ignitable liquid residues were identical and because Mr. Lee’s defense was that the daughter had set the fire, the prosecutor argued that somebody had mixed the accelerants together and a mentally ill girl was not smart enough to do that.

Over the next 21 years, Mr. Lee, who had been sentenced to life in prison, continued to serve his life sentence. After many back-and-forth trips to court, including a five-year hiatus during which the state simply ignored the case and refused to respond, the Third Circuit Court of Appeals ruled that the evidence must be given up for reanalysis. It turns out that the chromatography, which could have been examined back in the early 1990s when it was first requested had been “lost” by the district attorney, who had inexplicably taken custody of this data which would normally have been stored in a state archive. Testing of the samples in 2012 revealed significant differences among the three samples and undermined the argument that somebody had mixed up several gallons of accelerant prior to the fire.

The original fire investigation was replete with the types of errors that were common in those days, as well as some additional errors that defy understanding.

The final ruling, issued June 13, 2014, by Magistrate Judge Martin Carlson gives meaning to the term “poetic justice.”

Arson Mythology, Expert Errors, New Science



F6 Misinterpretation of Common Fire Behaviors and Its Effect on Criminal and Civil Litigation

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The goals of this presentation are to: (1) increase awareness of the propensity for misinterpretation of the effects of commonly encountered fire scenarios leading to improper conclusions as to fire origin; (2) demonstrate useful techniques to identify fire-spread behaviors; and, (3) expand awareness of frequent mistakes made in investigating common fire scenarios.

This presentation will impact the forensic science community by increasing awareness of misinterpretation of fire scene evidence which will reduce the likelihood of baseless litigation in both criminal and civil cases.

For more than 20 years, the fire investigation community has undergone a metamorphosis, transitioning from widespread adherence to an art-based investigative approach to a more science-based style. Historically, explanations offered to explain the creation of burn patterns were based less on scientific fact than on anecdotal beliefs.^{1,2} Guidance from documents such as the National Fire Protection Association (NFPA) 921, Guide to Fire and Explosion Investigation and other references along with training and certification programs have emphasized reliance on scientific principles and have nudged investigators along that path of change.³ Interim developments in federal case law also now require that fire investigation be based on solid scientific principles rather than novel, untested techniques.

In 2005, while presenting a seminar on fire dynamics, Special Agents from the United States Bureau of Alcohol, Tobacco, Firearms and Explosives (ATF) designed an exercise to evaluate the information relied upon by investigators in identifying fire origins. Two identical, bedroom-size burn cells were each burned for seven minutes, reaching full fire involvement. Fifty-three fire investigators who had not observed the fires briefly examined the cells to identify which quadrant of the cells they thought each fire had started. Upon evaluating the first cell, only three of 53 investigators (5.7%) correctly selected the quadrant of fire origin. The same percentage (although different investigators) correctly identified the quadrant-of-origin in the second cell.⁴

In similar exercises conducted over the previous 16 years at the Federal Law Enforcement Training Center in Georgia, only 8%-10% of students correctly located the origins of fully involved fires. Investigators not identifying the correct origins typically reported being misled by burn patterns generated during full involvement under ventilation-limited conditions. This was despite such conditions frequently occurring in structure fires.

Follow-on studies of ventilation-limited fires were conducted in places such as the ATF Fire Research Laboratory in Amundale, MD, and elsewhere. Computer analyses have been used to study such fire behaviors and to explain ventilation flows and the related fire spread. Examination of heat flux, temperature, and gas concentration data as well as the burn patterns have enabled better understanding of the various mechanisms involved. Transfer of such information to the fire investigation community, albeit a seemingly slow process at times, is crucial for improving the accuracy of fire origin determinations.⁵

Several regularly encountered fire scenarios have been shown to result in conditions often at odds with commonly accepted ideas. One such popular belief is that the area of worst fire destruction is indicative of the place the fire burned the longest. Post-flashover and elevated fires are examples of scenarios where this idea is frequently proven wrong. In each case, areas of extensive fire damage may be generated well apart from the origin and misinterpreted as being the actual origin. Techniques such as computer modeling are available to test hypotheses but their use remains limited.⁶⁻⁸

Examples of actual investigations will be discussed in which the behaviors of such fires were misinterpreted. In at least two instances, homicide charges were proposed in suspected arson fires later shown to have started on a stovetop. Incorrect fire cause determinations behind convictions that were later overturned will also be reviewed.



Jurisprudence Section - 2015

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Ventilation-Limited Fires, Fire Patterns, Origin Determinations



Jurisprudence Section - 2015

F7 A Perfect Storm Brewing for Fire Investigators in Court

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After attending this presentation, attendees will understand the significance of three forces that are converging into a perfect storm for fire investigators. Included will be an explanation of the hypothesis that the culmination of these three forces is resulting in a reinvigoration of challenges based on the Supreme Court decision in *Daubert v. Merrell Dow Pharmaceuticals Inc.* (or state equivalents), including heightened inquiry into the extent of subjectivity and bias by experts in fire cases.

This presentation will impact the forensic science community by providing information relevant to anyone involved with civil or criminal fire litigation. Extensive research has been conducted into cases across the United States and Canada where industry standards such as the National Fire Protection Association (NFPA) 921 are used in court to either support or challenge expert testimony. This research has revealed trends toward a closer scrutiny of the reliability of expert testimony and sufficiency of experts' qualifications. The detailed description of these trends (as supported by this presentation) provides attorneys, judges, and fire experts with a road map for ways to deal with fire experts in court. Even though the 2009 National Academy of Sciences (NAS) Report devoted but a short paragraph to fire investigations, attendees will learn why it is responsible for a huge impetus toward closer scrutiny of the qualifications and opinions of experts in fire cases. Together, the NAS Report, growing publicity about wrongful convictions in arson cases, and changes in industry standards for fire investigators are putting fire experts under a microscope. This presentation will help members of both the legal and the fire investigations communities to prepare for the perfect storm on the horizon that is being precipitated by these factors.

This presentation will review the three forces behind the big changes in the offing for fire investigators: (1) the ongoing movement by courts across America to scrutinize more closely the reliability of expert testimony under *Daubert* and its progeny; (2) a growing apprehension about wrongful convictions stemming from faulty forensic evidence and problems in fire investigations culminating in the revolutionary 2009 NAS Report, *Strengthening Forensic Science in the United States: A Path Forward*; and, (3) the continuing development of industry standards that are raising the bar for fire investigators, particularly revisions in the 2014 editions of NFPA 1033, *Standard for Professional Qualifications for Fire Investigator* and NFPA 921, *Guide for Fire and Explosion Investigations*.

This presentation will first explain the growing demand that experts be able to demonstrate the reliability of their opinions in court under *Daubert* and its state equivalents. Next, the problems caused by faulty forensic science, including weaknesses in fire investigations and the response by various sectors of American society to address these problems will be considered. The responses have come in the form of reports examining weaknesses in forensic science and fire investigations, together with recommendations to address these issues. The most significant of these reports are highlighted from the viewpoint of their importance to the fire investigation field.

Finally, this presentation will address the heart of this study's hypothesis and describe how these three forces are converging and causing a reinvigoration of *Daubert*. First to be considered is why *Daubert* and its progeny continue to have an influence in the coming of the perfect storm, even though investigators have had two decades to learn how to deal with *Daubert* challenges. The reasons why fire investigators have weathered the first part of the *Daubert* storm, but need to prepare for the next onslaught will be proposed. Further, an explanation of how the NAS Report and other forensic science reports are increasing pressure on investigators, thereby strengthening the potential of reliability inquiries under *Daubert* and its progeny will be presented. The session will conclude with recommendations for fire investigators planning to give expert testimony in court.

NFPA 921, NAS Report, Daubert



F8 Next-Gen Is Now: Legal Implications and Strategic Preparation for Massively Parallel DNA Sequencing in Forensic Science

Ted R. Hunt, JD, Jackson County Courthouse, 415 E 12th Street, Fl 7M, Kansas City, MO 64106*

After attending this presentation, attendees will understand the critical importance of multidisciplinary collaboration for the development and implementation of legal authorization and for making strategic preparations for Massively Parallel Sequencing (MPS) technologies.

This presentation will impact the forensic science community by explaining the legal and strategic challenges, opportunities, and potential threats to the forensic application of this cutting-edge technology and how these factors can be most effectively addressed through a coordinated interdisciplinary approach.

The forensic implementation of MPS has the potential to cause a sea of change in the criminal justice system's response to DNA evidence. This is because the technology has the capability to reveal an extraordinary constellation of genetic information about not only identity, but also the phenotypic characteristics, ancestry, lineage, and genetic predispositions of those associated with a criminal offense. It also has the potential to deconvolute currently indiscernible mixtures and detect genetic profiles from samples too degraded for fragment length-based detection methods.

It is precisely *because of* these new and distinctively different capabilities that the potential of MPS for criminal justice applications cannot be fully realized without the counsel and coordination of those both within and outside the forensic community. This includes attorneys, ethicists, policy makers, and industry leaders, among others. Importantly, this coordination must begin *now*, before critical choices are made about the forensic application of MPS that may ultimately prove to be legally indefensible or socially unacceptable.

From a legal perspective, statutory schemes that address constitutional concerns about law enforcement's collection, detection, retention, and comparison of sensitive genetic information must be drafted, debated, and enacted. This legislation must be adequate to withstand the heightened scrutiny that courts will surely apply to the Fourth Amendment "secondary search" analysis applicable to the detection and retention of the genetic data generated by MPS technologies.

State and federal legislation concerning the admissibility and use of DNA evidence in the legal system, which was largely drafted by law makers and interpreted by the courts in response to fragment length detection methods, must be reevaluated and revised to address the unique capabilities and concerns relevant to next generation sequencing technologies.

Existing legislation that prohibits the unauthorized dissemination and use of genetic information in government databases must also be considered and revised, as appropriate, to adequately address heightened ethical and privacy concerns applicable to this sensitive data.

From a strategic perspective, cases that cannot currently be solved with existing DNA technologies may well be cracked in the future by MPS analysis. As such, investigations now facing a forensic DNA impasse may be good candidates for future cold case review projects utilizing MPS. Therefore, America's crime laboratories must *currently* begin to systematically identify and document those forensic samples in their archives that are indiscernibly mixed or too degraded to yield a profile in response to contemporary testing technologies. This work must begin immediately to prevent future time-consuming and tedious retrospective searches for candidate MPS case samples. In other words, laboratories must stop throwing any more "needles" into the "haystack."

Crime laboratories, in consultation with law enforcement and prosecutors, must carefully weigh the risk of currently consuming challenging samples with present-day DNA technologies against the benefit of waiting to test that evidence with MPS-based systems. This dilemma is similar to the decisions labs faced in the past about whether to risk sample consumption with **Restriction Fragment Length Polymorphism** (RFLP) testing or wait for emerging Polymerase Chain Reaction (PCR) technologies.

Finally, evidence, case files, and third-party records from unsolved investigations that involve challenging forensic samples must be identified, properly preserved, and archived by crime labs, police agencies, and prosecutors. This will help ensure that these essential elements of a successful prosecution, a valid defense, or a meaningful post-conviction DNA analysis can be used to credibly support future judicial findings of guilt or innocence based on next generation technologies.

Massively Parallel Sequencing, Legal Implications, Strategic Preparation



Jurisprudence Section - 2015

F9 The National Commission on Forensic Science: Status Update

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After attending this presentation, attendees will better understand the activities of the National Commission on Forensic Science during the past year.

This presentation will impact the forensic science community by providing an update on the progress made by the National Commission on Forensic Science throughout its first year.

The development of a quality infrastructure for forensic science was a key component of some of the reforms anticipated in the 2009 National Academy of Sciences (NAS) Report entitled, *Strengthening Forensic Science in the United States: A Path Forward*. In response to the NAS Report, the National Institute of Standards and Technology (NIST) and the United States Department of Justice (DOJ) announced an interagency initiative to enhance forensic science which specified the establishment of a National Commission on Forensic Science (NCFS) and the development of “Guidance Groups” now termed Scientific Area Committees (SACs).

DOJ and NIST announced the membership in January 2014 and have since held five meetings in Washington, DC, (February 2014, May 2014, August 2014, October 2014, and January 2015). From more than 300 applicants, 37 individuals were selected to achieve a diversity of experiences, including: forensic science service providers; research scientists and academicians; prosecutors, defense attorneys, and judges; law enforcement; victim advocates; and other relevant stakeholders. The Commission is led by Co-Chairs Mr. James Cole, Deputy Attorney General, and Dr. Willie May, NIST Director and Under Secretary of Commerce for Standards and Technology. Nelson Santos, Deputy Assistant Administrator for the Office of Forensic Sciences at the Drug Enforcement Administration and Dr. John Butler, Special Assistant to the Director for Forensic Science, serve as the DOJ and NIST Vice-Chairs, respectively. The NCFS has seven subcommittees on the following issues: (1) Accreditation and Proficiency Testing; (2) Reporting and Testimony; (3) Scientific Inquiry and Research; (4) Training on Science and Law; (5) Interim Solutions; (6) Medicolegal Death Investigation; and, (7) Human Factors. All NCFS meetings are open to the public and materials are available at <http://www.justice.gov/ncfs>.

NIST developed the Organization of Scientific Area Committees (OSAC) to administer and coordinate support for the discipline-specific SACs (see <http://www.nist.gov/forensics/osac.cfm>). In September 2013, NIST issued a Notice of Inquiry (NOI) in the Federal Register to obtain national and international input on the establishment and structure of governance models. Eighty-two submissions were received in response to the NOI. The OSAC is designed to provide uniform administration for development, promulgation, and adoption of documentary standards in the forensic science community.

While NCFS is a DOJ advisory group to enact policies, OSAC will be an on-going community effort to improve forensic practices through developing documentary standards that can be used by accrediting bodies in future audits of forensic laboratories. This presentation will review progress made with NCFS. OSAC progress will be the subject of a separate presentation.

National Commission, NAS Report, Federal Advisory Committee



Jurisprudence Section - 2015

F10 **Leading a Horse to Water and Trying to Make Him Think: The Impact of the 2009 National Academy of Sciences Report (NAS) Report, *Strengthening Forensic Sciences in the United States: A Path Forward on America's Courts***

*Pamela A.W. King, JD**, 400 S Broadway, Ste 15, Rochester, MN 55904; and *Christine Funk, JD*, Department of Forensic Sciences, 401 E Street, SW, Washington, DC 20024

After attending this presentation, attendees will understand how the 2009 National Academy of Sciences (NAS) Report, *Strengthening Forensic Science in the United States: A Path Forward* has been received by the judiciary and has impacted the court system.

This presentation will impact the forensic science community by focusing on how cases around the country have addressed the NAS Report. It will describe how, in large part, the judiciary has found evidence to be admissible despite the concerns raised. This presentation will explore why the judiciary has been resistant to considering issues highlighted by the Report and why a more deliberate approach would be beneficial.

In February 2009, when the Committee on Identifying the Needs of the Forensic Science Community issued their report, *Strengthening Forensic Science in the United States: A Path Forward*, it raised serious concerns regarding the scientific underpinning of certain forensic disciplines, resulting in speculation about what impact this would have on forensic and legal communities.

The intent was “to chart an agenda for progress in the forensic science community and its scientific disciplines.”¹ Recommendations focused on reform to the forensic community, not the impact to the court system. How would the criminal justice system be impacted? One professor suggested, “The findings in the National Academy of Sciences report should spur judges to require higher standards.”²

Although not focused on legal reform, in March of 2009, the Committee Co-Chair said he expected, “courts [would] take the findings of the committee regarding the scientific foundation of particular types of forensic evidence into account when considering the admissibility of such evidence in a particular case.”³

In June, the United States Supreme Court cited this Report stating, “The forensic science system, encompassing both research and practice, has serious problems that can only be addressed by a national commitment to overhaul the current structure that supports the forensic science community in this country,” thus further suggesting that judicial review of the committee’s concerns might be warranted.⁴

As the Committee Co-Chair predicted, courts have been asked to address how the concerns raised by the NAS Report impact the admissibility of forensic evidence including fingerprints, ballistics, and hair comparison. Other cases addressed claims of ineffective assistance of counsel and newly discovered evidence.

Despite multiple challenges, case law suggests there has been little effect on how courts assess forensic evidence and written opinions tend to be cursory. Forensic evidence has rarely been deemed inadmissible. Courts have been reticent to engage in careful discussion of whether the concerns of the NAS Report are legitimate and, if so, what modifications to the admissibility of evidence are required to insure the integrity of the system. The changes the courts have made are usually in the form of limitations on the scope of expert testimony.

The court system has an important role, assuring the reliability and integrity of forensic science used in criminal cases that leads to the conviction of the guilty and the exoneration of the innocent. That is why the issues raised by the NAS affect the entire forensic family: defense attorneys, prosecutors, judges, and practicing scientists.

In 2010, the Committee Co-Chair said, “[T]here is still much more that can be done by members of the legal profession, bench and bar, within the existing legal framework, to insure that forensic evidence is properly assessed and admitted only when it will serve the ends of justice. If we insist on valid and reliable forensic methodologies and practices, and qualified practitioners, change will happen. And our systems of law enforcement and criminal justice will be better for it.”⁵



Jurisprudence Section - 2015

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NAS, Court, Evidence



Jurisprudence Section - 2015

F11 Forensic Science Leaders on the Path Forward

Sarah Chu, MS, 40 Worth Street, Ste 701, New York, NY 10075*

After attending this presentation, attendees will understand the various efforts advanced by the forensic science community and supported by the Innocence Project that can be implemented by laboratories to advance forensic science.

This presentation will impact the forensic science community by identifying and sharing model efforts that forensic science leaders have implemented in their laboratories.

In 2009, the National Academy of Sciences published an authoritative Report on the state of forensic science in the United States. The forensic science community advocated for this Report and leaders within the community began to advance policies and strategies to improve forensic science even before the publication of the Report. While members of this community may disagree on aspects of this Report entitled, *Strengthening Forensic Science in the United States: A Path Forward*, the Report has served to broaden the national conversation on forensic science, emphasize the critical need to act urgently, and has also served as a rallying point for federal resources.

In the six years since the release of the NAS Report, a number of developments have been driven by its release. Eight hearings in the Science and Judiciary Committees of both houses spurred discussions that have resulted in the introduction of three bills in the 113th Congress focused on establishing a federal infrastructure to support forensic science. Additionally, the Department of Justice (DOJ) and the National Institute of Standards and Technology (NIST) entered into a Memorandum of Understanding (MOU) to collaborate in the creation of a National Commission on Forensic Science (NCFS) and a forensic science standards setting infrastructure now named the Organization of Scientific Area Committees (OSAC). The creation of the NCFS was announced in February 2013, its members were appointed, and the first meeting held in February 2014. While the NCFS is co-chaired by NIST and DOJ, its organizational center is within the DOJ. NIST and DOJ are also collaborating on the establishment of the OSAC. Appointments of OSAC members are in process. To date, the appointments have incorporated members of the relevant scientific communities and their standards-setting work was anticipated to begin before the end of 2014.

Many recommendations of the NAS Report, such as research funding and setting national standards, can only be accomplished or are most efficiently addressed at the federal level. Among issues that can be effectively addressed at the state, local, or laboratory level are those that focus on quality assurance and quality control, processes to address cognitive bias, and improvements to transparency. This presentation will focus on strategies that specific laboratories or forensic science groups have developed to address these issues in the absence of national standards and policy recommendations from the NCFS. Among the efforts that may be highlighted are the implementation of root cause analyses, comprehensive reporting, and transparency measures. These accomplishments are notable in that these forensic science leaders are proactively advancing their laboratories' scientific practice which will better prepare them for them for the road ahead.

Policy, Laboratory Quality, NAS Report



Jurisprudence Section - 2015

F12 Got Forensic Science Standards? — How the Organization of Scientific Area Committees (OSAC) Activities *COULD* Impact the Courtroom...

Mark D. Stolorow, MS, MBA, NIST Office of Special Programs, Organization of Scientific Area Committees, 100 Bureau Drive, MS 8102, Gaithersburg, MD 20899-8102*

After attending this presentation, attendees will understand how the efforts of more than 600 subject-matter experts (volunteers) appointed to the Organization of Scientific Area Committees (OSAC) and others could impact laboratory protocols, accreditation efforts, and testimony in forensic science.

This presentation will impact the forensic science community by educating attendees on the processes employed by the OSAC to identify, foster the development of, and formally approve forensic science standards through publication on the OSAC Registration of Approved Standards and Registry of Approved Guidelines. These standards development efforts will ultimately affect methods employed in forensic science laboratories, standards used during the accreditation process, report writing, and testimony offered by experts.

As the forensic science community is aware, the development of a quality infrastructure for forensic science was a key component of some of the reforms anticipated in the 2009 National Academy of Sciences (NAS) Report, *Strengthening Forensic Science in the United States: A Path Forward*. In response to the Report, the National Institute of Standards and Technology (NIST) and the United States Department of Justice signed a bilateral agency Memorandum of Understanding (MOU) in March 2013 which specified the establishment of “Guidance Groups,” now termed Scientific Area Committees (SACs). NIST created the Organization of Scientific Area Committees (OSAC) model to promulgate NIST’s responsibility to administer and coordinate support for the SACs and subcommittees that represent specific forensic science disciplines.

The consensus-based documentary standards and guidelines approved for posting on the OSAC Registry of Approved Standards and Registry of Approved Guidelines will be considered: (1) by laboratories as standard methods for specific analyses; (2) as potential discipline-specific standards for consideration by accrediting bodies offering accreditation services in the forensic industry; and, (3) by officers of the court when evaluating processes employed and testimony given by forensic science experts.

The OSAC design employs the essential requirements of developing consensus-based standards which include openness, transparency, balance of interest, due process, and an appeals process that ensures each stakeholder’s viewpoints are properly considered. In addition, the OSAC infrastructure will bring a uniform standards recognition platform to the community, enhance scientific rigor, and increase communication among forensic scientists, research scientists, academicians, statisticians, attorneys, managers, and quality assurance specialists. The OSAC structure currently consists of a Forensic Science Standards Board, three resource committees, five scientific area committees, and 23 subcommittees.

Standards, OSAC, Testimony



Jurisprudence Section - 2015

F13 Strawberry Fields Forever: How a 73-Year-Old Birdwatcher Helped Nab a Sexual Predator in Central Park

Melissa Mourges, JD, New York County District Attorney's Office, One Hogan Place, New York, NY 10013; and Martha Bashford, JD*, New York County District Attorney's Office, One Hogan Place, New York, NY 10013*

After attending this presentation, attendees will learn how a good samaritan who took pictures of a deviant predator was later viciously sexually assaulted by this same man as punishment for her actions.

This presentation will impact the forensic science community by illustrating how the quick thinking of a crime victim and quick actions by the police led to the apprehension of a rapist and the collection of five different types of evidence leading to his conviction.

Beth B. is a dyed-in-the-wool New Yorker, a dedicated nature lover, and feisty as hell. At 73, she routinely walked through New York City's Central Park, enjoying the weather and taking pictures of birds. In September 2012, though, she saw something very disturbing through her camera lens — a heavily tattooed, creepy-looking man who was openly masturbating in the park. Beth took a picture of his face, in case he caused trouble.

Two weeks later, she was back in the park near Strawberry Fields (made famous as a tribute to Beatle John Lennon) with her camera. A man approached her. "Remember me?" he asked. She said no, but in fact, she remembered him well — he was the sinister man whose photo she had snapped earlier in the month. She walked away from him as quickly as she could, but he jumped on her back, pulled her to the ground, choked her, and pounded her face into the dirt. In broad daylight, he raped and sodomized her, then told her to count to 100. He fled through the park, taking her camera bag with him.

Beth reported the crime and went to the hospital, where a sexual assault evidence kit was taken. Surveillance cameras in the park caught the man moving away from the crime scene, and an All Points Bulletin went out. Patrol officers caught 42-year-old David Mitchell several hours later, still wearing the clothes he had on during the attack.

Beth picked him out of a lineup. Nevertheless, Mitchell claimed HE was the victim — of mistaken identification — and insisted the police had the wrong man. Because Mitchell was caught so soon after the crime, investigators took the unusual step of having doctors complete a "suspect evidence kit" including swabs from the defendant's genitals, looking for evidence of rape. Although police never found the stolen camera, computer forensics experts were able to recover Beth's photos, which she had downloaded, but deleted, two weeks before.

Mitchell, it turns out, had a frightening history. A drifter who terrified the other homeless people living in the park, Mitchell had confessed to the murder of his West Virginia neighbor, an 83-year-old widow, when he was only 16. He was acquitted after a psychiatrist said he was too cognitively impaired to understand either his rights or his confession. He was later charged with raping and robbing another woman in her 70s and pleaded guilty to robbery charges in that case. He then served eight years in jail for abducting yet another woman.

Attendees will see the evidence, learn how it was recovered, and what it proved. Actions of the quick-thinking and courageous victim, with an assist from video, computer forensics, and DNA, put a career criminal behind bars, far away from New York City's back yard, Central Park.

Sexual Predator, Photographs, New York City



F14 Mental Maps: Why You Should Worry

Katherine Ramsland, PhD, DeSales University, 2755 Station Avenue, Center Valley, PA 18034*

After attending this presentation, attendees will recognize how a personal Cognitive Positioning System (CPS) affects legal decision-making and case analysis.

This presentation will impact the forensic science community by illustrating the need to be educated about mental defaults that subconsciously filter information and distort judgments that guide decisions.

A CPS develops from experience and education, both of which are filtered through the brain's cognitive defaults. A person's CPS is their personal perspective, which is comprised of a unique network of influences and shortcuts that affect (and infect) mental processing. All interpretations and subsequent decisions are made within this frame. Everyone prefers familiarity, simplicity, and closure, and their mindsets comply with the feeling, "That's right!" This can hinder thorough analysis.

For example, in an experiment, 50 homicide investigators and 68 undergraduates were given evidence from a homicide case and offered two potential hypotheses. The students showed bias in accord with their initial hypothesis, but were open to disconfirming evidence. Investigators — especially those with a high need for closure — were more likely to automatically view evidence as incriminating. They accepted disconfirming evidence *only* if it aligned with their initial hypothesis.¹

Contrary to common belief, rational thinking is not automatic. Some information goes through rational channels and some through intuitive channels. The latter are faster, so they have the first impact. Intuitive responses are automatic, subconscious, and highly influenced by emotion. Conclusions form quickly, without analysis. Thus, they are vulnerable to the errors that form from personal bias and limited experience or education.

Everyone makes automatic judgments from within their frames of reference. Constructs learned from social and familial milieu, such as gender roles, rules of thumb, and racial stereotypes, have a significant subconscious impact on how the world is seen. Repeated experience with these constructs forms mental schemas. Then situations are "scripted" with construct-specific expectations.

These constructs feed into mental maps.² "Mental mapping" describes how people become habituated to their perspective. It goes by other names, such as cognitive map, perceptual set, or frame of reference. Individuals encode, recall, and recognize their "situated existence" according to a familiar frame. That is, people learn things from their families, culture, and subcultures that subtly thread throughout their personal interpretations.

How the world is physically and emotionally processed with mental maps guides people's decisions and behaviors. The brain then links perceptual sets with physiological systems, so that habits become encoded. This "embodied cognition" or "body memory" explains "gut feelings." Individuals allow even uninformed notions to guide them.³

However, what *feels* right is not necessarily right. Gut instinct can become a threshold diagnosis that averts a full analysis. People tend to see what they expect to see, and their recall is usually more consistent with personal beliefs and feelings than with facts, especially as it supports an intuitive hypothesis. Contradictory information is generally ignored. Sometimes, it is not even perceived.⁴

In sum: Research in cognitive psychology shows that certain types of mental maps present a persistent challenge for those involved in the legal/investigative process. Investigators arrive at scenes with mental maps that frame their investigation (or lack thereof).⁵ Attorneys, judges, and jurors approach cases via their personal CPS. This presentation will identify common cognitive biases and offer suggestions for minimizing their damaging impact.⁶



Jurisprudence Section - 2015

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Mental Maps, Threshold Diagnosis, Cognitive Bias



Jurisprudence Section - 2015

F15 Three Shots to the Head: A Case Study of a Murder Prosecution in Indiana

Christine Haskell, JD, 128 N Cullen Street, Rensselaer, IN 47978*

After attending this presentation, attendees will understand the importance of collecting and analyzing forensic evidence present at a murder scene and the importance of the forensic evidence used by the prosecutor in the eventual prosecution against the accused.

This presentation will impact the forensic science community by providing a case example of the importance of collecting forensic evidence by law enforcement officers, the processing of that evidence, and explaining the results of the analysis to ensure that the prosecutor's case is sound. In addition, it will demonstrate how important it is to utilize multiple forensic science disciplines to corroborate the theory of the prosecution's case. The case example will illustrate how vital it is for the prosecutor to have a firm understanding of the science in order to comprehend the implications of the forensic scientists' conclusions.

The primary goal of this presentation is to present a case study of a homicide prosecution in a rural county in Indiana. In this particular case study, law enforcement officers responded to a call from a young woman who reported that her brother had been shot. When law enforcement arrived, the victim was found lying dead on his bed with a wound to his head. The officers moved the body to see if medical attention could be rendered and to try to locate a weapon. When no firearm was located, the officers contacted a crime scene technician and began processing the scene as a homicide. Emphasis was placed on how the body was positioned when the sister and the first responding officer initially entered the bedroom. The crime scene technician arrived and processed the scene, collecting forensic evidence for analysis, specifically photographs of blood spatter patterns on the wall and the bedding with which the individual was covered. Law enforcement officers continued the investigation of the crime by questioning friends and relatives. In addition, an autopsy was performed and it was concluded that the victim had been shot in the head three times. In addition, toxicology results revealed the victim tested positive for various chemical substances in his body. Also, law enforcement officers located a firearm believed to be the murder weapon. Subsequently, a firearm examiner performed bullet comparison tests to determine if this was the murder weapon. The weapon that was used in the crime was a .22 caliber single-shot bolt-action rifle, which required the manual placing of a cartridge into the chamber for each shot. Law enforcement officers identified a person as the murder suspect. He was arrested, but claimed self-defense; however, based on the conclusions derived from the toxicology results, the blood spatter patterns, and the operating mechanism of the firearm, this claim was definitely not plausible.

With the corroborating forensic evidence from several forensic science disciplines, the prosecution was able to refute the claim of self-defense, which resulted in the accused pleading guilty to the murder.

Criminal Justice, Evidence Collection, Case Study



F16 How an Innocent Person's DNA Turned Up at a Murder Scene: Case Study of Lukis Anderson

Kelley Kulick, JD, Office of the Public Defender, 120 W Mission Street, San Jose, CA 95110*

After attending this presentation, attendees will have a basic understanding of primary, secondary, and tertiary transfer of DNA and how inadvertent transfer relates to criminal cases. The goal of this presentation is to review a case in which inadvertent, secondary transfer of DNA evidence in a homicide led to the arrest, accusation, and incarceration of an innocent man.

The facts of the case study confirm the reality that inadvertent transfer does indeed occur in criminal cases. This presentation will impact the forensic science community by raising questions about the likelihood and ease of inadvertent transfer of DNA and how the increased sensitivity of DNA testing heightens the risk that small amounts of transfer DNA from innocent people will be detected. This presentation also serves to educate lawyers, judges, law enforcement, and experts about the serious risks inherent in the introduction of DNA match evidence into an investigation and ultimately the courtroom, when corroborating evidence is weak or nonexistent.

Sometime between 10:30 p.m. on November 29, 2012, and 1:35 a.m. on November 30, 2012, assailants entered the home of Harinder and Raveesh Kumra. The couple were beaten, bound, and gagged as the home was ransacked. Mr. Kumra died from asphyxiation as a result of the binding and gagging. The prosecution alleged that the murder was committed by Lukis Anderson and three other African American co-defendants. The state charged Mr. Anderson with murder and the special circumstance of a murder in the course of a robbery, making him eligible for the death penalty. He was also charged with robbery, assault with a deadly weapon, terrorist threats, and false imprisonment.

During the police investigation, fingernail clippings of the right hand of the decedent were collected for forensic testing. A mixture of DNA from at least three individuals was detected in the swab of these clippings. Both Raveesh and Harinder Kumra were included as possible contributors to this mixture of DNA. On December 21, 2012, the remaining DNA types were searched against the Combined DNA Index System (CODIS) which returned a match for Mr. Anderson. The county crime laboratory calculated the random match probability for this DNA as 11,000 to 1 for African Americans.

The prosecution's case against Mr. Anderson rested entirely on the DNA evidence. The surviving victim was unable to identify the perpetrators. Even under great pressure, Mr. Anderson never confessed. The prosecution took great efforts to try to connect Mr. Anderson to the other co-defendants, but Mr. Anderson was an outlier.

Defense investigation revealed that on the night of the homicide, a clerk called the police to report that Mr. Anderson was intoxicated in front of a market. The local fire department was the first responder to the call and arrived on scene at 10:29 p.m. The paramedics determined that Mr. Anderson was unable to care for himself so an ambulance transported Mr. Anderson to the county hospital at 10:45 p.m. Mr. Anderson's blood alcohol level was approximately .41 when he arrived at the emergency room. This level was consistent with his fully intoxicated state of being non-ambulatory, flaccid, and totally somnolent. Mr. Anderson was not discharged from the hospital until the next morning at 9:50 a.m., well after the crime was committed.

Based on the presentation of hospital records and defense interviews of pertinent witnesses, the prosecution dismissed all charges against Mr. Anderson after he spent five months in custody. Further investigation by the prosecution showed that the ambulance that transported Mr. Anderson to the hospital was the same ambulance that responded to the homicide. In addition, the pulse oximeter applied to Mr. Anderson's finger to monitor oxygen saturation was also applied to the decedent's finger and is thought to be the source of the secondary DNA transfer.

Without the presentation of the documentary evidence establishing a complete alibi, Mr. Anderson would likely have been convicted of capital murder. This presentation will be an opportunity to learn and discuss the far-reaching implications of inadvertent transfer of DNA in criminal trials.

DNA, Inadvertent Transfer, Bias



Jurisprudence Section - 2015

F17 Crime Scene Investigator: Assessment of Expert Credentials

Kevin J. Parmelee, MPA, Somerset County Prosecutor's Office, Forensic Lab, 40 N Bridge Street, Somerville, NJ 08876*

After attending this presentation, attendees will be provided with a thought-provoking evaluation of the many variables that are considered for the determination of a Crime Scene Investigator (CSI) as an expert for the court. Variable perspectives will highlight the many aspects of a CSI's qualifications when considered as an expert witness. Information will include regional variations in the qualifications required to become a CSI, disparity between the qualifications of CSIs in large urban agencies vs. smaller rural agencies, comparison between sworn law enforcement officers vs. civilian CSIs, and an assessment of certified vs. non-certified practitioners.

This presentation will impact the forensic science community by highlighting the many facets considered when identifying the qualifications necessary for a CSI to be distinguished as an expert witness.

After the first responding patrol officer arrives at a crime scene, the CSI is commonly the first individual specially trained to conduct an in-depth investigation for recognizing the value of relationships between statements, physical evidence, and crime scene observations. In particular, the CSI functions to recognize, document, collect, and preserve relevant physical evidence in order to generate a representation of events and circumstances about the crime. The information, physical evidence, and data obtained from a successful crime scene investigation provide the preliminary basis for any subsequent forensic investigations, testing, and analysis. Essentially, the CSI establishes framework for which the investigation, both criminal and scientific, is based. With such a large responsibility for providing a successful crime scene investigation, there would appear to be the expectation that the CSI is specially trained and proficient in his or her skills. So what qualifications would be used in the determination of a CSI as an expert witness in court?

Job descriptions from various regions of the United States represent a disparity in the requirements sought to perform the duties of the position. Furthermore, the demands and resources of larger, more urban agencies may represent a significant difference from smaller, more rural agencies. There are organizations that provide certification options for CSIs, but does a certification provide all that is necessary? Does the lack of a certification eliminate a candidate from providing expert testimony? These variables and inquiries will be reviewed to generate thought-provoking and insightful dialog.

Crime Scene Investigator, Expert Witness, Expert Credentials



Jurisprudence Section - 2015

F18 Root Cause Analysis for Attorneys: Why? Why? Why? Why? Why?

Max M. Houck, PhD*, Consolidated Forensic Laboratory, 401 E Street, SW, Washington, DC 20024

After attending this presentation, attendees will understand the basics of root cause analysis as a tool for exploring potential failures of forensic service providers.

The presentation will impact the forensic science community by providing attorneys with a valid framework for inquiries directed at actual or perceived failures of forensic service providers. This framework will help the forensic science community respond to legal inquiries in a structured, focused manner.

Root Cause Analysis (RCA) is a method of problem-solving that tries to identify the necessary and sufficient reasons for an event, typically a fault, problem, or — in this case — a non-conformity in a quality system. Once a root cause is removed from the sequence, the non-conformity should be prevented from happening again.¹ RCA assumes that systems and events are related. An action in one area can create other actions or results in others, continuing on in what is known as the “causal chain.” RCA traces the remnants of the chain of events back to its source, the root cause.

RCA is an iterative process.² The process of RCA begins with the problem statement: What happened? Why is it a non-conformity? How is it outside the quality system requirements? This is done using a technique of continually asking “Why?” until the root cause is identified.³ Once identified, the chain of events leading to the root cause of the non-conformity is characterized as simple, complicated, or complex; a root cause may be characterized as chaotic, but this is rarely the case.⁴ The characterization will indicate the kinds of changes that need to be made to test if the identified root cause is the actual source of the non-conformity; additional observations may be needed to confirm this decision. The confirmed changes are then made to the root cause, taking the rest of the quality system into account, to see if the non-conformity is prevented from recurring. Periodic checks are made to provide feedback on the continual prevention of the non-conformity; that is, are the changes still preventing the non-conformity?

The RCA process is intended to be followed step by step; however, it may be necessary to revisit or repeat a step if something does not produce the expected results: (1) state the problem; (2) get to the root cause — the 5 “whys”; (3) the decision framework — simple, complicated, complex, or chaotic; (4) remediation; (5) feedback; (6) resume the process; and, (7) check for recurrence.

For example, if the problem statement is, “The analysis of a sample/specimen was not completed by the deadline,” then the root cause is determined by asking: (1) Why? The instrument failed to complete the run; (2) Why? The instrument ran out of carrier gas; (3) Why? The tank of carrier gas emptied mid-run; (4) Why? More gas was not ordered; and, (5) Why? An employee forgot to order more gas.

The “whys” provide a problem analysis (the problem has already been *stated*), a breakdown of effects and causes leading from the problem to the root cause. The remediation may be that a reminder is set in a calendar function to inform an employee when more gas needs to be ordered; the message is the feedback on the remediation. Testing can now resume with occasional checks for recurrence; that is, other samples not being completed by the deadline because of lack of carrier gases.

Thinking that only one cause exists is dangerous: *A cause never stands alone*. A good RCA will explore the causal chain thoroughly; other instances of the event may have occurred and the root cause may be more widespread and disruptive than initially assumed.

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Root Cause Analysis, Quality, Forensic Failures



Jurisprudence Section - 2015

F19 **LEGLER: LEGal Linguistic Evidence Rulings and Forensic Linguistic Evidence**

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After attending this presentation, attendees will better understand the current and historical state of rulings concerning linguistic evidence in American jurisprudence. Attendees will learn how these rulings fit into the “four corners of forensic linguistics” and where litigation-independent research is needed, based on the judicial record of admissible or inadmissible methods.

This presentation will impact the forensic science community by helping attendees learn to accurately understand legal rulings about the current admissibility of different methods in forensic linguistics and to access a toolkit for evaluation of cases and rulings in forensic linguistics.

Whenever an attorney hires a forensic expert, he/she enters into a potentially unfamiliar area of science. This unfamiliarity is intensified when the forensic expertise involves an academic discipline that rarely appears in court. Linguistics is a rather rare academic discipline, with many universities offering only a course or two related to a major in foreign languages or education. Unsurprisingly, most attorneys have never heard of “forensic linguistics” or “linguistic evidence.” Further, most attorneys need help in evaluating the “forensic linguistics” or “linguistic evidence” that is being offered in a case because most attorneys are not linguists who are professionally trained in the academic discipline. Yet American courts have been considering and making rulings about the admission of linguistic evidence for more than 100 years and recently several high-profile cases have attracted attention to the idea of language as evidence. The 2014 ruling about the “Redskins[®]” trademark mentioned linguists hired by both sides to opine about the semantics of “redskins.” The 2012/2013 *Paul Ceglia v. Mark Zuckerberg* case highlighted language-based methodologies for determining authorship of electronic documents. On the pseudonymous landscape of the internet, forensic linguistic evidence will play an increasingly important role in investigation and litigation. The LEGal Linguistic Evidence Rulings (LEGLER) project helps attorneys quickly access rulings about forensic linguistic evidence and provides expert evaluations of the rulings from both the legal and linguistic perspectives.

LEGLER is web-accessible software that contains published rulings from American jurisprudence starting in the 1800s and continuing to the current year. Rulings were first gathered by an experienced attorney using one of the available legal databases. The 300+ rulings were then “scrubbed” by a computational linguist who wrote software to remove any proprietary information from the legal database. The scrubbed rulings were then loaded into the LEGLER section of ILER, a database platform for conducting web-based experimental research in forensic linguistics.^{1,2} Research associates of the Institute for Linguistic Evidence and subscribers to ILER can access LEGLER through a web-browser, with password authentication for the user account. LEGLER offers information beyond the legal databases (i.e., annotations by at least one attorney and at least one linguist). These annotations help the user organize and evaluate the rulings.

LEGLER annotations include several features: (1) each ruling is categorized as being from one or more of the “four corners of linguistic evidence,” (i.e., identification, text-typing, intertextuality, and linguistic profiling); (2) within the general category, each ruling is subcategorized by a specific topic. For instance, identification includes speaker, author, and language identification, or text-typing includes authentication of a document as a real suicide note, real threat, ransom note, and so forth; (3) each expert in the case is listed; and, (4) the method used by the expert(s) is evaluated for its relationship to linguistics. Methods related to linguistic evidence may be rooted in linguistics or other approaches to language such as literary criticism, prescriptive grammar, or computational stylometry. Only a trained linguist can accurately evaluate whether a method is rooted in linguistics or not, just as only an attorney can accurately evaluate the legal implications of a ruling. LEGLER annotations provide an interdisciplinary perspective on a ruling, search and organize rulings by all the usual legal parameters as well as linguistic methodology, and thus prepare for a case involving linguistic evidence with insight from both the law and linguistics.



Jurisprudence Section - 2015

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Forensic Linguistic Evidence, Admissibility, Legal Databases



Jurisprudence Section - 2015

F20 Trust — But Verify: The Reliability of Radiograph Techniques in the Courtroom

Jeffrey M. Jentzen, MD, University of Michigan, 300 N Ingalls, NI2D19 - SPC 5452, Ann Arbor, MI 48109*

After attending this presentation, attendees will understand some of the limitations of conventional radiographic techniques and the need for confirmation by another radiographic technique and/or pathological examination and interpretation by trained experts in the field.

The presentation will impact the forensic science community by illustrating the need for pathological documentation of radiographic findings for courtroom testimony and for insisting on a multidisciplinary approach to injury interpretation.

The routine use of radiographic imaging in forensic medicine and its presentation in the courtroom is constantly increasing. Radiographic documentation of injuries in child abuse and gunshot wounds, for example, has become standard practice.¹ Proponents of the routine use of radiographic imaging have argued that its use could eventually replace standard autopsy examinations. Recent studies have questioned the reliability of radiographic imaging and interpretation in the medicolegal environment. Molina et al. have demonstrated that Computed Tomography (CT) scans have a low rate of accuracy and sensitivity to provide definitive diagnosis and description of injuries at a legal standard for the courtroom.² This presentation will discuss representative cases where radiographic and clinical interpretation of injuries were overturned by the autopsy findings.

Case One: A 19-year-old male, distraught over the break-up from his girlfriend, sustained a self-inflicted gunshot wound to the head. The patient received CT imaging on admission to the hospital and was removed from life support five days following admission. The family was opposed to autopsy and a limited examination was performed. The CT report opined that the entrance wound was located on the left temple. The examination documented the entrance wound on the right temple.

Case Two: A 4-month-old infant sustained a traumatic head injury with skull fracture. In court, the radiologist was unable to opine whether or not the fracture had crossed the suture line. The autopsy convincingly demonstrated the extension of the fracture in the suture with extension. The finding had significant medicolegal importance on the amount of force and mechanism of injury sustained by the infant.

Case Three: A 3-month-old infant had been hospitalized since birth for congenital heart problems, had been recently discharged, and had gone home. The infant was examined in the emergency room after being found dead. The radiologist and Emergency Room (ER) physician diagnosed an “acute, comminuted depressed fracture to the occipital skull.” A subsequent autopsy revealed overlapping of sutures due to chronic compression of the skull.

Case Four: A 5-month-old infant was found dead. Pre-autopsy radiographs and CT scan failed to identify any fractures. An autopsy confirmed the presence of abdominal trauma as well as a recent fracture of the inferior occipital skull.

Case Five: A 35-year-old male sustained a gunshot wound to the head during an altercation. He remained in a coma and died three weeks later. On receipt of the autopsy report, the prosecutor questioned the identification of entrance and exit wounds because they were “not consistent with the ER physician statement.” Upon further review, despite CT scans and radiographs, the ER physician had misinterpreted the wounds.

Radiographic identification and documentation provides an important adjunct to the forensic autopsy. The low sensitivity, reliability of interpretation, and radiographic resolution of injuries requires pathological confirmation to attain the legal standards. The traditional autopsy remains the gold standard for the identification and documentation of injuries. The admission of a single piece of radiographic evidence in the courtroom should be confirmed by another method. Radiographic evidence submitted in the courtroom should be interpreted by trained experts in the field.

References:

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X-Ray, CT Scans, Injury Interpretation



Jurisprudence Section - 2015

F21 Is the Medical Examiner's or Coroner's Official Cause of Death the Last Word?

Glenn G. Hardin, MPH*, Hamline University, MB 239, 1536 Hewitt Avenue, Saint Paul, MN 55116

After attending this presentation, attendees will understand the importance of an effective evaluation of the circumstances surrounding a questioned death and the findings at autopsy.

This presentation will impact the forensic science community by describing four cases in which review of the evidence regarding questioned deaths — medical records, witness statements, the results of external and internal examinations at autopsy, and postmortem toxicology analyses — either supported or refuted the medical examiner's or coroner's official Cause Of Death (COD).

Case 1: Involved a subject who died while lying prone with a police officer's knee placed on his back. The Medical Examiner (ME) determined that the COD was cardiopulmonary arrest and mechanical asphyxia, and the manner of death was homicide although the toxicology findings included dextromethorphan at 2.0mg/L in blood. The decedent's heirs sued the city for wrongful death. The city attorney hired an ME from another state who opined that the subject died from respiratory arrest due to dextromethorphan toxicity, even though in the months prior to this event, the subject survived being admitted to various emergency departments after ingesting 30 tablets of a dextromethorphan over-the-counter preparation. A major weakness in the city's case was that the police officer's partner recorded the death event with his cellphone.

Case 2: Involved a subject who died one morning after injecting himself with heroin the evening before and ingesting methamphetamine the day before. At autopsy, signs of respiratory depression and arrest were present that are associated with death due to morphine (heroin) toxicity, including pulmonary edema in both lungs, lungs weighing >500g, and pulmonary vasculature congestion. Additionally, there were no signs of cardiovascular issues. Before the toxicology results were reported, a suspect — a methamphetamine dealer — was identified. The heroin dealer was never discovered. The toxicology results revealed methamphetamine at 0.70mg/L, amphetamine at 0.16mg/L, and free morphine at 0.05mg/L in the blood. The coroner opined that the COD was methamphetamine overdose. The suspect was then charged with murder for providing the lethal dose. Prior to trial, the coroner's COD opinion was challenged by a medical examiner and a toxicologist. On the very eve of trial, charges were dismissed.

Case 3: Involved a subject who was admitted into the hospital one evening with severe back pain. Multiple Central Nervous System (CNS) depressant medications were administered, including morphine, cyclobenzaprine, hydromorphone, lorazepam, and tizanidine. The next day, the patient appeared to be tolerating the drug regimen; however, the following morning he was found dead with a mouthful of emesis. Autopsy indicated bilateral congestive pulmonary edema, right lung weight of 1,050 grams, left lung weight of 980 grams, and a bladder filled with urine. Toxicology results revealed a free morphine level of 0.16mg/L and cyclobenzaprine level of 0.02mg/L in blood. The coroner ruled the COD was morphine toxicity and the heirs sued for wrongful death. The hospital disputed the coroner's finding, arguing that the medical personnel administered therapeutic doses of the medications.

Case 4: Involved a subject who suffered a serious injury at work, for which he was prescribed oxycodone. For at least six months, he abused prescriptions for oxycodone, during which time he demonstrated tolerance to the increased doses by engaging in a variety of normal activities. One morning he was found dead in bed. Autopsy showed that the right lung weighed 495 grams and the left lung weighed 465 grams. The upper airways were clear of debris and foreign material with normal-appearing mucosal surfaces. The urinary bladder contained 50 cubic centimeters of urine. The heart weighed 500 grams. There was 90% of focal non-calcified narrowing of the proximal left anterior descending coronary artery and 80% narrowing of the first and second diagonal coronary artery. The blood oxycodone level was 0.66mg/L. The ME initially concluded that the COD was atherosclerotic cardiovascular disease; however, after a visit from one of the heirs, the ME two months later changed the COD to oxycodone toxicity.

Cause of Death, Forensic Toxicology, Evidence Evaluation



Jurisprudence Section - 2015

F22 Forensic Field Testing: Curse or Cure?

Vincent J. Desiderio, Jr., MS, 33 Potterfield Drive, Lovettsville, VA 20180*

After attending this presentation, attendees will be aware of both the value and potential pitfalls of field tests in the forensic context.

This presentation will impact the forensic science community by bringing to the surface the various potential issues involved with forensic field tests.

Field testing of evidentiary materials is not a new concept. From the beginning stages of the first scientifically driven examinations, investigators have sought methods to rapidly and reliably extract information from materials encountered in the field. For the most part, such testing has been regarded as presumptive in nature and generally required confirmation through more elaborate laboratory testing in a controlled environment.

Examples of such testing has included simple oxidative tests for the presumptive indication of blood (e.g., o-tolidine, Kastle-Meyer, luminol, etc.), wet chemical field test kits for the presumptive indication of controlled substances, and canine detection of ignitable liquid residues. Technological and engineering advances have now enabled the miniaturization of powerful instrumental analysis techniques to the point that it is possible to carry such instruments to a scene with little effort.

At this point in time, there are various manufacturers of field-portable Fourier Transform Infrared (FTIR) spectrometers, Raman spectrometers, and gas chromatographs with various detectors including mass spectrometers. Such instruments are often equipped with extensive data libraries that provide a best hit to the operator. In the not-too-distant past, the only place that such instrumentation with their accompanying libraries could be found was in an analytical laboratory where they were operated, for the most part, by individuals with in-depth scientific backgrounds under stringent operating protocols.

As these instruments have become smaller and easier to produce, their price points have dropped accordingly. It is now economically feasible for both large and small well-funded law enforcement and first responder entities to purchase and distribute such instrumentation for use. When combined with delays in laboratory analysis due to backlogs, it should be no surprise that there is a growing push to place such powerful instrumentation into the hands of the masses.

Although issues with the various forms of field tests are not new (there are still many issues with the older forms of field testing), the deployment of such powerful technologies appears to be outpacing the ability of the field to fully evaluate the impact that such technology might have. This should be a concern that raises several issues/questions including: (1) the fact that conflicts which have arisen in the past with the older technologies have not yet been fully addressed; (2) are the people that will be using such instrumentation qualified to make conclusions based on the findings they are receiving; and, (3) are any quality measures comparable to those expected in the laboratory being employed to validate and ensure proper operation at the point of use?

This presentation will discuss the various merits and potential pitfalls of the different techniques available for use in the field from a historical perspective. Parallels will be drawn between the measures that are taken in the laboratory compared to those that should be taken in the field. Although this presentation may lean toward a cautious approach for the implementation of these newer technologies, the primary purpose is to create awareness of some of the pros and cons of these approaches. In order to illustrate some of the points that are discussed, specific examples from both the literature and from real-life experience will be presented.

Field Tests, Chemical Tests, Field Portable Instrumentation



Jurisprudence Section - 2015

F23 Technical Investigations Not Repeatable in the Italian Code of Criminal Procedure: The Guaranteed Anticipated Contradictory of the Defense

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After attending this presentation, attendees will understand that the Italian rules governing the activities of technical investigations about people, things, or places are subject to rapid change. In these cases, during the preliminary investigation conducted by the Public Ministry and carried out by the judicial police, the Italian Code of Criminal Procedure provides for the participation of other parties to the proceedings with the help of a consultant in order to establish a contradictory with the prosecutor.

This presentation will impact the forensic science community by underlining the usefulness of the presence and participation of legal experts appointed by the crime victim and the suspect to a plea that cannot be presented at trial. In this way, the private parties can contribute their own remarks to the technical investigation, rather than contend with the conclusions of the district attorney's consultant.

Italian rules of technical investigations not repeatable. When the investigations relate to people, things, or places whose state is subject to modification, the prosecutor immediately warns the defendant, the victim of the crime, and the defense of the appointed day, time, and place and of their right to appoint technical advisors. The defense as well as the appointed technical consultants shall be entitled to attend the meeting, to participate in the investigations, and to make comments and reservations. If, before the meeting, the defendant initiates an evidentiary hearing before the judge, the prosecutor cannot proceed unless these investigations, if delayed, may not be more usefully made. If the prosecutor, despite the express request of the defendant and not under the condition of urgency, has also carried out an investigation, these results cannot be used in the trial.

The classic example of non-repeatable technical assessment is the autopsy of a corpse or, in cases of murder or involuntary manslaughter (e.g., medical malpractice, car accident, or a work-related accident).

In cases of murder, rarely is there already a suspect; however, in those rare cases (e.g., murder committed within a family), the suspect may participate in a fundamental investigation, offering comments and reservations, and perhaps stating his defense, such as self-defense. In wrongful death cases, the suspect is generally known (e.g., the responsible physician, the driver of the car, or an employer). In these cases, experts appointed by the suspect can directly verify the correctness of the expert opinions, contributing to the investigation of the relevant facts (e.g., cause of death, relationship of cause and effect, and/or other contributing factors).

Technical Investigations, Anticipated Contradictory, Italian Criminal Procedure



F24 Communicating Error and Uncertainty in the Courtroom: The Language, Methods, and Psychology of Doubt and Belief

Ted W. Vosk, JD, 4040 Lake Washington Boulevard, NE, Ste 300, Kirkland, WA 98033*

After attending this presentation, practitioners will have a better understanding of: (1) how to effectively communicate forensic results and their uncertainty to fact-finders; (2) the psychological and cognitive dynamics created by the presence of error and uncertainty which may hinder rational decision making; (3) how these forces interact with the formation of belief or doubt in arriving at verdicts; and, (4) the creative use of language and analogies in educating fact-finders.

This presentation will impact the forensic science community by facilitating communication of forensic results in a manner intended to lead to legal outcomes that are consistent with scientific reality. Attendees will be provided tools for clearly illustrating what a scientific result represents, the role played by its uncertainty, and the limitations the uncertainty places on the conclusions supported by a result. These tools help dispel naïve notions of “scientific certitude” and facilitate informed, unbiased consideration of forensic results and the verdicts they support.

Scientific results do not permit us to know anything with absolute precision. Rather, due to the presence of error and necessarily imperfect and incomplete information, the conclusions supported by forensic results are always accompanied by uncertainty. A consequence of this is that forensic science cannot establish what is or is not true, but, rather, only what is more or less likely based upon the information possessed. Thus, relatively justified belief concerning a physical state rather than knowledge of the actual physical state itself is what science leads to. This holds for all fields of science and any results obtained no matter how good the procedures, equipment, or scientists involved. Only by properly understanding what conclusions scientific results actually support can fact-finders issue verdicts that consistently comport with the science underlying the result relied upon. As a result’s uncertainty conveys the conclusions the result supports, a critical aspect of the decision-making process is an adequate understanding of a result’s uncertainty and what it represents.

Unfortunately, many triers of fact don’t realize that uncertainty is a part of all forensic results and instead imbue them with an “aura of scientific infallibility,” interpreting them as a direct revelation of the truth of what is to be proved. This misunderstanding of what these results represent undermines the fact-finding process leading to legal outcomes that must be questioned even when the science relied upon is sound. Knowing the problem doesn’t solve it, though. Conveying these concepts so that they can be understood in the context of a trial or hearing by scientifically naïve decision makers can be difficult. Moreover, awareness of the existence of uncertainty itself creates psychological and cognitive impediments to rational decision making which creates further complications. The reaction of some to the presence of uncertainty is to reject a proposition altogether regardless of how certain it is. The reaction of others is to reject uncertainty altogether regardless of how uncertain the proposition is. Neither response is warranted in most contexts.

If the ultimate mission of our courts is the determination of factual truth, fact-finders must be taught to appreciate the role played by scientific uncertainty and to weigh scientific evidence in a rational manner consistent with science itself. In the courtroom, the responsibility for communicating what forensic results represent and the conclusions they support rests with scientific witnesses testifying about the results and the legal professionals presenting or challenging them. If forensic results are to be considered and weighed in a scientifically defensible manner by fact-finders, then these forensic and legal professionals must be able to act in the capacity of courtroom teachers, educating decision makers so that they are equipped to make informed decisions. This involves creative use of both language and analogies, translating scientific jargon and concepts into laymen’s terms and images. When used effectively, these tools not only facilitate understanding but diminish the impact of psychological and cognitive blocks to rationality.

This presentation will address the challenge of communicating scientific results and their associated uncertainty and will present tools that can be used to facilitate this process in a manner that both educates substantively as well as defusing psychological and cognitive blocks to rational decision making.

Uncertainty, Error, Communication



F25 Errors in DNA Testing: Lessons Learned — A Retrospective Look

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After attending this presentation, attendees will understand: (1) some of the limitations of DNA testing; (2) what types of errors may occur during DNA testing; (3) more about the detection, prevention, and remediation of errors in DNA testing; and, (4) current issues in testing and interpretation of DNA samples with small amounts of DNA and/or mixtures of DNA from three or more contributors.

This presentation will impact the forensic science community by providing information regarding the types of errors that may be encountered in forensic DNA testing, some of which could lead to misinterpretations of the data with false inclusions or false exclusions of known individuals.

There are errors inherent to all test systems and DNA testing is no exception. Since its introduction to the forensic science community almost 30 years ago, a great deal has been learned about the limitations and errors of DNA testing. It is true that there is a minimal chance of errors in testing and interpretation if evidence collection and handling are correctly performed, properly validated laboratory procedures are duly followed with appropriate controls being used and monitored, and analysts are well trained in the technical procedures and in the interpretation and reporting of the data. The types of errors that can occur in DNA testing, including human errors and errors related to limitations of the technology, will be discussed. Information regarding which type of errors may be detected and how they may be identified will be provided. Possible preventative steps and remediation activities will also be presented.

Examples of current problems in DNA testing of biological samples resulting from the dramatic influx of evidence items seized from property crime scenes will be provided. Stochastic effects that commonly occur when polymerase chain reaction technology is used for the amplification of small amounts of DNA, termed low template DNA, can result in DNA profiles that are not reproducible and do not reflect the DNA profile of the true contributor of the DNA sample. Many of these samples are complex mixtures of DNA from three or more contributors, some of whom may be related. Testing and interpretation issues resulting from these types of samples will be discussed, including the possibility of the reporting of false inclusions and exclusions of individuals and the difficulty in providing reasonable statistical frequency estimates.

It is important for the forensic science community to recognize and acknowledge the types of errors that can occur during testing. It is critical that a culture is developed and promoted where the detection and correction of mistakes is strongly encouraged and perhaps even rewarded. Laboratory management and analysts should be forthcoming regarding any errors made and any limitations to a test system to prevent a miscarriage of justice. The identification and acknowledgement of mistakes and errors provide a noteworthy opportunity for additional corrective training and for the introduction of preventative measures and improved procedures aimed at generating the best, most reliable, and defensible testing results possible.

Errors, DNA, False Results



F26 Culture of Infallibility: The Inability of the Criminal Justice System to Concede Scientific Uncertainty

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The goal of this presentation is to discuss new research which demonstrates how the investigative process by which cases come to court is frequently comprised of a mix of good and bad evidence that the judicial system cannot always adequately evaluate.

This presentation will impact the forensic science community by explaining how it is necessary to understand the adversary process by which the judicial system resolves claims and the effects this process has on the quality of evidence both as presented and as understood. The cooperation of all stakeholders — law enforcement, attorneys, forensic scientists, and judges — to understand the need to reform the practices of each must underlie true reform.

Social and behavioral scientific research demonstrates that bias infects the criminal justice system; forensic science, police departments, prosecutors, the criminal defense bar, and even the judiciary. It is directly related to unsafe judicial results and wrongful convictions. The revealing of motivational, relational, and confirmation bias that can affect forensic testing has incited obloquy from the forensic science community against anyone speaking of it, but that is far from the whole story. The conformational bias (tunnel vision) that can lead police investigators to pursue only the evidence most consistent with their pre-existing theory while rejecting confounding facts similarly leads to prosecutors failing to consider other possibilities (assuming a complete set of facts is even available). Similarly, prosecutors considered that their need for absolute guilt made any uncertainty in evidence, even what might be subject to ready explanation, intolerable as somehow indicative of reasonable doubt. Defense attorneys, accustomed to slam-dunk cases being brought against their clients, have learned not to seriously question forensic evidence, the reliability of which judges say can be “taken for granted” because they have been used for decades. The process by which evidence is evaluated to determine criminal liability is demonstrably unstable when the discernment of truth and falsehood are often most critical. Despite knowing about solvable problems, change is resisted.

Years ago, as prosecutors began to have confidence in the seeming certainty of forensic science, evidence technicians were trained to use phrases like “absolute certainty,” “100% accurate,” and “to the exclusion of every (fill in item) in the world.” Eyewitnesses were considered the “gold standard” of criminal trial evidence, particularly when they were victims. Driving while intoxicated/impaired cases, tried on the basis of “under the influence,” became inconvenient because of a flexible relationship between alcohol levels and impairment, necessitating the adoption of a “science-based” per se offense based on a number—the blood alcohol concentration. A mythological impression of infallibility accompanied the forensic expert to court, where prosecutors neglected to understand the evidence they presented, defense attorneys missed opportunities to question what might be questionable, and judges were happy with quick-moving cases that conformed to what were often their own preconceptions of the evidence. The adversary system masked what needed to be an open-ended search for the best evidence for the case.

Then came DNA. With its arrival, two important things were discovered: (1) what it looked like when laboratory-validated molecular science was practiced to rigorous standards and applied with equal statistical rigor to predict probable outcomes; and, (2) that in the face of irrefutably unique chemical bonding, when this new scientific evidence was used in old cases, it exposed the wrongful convictions of innocent persons. Since forensic evidence is a creation of the law, used by the law, and has little application outside the law, the fact that it had been used in so many cases proven to result in wrong outcomes began to draw scrutiny and the result of the scrutiny was not always favorable. There being no “tolerable” number of wrongful convictions, the traditions embodied in the law and its use of evidence in criminal cases, required reassessment. Inertial resistance to change, a “chicken little” attitude within forensic science, and a lack of widely-accepted standards for forensic science work and the way its results were expressed in court were all exposed as impediments to progress.

Recent books point to the influence of a great number of failings in the criminal justice system, many of which are so inherent to various police, forensic, and legal practices that they have escaped evaluation. Exposure breeds resistance by these actors. The need for the infallibility of the court system and proof beyond a reasonable doubt have created a culture that supports uncritical myths of infallibility. Resistance to change does not reward team playing, consistency of opinion, and critical thinking.



Jurisprudence Section - 2015

This presentation will discuss the directions in which legal scholarship is attempting to identify and correct many of these quite unintentional sources of error in judicial determinations. A message is that the overarching quest for justice is best served by cooperation and greater examination of best practices to avoid exonerating the guilty and convicting the innocent.

Change, Resistance, Judicial System



Jurisprudence Section - 2015

F27 To Err or Not to Err: How Judges and Jurors Apply “Margin of Error” and “Coefficient of Variation” to Their Determinations of Blood Alcohol Content in Criminal Cases Involving Blood Testing

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After attending this presentation, attendees will better understand the legal requirements of “margin of error” and “coefficient of variation” as applied in the courtroom.

This presentation will impact the forensic science community by providing the bases for “margin of error” and “coefficient of variation” as applied in the law.

In federal courts, federal trial judges apply the Daubert standard to make the preliminary assessment of whether an expert’s scientific testimony is based on methodology that is scientifically reliable and can be properly applied to the facts at issue. In state court jurisdictions, state trial jurisdictions vary on the application of which standard that state court applies for admitting scientific evidence: Daubert, Frye, or a combination of both standards, now coined as Fryebert, states the trial judge may apply each standard separately to the same factual methodology so that two analyses are conducted to cover both bases of Frye and Daubert standards.

When state trial judges apply the Frye standard to determine the admissibility of scientific evidence, the scientific theory must be “generally accepted” in the relevant scientific community. The Daubert standard allows trial judges to evaluate novel scientific evidence using a variety of factors in determining whether the expert’s methodology is valid, such as, but are not limited to, the following: (1) whether the theory or technique in question can be and has been tested; (2) whether the theory or technique has been subjected to peer review and publication; (3) what is the theory’s known or potential error rate; (4) whether there is existence and maintenance of standards controlling its operation; and, (5) whether the theory has attracted widespread acceptance within a relevant scientific community.

This presentation will discuss how “margin of error” and “coefficient of variation” as scientific and legal concepts are viewed by state trial judges, appellate court judges, and juries as reflected in their decisions regarding blood alcohol content in blood tests in criminal cases in a Frye jurisdiction such as Pennsylvania. How are these scientific and legal concepts viewed by these judges and juries in a Frye jurisdiction such as Pennsylvania where scientific methodology need only be “generally accepted” in the relevant field of science? In a Frye jurisdiction, is it an abuse of discretion for a state trial judge to view “margin of error” evidence or “coefficient of variation” evidence in a light most favorable to the commonwealth when the judge considers a challenge to the weight of the evidence? Is the jury “speculating” or trusting that the defendant’s actual blood content was .16% or higher within two hours when no direct or circumstantial evidence is presented to the jury at the time of trial regarding the possible source or basis for the application of a 10% “margin of error?” In a Frye jurisdiction, should the laboratory tests be considered scientifically valid when the testing is done by an approved testing facility in accordance with established “generally acceptable procedures” when the laboratory tests uses only one sample of blood? A judge will grant a new trial based on the weight of the evidence claim in cases where the factfinder’s verdict is “so contrary to the evidence that it shocks one’s sense of justice.” Should there be a grant for a new trial if the jury is not informed of the application of the 10% margin of error in the actual alcohol blood testing result? Does that verdict “shock one’s sense of justice?”

Margin of Error, Frye Jurisdiction, Scientifically Valid



Jurisprudence Section - 2015

F28 Lost and Gone Forever — Jeopardized Prosecutions and Denial of Due Process

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The goal of this presentation is to make attendees aware of the importance of early scientific involvement at crime scenes.

This presentation will impact the forensic science community by pointing out the value of having experienced scientists (criminalists) involved in crime scene investigations from the outset.

Events leave physical traces which form a physical evidence record of the event. Humans may be concerned about such physical evidence records extending over extremely vast timescales. Depending on the time frame, some past events may be the subject of investigations by cosmologists, astrophysicists, geophysicists, geologists, paleontologists, or archaeologists. The record is created by processes that are subject to physical laws. Cleland has characterized these investigative endeavors as “historical science.”¹ All are centered on developing a scientific understanding of the physical evidence record. Myriad physical evidence records are being created continuously. Some are the result of human activity. The vast majority of more recent events may be inconsequential; however, some small fraction of much more recent events can be of great concern in accident or criminal investigations.

To some extent, this record is written over the records of preceding events at the same location. In addition, this record will be overwritten by the record resulting from events that follow. This statement has important implications. A crime scene may contain hundreds, if not thousands, of items. Many of the most relevant and significant items will not be obvious. How can the remnants of antecedent events be accounted for and separated from the traces of the event of interest? What can be done to minimize the addition of traces from post-event activities?

Most well-read laypersons would be appalled to see reports of non-scientifically trained persons excavating some site of a past civilization with a power-driven excavator and loading the excavated material into barrels for shipment to an archaeologist at some university or other research center. Why doesn't the same concern seem to extend to the practice of the collection of evidence by non-scientist police officers at crime scenes?

Lay critics of crime scene work in specific cases tend to think in terms of errors or omissions made at the crime scene. This is overly simplistic conceptualization. Every crime scene is different. Each presents challenging scientific problems. Unfortunately, most crime scenes are not the subject of a scientific investigation. Most are treated with a “bag-and-tag” mentality. Evidence that is not recognized at a crime scene is lost and gone forever. This loss may allow the guilty party to go free or it may have been something that provided evidence that would have been effective in clearing an innocent person who is subsequently accused. In most jurisdictions internationally, law enforcement has the responsibility of the crime scene investigation. What are the implications for the defense in a criminal case? What rights does the defendant have? What control can the defendant have over the crime scene examination and investigation? In present practice, none at all. Can a defendant be irreparably harmed by the premature release of a crime scene? What remedies exist or can be envisioned?

Reference:

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Scientific Investigation, Crime Scene Investigation, Due Process



Jurisprudence Section - 2015

F29 Legal Update: Recent Court Decisions Declaring Testimony About Case Reviews Performed by Non-Testifying Experts to Be Inadmissible

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After attending this presentation, attendees will be better informed about some recent court decisions and the evidentiary grounds that have led courts to declare that expert testimony about case reviews performed by non-testifying experts is inadmissible.

This presentation will impact the forensic science community by educating all stakeholders in the administration of criminal or civil justice — experts, lawyers, and judges — about the evidentiary issues that preclude expert testimony about casework peer reviews by non-testifying experts.

Expert witnesses from various forensic disciplines have often testified that their casework is subjected to so-called “peer review.” Such testimony, generally elicited during direct examination, is supposedly introduced for the purpose of demonstrating the quality assurance protocol employed in the testifying witness’ forensic laboratory prior to the issuance of a final report. Lawyers and trial judges frequently assume that the mere mention of the words “peer review” equates to a comprehensive reexamination of the evidence and an independent verification of a given opinion or conclusion; however, sometimes the so-called “peer review” comprises little more than a “spell check.”

Some lawyers have attempted to challenge such testimony by establishing that the casework peer review was biased and unreliable because it was not performed blindly, e.g., the reviewer was a “friendly” colleague or coworker, working in the very same laboratory unit or office; however, as some recent court decisions indicate, testimony about case reviews performed by non-testifying experts has been ruled inadmissible on evidentiary grounds, and lawyers, judges, and testifying experts need to know the impact of these recent court rulings.

Casework Reviews, Inadmissible Testimony, Bolstering



F30 Reliability, Validity, Accuracy, and Bias in Forensic Document Examination: Results From an Interdisciplinary Study of Questioned/Known Signature-Comparison Tasks

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After attending this presentation, attendees will understand some of the theoretical principles of cognitive psychology and how the application of psychological principles and research methods to the question of how Forensic Document Examiners (FDEs) reach conclusions provided important information about some of the cognitive and physiological aspects of the evaluation of signatures. This presentation discusses some of the relationships among signature type, signature complexity, and the deployment of attention in signature comparison tasks as they relate to process and authorship decisions in a sample of handwriting comparisons.

This presentation will impact the forensic science community by demonstrating the importance of engaging in theoretically based, multidisciplinary research to reach an understanding of the nature of the methodology and expertise in forensic document examination.

This presentation reports findings from a national study of forensic document examiners concerning the deployment of visual attention as it relates to signature type and complexity.

A substantial portion of FDE training is devoted to signature comparisons, handwriting, and hand printing. FDEs seek those features and characteristics which may represent the document's identifying attributes. Examiners first determine the presence or absence of features, and then qualitatively assign these features some degree of evidentiary weight to reach their decisions. Examiners are trained to look for both substantial similarities and differences among writing samples and for repeated small characteristics which may sufficiently establish that writings are clearly the work of two individuals even though they may contain many general similarities. The number and quality of these features allow FDEs to make assertions about the authorship of the specimen and the extent of their confidence in their decisions.¹

Many current theories of attention propose that attention is based on the relationship between a bottom-up, saliency-based attentional system and a top-down, feature-specific selection mechanism. Attention is guided by relational information about the target or by information about how the irrelevant information of a non-target differs from the features of the target. Relational models of visual search demonstrate that visual attention can be guided by attending to specific feature values such as color, size, or intensity, by inhibiting attention to irrelevant features, or by directing attention to how stimuli differ. Relational models place the target in relation to its context, offering more specific (e.g., directional) information about differences.²

The features available for forensic evaluation are determined in part by the nature of the writing specimens. For example, compared to stylized or mixed signatures, text-based signatures may offer a greater variety of features for evaluation. Additionally, signatures vary in terms of their complexity (e.g., the number of turning points and crossing lines), their semantic content, and any number of additional features commonly recognized within the profession as indicators of the authenticity of the writing. Findings will be discussed in the context of Amos Tversky's "contrast model," which posits that most stimuli seem to be effectively described by the presence or absence of qualitative features. He and others argued that an object is represented by a set of features or attributes, and that judgments of similarity are achieved through a process of feature matching. The contrast model systematizes this "feature" approach and proposes that similarity depends on the proportion of features common to the two objects and also on their unique features.³

This research was supported by Award No. 2010-DN-BX-K271, National Institute of Justice, Office of Justice Programs, United States Department of Justice.



Jurisprudence Section - 2015

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Signature Comparison, Interdisciplinary Research, Reliability and Validity



F31 Prosecutorial Misconduct and Breaches in the *Brady* Doctrine

David M. Benjamin, PhD*, 77 Florence Street, Ste 107N, Chestnut Hill, MA 02467-1918

The goals of this presentation are to review the *Brady* Doctrine and examine cases of prosecutorial misconduct and breaches in the *Brady* Doctrine that have resulted in due process violations.

This presentation will impact the forensic science community by raising awareness of how *Brady* Doctrine violations prevent citizens from obtaining their due process rights.

The Registry of Prosecutorial Misconduct defines prosecutorial misconduct as any conduct, intentional or inadvertent, during the course of prosecution that: (1) violates the applicable code of professional ethics; (2) breaks a pertinent law; or, (3) prejudices, or appears to prejudice, the administration of justice.¹

A major claim against prosecutors is violations of the *Brady* Doctrine.² In *Brady v. Maryland*, the Supreme Court held that “the suppression by the prosecution of evidence favorable to an accused upon request violates due process where the evidence is material, either to guilt or to punishment, irrespective of the good faith or bad faith of the prosecution.”³ Most disputes over *Brady* issues focus on the definition of what is “material.” Recently, the Supreme Court defined evidence as “material” when “there is a reasonable probability that, had the evidence been disclosed, the result of the proceeding would have been a different.”⁴ In toxicology cases, failure to produce an exculpatory lab test occurs and the ramifications of this will be discussed.

Moreover, there are a number of questions related to the prosecutor’s duty to disclose material exculpatory evidence that are still unresolved: (1) what is the depth of the duty *Brady* imposes on prosecutors to look for and turnover material exculpatory evidence; (2) do a district attorney, attorney general, and United States attorney all share the same duty under *Brady*; (3) if a prosecutor fails to meet the duty under *Brady*, is the director of that operation vicariously liable for the omissions of subordinates; (4) can a prior defendant who was prejudiced by that action sue for civil liability; and, (5) in the absence of the right to sue for civil liability, what other legal remedies does the defendant have to right a prior breach? Filing a petition for a new trial and alleging both fraud against the government and ineffective assistance of counsel who represented the defendant are common. Failure to discover the withheld documents is ineffective, and the government’s failure to conduct an adequate search for the material exculpatory information or produce it in a timely manner rendered the trial fundamentally flawed.

Connick, District Attorney, et al. v. Thompson addressed the issue of vicarious liability of local government agencies.⁵ In *Connick*, John Thompson was convicted of attempted armed robbery. The Orleans Parish District Attorney’s Office conceded that prosecutors failed to disclose evidence that should have been turned over to the defense under *Brady*. Thompson was convicted. Because of that conviction, Thompson elected not to testify in his own defense in his later trial for murder and he was again convicted. Thompson spent 18 years in prison, including 14 years on death row. One month before Thompson’s scheduled execution, his investigator discovered that evidence had been withheld in his armed robbery trial. The reviewing court determined that the evidence was exculpatory and both of Thompson’s convictions were vacated.

After his release from prison, Thompson sued the Orleans Parish District Attorney, Harry Connick, in his official capacity for damages, alleging that Connick had failed to train his prosecutors adequately about their duty to produce exculpatory evidence and that the lack of training had caused the failure to disclose in Thompson’s robbery case.⁶ The jury awarded Thompson fourteen million dollars and the Court of Appeals for the Fifth Circuit affirmed. The Supreme Court ruled that local government liability for failure to train cannot be based on a single incident, but the plaintiff must show a pattern of similar constitutional violations.

Commonwealth v. Christina Martin, a case where the prosecution failed to turnover a negative confirmatory gas chromatography/mass spectrometry test will be discussed, and the analysis of the motions judge shared.⁷



Jurisprudence Section - 2015

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 6. *Ibid*
 7. *Commonwealth v. Christina Martin*, SJC-06789, 1998
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Prosecutorial Misconduct, The Brady Doctrine, Material Exculpatory Evidence



Jurisprudence Section - 2015

F32 Ethics in the Study of Forensic Science: Can Ethics Be Taught?

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After attending this presentation, attendees will consider how the formal study of ethics might be evaluated to determine the impact on ethical responses of forensic science professionals.

This presentation will impact the forensic science community by delineating how increased attention will be given to the ethics being taught to future professionals in forensic sciences. Suggestions will be garnered to improve the teaching of ethics. An additional result is that consideration will be given by academics and professional societies to strengthening the ethical standards and improving adherence to those standards.

This session will present an updated survey of ethics hours offered by forensic science programs that are accredited by the Forensic Science Education Programs Accreditation Commission (FEPAC). The session will also review the learning objectives, the textbook, and other class materials for a three-hour semester credit class. The class, "Forensic Science Ethics," taught at Purdue University was created as the capstone class for the forensic science minor. The impetus to create the class arose from the expense and disruption caused by problems at the Indiana State Department of Toxicology. The fact that other laboratories were facing challenges at that time added weight to the decision. As the current requirements for ethics instruction were reviewed with colleagues, some skepticism was voiced as to whether ethics can be taught. This presentation's counter argument, silence equals complicity, will be discussed.

The question of how the class impact might be properly evaluated will also be discussed.

Ethics, Teaching, Forensic



Jurisprudence Section - 2015

F33 Shaken Baby Syndrome: Current Evidence of a Pathological Entity

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After attending this presentation, attendees will recognize recent studies and publications on the proposed mechanism of sudden death associated with the Shaken Baby Syndrome (SBS). The presentation will discuss enhanced dissection techniques for the identification and documentation of neck injuries in suspicious infant deaths.

This presentation will impact the forensic science community by providing documentation of fatal neck injuries in infants suspected of abuse. It will provide insight into the injuries critical in explaining deaths due to SBS.

For the past 30 years, physicians have diagnosed SBS on the basis of the triad of subdural hematoma, retinal hemorrhages, and brain swelling. It is estimated that more than 1,000 infants are diagnosed with SBS in the United States each year. The entity was initially described by Caffey and others as unexplained subdural hemorrhage in infants without observable trauma or impact. Unable to demonstrate an acceptable mechanism of death in cases due to shaking, proponents have argued that the presence of associated trauma to the chest and ribs, lack of scalp impact, along with studies of post-conviction confessions supports shaking as a mechanism of injury.¹

Critics of the existence of SBS point to research using primates and inanimate models that show shaking alone provided insufficient biomechanical force to cause the formation of subdural hemorrhage as well as questioning the veracity of confessions. Defense experts routinely site incidental short-distance falls, resuscitation efforts, or rare medical problems as the cause of the subdural and retinal hemorrhage. Legal experts decry the lack of evidenced-based medicine and argue that until the mechanism of death in SBS is detected, it does not exist.

In 2008, the Wisconsin Court of Appeals supported a request for a new trial for Audrey Edmunds who had been convicted in the death of a 7-month-old child ten years earlier. During the testimony, the original pathologist volunteered that he could no longer support his prior testimony. Following the testimony of numerous experts, the court ruled for a new trial in that “a shift in mainstream medical opinion” had undermined the diagnosis of SBS.²

In 2011, Evan Matshes, a forensic pathologist, published a paper describing an enhanced dissection technique of the cervical spine of infants suspected of abuse. Using a matched control group, Matshes demonstrated the presence of hemorrhage in spinal nerve roots, especially in those responsible for respiration and heart rate. This study confirmed the findings of other forensic pathologists with similar cases. The findings of cervical spinal trauma is an acceptable mechanism to explain death in SBS.³

In addition to Matshes, forensic pathologists in the New York City Office of the Medical Examiner have confirmed other published studies, the presence of subdural hemorrhages, subarachnoid hemorrhage, and optic nerve hemorrhages and brain swelling without evidence of head impact after a thorough postmortem examination. In their study, 10/46 (22%) of infant homicide cases lacked an impact site. Of these, 29% demonstrated spinal nerve hemorrhage. It is argued that whiplash shaking without impact is the cause of death in a subset of infant homicides.⁴

In light of new techniques in the examination of an injured child, prosecutors, judges, and medical experts should evaluate all the existing evidence when deciding whether a child has been fatally shaken, especially when there is no other clinical context to explain what is otherwise a fatal head injury.

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Shaken Baby Syndrome, Abusive Head Injury, Child Abuse

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F34 Depraved Indifference — Murder of a Child in New York: Are Moms Getting Away With Murder?

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After attending this presentation, attendees will wonder whether those who cause the death of innocent children are being held to a proper legal standard as, over the years, New York's appellate courts have restricted the application of "depraved mind" murder (an equivalent in seriousness to intentional murder) for prosecutors.

This presentation will impact the forensic science community by illustrating deficiencies or "quirks" in the law of child abuse that prevent parents from being held responsible for the deaths of their children in egregious, "non-intentional" cases if they have attempted assistance in any way.

In the presented case study, the evidence overwhelmingly established that the defendant knew her son had sustained devastating, life-threatening injuries at the hands of her domestic partner and was in severe pain. Nevertheless, she did not call an ambulance or take her son to the hospital. Instead, she and the codefendant made worthless and belated efforts to treat the child with home remedies and children's acetaminophen. The defendant otherwise ignored her child's injuries over a period of seven hours. During this time, the defendant made casual telephone calls without mentioning the child's injuries, drank beer, and smoked, and then went to sleep. She finally called 911 at or around the time the child died. Even then, she took the time to dispose of potentially incriminating evidence before making the call. Furthermore, she admitted that she did not seek medical attention earlier because she was afraid of being blamed for the injuries. The fact that she deliberately placed her own interests ahead of her son's need for emergency treatment was strong evidence that her omission evinced depraved indifference rather than mere recklessness or negligence.

The mother's conviction was upheld at the intermediate appellate level; however, the highest appellate court reversed and remanded, finding that the treatment, given after the injuries were obvious, was a quintessential case of far too little, far too late; but to them demonstrated that it did not support a finding that she did not care at all. As for the fact that she attempted to conceal the crime, that also did not, in the court's view, evince a mental state of depraved indifference. Zahira Matos pleaded guilty to a new indictment charging her with Reckless Manslaughter.

Lawyers may be surprised to learn that even a studied indifference to the duty of care owed to a child by his mother can be considered merely reckless in New York, even when horrific injuries are demonstrated, as long as they cared to the smallest extent — even if the child is left to die.

This presentation introduces a case study of a child severely beaten by his mother's domestic partner, and then left without medical treatment in full view of both as they labored to cover up their crime. Experts at the trial of the mother found that the child died from child abuse syndrome and suffered for hours before death. When the two women finally called for police and medical assistance, the child had likely been dead for hours.

Child Abuse, Wound Interpretation, Homicide



Jurisprudence Section - 2015

F35 Child Psychological Abuse: Legal and Clinical Implications

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After attending this presentation, attendees will understand the definition of Child Psychological Abuse (CPA), clinical manifestations of CPA, legal implications of CPA, and criteria for reporting suspected CPA to child protection personnel. Attendees will also recognize how to distinguish parental estrangement (which typically involves physical abuse) from parental alienation (which involves CPA).

This presentation will impact the forensic science community by increasing the competence of mental health professionals in recognizing CPA in their clinical or forensic practice, thus helping them achieve greater confidence in treating and reporting CPA. Legal professionals will have increased competence in addressing CPA when it occurs in child custody cases and in dependency/neglect hearings.

Although the existence of child maltreatment can be traced to ancient times, it was not until the modern era that the mistreatment of children became a widely recognized social issue, which professionals in science and in law have addressed in order to protect children from harm. Psychologists, physicians, and social scientists have conducted research to analyze, identify, and understand this issue, while lawyers, judges, and social workers have been preparing directives and safety plans to protect children from maltreatment. The goals of science and law intersect to protect children from abuse.

Child maltreatment can be categorized into four types of abuse: (1) physical abuse such as kicking, biting, shaking, bruising, stabbing, or punching to cause physical injuries; (2) sexual abuse such as fondling, intercourse, exhibitionism, and commercial exploitation through prostitution and pornography; (3) neglect is the failure to meet children's basic needs for food, clothing, medical attention, or proper supervision; and, (4) emotional/psychological abuse such as social isolation, repeated unreasonable demands, ridicule, humiliation, intimidation, or terrorizing, which causes serious mental or behavioral disorders. Among these four types of abuse, the presenters will focus on the last form of child maltreatment — emotional/psychological abuse.

The federal definition of CPA refers to acts or omissions — other than physical abuse or sexual abuse — that caused or could have caused: conduct disorder, cognitive disorder, affective disorder, or other mental disorder. CPA frequently occurs as verbal abuse or excessive demands on a child's performance. CPA is a difficult topic for legal professionals and mental health practitioners to understand and address. The child protection agencies in the states have widely different approaches to identifying CPA; among the 50 states, the frequency of CPA ranges from 0.1% to 51% of substantiated cases of child maltreatment.

It is likely there will be an increased interest in this topic because CPA finally became an official diagnosis for mental health professionals in the United States when the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5), was published in 2013. The presenters will explain: (1) a brief history of CPA from the 1970s to the present time; (2) various definitions of CPA; (3) federal and state legal cases in which CPA was an issue; (4) clinical manifestations of CPA; and, (5) guidelines for reporting suspected CPA to child protection personnel. Also, there will be a discussion of the importance of distinguishing parental estrangement (when a child refuses contact with a parent because of a history of physical abuse, sexual abuse, or neglect) and parental alienation (when a child refuses contact with a parent because the child has been indoctrinated to fear or hate the rejected parent, which is a form of CPA).

Child Psychological Abuse, Parental Estrangement, Parental Alienation



F36 Hoarding Disorder: Whose Problem Is It, Anyway?

James P. Cho, MD*, 238 County Down Lane, Loveland, OH 45140; and Scott Bresler, PhD*, UC Medical Center, Division of Forensic Psychiatry, 260 Stetson Street, Box 670559, Cincinnati, OH 45219

After attending this presentation, attendees will be able to: (1) specify the *Diagnostic and Statistical Manual of Mental Disorders*, Fifth Edition (DSM-V) criteria of hoarding disorder; (2) describe the limited treatment options for hoarding disorder; (3) understand that mental health treatment for an involuntarily committed patient was ruled constitutional under the 14th Amendment by the United States Court of Appeals, Fifth Circuit; and, (4) identify that the issues of involuntarily committing and treating a person for hoarding disorder may be incompatible with attaining therapeutic benefit.

This presentation will impact the forensic science community by presenting several of the questions yet to be answered about hoarding disorder's place within the realm of involuntary commitment and court-ordered treatment.

Statement: Hoarding disorder is a new diagnosis in the DSM-V, but there are several questions yet to be answered about its place within the realm of involuntary commitment and court-ordered treatment.

Brief Synopsis: Hoarding disorder is a new diagnosis in the DSM-V that is generally understood to be potentially dangerous to one afflicted by it and perhaps to the community at large. The disorder presents with challenges to clinicians and the law as to where it fits within the context of involuntary commitment and treatment.

In the case of *Donaldson v. O'Connor*, the United States Court of Appeals stated, "We hold that a person involuntarily civilly committed to a state mental hospital has a constitutional right to receive such individual treatment as will give him a reasonable opportunity to be cured or to improve his mental condition."¹ Despite this, there are no accepted psychotropic medication treatments for this disorder, and psychotherapy is difficult at best with a patient that does not want to undergo treatment. Even with patients willing to accept treatment, improvements are difficult to achieve.²

In *Rogers v. Commissioner of Mental Health Department*, the Massachusetts Supreme Court ruled that in non-acute emergent situations, medications could not be given against the patient's consent unless they were ruled incompetent.³ Moreover, there must be treatment methods that are evidenced-based and widely accepted within the treatment community for the identified disorder leading to civil commitment; however, hoarding disorder is not generally associated with cognitive impairments, patients often demonstrate they have knowledge of their situation, and there are no accepted psychotropic medication treatments for this condition. Moreover, the hoarding behavior may be the cause of the identified danger, but is not or is rarely seen as acutely emergent.

In the situation of a hoarding-disordered patient involuntarily committed to a psychiatric unit, a number of questions arise: (1) is the person incompetent to understand their situation; (2) how can we treat them; and, (3) if there is no appropriate treatment, does that mandate release despite the continuing danger and mental illness?

If dangerous hoarding-disordered patients are not to be involuntarily held in a psychiatric unit, who in the community, if anyone, is responsible? The department of health or the police, or are the patients to be arrested or ignored despite the persistent danger? Adding to the confusion, law enforcement personnel are often encouraged to not arrest persons in the community that they suspect may be mentally ill as being incarcerated may hurt the person's ability to attain mental health care.

In this presentation, two hoarding-disordered patients who were held in a civil-psychiatric hospital for hoarding disorder are presented. In both cases, the patients were involuntarily committed; however, the court judgments did not explain how the rulings were compatible with the legal precedents established above. Both patients wished to return to their homes despite the fact that the homes were condemned by the department of health and declared too dangerous for habitation.

Hoarding disorder is a new diagnosis in the DSM-V that has gained popular attention due to the media, but the mechanics of involuntarily committing patients diagnosed with this disorder have not been sufficiently analyzed within academic forums or scientific literature. Many judges and magistrates entrusted with the gatekeeping responsibilities of civil commitment are involuntarily committing such patients without explaining how it can be appropriately done given prior legal precedents implying otherwise.



Jurisprudence Section - 2015

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Hoarding Disorder, Involuntary Commitment, Civil Liberties



F37 Why DNA Interpretation Has Become More Challenging in Recent Years

John M. Butler, PhD, NIST, 100 Bureau Drive, MS 4701, Gaithersburg, MD 20899*

After attending this presentation, attendees will better appreciate interpretation challenges being faced by forensic DNA laboratories.

This presentation will impact the forensic science community by providing a better understanding of how improved sensitivity has brought new challenges to forensic DNA interpretation.

DNA interpretation with Short Tandem Repeat (STR) markers involves utilizing genotyping software and laboratory Standard Operating Procedures (SOPs) to evaluate Capillary Electrophoresis (CE) data. Peaks in multi-colored CE electropherograms generated as CE mobility time points are translated into DNA size information and then to allele repeat number for each STR locus. In both evidentiary and reference samples, decisions are made for each peak above an analytical threshold regarding whether or not the peak is an allele or an artifact, whether or not alleles at an STR locus can be paired to form a genotype, whether it is possible for some alleles to be missing from the data, and whether or not the sample originated from a single-source or a mixture of multiple contributors. Validation studies are essential for setting parameters used in a laboratory's SOPs to make these decisions. Guidance on validation studies and data interpretation has been provided from organizations such as the Scientific Working Group on DNA Analysis Methods and the European Network of Forensic Science Institutes.

There are two areas of forensic DNA interpretations which are particularly challenging: (1) low-level DNA samples where sensitivity is an issue; and, (2) complex mixtures where specificity is an issue. The number of contributors to a sample and the amount of DNA available for testing can impact the uncertainty or ambiguity in the conclusions drawn. In many situations involving complex results where uncertainty in the interpretation is large, the only scientifically responsible conclusion is "inconclusive" to avoid the chance of inappropriately including or excluding a potential contributor from an evidentiary result.

It is important to keep in mind that some measurements and interpretations are more reliable than others. Thus, uncertainty in measurements and interpretation should be reflected in the reports generated in a forensic case investigation. Assumptions made during the interpretation process should be documented and conveyed as clearly as possible. This documentation will aid those individuals reviewing the lab report to appropriately assess the results obtained and the conclusions drawn.

This presentation will discuss principles behind why DNA interpretation has become more challenging in recent years with an increase in sensitivity being applied by modern polymerase chain reaction amplification techniques. Approaches taken for interpretation may be subjective in some cases and therefore can become the weakest part of the overall DNA typing process. This presentation will also provide some historical perspective on the impact of improved sensitivity with DNA analysis and the desire to extend the reach of forensic DNA testing.

Forensic DNA, DNA Mixtures, DNA Sensitivity



F38 Who and What: Providing Context to a DNA Profile

John Ballantyne, PhD, University of Central Florida, Dept of Chemistry, 4000 Central Florida Boulevard, Orlando, FL 32816-2366; and Erin K. Hanson, PhD, PO Box 162367, Orlando, FL 32816*

After attending this presentation, attendees will learn how forensic geneticists may be able to provide contextual activity-level information pertaining to what transpired prior to the deposition of a DNA profile.

This presentation will impact the forensic science community by demonstrating the need for forensic scientists to be aware of the alleged circumstances of the crime despite the possibility of contextual bias.

I keep six honest serving-men (They taught me all I knew); Their names are What and Why and When and How and Where and Who...Rudyard Kipling

The ultimate aim of forensic scientific evidence in a criminal investigation is to help establish a fact or facts that may be at issue. The questions involve what, why, when, how, where, and who as they pertain to the people, places, and things involved in the commission of the crime. The DNA technology revolution has significantly impacted the criminal justice system in that the individual who deposited crime scene biological trace or body fluid evidence can be routinely identified. This source level information has facilitated the identification and conviction of perpetrators and the exoneration of falsely accused individuals.

The forensic DNA community has concentrated its efforts in producing for stakeholders an error-free product with, arguably, less emphasis on completeness of analysis, including trying to answer questions that go beyond identifying the source (the “who” and to a lesser extent, the “where” questions). Specifically, forensic geneticists currently are rarely in a position to provide context to the DNA profile, namely what “activity” led to the deposition of the biological material. This pertains to the “what” (and perhaps “how”) question relating to the case events. For example, consider a sexual assault on a female victim with an object (recovered from the suspect) and the victim’s DNA is recovered from the object. He could claim that the victim handled the item during a casual encounter and this explains why her DNA was present; however, the significance of this evidence would increase if the DNA could be shown to originate from vaginal cells, a circumstance that would be consistent with a sexual encounter but not with casual handling. The ability to distinguish between these alternative propositions, sexual versus social contact, might prove to be critical to the investigation and prosecution of the case. Failure to provide this critical probative information could allow perpetrators to exploit the shortcomings in routinely used body fluid identification methods in order to provide reasonable doubt regarding the true circumstances of the crime. This presentation will briefly describe new methods that permit the definitive identification of activity-level-indicating body fluids such as vaginal secretions, menstrual blood, and skin as well as blood, semen, and saliva.

There has been some discussion in the scientific literature and in the popular press that forensic scientists should be blind to the circumstances and details of the crime due to the possibility of contextual bias influencing their interpretation of the scientific data. This study argues that forensic geneticists should, in addition to identifying the source of biological material, also concern themselves with trying to help deduce what occurred. In order to effectively assess the strength to be attached to the scientific findings, the alleged circumstances need to be taken into account and the scientist needs to be aware of them. The framework for evaluative reporting is the likelihood ratio, which measures the strength of support the findings provide to discriminate between propositions of interest.

DNA Profiling Context, Body Fluid Identification, Source/Activity Level



F39 Biological Evidence Storage and Disposition: A Discussion of Legal Implications, Trends, and Policy Recommendations

Shannan Williams, MA, 100 Bureau Drive, Gaithersburg, MD 20899*

After attending this presentation, attendees will be aware of the recent deliberations of the Technical Working Group on Biological Evidence Preservation in developing recommendations to policy makers regarding the issue of biological evidence storage and disposition.

This presentation will impact the forensic science community by providing insight on the legal and policy considerations in the improvement of biological evidence storage and management.

In August of 2011, the National Institute of Justice and the National Institute of Standards and Technology's Law Enforcement Standards Office convened the first meeting of the Technical Working Group on Biological Evidence Preservation. The primary objective of the working group is to establish best practices, based in science, to reduce the premature destruction and degradation of biological evidence, thus ensuring its availability for future analysis; however, a key dimension to the work of the group is the legal and legislative landscape of biological evidence retention. Following the publication of *The Handbook on Biological Evidence* in 2013, the group agreed to produce additional recommendations geared specifically toward policy makers to facilitate the implementation of best practices within law enforcement agencies. The purpose of this presentation is to bring awareness to these issues and introduce the preliminary recommendations being proposed by the working group.

Recent headlines have highlighted significant problems with the storage of potentially exculpatory biological evidence in property and evidence storage units across the country. Court orders for the location of evidence have demonstrated inadequacies in the packaging, storage, and tracking process of some evidence. Investigations into these inadequacies reveal underlying factors such as: capacity of the storage facility; laboratory backlog; materials available for packaging; geographic distance between the collecting and storage facility; and, the selected tracking system. While preserving and readily retrieving biological evidence from adjudicated and unsolved cases is a goal and has clear benefits for all members of the criminal justice system, the management of retaining and eventually disposing of biological evidence requires that each state and jurisdiction consider the relevant legal and policy issues.

Most states have laws that provide guidance for the evidence storage and disposition process but these laws vary widely. This process may include getting a court order, district attorney approval, notification of the law enforcement agency, or notification of the defendant/defense attorney or attorneys of record. Recent Supreme Court decisions including *Melendez-Diaz v. Massachusetts* and more recently *Bullcoming v. New Mexico* set out the importance of chain-of-custody documentation and the importance of evaluating the integrity of evidence and the circumstances of testing. Creating the appropriate sanctions for evidence destroyed in violation of relevant policy and ensuring remedies for the denial of access to biological evidence are also issues under consideration by the working group based on its analyses.

Evidence, Policy, DNA



Jurisprudence Section - 2015

F40 DNA Typing: Controls and Validation Updates to Support Current Testing

Christie T. Davis, PhD, Helix Analytical, Inc, 90 Saturn Street, San Francisco, CA 94114*

After attending this presentation, attendees will learn that current controls and validation used for optimum amounts of DNA are lacking in establishing scientific practice for low amounts of DNA, which deters accuracy in obtaining and interpreting DNA typing results. Suggestions will be provided for controls and studies needed to augment current procedures.

This presentation will impact the forensic science community by providing information that will improve DNA typing accuracy. While DNA typing kits and analytical equipment have rapidly changed to allow typing of low quantities of DNA (Low Level DNA (LLD)), there has been no matching change in requirements for validation studies and controls to address additional problems that arise in typing LLD. The goal of this presentation is to present techniques and studies to strengthen current testing results.

Existing validation studies of two-person mixtures and sensitivity have demonstrated that LLD typing may exhibit known problems such as stochastic effect, founder effect, allelic dropout, and allelic drop-in. These issues become exacerbated with the use of current kits and analyzers that can operate with very low quantities of DNA, particularly when mixtures consist of more than two persons. Studies will be discussed that demonstrate that allele sharing can confuse interpretation of the number of contributors present; the data may look like two persons, but are actually three. Crime labs using data from LLD typing can address these issues through directed validation and controls. The existing validation studies do not address peak height imbalance due to allele sharing and investigation-borne contamination. Current controls do not address detection of contamination or stochastic effect in mixtures.

Currently, LLD typing is treated no differently than sufficient DNA samples with regard to testing controls. Existing controls are made up of sufficient DNA (positive amplification and Quality Control (QC)) or no DNA (negative control). LLD controls should be added, such as a low-level single-source known control and low-level mixture controls. It would be advantageous to add blind LLD mixture controls. Some labs currently set up a QC sample where the result is unknown to the analyst who then types the sample and provides the profile to the laboratory QC supervisor. It would be of minimal disruption to add an unknown LLD mixture sample.

LLD sample results are also more susceptible to contamination picked up during scene processing, during autopsies at coroner laboratories, from a victim requiring medical procedures, and during crime laboratory processing. Cases will be presented in which contamination was “proven” after the fact. Validation studies would help ascertain where conditions are poor and what steps can be taken in advance rather than in the aftermath. Mixture results are particularly problematic in determination of whether investigation-borne contamination is present and at what levels. Validation studies addressing this issue will be discussed.

Additional cleaning and testing procedures can be established at the DNA laboratory, coroner’s autopsy rooms, and other potential evidence-handling locations to predetermine what contamination sources may exist. Suggestions will be made during the presentation on possible procedures to decrease contaminating DNA. Additional validation on pipetting accuracy will ensure more accurate results. LLD sample typing is more susceptible to amplification problems due to poor pipetting skills.

Validation, Contamination, Controls



Jurisprudence Section - 2015

F41 The Discovery Motion for “Scientific Stuff:” Don’t Expect to Get It, Find It, or Recognize It (Even if You Do Get It) if You Have No Idea What You’re Looking For!

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The goal of this presentation is to focus on documentation which should be available through a discovery motion for review by both sides as a part of pre-trial discovery. Specific technical documents and operational protocols will be identified. The objective will be to provide a lawyer with the answer to the question: What am I looking for and what could it mean?

This presentation will impact the forensic science community by enhancing the knowledge base of what to request in a discovery motion and what to expect from the forensic science laboratory. (Since the credibility of a forensic scientist’s testimony could be inversely proportional to the number of four-syllable words which are used without an explanation in a “lawyerly discussion,” the technical jargon in this presentation will be minimized.) There are required laboratory documents which the defense should request and which the prosecution should be aware of in order that both sides can fulfill their responsibilities to the courts in evaluating the elements associated with the case at hand.

Some of the more common questions lawyers wonder about in reviewing a forensic analysis or discussing the analysis with the “expert” are: (1) what does “get it” mean; (2) do I know exactly what I am looking for or looking at; (3) why didn’t someone tell us about this; (4) what do all those big words mean and are they important; (5) how am I supposed to cross-examine an expert when I have no idea what he/she is talking about or when I don’t understand what was done in my case; and, (6) standards, what are standards?

Most forensic science laboratories in the United States have nothing to hide and issue reports which reflect conformance to standards. Most laboratories have subscribed to the oversight of external accrediting bodies and state-wide commissions to maximize the probability that everything is transparent in the management and operational aspects of valid science. Disclosure of what is happening behind those doors with signs dictating “Authorized Personnel Only” is there for the asking; however, there are times when the defense and even the prosecution should be more inquisitive in seeking answers to questions which have a legal impact on what is happening behind those locked doors. The prosecution can usually pay a visit to the laboratory for a sit-down. The reality is that these “sit downs” with the prosecution occur far too infrequently. The “other side” faces a more daunting challenge. The defense, if they want to see anything, may be asked to a court order. Sometimes there may be valid reasons for denying admission to certain part of the laboratory (like the vault) to “unauthorized personnel.” Even when the defense is allowed into the laboratory, all they will usually see are white coats, dropper bottles, flasks, microscopes, humming machines with robotic arms picking up small rubber-capped vials, and row after row of computer monitors with images of straight lines, curved lines, or columns of numbers.

The properly worded discovery motion is one effective way to obtain the answers to the most important questions. The challenge for the lawyer is this: How do you get what you need if you don’t know what you are looking for and if “you do get it,” what does it all mean? Many discovery motions are couched in terms of “give me everything you’ve got.” Even if and when “everything” is provided, the lawyer probably has no idea what “everything” (all that paper with charts and lines and paragraphs with four- to six-syllable words) really means.

So the next step is to ask for a CV or Bio and proficiency test results. Nearly everyone will recognize an academic degree or the fact that the analyst passed a proficiency test. The issue here is that this information will provide very little if anything about the “case at hand.” Most analysts have college degrees and most analysts pass a proficiency test. If they don’t pass a proficiency test, they’ve probably been “rehabilitated.” Then there are the requests for “six months of calibration and maintenance records for those machines.” Even with the reams of paper which are produced for this request, there will be little information obtained with which to evaluate the relevance, reliability, and validity of what was done in the “case at hand.”



Jurisprudence Section - 2015

The factors in determining the reliability of any scientific analysis conducted in a laboratory can be “discovered” by evaluating documentation related to how the laboratory is managed; whether the laboratory is conforming to scientific standards which have become pro-forma requirements in forensic science laboratories; whether the data in the case at hand conforms to reporting protocols; whether all of the documentation supports the conclusions; whether alternative explanations are possible for those conclusions; and, whether all of the information which may be exculpatory is being provided. A properly worded discovery motion for specific documents can provide some level of assurance that the answers to the relevant questions related to the case at hand are actually being provided, remembering that nothing is absolute. Standards do exist and conformance to those standards is the foundation of a laboratory’s credibility.

Discovery Motion, Scientific Standards, Scientific Data



F42 It's My Toy and You Can't Play With It: Defense Counsel Problems With Access to CODIS

Alissa L. Bjerkhoel, JD*, 225 Cedar Street, San Diego, CA 92101

After attending this presentation, attendees will have an overview of the origins and scope of the Combined DNA Index System (CODIS), the requirements for obtaining access to CODIS, and the obstacles defense counsel face when attempting to use this investigative tool to establish their client's innocence.

The presentation will impact the forensic science community by identifying an existing problem with CODIS access, highlighting the lack of any substantiated counterargument for the denial of such access, and proposing the need for legal reform to make CODIS equally available to defense counsel in a criminal justice system that prides itself on being fair.

Over the past two decades, DNA technology has revolutionized the nation's criminal justice system. DNA has become the foremost technique for conclusively identifying and excluding criminal suspects in cases where biological material is left at a crime scene. As of June 2014, CODIS produced over 250,000 hits which helped in nearly 239,000 investigations.¹ Thousands of courts throughout the United States have reversed convictions of people who were wrongfully convicted.² Currently, there have been 1,406 individuals exonerated in the United States.³ Of those exonerations, 317 have been the result of DNA testing, and convictions taking place as early as 1974 have later been reversed through DNA testing.⁴ Of the 317 post-conviction DNA exonerations, DNA testing has led to the identification of the true perpetrator in a staggering 152 of the cases.⁵ There can be no denial that DNA is the leading tool for convicting the guilty and exonerating the innocent.

Sadly, only nine states — Colorado, Georgia, Illinois, Maryland, Mississippi, New York, North Carolina, Ohio, and Texas — have laws giving defendants access to CODIS.⁶ Defense counsel's success in accessing CODIS in other jurisdictions tends to be the result of luck, interested law enforcement, or the kindness of prosecutors. More often than not, law enforcement and prosecutors have outright blocked defense counsel access. The typical arguments against access is that the defense has no right to access, or that access would result in the disruption of the national database system altogether. Such claims appear to be largely unfounded and concerns could be satisfactorily dealt with by proper reform. Further, the denial of access is at odds with a defendant's right to due process and the government's obligation to disclose exculpatory evidence.⁷ Steven Benjamin, the National Association of Criminal Defense Lawyers' president, said, "Science doesn't belong to the government, but they act like it does."⁸ It should not be so.

In 2009, prosecutors charged Reggie Cole with possession of a razor blade in prison; a razor blade had been found inside his prison mattress. DNA testing was performed on the razor blade and Cole was excluded. Cole motioned for the profile to be run through CODIS. The motion prompted the DNA attorney for the California Department of Justice to personally appear in the remote county where the trial was taking place to successfully oppose the motion. Uriah Courtney's case was much different. In 2005, Courtney was convicted of kidnapping, rape, and other charges. He served more than seven years in prison before a forensic profile was run through CODIS and generated a match to the true perpetrator. Unlike Cole, the county prosecutor agreed the profile generated should be run through CODIS.

Other examples highlighting defense counsel's struggle and the need for improving the criminal justice system will be discussed.

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DNA, CODIS, Defense Counsel

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F43 “In God We Trust, All Others We Cross-Examine” — Cross-Examination in DNA Mixture Cases

Nicole Kubista, JD*, 7582 Currell Boulevard, Ste 212, Woodbury, MN 55125; and Rebecca A. Waxse, JD, 5669 147th Street, N, PO Box 548, Hugo, MN 55038

After attending this presentation, attendees will understand the theories behind cross-examination as a method for relaying information and uncovering biases and inaccuracies of this method as it relates to DNA mixtures.

This presentation will impact the forensic science community by highlighting the practical aspects of cross-examination, specifically where this tool meets and fails to meet its purpose of conveying accurate information to fact finders.

Cross-examination of witnesses is often a criminal defendant’s only hope of uncovering inaccuracies, relaying concerns, and testing the veracity of evidence against him. Depending on the initial presentation of evidence in the direct examination, cross-examination may offer a more comprehensive understanding of the evidence, allow juries to understand what parts of the analysis are subject to possible bias or multiple interpretations, help the jury assign weight and meaning to the evidence, and in some cases create confusion as to the nature of the testimony.

In the case of scientific evidence or evidence of a highly technical nature, such as DNA mixtures, the necessity for a defendant to convey the complexities internal to the case and within the forensic community is heightened. The important scientific discussions about interpretation of mixtures may make the difference between a conviction or an acquittal. Furthermore, the presentation of DNA mixture evidence may be given too much or too little weight in helping a jury decide the final outcome of a trial. Thus, for many defendants, cross-examination is the imperfect tool they must use to convey not only the complexity of the science but also the imperfections of human analysis.

For an expert witness, cross-examination is the moment where a perfectly laid out explanation of the scientific evidence is muddled by an attorney. The procedural demand for short explanations and “yes” or “no” answers leaves many witnesses with a sense that all the necessary information was not conveyed to the fact finder or worse, misinformation was relayed to the jury. This frustrating process appears to be in direct opposition to properly conveying scientific knowledge.

When it comes to explaining DNA mixtures, attorneys, witnesses, and defendants find themselves competing to control the information relayed to the fact finder. The tension between these parties is often complicated by the lack of limits on admissibility of scientific evidence. Without proper limits, both direct and cross-examination may attempt to push a witness into unsupported or contested scientific positions. This presentation will examine actual trial transcripts and draw from examples of DNA mixture litigation in criminal cases.

J. Wigmore in *Wigmore on Evidence* stated that cross-examination is “beyond any doubt the greatest legal engine ever invented for the discovery of truth.”¹ Other legal scholars view cross-examination as the principal means by which biases or inaccuracies of witnesses are uncovered; however, the real-world application of these theories creates highly variable results especially when a complex DNA mixture is the subject of cross-examination. Witnesses, defendants, attorneys, and everyone involved may feel frustrated because important information has been obscured or was unclearly communicated.

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DNA, Cross-Examination, Jurisprudence



Jurisprudence Section - 2015

F44 Interpreting Gobbledygook — What Lawyers and Forensic Scientists Can Do to Communicate Clearly About Scientific Evidence to a Jury

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After attending this presentation, attendees will understand the challenges that lawyers and forensic scientists face in trying to effectively communicate scientific evidence to a jury. Attendees will learn what works and does not work when communicating about scientific evidence and will receive practical suggestions as to the most effective means of communicating scientific evidence to a jury.

This presentation will impact the forensic science community by facilitating a discussion on what needs to be done by both lawyers and forensic scientists to increase the effectiveness of forensic science communication to a jury. This will enable juries to be able to use the evidence in a meaningful manner and will promote more informed results.

Lawyers and forensic scientists have all been told how juries give science in the courtroom a great deal of weight. Judges also place great weight on science. Science can be a very powerful tool in a court case; however, to best utilize this tool effectively, both lawyers and forensic scientists must be able to communicate about the science in an effective manner. Lawyers and forensic scientists each face challenges to ensure that the science is communicated clearly in the courtroom setting.

If a lawyer presenting a forensic scientist witness does not understand the language being used by the witness, the lawyer is not going to be able to ensure that the jury is understanding the witness' message. In addition, if a lawyer is cross-examining a forensic scientist witness and does not understand the language being used by the witness, the lawyer is not going to be able properly cross-examine the witness. To properly allow a witness to communicate scientific information, the lawyer must understand the information. The lawyer must be able to clearly communicate the scientific language and work to make sure the jury understands that language.

A forensic scientist's role in the courtroom is to clearly communicate how his or her testing was conducted and the results of his or her testing. Most forensic scientists could easily explain his or her process and results to other scientists without any issues; however, in court, the forensic scientist is not explaining the process and results to other scientists — the forensic scientist is explaining it to a jury. To complicate this further, the forensic scientist does not get to just get up and speak. He or she must answer the questions of the lawyers. This can be a very awkward manner in which to communicate information and often, due to this awkward manner of communication, a forensic scientist is not able to clearly communicate.

Forensic science has an important role in our justice system. This presentation will address what lawyers and forensic scientists need to do to ensure that the information is presented in an understandable manner so it can be used in the most effective way within the courtroom.

Language, Scientific Evidence, Jury



F45 Development of Small-Group Forensic Counsel in Greater Minnesota: Forensic Case Review in the Boonies

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After attending this presentation, attendees will understand the basic requirements, goals, and benefits of the establishment of a small, geographic-centered, forensic criminal case-review program for defense counsel. In addition, attendees will understand how such a group fits within a larger-scale statewide forensic program for defense counsel.

This presentation will impact the forensic science community by demonstrating the importance of initial and immediate forensic case review. This presentation will also demonstrate the need for involvement by defense counsel at the commencement of representation and throughout trial. Finally, this presentation will show how working within a small forensic unit assists in providing effective assistance of counsel and improved client representation.

In Minnesota's Ninth Judicial District, the public defender's office has created a template whereby cases involving forensic evidence are being identified immediately and assigned to individual lawyers who are trained in forensic litigation. There are six lawyers in the Minnesota Ninth Judicial District public defender's office who are trained in forensic litigation and comprise the unit. These individual lawyers act as a supporting mechanism for the trial counsel and work together as a group to review the cases and are also able to take part in larger statewide forensic training.

Although there have been many calls for the education and training of legal counsel in forensic fields, the actual implementation of programs are, for many, still aspirational rather than actuality. The initiation of a small forensic counsel group to review, assist, and advise trial counsel in greater Minnesota has provided a client base located in a geographically large area with a benefit that was previously much more difficult provide.

The establishment of small forensic groups to assist in initial review and assistance to trial counsel is now a necessity, rather than an option, for representation. With the widespread expansion and access of prosecution offices for inclusion of the forensic analysis of evidence, it is incumbent for defense counsel to provide for knowledgeable review of case-specific forensic evidentiary issues.

The involvement of forensic counsel at the onset of a criminal case provides for better representation and trial preparation for the individual criminal defendant because forensic issues are not only being identified, but actually being reviewed for issues that may directly affect the outcome of a client's criminal case, while also providing feedback and information with regard to the number of forensic cases being seen in a particular jurisdiction and related benefits because of forensic counsel involvement.

The specific challenges of a large geographic area of responsibility and non-centralized forensic counsel provides challenges that have been countered through the use and application of several technological issues. From simply conducting meetings or communicating through video conferencing, to large file-sharing programs, to detailed computer review of raw forensic data, technology is a cornerstone to a forensic group and an area that will need continual updating and training.

In conclusion, ensuring review of forensic case files for criminal defendants is essential in providing effective legal defense services for individual clients. Absent such involvement and review of individual forensic case issues, clients cannot be assured an adequate and effective defense, to which each is entitled.

Trials, Training, DNA



Jurisprudence Section - 2015

F46 In or Out? What is the Threshold for Admissibility?

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After attending this presentation, attendees will be more prepared to answer the question, what is the threshold for admissibility? Attendees will gain knowledge and an understanding about some of the tools which the forensic community uses and whether those tools are being used for investigative versus evidentiary purposes.

This presentation will impact the forensic science community by prompting defense and prosecution attorneys to be more careful in their examination of information gathered from the use of forensic tools. This presentation will urge attorneys to make a determination of whether information is being used merely as part of the overall investigation by law enforcement or whether that information is to be used at trial for acquittal or conviction.

The admissibility of forensic evidence has always created some challenging questions for the bench and bar. To be admissible, evidence must meet the standards set forth in the rules of evidence as well as the standards articulated by *Frye* and more recently, *Daubert*. Yet outside of the types of forensic evidence that meet these standards, there are forensic tools used for investigative purposes which are not intended to be and should not be considered evidentiary in nature. For example, the use of polygraphs to establish whether an individual is lying or preliminary breath tests to establish a blood alcohol concentration.

This presentation will explore an examination of the Rules of Evidence and the *Frye* and *Daubert* standards to help understand what the criteria are for evidence to be deemed admissible. It will further explore the category of forensic tools that are designed for “investigative purposes” and how these are and/or should be treated by criminal courts.

What makes a test “presumptive” versus “confirmatory” and why are these distinctions important? This presentation will explore the admissibility of other tools such as narcotic identification kits and “hits” to the DNA database. It will look at examples where these distinctions of “presumptive” and “confirmatory” are not fully appreciated by lawyers and the problems created when attorneys and judges do not think in these terms. The idea that “presumptive” does not equal “truth” must be considered when determining admissibility or inadmissibility.

The 2009 National Academy of Sciences Report, *Strengthening Forensic Science in the United States: A Path Forward*, found that lawyers “often lack the scientific expertise necessary to comprehend and evaluate forensic evidence.” Even six years later, this observation continues to ring true. Lawyers still struggle with how to understand and evaluate forensic evidence. Exploring the distinctions between what can and should be used for investigative purpose and whether such tools should be admissible may lead to a richer understanding of the interplay between forensic science and the courtroom, which can benefit the entire criminal justice system.

Admissibility, Investigative, Evidentiary



Jurisprudence Section - 2015

F47 How a Grant Manager Can Allow Scientists to be Scientists

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WITHDRAWN



Jurisprudence Section - 2015

F48 The Validity of Enzymatic Assay for Blood Alcohol Content (BAC) Determinations

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The goal of this presentation is to focus on the scientific issues concerning the limitations of Enzymatic Immunoassay (EIA) -based ethanol testing as well as the legal sufficiency and admissibility challenges that have occurred across the United States.

This presentation will impact the forensic science community by further spurring discussion in the legal community about the continued suitability of this assay when other, more specific assays are available.

BAC testing is crucially important in a Driving Under the Influence (DUI) prosecution. It is one of the fundamental pieces of, and often the only, evidence against a citizen who has been accused of a DUI. In the United States, there are three different types of methods used to provide for a BAC: breath testing, gas chromatography, and EIA (sometimes referred to simply as “hospital blood”).

Scientifically educated DUI defense attorneys have been successfully challenging the admissibility and the sufficiency of EIA evidence to form a conviction on a *per se* DUI BAC offense. These successful challenges simply point out the known limitations of the assay. The publication of these successful challenges has led many hospitals that offered EIA-based testing for BAC purposes to stop. In other jurisdictions, district attorney’s offices have ceased using EIA-based evidence due to various reasons such as an increase in costs due to the need to employ an expert to try to interpret and defend the EIA-based BAC evidence, a sense of justice and ethics, as well as the lack of expertise in their offices in successfully prosecuting such cases.

Mean ratio	± SD	Range	Sample Number	Reference
1.11	± 0.02	1.08-1.16	4	Hodgson, et al. 1985
1.14	± 0.02	1.09-1.18	50	Winek, et al. 1987
1.10	± 0.03	1.03-1.24	17	Jones, et al. 1990
1.15	± 0.041	0.88-1.59	211	Rainey 1993
1.14	± 0.04	1.04-1.26	235	Charlebois 1996
1.16	N/A	1.08-1.21	N/A	Iffland, et al. 1999

Table 1. Summary of alcohol concentration ratios for plasma and serum comparisons to whole blood

This chart shows the wide range of conversion factors. Such a wide range illustrates the difficulty in attempting to convert non-whole blood results to whole blood expressions. Additionally, it demonstrates that there is insufficient agreement in the scientific community as to an acceptable range of values.

It is incumbent upon the legal and medical communities to begin to understand the fundamental differences in the uses of BAC testing. In situations that require high degrees of confidence in the measures of BAC, such as in the case of criminal trials, only the most accurate and selective methods should be used to ensure that the guilty are not needlessly acquitted nor are individuals falsely convicted.

Enzymatic Assay, DUI, BAC



Jurisprudence Section - 2015

F49 Driving Under the Influence of Drugs in Florida

Nicholas B. Tiscione, MS*, 3228 Gun Club Road, West Palm Beach, FL 33406; Xiaoqin Shan, PhD, Palm Beach County SO, Crime Laboratory, 3228 Gun Club Road, West Palm Beach, FL 33406; and Dustin Tate Yeatman, MS, 3228 Gun Club Road, West Palm Beach, FL 33406

After attending this presentation, attendees will understand the potential disadvantages of Driving Under the Influence of Drugs (DUID) laws that list specific compounds rather than using more general “any impairing substance” language.

This presentation will impact the forensic science community by offering insight into challenges of combating the incidence of DUID when specific compounds are listed in laws. Overall statistics as well as specific case examples will be presented.

The laws in Florida for DUID list specific compounds (controlled drugs) that must be proven to cause the observed impairment. Many prescription and over-the-counter drugs that can cause significant impairment are not included in this list. Therefore, according to Florida law, one is not guilty of driving under the influence if the observed impairment is due to a drug not listed in the Florida statutes. The Palm Beach County Sheriff’s Office (PBSO) Toxicology Unit analyzes and reports all impairing substances, within the capabilities of the laboratory, regardless of the scheduled status of the drug.

In Florida, urine samples are routinely collected when breath alcohol results are below 0.08g/210L for DUID investigations. Blood samples are only collected when it is impossible or impractical to perform a breath alcohol test or if there is a serious bodily injury or death involved. From January 2007 to January 2014, 1,361 urine specimens and 914 blood specimens were analyzed for drugs by the PBSO Toxicology Unit. Urine specimens were screened by a basic extraction with scan Gas Chromatography/Mass Spectrometry (GC/MS) and an eight-panel Enzyme-Linked Immuno-Sorbent Assay (ELISA) for barbiturates, benzodiazepines, buprenorphine (2013 to 2014), carisoprodol, cocaine/Benzoyllecgonine (BE), opiates, oxycodone/oxymorphone, and cannabinoids. Blood specimens were screened by a basic extraction with scan GC/MS and a ten-panel ELISA for amphetamines, barbiturates, benzodiazepines, buprenorphine (2013 to 2014), carisoprodol, cocaine/BE, methamphetamines, opiates, oxycodone/oxymorphone, and cannabinoids. All positive results were confirmed with GC/MS.

Over the past seven years, 25% of all drug-positive blood specimens and 46% of all drug-positive urine specimens contained at least one non-controlled drug, often mixed with controlled drugs. The top ten non-controlled drugs excluding Selective Serotonin Reuptake Inhibitors (SSRIs) identified in both blood and urine are listed in Table I. The driving under the influence charges in many if not most of those cases with non-controlled drugs were either dropped or not even filed.

Table I. Top ten non-controlled drugs identified in blood and urine (excluding SSRIs)

Blood			Urine		
Analyte	# of Cases	% of Positive Cases	Analyte	# of Cases	% of Positive Cases
Diphenhydramine	37	5.4%	Diphenhydramine	112	9.7%
Zolpidem	29	4.3%	Zolpidem	83	7.2%
Tramadol	17	2.5%	(d) Methorphan	52	4.5%
(d) Methorphan	15	2.2%	Cyclobenzaprine	46	4.0%
Cyclobenzaprine	10	1.5%	Tramadol	41	3.6%
Trazodone	9	1.3%	Trazodone	40	3.5%
Topiramate	9	1.3%	Chlorpheniramine	32	2.8%
Chlorpheniramine	6	0.9%	Doxylamine	32	2.8%
Lamotrigine	6	0.9%	Nortriptyline	22	1.9%
Phenytoin	5	0.7%	Amitriptyline	20	1.7%

DUID, Non-Controlled, Florida

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F50 Evaluating Forensic Evidence in Drug Impairment Cases: Pitfalls and Complexities of Testimony by Non-Medical Witnesses

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The goal of this presentation is to provide attendees with an overview of: (1) why non-medical witness testimony interpreting human performance as drug impairment can lead to an unfair application of justice; and, (2) the common pharmacokinetic and pharmacodynamic issues of the seven distinct drug classifications used in American jurisprudence.

This presentation will impact the forensic science community by explaining how, in order to accurately determine if a human subject is impaired by drugs, the person making the determination must be educated in the interactions of the chemicals found in the drugs with the biological receptors in the brain (pharmacodynamics). The person making the determination must also understand fully how the chemicals from the drugs are absorbed, distributed, and eliminated in the body (pharmacokinetics) and how long that process takes.

Drugs in the body can be broadly classified into two types: (1) zero order drugs; and, (2) non-zero-order drugs. Essentially, this means that for zero-order drugs, the elimination rate is constant and not dependent on any biological variability. Conversely, the elimination in non-zero-order drugs is variable. This non-zero classification is a significant departure from that of ethanol metabolism. Absent a complete subject (patient) medical history, the reported drug concentrations and subjective observations reported by the non-medical witness are incomplete and can be misleading.

Pharmacodynamic effects reported by a non-medical witness require considerable education about the central nervous system's physiology and neurotransmission. The witness must be able to testify about the synaptic vesicle, reuptake, receptors, and the transfer through the synaptic cleft. The witness must also be able to distinguish between the various sites of synthesis as they relate to the class of transmitter.

To understand the significance of transmission differences, the witness must recognize that some drugs stimulate the receptor by fooling the neurotransmitter to respond. Alternatively, another type of drug may depress the site by acting as a wall, preventing the cell from stimulation or engaging with its receptor. The individual responses of both stimulation and depression of neurotransmitters vary from person to person. This makes dosage and interpretation of toxicology report values variable and complex. The non-medical witness must recognize that drug variability is affected by dosage, route of ingestion, length of time taking drug, interaction with other drugs, drug potency, gender, body mass, age, and genetic makeup, including ethnicity.

The non-medical witness must also understand the bioavailability of the drug. This means that the witness must understand what percentage of the consumed drug is free vs. bound. How much of the drug reaches the effected receptor is dependent on how the drug is ingested. Was it a tablet taken orally, an injection administered intramuscularly or intravenously, etc.? Is the drug protein-bound? Is there more than one drug of similar polarity attempting to bind with the same protein or is the drug free to enter the cell? These are all questions that the witness must be able to answer if they are attempting to correlate a drug level with impairment.

Opinion testimony about a drug concentration by a witness that does not possess the necessary medical background in understanding of free and bound drugs and the specifics about the individual drug polarities can be misleading to the jury and the judge. A hearing to determine the admissibility of such opinion evidence should always be required but often is overlooked and instead labeled as fodder for cross-examination.

The non-medical witness opinion evidence is often based on an incomplete scientific picture. This fact requires that the attorneys and judges educate themselves about the considerable limitations of the drug recognition evaluation and stop unequivocal testimony that a certain drug concentration in the body equals impairment universally without considering any medical or physiological information about the individual subject.

Non-Medical Witness Testimony, Pharmacodynamics, Pharmacokinetics



Jurisprudence Section - 2015

F51 Forensic Sciences as a Contributor to Miscarriages of Justice in Western Europe

Joelle V. Vuille, PhD, University of Neuchatel, School of Law, Neuchatel 2000, SWITZERLAND*

After attending this presentation, attendees will understand how inquisitorial and adversarial criminal justice systems operate with regard to forensic science evidence. Attendees will also understand how the specificities of each system affect the use of scientific evidence in criminal prosecutions, both positively and negatively.

This presentation will impact the forensic science community by helping build a context in which the role of scientific evidence in miscarriages of justice in the United States and in Europe can be better understood. This will be accomplished by highlighting structural and operational differences between inquisitorial and adversarial criminal justice systems and linking them to the use of scientific evidence in those jurisdictions.

After being hailed as a miracle tool in the fight against crime, the forensic sciences have slowly come under scrutiny as a possible contributor to miscarriages of justice in the United States. Between honest mistakes, voluntary manipulations, and a general lack of experimental validation, many cases can now attest to the role played by the various forensic sciences in the prosecution and conviction of the innocent; however, the phenomenon has so far curiously remained practically unknown in Western Europe. Only a handful of cases have come to light in which flawed scientific evidence contributed to a miscarriage of justice. How can one explain such a stark contrast?

Two main hypotheses will be considered: (1) it could simply be that forensic science has never been identified as a contributor to miscarriages of justice in Western Europe, but plays an important part nonetheless; the reasons possibly preventing the detection of errors will be discussed, such as varying policies in the preservation of evidence, lengths of sentences, attitudes of defense attorneys, etc.; (2) forensic science does not contribute to miscarriages of justice in Europe in the same proportions as in the United States. Different variables could be at play in this context, such as the structure of the adversarial and inquisitorial criminal justice systems, the possibilities of appeal and review of convictions, the funding of laboratories, the education of scientists, and the ways in which forensic science is used by law enforcement to solve crimes.

The goal of this presentation will be to discuss these various propositions and present the (minimal) data available in Western Europe on this topic. The relevance of various research options will be debated, as well as the potential impact of such a line of research on the field.

Miscarriage of Justice, Adversarialism, Inquisitorialism



Jurisprudence Section - 2015

F52 Defending Veterans: Teaching Juries the True Costs of War, Post-Traumatic Stress Disorder (PTSD), Adrenaline Addiction, and Deadly Drug Cocktails

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After attending this presentation, attendees will learn about the nature of conveying to juries the psychology of returning from war, toxicological challenges, and overcoming bias against veterans in the criminal justice system.

This presentation will impact the forensic science community by informing forensic science practitioners, lawyers, and other key criminal justice system stakeholders how to effectively tell a veteran's story to judges and juries.

This presentation will provide better understanding of the challenges facing many veterans returning from war when they enter our criminal justice system. According to Department of Justice statistics in 2004, one in ten of all the inmates in the United States have served in the military. One out of every five combat veterans returning from war is diagnosed with PTSD. As one commentator stated: "A certain number of veterans suffering from mental-health issues will, invariably, end up in jail or prison. After Vietnam, the number of inmates with prior military service rose steadily until reaching a peak in 1985, when more than one in five was a veteran. By 1988, more than half of all Vietnam veterans diagnosed with PTSD reported that they had been arrested; more than one-third reported they had been arrested multiple times. Today veterans' advocates fear that, unless they receive proper support, a similar epidemic may befall soldiers returning from Iraq and Afghanistan."

Other veterans suffer from anxiety, drug, and even adrenalin addiction. This has prompted the emergence of specialty "veterans' courts" popping up across the country in state and federal jurisdictions, often with grant assistance from the government. Unfortunately, these intensive programs do not normally accept those veterans charged with violent crimes, leaving veterans to face judges and juries while dealing with a myriad of psychological and toxicological issues.

This presentation will discuss the landmark case of *United States v. Gabriel Brown*, a former Green Beret sniper turned serial bank robber, as well as other cases that shed light on the best practices on identifying and presenting to judges and juries the stories of those soldiers who went from fighting for our freedom to fighting for their very own. Best practices related to presenting mitigating information to judges and juries will be discussed.

Veterans, PTSD, Adrenaline Addiction



F53 Full-Scale Intelligence Quotient Test Scores and the Impropriety of “Ethnic (or Socio-Economic) Adjustment” in *Atkins* Cases

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After attending this presentation, attendees will gain new information regarding developments in epigenetics which relate to the validity of Full-Scale Intelligence Quotient (FSIQ) scores in determining intellectual disability for the purpose of eligibility of a criminal defendant to be executed if otherwise subject to the death penalty.

This presentation will impact the forensic science community by squarely challenging the scientific validity of testimony accepted by the courts — most recently, the California Supreme Court — to the effect that the FSIQ scores of African American defendants should be “ethnically adjusted” to make such defendants eligible to be executed.

In certain capital cases, a defendant who qualifies as “intellectually disabled” (“mentally retarded”) cannot be executed under the Constitution as held by the United States Supreme Court in the *Atkins* case. The first criterion of the legal definition of “intellectual disability” is a limitation in intellectual ability, primarily as measured by standardized tests of the person’s FSIQ. If the FSIQ score is two standard deviations below the norm and if the person meets the other two criteria (deficits in adaptive functioning and onset before age 18), the person cannot be executed. Failure to meet any of the criteria, including this first criterion based on FSIQ, generally means that the person will not qualify as “intellectually disabled” and, if otherwise subject to the death penalty, can be executed.

In cases in which the defendant is African American, prosecutors have recently proffered “expert” testimony that the defendant’s scores should be “ethnically adjusted.” The “adjustment” adds five to 15 points to the defendant’s FSIQ. In stark terms, this means that given two identically situated defendants with identical FSIQ scores, one Black and the other White, the government can execute the Black defendant and not the White defendant. This process of “ethnically adjusting” the FSIQ scores of African Americans has been accepted by some courts including, most recently, the Supreme Court of California.

This presentation will establish that “ethnic adjustment” is not supported by the scientific evidence. Deviations from the norm of various cohorts — specifically considered here, those of African Americans — are not explained by “ethnicity.” Instead, established research shows that the deviation from the norm is related to socio-economic deprivation which has an unfortunate sociological correlation with race. In other words, race in this context is a proxy for socio-economic deprivation.

That fact has not ended the controversy. Instead, certain prosecution experts have modified their testimony to claim that there should still be an upward adjustment for defendants (disproportionately African American) who lived in poor socio-economic circumstances. This argument is based on the contention that such defendants are “poor test takers” and that the deficit in the FSIQ score is learned/behavioral, not phenotypical/biological and therefore not a limitation. The result, according to these experts, is to add the five to 15 points which, again, permits the government to execute such defendants.

This presentation will review significant scientific evidence that supports the hypothesis that the lower IQ scores of the cohort of people who have suffered childhood abuse, neglect, stress, poverty, and trauma are epigenetic. In fact, exposure to such factors may engender multigenerational epigenetic effects. The lower IQ scores of the cohort of people who have suffered childhood abuse, neglect, stress, poverty, and trauma have a biological basis and therefore a physical limitation on intellectual disability, not a transient behavioral impairment in test taking.

This presentation will discuss the scientific literature documenting epigenetic effects of abuse, neglect, stress, poverty, and trauma resulting in changes in the pattern of gene expression. Longitudinal studies have shown that these effects are heritable to children and even grandchildren. These changes are in addition to other adverse biological influences like exposure to toxins in poorer environments and to the effects of fetal alcohol spectrum disorder and other insults to the fetus *in utero*.

Based on the scientific evidence, it is not proper to “ethnically” or “socio-economically” adjust the FSIQ scores of defendants. Epigenetic effects on genomic imprinting biologically alter cell development which can contribute to the intellectual disability reflected in FSIQ scores within the meaning of the *Atkins* case, preventing execution of the intellectually disabled. This presentation suggests that further research needs to be done; however, what is known right now specifically conflicts with the hypothesis of “ethnic (or socio-economic) adjustment.”

Death Penalty, FSIQ, *Atkins*



Jurisprudence Section - 2015

F54 Defining Depravity in Crime: Approaching a Standard for Sentencing Application

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WITHDRAWN



Jurisprudence Section - 2015

F55 Sexual Abuse Case Study — Forensic Science and the Age of Social Media

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The goal of this presentation is to demonstrate the effectiveness of the forensic sciences in identifying fabricated testimony.

This presentation will impact the forensic science community by challenging attorneys to analyze their cases with greater sensitivity to potential forensic science issues.

Several forensic disciplines contributed to exposing false accusations in this case of alleged sexual abuse. Voluminous records of text messages provided the defense with extremely fine detail concerning the complainant's allegations; scrutiny of these messages revealed the stories to be impossible.

The complainant was 18 years old and living at her parents' home after graduation from high school. One day, she appeared at a sheriff's department substation accompanied by two friends. One of the friends advised that the complainant had been sexually abused by her father; the police received several pages of Facebook® messages in which the complainant described being raped. The complainant was unwilling to speak with detectives, but eventually wrote several statements describing sexual abuse. Her father was arrested and the complainant's siblings, aged 17 years old and 12 years old, were taken under court jurisdiction.

Detectives obtained a search warrant for the complainant's Facebook® account and more than 5,000 pages of records were produced. The messages described unprotected vaginal, oral, and anal penetrations that included ejaculation. This was to have taken place in the family home on a daily basis over a period of one-and-one-half years.

Interviewed using standardized child abuse investigative techniques, the siblings denied that any abuse or improper touching had occurred. At the home, only one suspected biological stain was located; collected from an old mattress in the basement, the stain tested negative for seminal fluid.

The prosecution proceeded to preliminary examination. The complainant's testimony was contradictory, but the matter was bound over for trial on several counts of sexual assault. The defense investigation then focused on the details contained in the text messages. The complainant described being beaten and kicked on numerous occasions. In one incident she claimed she was kicked so badly that she suffered extensive bruising, possible broken ribs, and missed "like three weeks of school." Attendance records showed she never missed more than two consecutive days of school.

The complainant described being forced to take Valium® and Vicodin®. Her descriptions of the effects of these drugs included violent seizures and colorful hallucinations. A toxicologist and neuropsychopharmacologist concluded the complainant's descriptions were inconsistent with the known effects of these drugs. Records subpoenaed from the Michigan Automated Prescription System confirmed that neither parent had prescriptions for any controlled substances for the relevant time period. When the home was originally searched by police, no prescription drugs had been found.

A recurring theme was that the complainant's father would drag her to the basement, lock her in, and sexually assault her. She described being locked in the basement all night. Examination by the defense crime scene investigator revealed that it was impossible to lock a person in the basement. There was no lock, latch, or hook of any kind on the door. The family home was a 1,200-square-foot dwelling. Examined with specific-wavelength ultraviolet light, the interior exhibited patterns of biological staining consistent with habitation by five people, two dogs, and a housecat. There were no biological stains consistent with the complainant's stories of sexual assault having occurred in the complainant's bedroom, the family room, and basement.

Reports from the defense experts were furnished to the prosecution. The prosecutor met with the complainant and discussed the findings. The complainant admitted to fabricating the stories and all charges were dismissed. At that point, it was unnecessary to explore how the complainant came to invent the stories; however, had the case proceeded to trial, it would have been useful to have employed a forensic psychiatrist to assist in crafting the cross-examination of the complainant.

In testing the credibility of witnesses, attorneys would do well to consider all the tools of forensic science at their disposal. The proactive employment of forensic science professionals gives lawyers the power to expose fabricated testimony that might otherwise go undetected.

Fabricated Testimony, Biological Stain Detection, Neuropsychopharmacology



Jurisprudence Section - 2015

F56 Digital Evidence 101 — The Evidentiary Value of Digital Evidence: The Impact of Digital Evidence on the Investigation and Brief of Evidence

Paul Reedy, BS, DC Department of Forensic Sciences, 401 E Street, SW, Washington, DC 20024*

The goal of this presentation is to assist the forensic science community in understanding recent developments in information and communications technology, its use by perpetrators and victims in the context of criminal activity, and its evidentiary value in investigations and criminal proceedings.

This presentation will impact the forensic science community by providing attendees with a practical understanding of the role of information technology in various criminal activities plus anticipation of possible future directions.

Information (and communications) technology is ubiquitous to modern human existence. It follows that this technology is involved in nearly every facet of human activity, including criminal activity, whether it is simply the act of a person carrying a phone, a computer used to transact business, or a device that informs about an individual's state of mind and interests. Consequently, any investigation of criminal activity will potentially have lines of inquiry that include digital evidence. Digital evidence can be used for the investigation of homicide, extortion, assault, sexual assault, online child exploitation, stalking and intimidation, fraud, arson, sudden infant death syndrome, and others; it can include exculpatory as well as inculpatory evidence.

It is no secret that information technology evolves at a rapid rate with the occasional revolution thrown in for good measure. Criminals are notorious for being early adopters of technology, comfortable in the knowledge that law enforcement agencies will struggle to attain the capability and capacity to keep up.

The challenge for digital evidence experts is understanding and deciphering new technologies, including "the latest app," as critical evidence is invariably present in these new formats. The digital evidence analyst must be sufficiently qualified and experienced to reflect an understanding of the evidence under examination.

The challenge for the prosecutor is to understand the probative value of the evidence and to ensure that it is presented in a manner that is easily understood by the court.

The challenge for the defense counsel is to understand the nature of the evidence under examination, identify any insufficiencies in the forensic examination that lead the conclusions, and to identify alternative credible hypotheses based on the available evidence.

This presentation will provide descriptions of places digital evidence may be located; several case examples where digital evidence is critical to, or augments, the investigation and/or brief of evidence; the applicability of international standards to digital evidence; anticipated developments in information technology; and, some introductory remarks concerning the current legal environment.

Digital, Evidentiary, Case



G1 Non-Traditional Positive Dental Identification

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After attending this presentation, attendees will understand both traditionally utilized methods used in the positive identification process and non-traditional normal anatomical variants and pathological conditions in dento-facial remains and restorations that may provide a means of dental identification.

This presentation will impact the forensic science community by demonstrating non-traditional dento-facial therapeutic evidence as well as normal and abnormal anatomical evidence that may provide forensic odontologists with successful dental identifications.

Positive identification of the decomposed, skeletal, disfigured, or incinerated victim is the goal of forensic odontology and the foremost requirement of forensic pathology. Routine positive identification methods rely on tried and true comparison of antemortem to postmortem radiographs for restoration assessment. Such identifications are only as reliable as antemortem radiographic records, which document those dental and alveolar conditions as well as different composite resin materials, amalgams, gold inlays, endodontic conditions, and prosthodontic devices; however, at times the forensic dentist does not have those restorations in the decedent's mouth. The oral cavity may present as edentulous or contain 32 virgin teeth resulting in documentation of crown and root and/or alveolar bone peculiarities. Frequently, a more non-traditional method of comparing other unique conditions not related to restorative materials may be used.

The forensic dentist can utilize some quite unusual means of comparisons, such as amalgam tattooing, in a pattern reproducible in the postmortem radiograph. Endodontic sealer "poof" marks have been used for identification purposes where root canal sealer extruded from the apical foramen, displaying an unusual shape useful with antemortem-postmortem radiographic comparison.

Occasions also arise where oral surgeons reapproximate jaw fractures with metal stabilizing plates that exhibit unusual shapes, allowing forensic dentists to compare X-rays of like kind from the decedent. With numerous types and brands of implants on the market today, the structure of the implant can even be used, especially those from decades ago where endosseous blade implants exhibited various shapes and designs of the device's struts. These can readily provide an interesting basis for comparison.

Further examples will be demonstrated where an edentulous decedent was found wearing a broken upper denture, with a rather large piece of the denture missing. No other identifying characteristics could be discovered. Several months later, detectives were interviewing a family who had reported a missing person. The family related that their missing family member was edentulous and when shown the discovered denture fragment found on the body, did allege that the shape of the denture looked similar to the decedent's smile; however, this is not conclusive evidence, but rather a suggestive clue that would encourage investigators to look further.

In searching through the personal effects of the decedent, a denture fragment was found in the bathroom drawer, which was used to reapproximate the main denture fragment found on the body of the decedent. The fracture halves, which were irregular and jagged, matched perfectly. With the fracture lines of the two denture halves from two different locations, one from inside the house of the missing person and the other at the crime scene miles away, enough information was provided for the forensic odontologist and the medical examiner to confirm identity of the body.

Several comparisons have been submitted showing iatrogenic conditions such as a broken bur embedded within the alveolus or a separated endodontic file retained in the jaw. These less-often-used criteria are equally discriminating in securing positive identification.

Attendees will recognize several unorthodox means available to positively identify victims that have seldom been used. These cases will challenge the attendee/forensic scientist to delve further into the possibilities of unusual identifying characteristics.

Positive Identification, Amalgam Tattooing, Endodontic "Poof"



G2 X-Ray Photoelectron Spectroscopy (XPS) Analysis of Etched Dental Crown Metal Surfaces Demonstrates Prior Immersion in Hydrochloric Acid

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After attending this presentation, attendees will be aware of a technique that will allow for quantitative elemental analysis of material surfaces with the ability to determine oxidation states and infer molecular structure. This technique has been used in this study to determine that the specific corrosive in which a dental crown was immersed was hydrochloric acid. It may have uses in other forensic situations in which quantitative surface analysis becomes important.

This presentation will impact the forensic science community by illustrating the use of a technique that potentially extends the analytical repertoire available to forensic odontologists and other practitioners.

Four distinctive dental Porcelain Fused to Metal (PFM) crown artifacts were received in connection with a forensic matter. It was suspected that these crowns had been immersed in hydrochloric acid for seven days. Previous analysis using scanning electron microscopy and Electron Dispersive Spectroscopy (EDS) had demonstrated that the items had been immersed in a corrosive substance but could not determine specifically what the corrosive had been.¹

XPS is a surface analysis technique that permits both quantitative estimation of the elements present in a sample and determination of their oxidation state. Depth of information is only from the outer five to ten nanometres of the surface being analyzed. It operates by using a high-energy X-ray beam to excite electrons and etch a thin layer (approximately ten nanometres) of the sample surface. The number and kinetic energy of these electrons is measured and the machine produces a graph specific for the chemical and electronic state of each of the elements present. This study hypothesized that it would be capable of determining the specific chemical identity of the corrosive in this matter; specifically, finding evidence of Cl⁻ ions would be sufficient to permit this conclusion. No prior preparation of the surface was required.

One of the crowns was selected and an area of the crown was exposed to the X-ray beam in a hard vacuum. The kinetic energies of the resulting electrons were analyzed and charted by the machine.

This study was able to determine the elemental composition of the crown at the scanned location. It confirmed the makeup of the crown as first determined by EDS. Crucially, it also showed the presence of chlorine.

To test the hypothesis that chlorine would be found only in the surface (indicating immersion in HCl), an *in situ* argon ion gun was used to etch 20 nanometres of the surface from the sample and then the surface was re-analyzed. The comparison between the two scans confirmed the presence of the two peaks characteristic of chlorine, demonstrating its presence in the surface layer, probably as the chloride of nickel, molybdenum, or both. The post-etch analysis showed a greatly reduced level of chlorine, confirming the hypothesis that the chlorine was confined to the surface level only.

The presence of chlorine in the surface layer of the metal component of the dental crown, and the reduction in its level as the test moved more deeply into the metal, confirmed the hypothesis that the corrosive substance in which this crown had been immersed was Hydrochloric Acid (HCl).

This presentation provides information on the use of XPS to determine the surface composition of a dental crown believed to have been immersed in hydrochloric acid, and demonstrates that the technique can be used to demonstrate this fact. This analytical technique may be of relevance to forensic analysts who are required to make similar determinations.

Forensic Odontology, Analytical Technique, XPS



G3 Reassessing the Dental Features of Lamendin's Age-Estimation Method

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After attending this presentation, attendees will be more aware of each dental feature of Lamendin's method and its accuracy in age estimation. Repeatability, high accuracy, and high correlation with age are traits of a good age indicator.

This presentation will impact the forensic science community by helping forensic practitioners decide if the new equations derived only from certain dental features should be used for aging human remains.

Age estimation forms one of the most important steps in forensic anthropological analysis. Teeth, being very resistant to postmortem destruction and fragmentation, can be used as an alternative biological material for age estimation, especially when bones are not well preserved. Gustafson was the first to develop an aging method based on six features of dental structure.¹ Modifications of this method were later developed, including the technique of Lamendin et al.² The authors of this method established an equation for estimating age-at-death in adults based on the measurement of periodontosis (gingival regression) and root transparency height as related to the overall height of the root, taken from the labial surface of single-rooted teeth. The goal of the present study is to reassess the significance of these dental variables in estimating age-at-death using the canines from a modern European skeletal sample. A new equation for age estimation is derived for each canine using only the variables presenting significant correlation with age. The canines of 73 individuals (43 males and 30 females) from the Athens Collection were examined. This skeletal collection consists of individuals of known sex, age, occupation, and cause-of-death that died in the second half of the 20th century in Athens, Greece. The mean age was 46.02 years (range: 24-96 years) and 48.30 years (range: 20-85 years) for males and females, respectively. Periodontosis, root transparency, and maximum root height were measured at the mesial, distal, labial, and lingual surface of each canine, using a digital sliding caliper. Pearson's rank correlation statistics were applied to identify relationships between each measurement taken and chronological age. Multiple Regression Analysis (MRA) was applied in correlation with age variables in order to produce the best predictive model for age estimation. In addition, bias and inaccuracy tests as well as a Wilcoxon test were performed to assess the accuracy of the method. Age was estimated using the prediction equation for each canine. Only periodontosis and root transparency presented a statistically significant positive correlation with age. MRA produced four different models for each canine with R² ranging from 67.6% to 79.2%. Bias results showed that there was a small overestimation of one to two years for ages 20-59 years old; however, in ages more than 60 years, there was an underestimation that reached 17 years. The Wilcoxon test showed a significant difference between estimated and real age only for individuals over 60 years of age.

In conclusion, periodontosis and root transparency are robust age indicators as they present a high correlation with age. The new regression equations were found to be suitable for an accurate age estimation with the most accurate results to be observed among individuals 20-59 years of age. The low values of mean error (bias) from the real age make canines reliable for estimating age-at-death using only the dental features of periodontosis and root transparency.

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Forensic Odontology, Forensic Anthropology, Age Estimation



G4 Evolution of Forensic Odontology Services in Queensland: 1994-2014

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After attending this presentation, attendees will be able to evaluate the evolving pattern of casework undertaken over a 20-year period at the Forensic Odontology Unit, Forensic and Scientific Services (FSS), Department of Health Queensland Government in the state of Queensland, Australia. The single largest change in the pattern of practice has resulted from the introduction of a Toshiba® Aquilion™ 16-Slice Computed Tomography (MSCT) scanner in 2009. It will also be clear that increasing recognition by forensic pathologists of the role forensic odontologists can play in the investigation of facial and dental trauma has led to a change in the types of casework forensic odontologists undertake. An increasing recognition of the expertise possessed by forensic odontologists has also led to increased consultation in cases where oro-dental pathology may have contributed to the cause of death.

This presentation will impact the forensic science community by showing the changing nature of the caseload in forensic odontology in Queensland, Australia, and underlines the importance of incorporating CT technology in forensic odontology casework.

FSS is a large organization that exists under Health Support Queensland (Department of Health Queensland Government) to provide expert analysis, advice, and research in forensic matters including forensic molecular biology, forensic chemistry and toxicology, and forensic pathology and odontology. It is tasked with supporting a Queensland Government response to threats to public health and the environment, epidemics, civil emergencies, criminal investigations, and coroners' inquiries into reportable deaths. The Forensic Odontology Unit is part of the Forensic Pathology Department in FSS.

The Forensic Odontology unit at FSS deals with only coronial cases. These include routine coronial identification and dental age estimation, as well as assisting in coronial death investigations by consultation with forensic pathologists. Examination of submitted items is also undertaken when this is requested by the Queensland Police Service (QPS) or from other departments within the Department of Health Queensland Government, such as the Environment and Food Testing section.

In the 1990s, postmortem dental identification routinely involved resection of both the maxilla and mandible. Accomplishing this important work and recognizing that avoiding mutilation out of respect for the deceased is a guiding principle in all undertaken work. Consequently, this study has adapted techniques to utilize imaging and image comparison wherever feasible to negate the need for jaw removal. This has been very successful — today, if jaw resection is required, permission needs to be sought and strongly justified in writing from the Queensland State Coroner, and degloving techniques are used to ensure that the replaced bony and soft facial tissues look undisturbed at the end of the examination. Statistics show that since the end of 2006, only two jaw resections have been required for access to the dentition. While the MSCT has been extremely useful in obviating the need to perform resections, this was being achieved some years prior to its introduction at FSS, reflecting this study's underlying philosophy.

Since 2009, the MSCT has been used to provide 3D image data of all forensic cases admitted to the FSS. This data can be sliced and reconstructed (Multi-Planar Reconstruction (MPR) imaging) to simulate plain-film radiographs including orthopantomograms. These MPRs are useful in comparing the features of the postmortem dentition with available antemortem dental records and radiographs. It also plays a role in disaster victim identification responses for both dental identification and age estimation in children and adolescents. CT data can also be used for assessing injuries in the maxillofacial region when assessment and comment on injuries and injury patterns are required or requested.

Forensic Odontology, Computed Tomography, Forensic Services



G5 Forensic Dentistry and Malpractice Lawsuits in Turkey

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After attending this presentation, attendees will be familiar with Turkish malpractice laws, cases that have been brought against dentists, and general statistical data about dentistry in Turkey.

This presentation will impact the forensic science community by presenting a history of forensic dentistry in Turkey.

The 2012 census shows the population of Turkey to be 75,627,384 with 24,725 practicing dentists. This gives a ratio of 27 dentists per every 100,000 Turkish citizens. Dentists complete their education in a five-year curriculum in one of the 55 universities which offer the course. Article 10, document number 1219 of Turkish law forbids foreigners from practicing medicine in Turkey; however, with recent changes to the law, if they meet standards set forth by the law, their practice in dentistry is lawful. The Ministry of Health's data shows that 89.3% of individuals between the ages of 35 and 44 years had at least one tooth missing and there were seven million fillings performed by dentists working for the ministry.

The number of malpractice lawsuits is on the rise in Turkey. Some of the reasons for this increase include an increase in the number of people seeking medical services, higher patient expectations, media obsession with these cases, and the fact that some attorneys solicit and recruit patients for filing malpractice lawsuits.

Below are two Turkish Penal laws as they are written in the statutes:

Reckless Homicide Article 85: the punishment of an individual who commits reckless homicide will be imprisonment for two to six years.

Willful Injury Article 86: (1) the punishment of an individual who causes pain or detriment to someone's health or ability to function normally will be imprisonment for one to three years; and, (2) provided that the willful injury has a mild effect on the victim's health and well-being and can be remedied by a simple medical intervention, the perpetrator is punished with imprisonment of four months or a judicial fine.

Patient confidentiality and obtaining consent for treatment are the dentist's responsibilities. Some of the cases brought against dentists in respect to these areas include: forcing the patient to give consent; medical intervention of an incapacitated person without consent of their guardian, curator, or the court; asking the patient to sign a blank piece of paper or write, "I accept any intervention;" and, so forth. As for malpractice cases against dentists in Turkey, reasons for these lawsuits include not meeting medical standards due to lack of knowledge or neglect and medical device malfunction. According to a review of judicial files, there were 101 malpractice lawsuits filed against dentists between 1984 and 2008: 44% of the cases were for faulty dentures; 29% for wrongful surgery; 9% for wrongful orthodontic treatment; 8% for faulty implants; 5% for wrongful periodontal treatment; and, 5% for other dental treatments.

Forensic Dentistry, Malpractice, Turkey



G6 What You See Is Not Always What You Should Believe: A Presentation of the Inconsistencies Commonly Seen in Antemortem Dental Charting

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After attending this presentation, attendees will gain understanding of antemortem dental charting practices and the inconsistencies, both intentional and unintentional, by a treating dentist as well as the potential negative effects on the dental identification process of the deceased.¹

This presentation will impact the forensic science community by providing examples of how charting inconsistencies, whether intentional or unintentional, can have significant effects on the human identification of dental remains by forensic dentists. This presentation will also strive to broaden the understanding of the importance of a systematic means to document and interpret dental charting in cases where a dental identification is necessary and will emphasize and enable an increased awareness that documentation inconsistencies can occur, demonstrating some of the most common charting inconsistencies according to the literature.

According to Zahrani, dental identification has become a vital resource for identification of human remains of mass disasters, acts of terrorism, severe mutilation, or burning of bodies. The identification of human remains has become essential for both legal and humanitarian issues.²

The human dentitions' uniqueness is what makes it such a benefit for identification purposes; the dentitions can also be so unique that charting a patient's dentition can make patient dental charting in the antemortem setting a somewhat subjective task to even the most scientific of dental practitioners. Such charting dilemmas can evolve as a result of irregular tooth development, shifting of dentitions, adaptive changes, and restorative work done by previous practitioners.

Problems in human dental identification frequently involve the lack of adequate dental charting, as some practitioners do not chart existing restorations but only chart work to be done or work that was completed. As the medical field is gradually moving to electronic health records, many dental practitioners still utilize handwritten records. Radiographic imaging is also a factor in regard to the antemortem dental records. Some patients may limit the amount of or even refuse to have radiographs taken. Some radiographs are taken with poor angling techniques or, in some cases, could be mounted or moved digitally to an improper position on the mounts or templates. The other major factor is human error. Human errors can cause significant inconsistencies in comparison analysis for dental identifications.

The problems and possible inconsistencies that will be identified in this presentation can result in delaying for many hours or possibly even days a positive identification of the deceased in order to provide a grieving family closure of a loved one's death.

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Dental Identifications, Dental Charting, Inconsistencies



G7 Analysis of Human Bitemarks in Food and Beverages Using Metric and Biological Analysis

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After attending this presentation, attendees will understand the importance of analyzing bitemarks by both physical and biological methods in criminal cases involving forensic odontology.

This presentation will impact the forensic science community by presenting different tests which will achieve human identification in simulated cases involving bitemarks and evidence found at crime scenes.

Multiple methods can be used for the comparison analysis of a suspect dentition to a suspected bitemark injury. The two most common are metric or biological analysis. Metric analysis involves the overlaying of a replica suspect's dentition above the pattern injury. This can be done physically, via caliper measurements, or virtually, using computer software. Biological analysis involves the comparison of the deposited salivary cell's DNA with a reference sample; however, the effectiveness of these methods can vary greatly depending on the impression substrate.

Study Goal: The goal of this study is to compare the effectiveness of four metric analysis methods on bitemarks produced in cheese and chocolate. A second goal was to determine if a biological method using DNA extracted from the saliva residue left on bitten food or water consumed from a bottle could also be used for comparison. Additional variables, such as the storage temperature of the substrate following the biting, as well as the time interval between the time of biting and time of analysis would have an effect on the analysis.

Study Design: The study consisted of 20 volunteers, ten males and ten females. The subjects were asked to bite into five samples of soft cheese, five samples of chocolate, and to drink from five bottles of water. This produced 15 samples from each of the 20 participants. One sample of each type was analyzed immediately while the remaining were stored at either room temperature (25°C) or in a refrigerator (4-8°C). Those samples were later divided so that one set was analyzed at three days and the other at seven days. Impressions were made of the bitten foods as well as the maxilla and mandible of all the participants utilizing alginate. Plaster casts were fabricated from all the impressions. Finally, reference DNA sample were obtained from each subject by collecting 2mL of saliva *in natura*.

Analysis: Four different metric analyses were performed on the food, one using a digital caliper, one using a manual overlay of the dental casts opposing the casts of foods, and two using a digital imaging overlay method and Adobe® Photoshop®. Metric comparisons were performed by a single forensic odontologist. For the biological analysis, a sample of the DNA was collected from cheese, chocolate, and water using the double-swab technique. The DNA was extracted according to the protocol of the QIAGEN® QIAamp® kit, quantification of the recovered DNA was performed using a NanoDrop™ spectrophotometer. Amplification was then performed using Identifiler® PCR Amplification AmpFISTR® kit (CSF1P0, D2S1338, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D19S433, D21S11, FGA, TH01, TPOX, VWA, and amelogenin) and agarose gel electrophoresis.

Results: The results showed that there was no significant difference between the four metric methods used. Manual overlay comparison yielded the highest number of matched subjects with 58% for both sexes. The Adobe® Photoshop® method yielded the lowest number with only 32% of the female samples matching and 44% of the males. For the biological analysis, DNA samples from saliva deposited in water, cheese, and chocolate had concentration values ranging from 26.66±12.32 to 9.38±3.42, showing that there was sufficient DNA present for amplification and later comparison. Thus, it was concluded that the sampling of food products at crime scenes can be important to investigators in identifying a suspect by utilizing metric and biological analysis.

Bitemarks, DNA, Saliva



G8 How the Characteristics of One Tooth Were Used to Make a Dental Identification in a Burn Victim: A Case Report

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The goal of this presentation is to show how the characteristics of a single tooth were used to help make a positive dental identification of the partial remains of a burn victim.

This presentation will impact the forensic science community by sharing an interesting case where a partial maxilla and seven teeth were the only oral structures found on charred remains and by discussing how a single tooth's characteristics and dental treatment helped make a positive identification of the body. Other single tooth dental identifications have been reported by the forensic dentistry community and it has been noted that said tooth should possess a quality that would set it apart from others, thus eliminating the possibility of a misidentification.

A burned body was discovered at a beach in Puerto Rico inside the trunk of a burned car. The personnel from the Institute of Forensic Sciences of Puerto Rico were called to the scene and recovered the charred remains. These were believed to be the remains of a young man who had recently been reported missing. Using the car's license plate, the car was traced to a family member of this young man, thus reaffirming the possibility of the remains belonging to this person. An interview with the family of the presumed deceased provided the name of the dentist who treated this person. Personnel from the Institute of Forensic Sciences in Puerto Rico requested the dental record and radiographs from the dentist.

Upon examination, the remains were found to be mostly broken down into small, very burned pieces. The anterior part of the maxilla was found in two pieces with some teeth still in their sockets. Other teeth, including three maxillary molars whose crowns were observed to be partially destroyed by the fire, were found in the remains. Digital radiographs, using a hand-held X-ray machine, were taken of these oral structures. A dental autopsy was performed. The antemortem and postmortem radiographs and dental information were compared. Only one tooth, #11 (using the universal numbering system), was found to be in both sets of radiographs and have enough structure left to make a comparison. Antemortem radiographs showed that a root canal treatment had been performed by the dentist. They also revealed a root dilaceration. Both of these notable features provided comparison data, leading to the conclusion that the anatomy and dental treatment performed on this one tooth should lead to a positive identification of the victim.

Dental Identification, Single Tooth, Puerto Rico



G9 Methodology and Interests of 3D Modeling of Bitemarks

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After attending this presentation, attendees will understand the methodology used and the interests of the optical impression of the bitemarks on a victim's skin and on the dental arches of the suspects. This technology offers a color 3D view of injured tissue and teeth and allows the backup of all data later submitted for analysis.

This presentation will impact the forensic science community by explaining how this new protocol and advanced technology will make experts aware that the recording of the shapes of bitemarks and the development of their features can be achieved in good condition either at the crime scene before any manipulation of the body or at the forensic medical institute with no risk of image distortion (poor angle of the photographic view taken) or skin deformation by overload stemming from the application of a conventional impression material (alginate, silicone). This protocol, which was laboratory tested, can be used for expert bitemark analysis.

As long as the protocol of modeling of the dental arches in 3D exists, the use of optical imprint technology is enough to reproduce the real shape of bitten human skin.

Material: The material used for the modeling of the 3D dental arches and for the modeling of injured tissues is unique. A laptop is necessary and works well to gather real-time 3D image analysis, reconstruction, and data storage software programs. An optical camera linked to the laptop by a USB port provides for the taking of impressions.

Method: All the tests carried out check that the imprint of the bitemarks on the victim and the imprint of the dental arches of the suspects are automatically made in the same scale. Repetitive results are also verified, thus allowing the superimposition of teeth and bitemarks without any risk of unmanaged enlargement. Moreover, modeling and image processing must allow for the 3D superposition of bitemarks and of the dental arches. The color of the lesions are verified, as well as that the teeth are of optimal quality, and that the measurement tools available meet the specific needs and are reliable. This is the process for cuts made on teeth before being superimposed on bitemarks.

Conclusion: Dental computer-aided design/computer-aided manufacturing used in forensic dentistry provides reliability in the study of bitemarks. It avoids the errors related to the photographic shots at a bad angle and the consequent change of data. The 2D image replaced by a 3D image becomes a concrete support and the superposition of the teeth and bitemarks provides a more accurate analysis. The latter, presented to a magistrate, will facilitate the comprehension of the bite process.

Dental CFAO, Bitemarks, Postmortem



Odontology Section - 2015

G10 Bitemarks From the Emergency Room to the Courtroom: The Importance of the Expert in Forensic Odontology

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After attending this presentation, attendees will be aware of how forensic odontology plays a vital role in many arenas, especially when crimes against children are involved. By identifying victims of child abuse as well as their abusers, forensic odontologists provide valuable services at every phase of the criminal justice process.

This presentation will impact the forensic science community by communicating how, through forensic case examples and legal case citations, forensic odontology aids in the detection, prevention, and prosecution of child abuse and other serious crimes.

Lost in the debate over bitemark testimony is the undeniable fact that forensic odontologists provide vital information at every phase of a criminal investigation and prosecution. This is especially true for cases involving society's most vulnerable victims, the children who are all too frequently abused in the unwitnessed privacy of their homes by their own caregivers.

Case examples will demonstrate the importance of fast response by a well-trained forensic odontologist to a pediatric Emergency Room (ER) or more tragically, the morgue, where evidence of both old and new bites and other abuse may be visible. Forensic odontologists can examine suspected bitemarks and determine whether they are human or animal or not bitemarks at all.

The ability to quickly include or exclude a suspected biter, or to reliably identify a biter, especially in the closed population of a family or daycare group, is of vital importance. Sometimes, the "usual suspect," who is often the mother's boyfriend or other adult male in a household, might be charged and jailed, or at a minimum, barred from the home during the investigation. A timely examination, analysis, and comparison can determine that the culprit is actually a child who might be large for his age. Once the bite is recognized as non-criminal, the initial suspect is exonerated. Similarly, when a child in the ER with human bites comes under the care of a forensic odontologist and the perpetrator is identified as the mother, the victim and other children in the household can be removed from the specter of further harm.

While critics of bitemark testimony cite the 2009 National Academy of Sciences (NAS) Report, *Strengthening Forensic Science in the United States: A Path Forward*, as authority for keeping forensic odontologists out of the courtroom, federal and state court judges reject that approach. A survey of both federal and state court opinions confirms that courts have not read the NAS Report as authority to scuttle long-accepted forms of forensic evidence, including forensic odontology. Judges, especially at the trial level, appreciate the value well-trained and careful forensic odontologists bring to the fact-finding process.

Just like fingerprint analysts who can match a latent to an inked print, a forensic pathologist who can determine cause and manner of death, or a forensic psychiatrist who retroactively evaluates a defendant's state of mind at the time of a past crime, forensic odontologists bring crucial expertise into a courtroom. A jury faced with 8"x10" color photos of a child grievously injured with bitemarks and the daunting responsibility of determining whether the charged defendant inflicted them, deserves every item the criminal justice toolbox has to offer.

Forensic Odontology, Criminal Justice, NAS Report



G11 An Objective, Dynamic Bitemark Overlay Technique

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After attending this presentation, attendees will be informed about a method of producing bitemark overlays that are objective, reproducible, and aid in the analysis of the 3D interaction between a dentition and a bitten surface. Attendees will understand the principles behind the overlay's creation and use.

This presentation will impact the forensic science community by demonstrating how many of the complexities inherent in the production and imaging of traditional bitemark overlays can be addressed and will understand how to make and work with objective overlays that eliminate imaging problems and subjective decisions. Attendees will also understand how these overlays can be used to build a 3D appreciation of the interaction between a dentition and a bitten surface.

Bitemark templates or overlays (also known as “exemplars”) are traditionally considered to comprise outlines of incisal edges of teeth used in the comparison process. The use of only the incisal edge outlines ignores the mechanics of the wide biting process and fails to document the palatal anatomy of the upper incisor teeth. Further, many of the techniques used to produce them have been shown to be subjective, introducing a potentially uncontrolled source of error to an already error-prone process.

This presentation seeks to address the subjective elements of overlay production and additionally creates a series of contour slices through a model that can be used to examine the 3D interaction between a dentition and a bitten surface.

Initially, an accurately cast dental model of a dentition to be compared with an alleged bitemark injury is obtained and a digital model of it is produced by rendering isosurfaces from a high-resolution laser scan of the model. Laser scanners with a resolution of 20 microns or better are becoming increasingly common in dental offices and laboratories and a typical scanner will produce a model of a dental cast in approximately 75 seconds.

The mean angle of the occlusal plane to the substrate at the time of biting is estimated and the occlusal plane of the virtual model is positioned accordingly to the horizontal. If the plane is not evident, then two comparisons are suggested, one with a horizontal occlusal plane and one with the maximum estimated angle to the bitten surface. Both are used, and it is said that the true angle lies somewhere between these extremes.

The digital model can then be cut into virtual slices at whatever interval is deemed appropriate, commonly 0.5 millimeters. Each slice can be rendered as a 3D structure or as an outline drawn around the bottom of the slice. Each of these slices represents the tooth surface at the relevant distance from the occlusal plane and can be used separately or assembled in any combination to examine the 3D interaction between the injury and the overlay, addressing the “dynamic” aspect of the technique.

When different individuals are provided with the scan resolution and the original cast (or an accurate copy) and they agree on the position of the occlusal plane and the slice interval, they will end up with the same result, addressing the objective aspect of the technique. The rendering of outlines from the base of each slice creates objective hollow-volume contour overlays that can be reproduced by different operators.

When the successive slices are used, either in sequence or assembled, comparison with impressions or marks resulting from contact between the substrate and the palatal surfaces of upper incisors and marks resulting from dragging of the dental arch across the bitten surface becomes possible, often increasing the number of characteristics that can be used for comparison.

The technique is illustrated and its application in reducing uncontrolled errors in overlay production phase of the bitemark comparison process is demonstrated.

Forensic Odontology, Bitemark, Bitemark Overlay



G12 Bitemark Evidence

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After attending this presentation, attendees involved in bitemark analysis will better understand distortion of bitemarks resulting from infliction on different parts of the porcine anatomy.

This presentation will impact the forensic science community by making the attendee aware of the various potential problems involved in human bitemark interpretation, analysis, and comparison with suspect dentitions.

In vivo porcine skin is considered as a representative model for the study of human bitemarks.¹⁻⁵ Error rates have also been calculated using the porcine skin model as a substitute for human skin.⁶ The current study compares 20 bitemarks inflicted by the same dentition (known biter) on different parts of the porcine anatomy. The bite sites include the neck, the thorax, the axilla, the thigh, the stomach, and the back.

The type of photographs studied were color and alternate light imaging and all were lens and metrically corrected prior to comparison. The photographs were taken *in vivo* at the time of bitemark infliction and on the third day postmortem. The latter scenario was chosen to mimic a potential real-life encounter of a Friday body recovery with a Monday autopsy. Participants will have an exclusive and rare opportunity to observe the amount of change in the bitemark pattern from antemortem infliction to postmortem observation on the third day. The upper and the lower arches of the bitemark were compared separately and serially. The same exercise was performed comparing the bitemarks to the dentition that created them.

Conclusions will be drawn from this exercise in order to minimize potential difficulties of interpretation, analysis, and comparison with a potential suspect dentition in a human.

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Forensic Odontology, Bitemark, Dentition



G13 Paradigm Shifts on the Validity of Bitemark Evidence and the Dramatic Connection With Best Practices in Forensic Technical Analysis Via the Poma Murder in Rome: A Case Report

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After attending this presentation, attendees will have an understanding of the Italian judicial penal system and the appointing of expert witnesses in court.

This presentation will impact the forensic science community by highlighting the importance of specialist training in forensic science and medicolegal doctrines for dentists before involvement in any lawsuits.

Medicolegal evaluation in civil and penal cases of dental and/or odontology case works require expert witnesses with biological and technical knowledge of dentistry and a sound medicolegal education and experience. During the 2013 International Organization of Forensic Odonto-Stomatology international congress in Florence, Italy, the civil law judicial system on the designation of expert witnesses in six states were investigated, highlighting the need to work toward harmonization with more rigorous standards to become eligible as an expert witness in court, preventing spontaneous involvement of dental practitioners, and to improve their forensic and law teaching. These issues are even more relevant to penal case works.

In this presentation, a 20-year-old homicide cold case with a pattern injury suggestive of partial bitemark evidence is presented. Raniero Busco was accused of having murdered Simonetta Cesaroni, his ex-girlfriend, on Via Poma in Rome in 1990. A pattern injury on the breast of the victim was assessed as a “bitemark” by the forensic pathologist performing the autopsy. After 18 years, the case was reopened for new DNA analysis and the pictures of the “bitemark” on the breast were re-evaluated by dentists, pathologists, and odontologists. The injury suggestive of a partial bitemark is presented, demonstrating the concerns around the paradigm shifts on the validity of bitemark evidence and the dramatic connection with the technical analysis which is applied. The Italian judicial penal system and the analysis of the expert witnesses are presented. Questions and concerns on quality assurance and the criteria of forensic analysis of patterned injury are raised. Raniero Busco was found guilty in the first trial in 2011 and sentenced to 24 years in jail; he was subsequently acquitted on appeal in 2012. The acquittal of the accused was confirmed by the Supreme Court in 2014, which turned down the appeal by the Attorney General.

The purpose of this presentation is to promote a discussion within the dental community about who should perform forensic analyses in order to reduce errors and observer bias. The responsibility of forensic experts is to emphasize to colleagues who want to work within the judicial systems that forensic cases must follow precise legal, ethical, and scientific rules, which go beyond everyday dentistry and training. Pitfalls and errors can lead to erroneous attribution of responsibilities in professional liability cases and faulty accusations of innocent people. Dentists without specialist training should refrain from involving themselves in such lawsuits, leaving expert witness testimony to those dentists qualified in forensic sciences and medicolegal doctrines.

Bitemark Analysis, Forensic Odontology, Expert Testimony



G14 Construct Validity of Bitemark Assessments Using the ABFO Bitemark Decision Tree

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The goals of this presentation are to help attendees understand both the nature of agreement with respect to decision making and the importance of construct validity in the assessment of bitemarks.

This presentation will impact the forensic science community by informing attendees that bitemarks are currently under considerable scrutiny from judicial and scientific communities and by providing the result of an assessment of 100 injuries to determine the degree of agreement.

Since the criticisms within the 2009 National Academy of Sciences Report, Strengthening Forensic Science in the United States: A Path Forward, bitemarks have been an area of odontological practice that continue to be under considerable scrutiny.

The American Board of Forensic Odontology (ABFO) has developed a decision tree to help odontologists navigate the assessment, analysis, and conclusion levels that should be applied to bitemarks. The first portion of the decision tree is to assess a patterned injury to determine if it is a bitemark, suggestive of a bitemark, or if biting can be excluded as a cause. If the injury is determined to be a bitemark, then the next step is to determine if individual arches and tooth marks are identifiable within the injury. Following this, the decision tree goes on to assess the analysis and comparison of the injury.

This research study was concerned with the first two stages of the pathway — if these are not reliable or valid then the rest of the decision tree is rendered invalid. In order to assess the level of agreement using the decision tree, the following was undertaken: 100 injuries, comprised of suspect bitemarks and other patterned injuries, were presented, using a web-based system, to ABFO diplomates. Each image set included at least one scaled and one orientation image. No contextual information was provided. Respondents were asked to rate each image as either a bitemark, suggestive of a bitemark, or not a human bitemark. For those responses where the injury was determined to be a bitemark, an additional question was asked concerning the identification of individual arches and tooth marks.

In total, 39 diplomates completed all 100 questions out of 103 diplomates contacted. As there is no established reference standard for these data, a modal approach was adopted with percentage agreement established between the respondents. Data will be presented on the each of the decision elements, including the dichotomous decision to render an opinion, the three options for stating if the injury was a bitemark, and the level of forensic significance associated with the injury.

Bitemark, Agreement, Reliability



G15 Bitemarks: To Profile or Not to Profile — So What's the Question?

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After attending this presentation, attendees will know the value and importance of profiling as a part of the initial phase of bitemark analysis. The significant differences with actual case material versus experimental bites on cadavers and live volunteers will be shown and explained.

This presentation will impact the forensic science community by sharing the experience of profiling bitemarks in experimental cases and comparing them with actual case material. The importance of analyzing a bite pattern and forming a profile of the biter may be necessary to obtain a court order or search warrant in order to proceed with securing records from a defendant. The bitemark profile will provide the necessary “probable cause” to justify a search warrant or court order.

A bitemark left in human flesh by another human will not only leave a pattern injury but, most importantly, DNA evidence. The forensic odontologist will be responsible for first analyzing the injury, then providing an opinion as to the origin, such as human or animal teeth or a pattern that mimics a bitemark. If the injury is indeed a bitemark left by human teeth, the investigator should be able to classify the bitemark based on its appearance and detail or lack of detail.^{1,3}

A Class I bite exhibits no details of individual teeth and has been described as a “smoky ring,” but it still is of evidentiary value. It may yield DNA evidence and may be helpful in the elimination of suspects. A Class II bite pattern will have class characteristics but limited individual tooth pattern recognition and may have only one arch identifiable. A Class III bite pattern has been referred to as a “classic” bitemark. Both arches are present and individual teeth can be identified. A Class IV bite is one that produces total avulsion of the body part such as an ear, finger, or nose and usually does not leave a Class or individual pattern. There may be another area of the body with a Class I, II, or III bite pattern.^{1,4}

The analysis of a bitemark should include an opinion as to the arrangement of the biter's teeth, the position of the biter vis-à-vis the victim and, in some cases, whether the bite was left by a child (deciduous teeth) or an adult. This is all part of the analysis of a bitemark and specifically profiling of a bite with real teeth in a real (not experimental) situation will be helpful to the authorities and indeed may be essential in order to obtain a search warrant or court order.^{1,2,4} A bite profile need not be very specific and should leave room for modifications. An example is a bite that shows a space between the maxillary incisors or where the pattern shows clearly crooked teeth. Distortion plays a part in almost all bitemarks and needs to be taken into consideration. Profiling may be possible with all classes of bites except the Class IV. Profiling with experimental bites in cadavers is usually not possible, largely because of the distortion factor. Indeed, false profiles and mismatches are the usual result. Likewise, experimental bites on live volunteers after only a short period of time leave no pattern for profiling. This presentation will show profiling with all three classes of bites in real life cases, the effect of distortion on the bite pattern, and a comparison with experimental cadaver and volunteer bitemarks.

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Bitemarks, Analysis, Profiling



G16 Lip Prints: Inter-Rater Reliability

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After attending this presentation, attendees will recognize the similarities and differences in lip print analysis and appreciate the need for rater calibration methods for reliability.

This presentation will impact the forensic science community by demonstrating the error rate in an inter-rater reliability test for the analysis of lip prints.

Lip prints and their study, cheiloscropy, have been the topic of research and are also occasionally mentioned in literature by a detective, a crime scene investigator, or an examiner in another field of evidence comparison.¹ Subject comparisons have been recorded in the thousands. Research studies on lip prints have been contributed mainly by forensic dentists and anthropologists in countries throughout the world.

Literature and studies claim the uniqueness of human lip prints.^{2,3} Investigations may be able to rely on lip prints to identify possible suspects or to support evidence gained in investigations. A standard and uniform procedure has been put forth for the analysis of lip prints. The need to develop one cohesive cheiloscropy system, practicable in forensic dentistry, has been published as the one defined by Tsuchihashi in 1970.^{4,5} This method is published in the 2013 American National Standards Institute/National Institute of Standards and Technology (ANSI/NIST) revised dental standard ITL-1-2011.⁶

The legal community demands a reliable method of analysis and reliable experts to interpret patterns. This study sought to identify the amount of inconsistencies regarding the practice of lip print analysis, the results of which would determine the need for rater calibration. To prove the evidential value of lip prints in a court of law, a standard and uniform procedure has to be developed for the collection, development, and recording of lip prints and the ensuing comparison. The practice of analyzing lip prints is in the infant stage. Forensic odontologists are novice at it and have very little, if any, experience in the practice. Dentists with other forensic experience analyzed sets of lip prints using lip photos and prints recorded on paper. The results reveal the need for practice, experience, and further research to gain a status of reliability in the forensic community.

Many conclusions can be questioned when critical appraisal skills are applied, as in the calibration of examiners and also the inconsistency of methodologies in recording and analyzing prints. Establishing that the metric can be used consistently and reliably is attempted in this study. A consensus of uniqueness of lip prints must be reflected in the quality of the research and the number of prints analyzed to contribute to determining uniqueness. In addition to this study, more quality-designed studies using forensic professionals in the analysis of lip prints are needed. This further research of lip prints analysis should continue with the goals of meeting the *Daubert* standard and validating its usefulness in forensic identification for applicable legal matters.

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Lip Prints, Cheiloscropy, Inter-Rater Reliability



G17 Morphological Patterns of Melanoderm Lip Prints In Dakar, Senegal

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After attending this presentation, attendees will be educated on a variety of lip prints among the native Senegal population and the differentiation of lip prints for identification purposes.

This presentation will impact the forensic science community by describing a system which is seldom used but could be beneficial in the identification of unknown persons or suspects in criminal investigations.

In recent decades, the imprints of lips (cheiloscropy) attracted the attention of many scientists as a new tool for human identification in both civil and criminal matters. The present work was the in-depth study of lip impressions of men and women living in Senegal. A total of 200 people, including 96 men and 104 women, were included in the study. Red or black lip coloring and cellophane were used to print the lips. Each lip print was divided into six topographical areas and examined by magnifying lenses. They were then photographed and analyzed with greater magnification. Renaud classification in 1973 was used to classify the types of grooves and the results were analyzed statistically. Throughout the study, no identical lip print occurred in any two subjects. In total, impressions of lips (10.19% for both sexes) showed the same type of groove in all six areas of the lip (11.15% men and 12.56% women). It was found that 44.5% of lip prints (94 people) showed the same types of grooves in the Upper Right (UR) zone (46.88% men and 47.12% women); 45% (90 people) in the Upper Middle (UM) zone (52.08% men and 38.46% women); 52.5% (105 persons) in the Upper Left (UL) region (52.08% men and 52.88% women); 41% (82 people) in the Lower Right (LR) area (40.63% men and 41.35% women); 37.5% (75 people) in the Lower Middle (LM) zone (39.55% men and 35.58% women); 45.5% (91 people) in the Lower Left (LL) region (47.79% men and 46.15% women).

All ten types of grooves were recorded during this study. Groove type B (partial path from an edge, but not reaching the other side) was significantly more recorded (21.8%; 21.9% in men and 27.7% in women) followed by groove G (cross-linked forms) (21.3%; 21.2% in men and 27.4% in women); groove F (groove-shaped tree branch from an edge but not reaching the other side) (16.1%; 16.3% in men and 20.6% in women); groove D (fork or trident from an edge but not reaching the other side) (14.9%; 14.3 for men and 19.6% in women); groove A (complete path from one end to the other slot) (9.3%; 9.2% in men and 12.1% in women). The least observed grooves and I were in descending order (horizontal furrow or approaching the horizontal) (8%; 8.2% for men and 9.9% in women), followed by the groove J (ellipse, triangle, or V, while minor groove) (5.3%; 5.4% for men and 6.6% in women); H (intersection of groove sign of X or + sign) (1.8%; 2.5% for men and 1.6% in women); C (fork or trident from one side of the lip to the other) (0.8%; 0.7% for men and 0.8% among women), and groove E (shaped groove branch tree from one edge to the other of a lip) (0.7%; 0.4% in men and 1.1% in women). This study describes in detail the prints from the lips of melanoderm Senegalese men and women and seems to confirm that the “footprints” of lips are unique to each individual, even among twins and members of the same family. According to this finding, it is recommended that a database be set up for all individuals in the hope of being a reference in civil and criminal litigation. Further studies on a population with a larger effect on members of the same family and identical twins are recommended.

Lips, Print, Sengal



Odontology Section - 2015

G18 Honoring My Mentors — Drs. Levine, Snow, Maples, Stimson, and Stein

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The goals of this presentation are to abide by Dr. Martell's meeting theme and to pay tribute to the forensic scientists who helped shape my 38-year career as a forensic odontologist.

This presentation will impact the forensic science community by stressing the importance of mentors and mentoring for all American Academy of Forensic Sciences (AAFS) members, not only in a specific section but also in the wider forensic community.

Beginning in October of 1976, I have had the good fortune to learn from and work alongside four of the most preeminent forensic scientists in the world: Drs. Lowell Levine, Clyde Snow, Bill Maples, and Robert Stein. Three of the four, most recently Dr. Snow, have passed away.

I first met Dr. Levine in 1976 at The Armed Forces Institute of Pathology (AFIP) while taking my first course as a "newbie" in the field of forensic dentistry. In May of 1979, I had just finished attending my second AFIP course, Aircraft Accident Investigation, and would return to Chicago from Washington, DC, on the night of May 24. The next day, the American Airlines DC 10 crash occurred. As one of the first forensic dentists on the scene, I was tasked by American Airlines to "find Dr. Levine" who had worked with American Airlines on an accident in the United States Virgin Islands a few years earlier. I had the good fortune to work alongside and learn from Dr. Levine in a major mass disaster when morgues had to be assembled from scratch, long prior to the current instantly operational Disaster Mortuary Operational Response Team system.

At the same disaster incident, I met Dr. Clyde Collins Snow, probably the original "Indiana Jones." Even in the midst of the chaos of a 273-person mass fatality incident, Clyde was a patient teacher of forensic anthropology. In the morgue at his table, he would carefully measure the bones that had been cleaned by the Chicago Police Department Homicide detectives. Clyde would explain in detail why and how he was doing his examinations. I even learned that it was possible to determine if a female had given birth based on the scarring of the pubic symphysis. We had a birthday party on Independence Day for Clyde, complete with a sheet cake and champagne, courtesy of Dr. Stein, the Chief Medical Examiner for Cook County. Unfortunately, July 4 was not Clyde's birthday. He entered his birthday into the American Airlines ID Database in European fashion, month-day-year, so actually he was born April 7, not July 4. He never let on until years later.

In 1984, I became the consultant odontologist for the Cook County Medical Examiner's office and Clyde, having worked both the Gacy case and the DC 10 crash, was the forensic anthropologist now used by the office. He would fly to Chicago each month and spend a few days on skeletal cases. I worked alongside him again on numerous cases when he came into town. He never stopped teaching, asking questions, and also learning some forensic odontology from me.

I met Bill Maples at AAFS meetings. My first real work with him was on the Forest Lawn cremation case. I was invited to a meeting at the Cook County Medical Examiner's Office to assist with the dental radiography of a metal dental post that was found in interred cremains and that became critical to the correct identification. Dr. Maples was the lead anthropologist on the case. I would work another cremains case in Chicago with him and testify for the defense with him on a third case.

The final mentor I wish to mention is Dr. Robert Stein, the first Chief Medical Examiner of Cook County. In August 1984, I received a call from his secretary, asking if I could come to the medical examiner's office the next morning to assist with a case. The victim had been assaulted, raped, and strangled. As she lay on the morgue table, her shorts were pulled part way down her legs. I remember Dr. Stein's first gruff words to me, "You did not touch the body, did you?" I replied no. Later that morning, he would say to send him a bill. I said no, but he said to do so as I would be working there in the future. I would learn much from him over the years and he always treated me with respect as a peer forensic scientist.

Mentors, Levine, Snow



G19 Complex Dental Restorative Techniques: Are They Recognizable and Do They Survive Extreme Conditions?

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After attending this presentation, attendees will better understand how complex dental restorations withstand extreme conditions and how this knowledge may aid in routine dental identification or in Disaster Victim Identification (DVI).

This presentation will impact the forensic science community by providing the forensic odontologist with chemical and structural information of restorative materials and their interaction at high temperatures.

Recent research has shown that restorative dental materials can be recognized by microscopy and elemental analysis (Scanning Electron Microscopy/Energy Dispersive X-Ray Spectroscopy (SEM/EDS) and X-Ray Fluorescence (XRF)) and that this is possible even in extreme conditions such as cremation. These analytical methods and databases of dental materials properties have proven useful in DVI of a commercial plane crash in 2009 and in a number of other victim identification cases.

Dental materials appear on the market with ever-expanding frequency. With their advent, newer methods of restoration have been proposed and adopted in the dental office. Methods might include placing multiple layers of materials having different properties including adhesion, viscosity, working time, or curing methods. Use of different materials such as filled adhesives, flowable resins, glass ionomer cements, composite resins, liners and sealants is current.

In the normal process of forensic dental identification, restorations that exactly replicate tooth structure and color complicate the task for the forensic odontologist. With possible combinations of different materials in these restorations, the forensic odontologist is now confronted with a new difficulty: how to recognize each individual material. The question might be posed if it is even possible to perform this task.

Furthermore, an odontologist might be called upon to identify a victim under difficult circumstances, such as when presented with fragmented or incinerated remains. In these circumstances, the ability to identify specific dental materials could assist in the identification of the deceased.

Key to use of this information is whether these new materials and methods are detailed in the dental chart. Visual or radiographic inspection may not reveal the presence of a restoration, let alone the possible complex nature of that restoration.

Materials and Methods: Extracted teeth obtained from the State University of New York (SUNY) at Buffalo School of Dental Medicine were utilized in this *in vitro* study. Class II preparations in posterior teeth and Class IV preparations in anterior teeth were made. Restorations were completed using both single materials and materials in various combinations. The restorative materials used were the following: Composite resins (Grandio SO, Grandio Heavy Flow, Tetric EvoFlow, Tetric EvoCeram), glass ionomer cement (Fuji IX), and calcium hydroxide liner (Ultra Blend plus). Combinations of materials placed included Grandio Flo and Grandio SO, Fuji IX and Tetric EvoCeram, Tetric Flow and Grandio SO, and Ultrablend Plus and Tetric EvoCeram. Control samples of all dental materials were made in a ring mold. Cross-section fractured surfaces were analyzed by SEM/EDS. Elemental composition and microstructure of each material was recorded. Prepared teeth were then incinerated at 900°C for 30 minutes. As expected, these conditions caused separation of enamel from dentin and variable adhesion to, or separation of, dental materials to tooth structure. Resulting fragmented specimens were inspected by stereomicroscopy and analyzed by SEM/EDS.

Results: Post-incineration, dental restorations could be distinguished from burnt tooth structure by optical microscopy; however, it was not possible to differentiate one material from another by this means. With SEM/EDS, it was possible to differentiate the different dental materials in restorations using a combination of backscattered electron imaging and EDS analysis. Post-incineration elemental compositions matched those of pre-incinerated controls.



Odontology Section - 2015

Conclusions: Complex dental restorations can survive incineration conditions. Each material retains its individual elemental properties, and is easily distinguishable using SEM/EDS. In everyday dentistry, dental restorations are becoming more complex, especially as dentists are now utilizing different dental materials to achieve “perfect” dental restorations. This presentation is to alert forensic odontologists to these challenges and to transfer awareness to general dentists to maintain accurate dental records, as each dental material used during dental restoration could be important in the outcome of dental identification.

Forensic Odontology, Disaster Victim Identification, Dental Identification



G20 Electron Microscopy of Etched Dental Crown Surfaces and Electron Dispersive Spectroscopy (EDS) Studies Following Immersion in Hydrochloric Acid

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After attending this presentation, attendees will be aware of changes in the surface appearance at the Scanning Electron Microscope (SEM) level that indicate the exposure of porcelain-fused-to-metal (PFM) dental prostheses to commercially available concentrated hydrochloric acid. This presentation documents the changes observed and this may permit other practitioners to recognize similar features should they encounter cases in which disposal of human tissues has been undertaken using similar methods.

This presentation will impact the forensic science community by providing information about the changes noted in porcelain-fused-to-metal dental prostheses when they are exposed to concentrated hydrochloric acid for a period of several days.

A number of dental PFM crowns (the original crowns) were submitted for analysis. Information was provided that it was suspected that these items had been immersed in Hydrochloric Acid (HCl) for seven days and was asked if that could be confirmed. Perusal of the literature revealed no publications that documented the changes in appearance of materials commonly used in dental crown and bridge fabrication after prolonged exposure to HCl. The outcome was therefore tested by experimentally immersing a test bridge made by the same dental laboratory using the same materials and the changes noted in the test bridge surfaces were compared with the surfaces of the original crowns.

A sample test bridge made by the same dental laboratory as the submitted (original) crowns was obtained and both the metal and porcelain parts were examined under an SEM.

The elemental composition of the metal components of both the test bridge and the original crowns was determined by Electron Dispersive Spectroscopy (EDS) which showed that the principal components in the metal structure of both prostheses were Nickel (Ni) and Chromium (Cr).

The original appearance of the metal surface of the test bridge was examined and documented as a series of images. The test bridge was then immersed in the same brand and concentration of HCl believed to have been used for the original crowns and for the same period (seven days). The same area of the test bridge was then re-examined to determine the effect of the HCl. Changes consistent with corrosion were observed in the metal. The porcelain component of the test bridge was also examined before and after immersion in the HCl and the resulting SEM scans also exhibited distinctive changes. The original crowns were then examined with the SEM. The appearance of the metal and porcelain surfaces in the original crowns were observed to show the same features as those seen in the test bridge after exposure to HCl for seven days.

The goal of this study was to determine and document the changes occurring in dental crowns, bridge metal, and ceramic materials caused by immersing them in HCl for seven days. When the resulting changes were compared with the features noted in the original crowns, they were found to be very similar, indicating the likelihood that the original crowns had also been immersed in a similar corrosive.

The identity of the specific corrosive in which the original crowns were immersed was not identified by the technique used because EDS did not identify chlorine or any other substance indicating a corrosive material as a surface component of the material being examined.

Forensic Odontology, Analytical Technique, SEM



G21 Chronological Evaluation of Bruising in Bitemarks and Blunt Trauma: Validation of the Validation of the Nuzzolese-Neri-DiVella (NNDV) Colorimetric Scale

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After attending this presentation, attendees will have an understanding of the standards and accuracy of a new colorimetric scale for the standardization of photographic imaging of bruises and ecchymoses.

This presentation will impact the forensic science community by presenting the results of a new and very useful tool for forensic photographic imaging and chronological assessment of bruising.

One of the more controversial aspects in the evaluation of a wound caused by trauma in a living subject is determining the time in which the trauma was inflicted; this is accomplished by evaluating its age determination according to repeatable standard procedures which are able to be measured, uninfluenced by subjectivity. Observational standardization, combined with a chromatic evaluation of the areas affected by ecchymoses, are necessary in order to obtain such a result.

The goal of this study is to verify the validity of the innovative NNDV colorimetric scale as an aid to the development of a photographic and analytic standard in the field of forensic photography, targeting chronological determination of one or more ecchymosis caused by trauma or human biting. The discriminant factor of the current technique and subjective procedure has been evaluated using the NNDV colorimetric scale, relating to the changing colors of the ecchymosis due to the passing of time by using an innovative color index. A total of 17 subjects and 22 ecchymoses were assessed and rated using this scale. Validation was performed through statistical analysis via software for the analysis of Red/Blue/Green (RBG) colors on the DigitalColor Meter. The RGB percentage value present in different areas of the ecchymosis was assessed and compared to the RGB value present on the color sections of the colorimetric scale located on the same photograph, using the same light exposure. This allowed identification of the two RGB percentage values which were most similar to each other in order to facilitate evaluation of the chromatic variants and validate the deductions obtained during the first stage of the method.

Chronological determination was accomplished by using both the subjective and objective discriminant power of each method. The power increases by combining the objective technique to the validation of the method via the use of a photo-editing software, reaching 86.36% percentage of success.

The colorimetric scale shows promise as a valuable and reliable tool for medical examiners and forensic odontologists for the assessment and standardized interpretation of bitemark and bruising age evaluation through the use of a photo-editing software.

Forensic Photography, Blunt Trauma, Bitemark



G22 Ethical Proceedings Involving Dentists in the State of Minas Gerais, Brazil

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After attending this presentation, attendees will have an overview of the annual development of ethical proceedings judged by the Regional Council of Dentistry in the state of Minas Gerais, Brazil. Specifically, the present research relates the reasons for justifying ethical proceedings and its respective sentences and penalties.

This presentation will impact the forensic science community by exposing the most common ethical violations in one of the most populous states of Brazil. The presentation specifically illustrates the relevance of properly following ethical guidelines in daily clinics in order to avoid potential ethical and legal convictions.

The Brazilian labor market annually increases with approximately 9,000 new dentists.¹ Currently, more than 250,000 dentists perform legal activities in a country with about 200 million habitants. This statistical data supports a competitive professional panorama and directly influences potential increases in the amount of daily ethical violations.^{2,3} In order to guide adequate ethical behaviors in dentistry routines, Federal and Regional Councils of Dentistry were created, including special sections for the investigation and judgment of dental ethics.⁴ Specifically, a total of 10% of the Brazilian population lives in the southern state of Minas Gerais, consequently representing a large demand for dental treatment. In this context, this study investigated the ethical proceedings prosecuted and judged at the Regional Council of Dentistry of the state of Minas Gerais, Brazil, in the period between 2005 and 2011.

A retrospective observational transversal research was designed in the database of the section of Dental Ethics at the Regional Council of Dentistry of Minas Gerais, Brazil. The sample consisted of 955 ethical proceedings detected in the period between 2005 and 2011. All data was systematically analyzed by a single observer in order to extract information concerning: the related reasons justifying ethical proceedings and the respective sentences and penalties. Descriptive statistics were performed to obtain an overview of the outcomes and an association Chi-square test was applied to assess the relationship between ethical reasons and sentences. The outcomes revealed a gradual annual increase in the amount of proceedings. Specifically, in the first year studied (2005), 49 proceedings were observed, while 249 were detected in the last year (2011). The most prevalent reasons justifying ethical suits were: (1) irregular marketing and advertising (30.4%); (2) lack of adequate technical performance (30.4%); and, (3) dental clinics not registered in their respective Regional Council (25.5%). The investigation of sentences revealed that approximately 67% of dentists were acquitted. Convicted dentists (33%) were predominantly sentenced to confidential notice (19.1%). Association tests revealed a positive relationship between reasons and sentences, indicating that proper judgments are performed once the illegal practice of dentistry, such as irregular marketing or through unregistered facilities, is highly condemned. Additionally, the conviction of inadequate technical performances highlights the need for constantly updating and improving technical knowledge.⁵

A linear growing trend of ethical proceedings was observed during the period of investigation, revealing that dental surgeons are convicted in the face of major ethical infractions. It indicates the essential role played by the section of Dental Ethics from the Regional Councils of Dentistry inspecting routine professional activities. On the other hand, surveillance is also necessary during the design of undergraduate programs in dentistry, which must contain specific education on dental ethics, dental law, and forensic odontology, properly supporting the clinical behavior of future professionals.



Odontology Section - 2015

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Ethics, Dental Law, Forensic Odontology



G23 Dental Identification Using Facial Reconstruction on a Train Collision Victim's Mangled Body

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The goal of this presentation is to inform attendees that the use of a positioning device can make dental identification easier, faster, and safer, as well as improving the quality of the postmortem records.

This presentation will impact the forensic science community by illustrating the advantages of dental comparison even in “extreme” cases of identification.

In Bari, southern Italy, in February of 2014, an extremely fragmented body was found scattered along the railway connecting Bari to Lecce. The body pieces were collected along 100m of train tracks in both directions, allegedly after being hit by several trains. The skull was extensively fractured with some portions of the base still present; the trunk skeleton was massively fractured, lacking its anatomical profile; and the superior and inferior limbs were reduced to several pieces. No documents or personal belongings were collected from the body; therefore, the police assumed the body was a clandestine immigrant.

The first medicolegal investigation revealed the corpse belonged to an unknown 20-to-25-year-old Caucasian man. After several days, a report was presented to the police station by people claiming a relative of theirs had disappeared several months earlier. Generic information revealed he was a 22-year-old young man affected by leukoderma, who had previously attempted suicide. The report was presented by his distant relatives with whom the man had lived several years before and with whom he was occasionally in touch.

No DNA analysis was possible for identification and no reconstruction of the soft tissue of the face or facial skull superimposition could be performed. Given that the investigator had to proceed with personal identification procedures, the police were asked to visit the relative's house and search for evidence of the victim's DNA or some of his old medical files. Luckily, a five-year-old Ortopantomography (OPT) was found; however, it was not possible to compare it with Postmortem (PM) data due to the extensiveness of the mandible and maxilla fractures. In fact, there were numerous missing structures and only a few pieces of the facial skull were still present.

It was decided that the facial skull fragments would be placed in correct anatomic position on a polystyrene head model. The model obtained was X-rayed, and the Antemortem (AM) and PM data were compared. Neither the OPT nor the X-rays showed any restored teeth. The PM radiograph of maxillary left fragment (with teeth 26-27-28) was compared to the AM panoramic X-ray. The comparison revealed a morphological compatibility between the particular radicular shape of the third molar and also of the pulpal chamber of the second molar. Moreover, there was evident compatibility of the bone profile.

In this case, it was possible to reach a positive identification through the comparison of images taken from similar angles with the positioning of the fragments on the polystyrene head model. This positioning device allowed performing a more precise morphological comparison. A better PM radiograph leads to a better AM/PM comparison.

Mangled Body, Identification, Skull Face Fragments



Odontology Section - 2015

G24 Scanning Electron Microscopy (SEM) and Energy Dispersive X-Ray Spectroscopy (EDS) of a Supposed “Gold Dental Crown”

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After attending this presentation, attendees will better understand the role of the SEM and EDS used in the analysis of a dental crown that was the subject of an investigation. Attendees will also learn the role of SEM/EDS in the field of forensic odontology.

This presentation will impact the forensic science community by providing an option for the analytical examination of dental materials in areas other than forensic human identification purposes. This presentation also demonstrates the application of SEM/EDS in the standard-of-care field in forensic odontology.

In today's world of global marketing, it is easy for a person to buy and sell any items overseas. It is also cheaper to have an item made overseas and subsequently imported into the country for sales; this is widely used in many industries, dentistry included.

There are several news articles about local dental laboratories outsourcing dental lab work to another country for manufacturing or of dentists sending their dental lab works overseas for fabrication of the dental work. The main reason is due to cost-cutting and is profit driven. Local dental laboratories have to comply with the rules and regulations set out by the state or country; however, overseas laboratories may not comply with any regulations at all. Dentists or the local laboratory owners will never know what the actual ingredient or actual content of the requested manufactured dental laboratory work is, until an issue is raised that requires further investigation. Is the received dental gold crown actually made of gold?

This study was consulted by a dental colleague about a full gold dental crown that discolored in less than two weeks post-cementation. The patient was unhappy and the gold dental crown was removed and refabricated. The dental colleague was told by the dental ceramist that the particular gold dental crown was made of high precious metal dental alloy (the piece of dental alloy was manufactured by a particular dental company) and was curious why the gold dental crown discolored.

SEM/EDS examination revealed that this particular “gold dental crown” was not made from high precious dental alloy and that the discoloration of the gold-colored dental crown was actually copper oxidization.

Recent research has shown that restorative dental materials can be recognized by microscopy and elemental analysis. These analytical methods of dental materials properties have proven useful in Disaster Victim Identification (DVI) and in a number of other victim identification cases. This report provides another role that SEM/EDS analysis can play in forensic odontology and dentistry in general.

This presentation highlights the importance of dental material science and scientific analysis in the field of forensic odontology.

Forensic Odontology, Dental Materials, SEM/EDS



G25 A Hot Air Balloon Crash in Wairarapa, New Zealand: A Forensic Dental Perspective

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After attending this presentation, attendees will understand the importance of preparation, planning, and appropriate training for Disaster Victim Identification (DVI) work. Attendees will also understand the role of the forensic odontologist in the identification of badly damaged human remains.

This presentation will impact the forensic science community by outlining the DVI response and problems associated with a hot air balloon crash in New Zealand.

On January 7, 2012, on a perfect summer day, a scenic hot air balloon trip ended in disaster when the basket collided with power lines, caught fire, and eventually crashed onto farmland near the rural town of Carterton, North Island, New Zealand. This was one of the deadliest air disasters to occur on mainland New Zealand in decades; 11 people (ten passengers and the pilot) lost their lives. Two of the passengers jumped from the balloon to their deaths; the remainder were trapped and engulfed in flames. Emergency services were at the scene very quickly and witness reports along with camera footage taken by the balloon ground crew would help to reconstruct the final moments of the flight.

This accident was managed as a DVI incident with multidisciplinary participation. One of the forensic odontologists and the regional forensic pathologist attended the scene to locate, preserve, document, and assist with the safe transportation of the remains. The badly damaged and burned bodies were taken to Wellington Regional Hospital mortuary where autopsies and identifications were undertaken. At the mortuary, forensic odontologists, fingerprint teams, and DNA teams worked alongside forensic pathologists with police and coronial support for the next week. Crash investigators, fire services, and police worked together at the scene. Surprisingly, all victims were from the region, simplifying the collection of antemortem information in readiness for comparison with the postmortem findings. Being a “closed” disaster, there was a readily available passenger list. Toxicological findings indicated the presence of tetrahydrocannabinol (a constituent of cannabis) in the body of the pilot.

Events surrounding the crash and the difficulties encountered by the DVI teams and relatives of the deceased will be outlined in this presentation. It is essential that all agencies and individuals working in this field manage the situation effectively and sensitively and learn from the process. At times of tragedy, relatives need excellent communication from those assisting to help them understand why this event claimed the lives of their loved ones. The post-incident debriefing was invaluable for improving the response for future events. An inquiry into the accident has been conducted by the Transport Accident Investigation Commission. At inquest, the families of the deceased requested that recommendations should be acted upon to prevent this type of tragedy from happening in the future. Will anything be done?

All victims were identified in the following days. After attending this presentation, attendees will understand the importance of preparation, planning, and appropriate team training for timely action when prevention fails and incidents occur. This presentation also emphasizes the role of the forensic odontologist when remains are badly damaged and commingled.

Balloon Crash, DVI, Dental



G26 Isle-Verte Disaster

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After attending this presentation, attendees will understand the importance of a well-orchestrated operations plan in order to navigate the unexpected conditions at a mass disaster site and the need for collaboration between forensic experts for a successful identification outcome.

This presentation will impact the forensic science community by helping attendees gain information on both procedural solutions to complicated climactic and site conditions in a mass disaster situation and on the lessons learned from two mass disasters occurring within months of one another, each with unique environmental issues.

The fatal fire of Résidence-du-Havre, Isle-Verte, Quebec, Canada, which occurred on January 23, 2014, struck the seniors' residence at 12:05 a.m., resulting in 32 deaths. Firefighters were called to the Résidence-du-Havre in sub-zero temperatures with a wind chill factor of -35°F which culminated in a cascading ice castle encasing the building's debris and victims alike. This presented major challenges for the recovery team.

The Isle-Verte tragedy fell on the heels of the Lac-Mégantic, Quebec, fatal train derailment in 2013, less than six month's earlier, where an unattended 72-car freight train operated by the United States-based "Montreal, Maine and Atlantic Railway" (MMA) carrying over two million United States gallons of crude oil broke away, derailed, and caught fire with multiple explosions in the center of the town resulting in 47 deaths.

The Montreal-based forensic team was cohesive and well coordinated following the Lac-Mégantic Disaster and cross-functional teams were well established to face the Isle-Verte tragedy.¹

The on-site forensic identification team included two pathologists, an experienced denar, and an anthropologist. Participants will learn the complex process of extracting cremated human remains under extreme environmental conditions. Once extracted, the human remains were bundled, packaged, numbered, and shipped to Montreal where pathologists, dentists, and DNA experts further analyzed the remains at the central forensic laboratory. The average age of the victims was 89 and most had few or no teeth. Two victims were 99 years of age. All human remains were photographed, examined, underwent fluoroscopy, and medical and dental radiography. Through the collaboration of the pathologists, odontologists, and DNA experts, 28 of the 32 victims were successfully identified. Dental identification was primarily based on trabecular bone pattern.

The Lac-Mégantic and Isle-Verte tragedies will be compared statistically and lessons learned will be shared.

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Forensic Odontology, Mass Disaster Management, Positive Identification



Odontology Section - 2015

G27 Dental Identification of 530 Landslide Victims: Lessons Learned

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After attending this presentation, attendees will better understand the nature of this landslide, how this type of environment impacts victim recovery and victim identification utilizing dental records. Additionally, attendees will learn how one agency utilized their local forensic odontological community to successfully identify victims.

This presentation will impact the forensic science community by further illustrating the importance of a sound working relationship between the forensic odontologist and medical examiner. Prior to the landslide on March 22, 2014, the forensic odontologists already had an established working relationship with the medical examiner's office. In addition, the local dental identification team had been trained, had experience in the medical examiner's office, and were already known to the chief medical examiner.

On Saturday, March 22, 2014, a massive landslide occurred near the small rural town of Oso, WA, obliterating a residential community along the Stillaguamish River. The mudslide also created a soft earthen dam across the river, creating a lake in the river's path upstream and a potential flooding risk downstream. Debris covered approximately one mile of State Highway 530, effectively isolating the neighboring town of Darrington. The debris field was extensive and deep: areas were covered by 10 to 60 feet of debris. The forces involved in the mudslide dismembered many victims and displaced them over an estimated one square mile. Miraculously, several people survived.

The Snohomish County Medical Examiner's Office was responsible for all victim identifications. Multiple agencies assisted in the recovery of the victims over the next several weeks. Individuals from multiple agencies also assisted directly with the medical examiner's office. During the initial days of the response, the number of missing was at times estimated to be as high 170 people. Careful follow-up of all missing person's reports (more than 500) ultimately trimmed the death toll to the more realistic 43 individuals.

Not surprisingly, the 530 Landslide was a highly publicized incident, both locally and nationally. By design and otherwise, there were many local residents volunteering onsite with recovery. Media updates of a day's events were virtually instantaneous. Appropriate scientific means of identification were viewed by some as causing unnecessary delays. In some cases, decedents had actually been recovered by local residents. At times, it was frustrating to these volunteers to accept that facial recognition alone as a means of positive identification was insufficient. The Chief Medical Examiner for Snohomish County chose to adhere to scientific methods of identification which included dental records, fingerprints, and DNA for positive identification.

While federal assets such as the **Disaster Mortuary Operational Response Team (DMORT)** would likely have been available, the Chief Medical Examiner chose to work with the forensic odontologists with whom he was already familiar and with whom their office had long-standing working relationships. Federal assets (DMORT) can be deployed rapidly; however, there would not have been the established working relationship between those forensic odontologists and the medical examiner's office. Even with a rapid DMORT deployment, that response would likely not have been as quick as what local odontologists were able to achieve. With the operating framework already in place, the medical examiner's office was able to respond to the public's need for information (i.e., identifications) promptly.

The lead forensic dental consultant for the Snohomish County Medical Examiner's Office was contacted directly by the medical examiner a few hours after the mudslide. Given the nature of the event, the Chief Medical Examiner felt that dental identification would be an important part of the victim identification process. Starting on the Monday after the mudslide, forensic dentists were at the medical examiner's office almost daily. Standard clinical dental examinations, photography, and digital dental radiography were performed on almost all of the victims. In many cases, positive identification was accomplished via dental records, providing timely information for surviving friends and family, as well as information for directing further recovery efforts.

By July 22, exactly four months after the 530 Landslide, all 43 known victims had been recovered and positively identified. Twenty-seven were identified via dental records. The remaining victims were identified by other scientific means (fingerprints, DNA, skeletal, and/or medical devices).

Dental Identification, Mass Fatality Incident, Landslide



Odontology Section - 2015

G28 Dental Identification of the 2008 Peruvian Andes Helicopter Crash Victims

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After attending this presentation, attendees will understand two of the techniques used in the identification process of a decedent.

This presentation will impact the forensic science community by demonstrating the importance of proper protocols used in the identification process to assure reliable results.

This presentation will provide an overview of the efforts made in the recovery and identification process of the victims of the 2008 *Rio Tinto Copper Mine* helicopter crash in the Peruvian Andes Mountains and will demonstrate the use of death mask reconstruction and established forensic odontologic protocols.

All too often, recovery efforts can be challenging due to environmental factors including weather, terrain, and lack of manpower. This unfortunate disaster occurred in a nearly inaccessible mountain range, in heavy weather requiring the use of specialized forces to recover remains. These factors will be discussed and the value of precise antemortem records and their interpretation will be presented. Additionally, this presentation will discuss the difficulty encountered when instrumentation and technology are limited.

The discussion will stress the importance of understanding other cultures and their impact on the identification process. This presentation will also demonstrate the need for those of lesser forensic experience to be given the guidance and knowledge necessary to perform a proper forensic dental identification — this includes understanding the value of separating and labeling personal effects to minimize commingling and, in so doing, potentially facilitate the identification process. Emphasis will be placed on the necessity of “guest” odontologists to conform to the protocols of the “host” while gently introducing techniques that as yet may be unfamiliar and, by doing so, advancing the level of knowledge and ability of all, in an effort to reach positive outcomes.

The interaction between forensic professionals and the importance of mutual respect will be discussed as it applies to sharing of ideas and techniques that may be foreign to non-dental investigators. Additionally, attendees will come to understand the necessity for modifying protocols and demonstrating flexibility when called upon to act as a forensic odontologist rendering proper identifications in a difficult environment.

Forensic Odontology, Unidentified Persons, Helicopter Crash



Odontology Section - 2015

G29 The Reno, Nevada Air Race Accident

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After attending this presentation, attendees will be acquainted with information regarding the Reno, NV, Air Race accident which occurred at 4:25 p.m. on September 16, 2011, and resulted in 11 fatalities and approximately 60 injuries.

This presentation will impact the forensic science community by providing an understanding of the vital role the dental team plays in the management of the identification of the victims of a Multiple Fatality Incident (MFI).

On the afternoon of September 16, 2011, at a popular annual air show in Reno, NV, located in Washoe County, a modified World War II-era fighter plane crashed into the edge of the VIP grandstands. Eleven people, including the pilot, were killed and approximately 60 spectators were injured, many suffering amputations.

This incident occurred within the jurisdiction of the Washoe County Medical Examiner's Office (WCMEO). The WCMEO, located in Reno, NV, is responsible for forensic death examinations for all of the State of Nevada (with the exception of Las Vegas and several southern Nevada counties) as well as a few northern California counties. The office covers an area of approximately 100,000 square miles and a population of 800,000.

The WCMEO was notified and responded to the former Stead Air Force base, approximately 12 miles from the morgue. An initial site assessment was completed, and recovery, antemortem data collection, and postmortem data collection teams were activated. This scene assessment led to the discovery of many fragmentary remains and an indeterminate number of deceased victims. Survivor and decedent dismemberments were commingled with the crash site debris.

This presentation will illustrate how the WCMEO recovered and made scientific identifications of these remains. Specifically, it will demonstrate how the dental team, composed of one forensic odontologist and one WCMEO death investigator, positively identified victims using traditional methods of comparison of antemortem and postmortem dental records and dental radiographs. One intact body from the scene as well as 16 individual dental fragmentary remains, which included teeth, were identified. All positive dental identifications were completed by September 21, 2011. Subsequent DNA sequencing, one to two days later, confirmed the dental identifications.

Information will also be provided on how other postmortem modes of scientific identification (fingerprints, radiology, and DNA) were used to identify and to re-associate remains. All remains were able to be released within one week of the incident. This rapid release highlights the teamwork required of law enforcement, first responders, medical examiner office personnel, and members of the health care community when dealing with the aftermath of an MFI. It also clearly demonstrates the respect the forensic science community has for victims and families during a very stressful and emotional time.

Forensic Science, Forensic Odontology, Multiple Fatality Incident



Odontology Section - 2015

G30 Identification of an Unknown Sailor From the December 7, 1941, Attack on Pearl Harbor

John A. Lewis, Jr., DDS, 407 Heritage Place, Flowood, MS 39232*

After attending this presentation, attendees will have gained an understanding of the forensic identification procedures used to identify the skeletal remains of a previously unidentified American sailor who was killed during the December 7, 1941, attack on Pearl Harbor. Attendees will also learn of efforts made to identify other previously unidentified victims of the Pearl Harbor attack.

This presentation will impact the forensic science community by describing the forensic identification procedures used to identify Americans lost during past conflicts.

On Sunday, December 7, 1941, the tranquility of the island of Oahu, HI, was shattered by the Japanese attack on the American Pacific Fleet anchored at Pearl Harbor. By the end of the day, close to 2,400 military and civilian personnel were dead, killed in the attack which launched the United States into World War II.¹

One of the 162 ships and vessels within Pearl Harbor proper on that day was the U.S.S. *Curtiss* (AV-4), a seaplane tender. During the attack, the *Curtiss* was hit twice by Japanese aircraft.¹ The second contact resulted in the deaths of 21 sailors from the *Curtiss*. Two of the *Curtiss*' crew members were unaccounted for. One body was unidentifiable at the time and was ultimately buried as an unknown at the National Memorial Cemetery of the Pacific, also known as Punchbowl.

Pearl Harbor survivor Ray Emory was a young Seaman 1st Class stationed aboard the U.S.S. *Honolulu* which was in port at Pearl Harbor on December 7, 1941. In recent years, Emory began researching the unknown victims of the attack on Pearl Harbor who were buried at Punchbowl. Through the efforts of Mr. Emory, the unknown remains of a sailor was disinterred and positively identified using forensic odontology, forensic anthropology, and mitochondrial DNA testing.

The American military has a solemn pledge to never leave one of its own on the battlefield. This includes using every available tool to identify its unknown war dead from past wars. Even though the attack on Pearl Harbor occurred almost three-quarters of a century ago, forensic science was used to identify an unknown American killed in the attack which launched the United States into World War II.

Reference:

1. Naval History and Heritage Command

Odontology, Pearl Harbor, Identification



Odontology Section - 2015

G31 Forensic Dental Examination of Disinterred 19th-Century Archaeological Remains

Thomas J. David, DDS, 1000 Johnson Ferry Road, Bldg H, Marietta, GA 30068; and Adam J. Freeman, DDS, 22 Imperial Avenue, Westport, CT 06880*

The goals of this presentation are to distinguish between archaeological remains and more recent burials and to compare and to contrast dental restorative treatment from the 19th, 20th, and 21st centuries.

This presentation will impact the forensic science community by discussing the historical significance of forensic dental information from 19th-century dental remains.

The discovery of gold along Whitewood Creek in the fall of 1875 led to the settlement of Deadwood, SD. Deadwood's first cemetery was established out of necessity for the proper disposal of human remains. This cemetery was originally located about one-quarter mile from Deadwood's central business district. It is unknown who established the cemetery or when the first burial occurred, since newspapers in the town were not established until June of 1876. Nevertheless, many of the town's newspapers provided news articles and obituaries about individuals buried in the original cemetery. The first cemetery did not have a formal name and was referred to by many names, including "the Deadwood Cemetery," "cemetery on the hill," "City Cemetery," and "old graveyard in South Deadwood." As a matter of fact, a newspaper article once referred to the cemetery by two different names on the same day. The creation of Mt. Moriah Cemetery in 1878 led to the downfall of Deadwood's first cemetery; however, after the establishment of Mt. Moriah Cemetery, Deadwood's first cemetery would be called "Old Deadwood Cemetery."

Approximately 122 individuals were buried in Deadwood's first cemetery from 1875 to 1878. In June of 1877, one newspaper reported there were 80 burials in the cemetery. Two noteworthy individuals buried in the first cemetery included James Butler "Wild Bill" Hickok and Methodist minister Henry Weston Smith. Both of these individuals were later exhumed and reburied in Mt. Moriah Cemetery in 1879 and 1883, respectively.

In the spring of 2006, construction workers discovered human remains while in the process of replacing a retaining wall and the unearthed remains were sent to Minnesota for forensic analysis. It was determined that the individual was an adult male who was approximately 25-34 years of age and stood 5'4"-5'8" in height. The ethnic origin was determined to be Asian, Mongoloid, or Native American. This individual was reinterred in the Mt. Moriah Cemetery on July 28, 2010. A monument was placed on the grave that acknowledged the possible Native American and Chinese heritage of this individual.

In March of 2012, construction workers unearthed a fully articulated coffin burial while replacing a retaining wall at 66 Taylor Avenue in Deadwood. At that time, both Deadwood Historic Preservation officials and South Dakota State Archivists were on hand to exhume the burial. They found approximately 99% of the skeleton, with the exception of one tooth and a few finger and toes. The remains were subsequently transported to a forensic anthropologist for analysis. It was determined that this individual was a white male of 18-24 years of age and was 5'4"-5'8" in height.

After a forensic anthropologic analysis, the remains were transported to Marietta, GA, for a forensic dental analysis. This analysis was conducted on March 1, 2014, and the results will be discussed in detail. Despite multiple forensic analyses, no cause of death has been determined.

Archaeological, Disinterred, Cemetery



Odontology Section - 2015

G32 Adam Mayes: America's Most Wanted — A Lack of Wisdom?

Edgar W. Turner, DDS*, 410 Farino Way, Somerville, TN 38068

After attending this presentation, attendees will understand the problems that may be encountered in a dental identification involving the presence, or lack thereof, of third molars in various stages of development. This case will demonstrate cross-arch variations that may occur in the development of third molars within the same individual.

This presentation will impact the forensic science community by explaining the premise that if a tooth is missing in antemortem dental records but is present in the postmortem dental records, this generally constitutes an exclusion in terms of dental identification.

In April 2012, Jo Ann Bain and her three daughters were reported missing from the rural town of Whiteville, TN. Although they were scheduled to move out of state the next day, an investigation by law enforcement officials led to the conclusion that the family may have been kidnapped by a family acquaintance, Adam Mayes. An Amber Alert was issued for the missing children. The nature of the case resulted in local, state, and federal authorities becoming involved. As the investigation continued, Mayes was placed on America's Ten Most Wanted Criminals list by the Federal Bureau of Investigation. Adam Mayes became the Most Wanted Man in America after the bodies of Jo Ann Bain and one of her daughters were found buried in a shallow grave in Mississippi, while the remaining two children continued to go missing.

The partially decomposed remains of the mother and one daughter were transported to the West Tennessee Regional Forensic Center in Memphis, TN, for a complete autopsy and identification. While the dental identification of the mother was straightforward, identification of the daughter became problematic when three of the decedent's third molars were found to be present in the antemortem panoramic radiograph; however, postmortem radiographs revealed that all four third molars were present. The absence of a tooth in antemortem radiographs, which is subsequently found in postmortem records, is generally recognized as an unresolved discrepancy and routinely results in an exclusion in terms of a dental identification. In this particular case, two forensic odontologists independently reviewed the case and concluded that a positive identification could be made in spite of the discrepancy with the third molars. This presentation will discuss the problems associated with the identification as well as developmental discrepancies in third molars.

Mayes was eventually located in Mississippi by state authorities along with the two missing children. The case was adjudicated in 2013. As part of this presentation, the final resolution of this case will be provided, including the status of Adam Mayes, the missing children, and the accomplices involved in this case.

Identification, Odontology, Exclusion



Odontology Section - 2015

G33 Two Teens, Midnight, and a Porsche® Carrera® — What Could Go Wrong?

John P. Kenney, DDS, MS, 101 S Washington Street, Park Ridge, IL 60068-4290*

The goal of this presentation is to discuss the importance of complete dental records being submitted for an identification.

This presentation will impact the forensic science community by educating both the odontology community as well as fellow forensic scientists as to why complete antemortem records are requested before beginning an identification.

In the summer of 2010, two 17-year-old males were returning to their home in the suburbs of Chicago around 11:30 p.m. in a Porsche® Carrera® titled in the driver's name. They were traveling at a high rate of speed (estimated at 100+mph) and sideswiped a Dodge® minivan along Interstate 88 near Winfield, IL. Fortunately for the van's passengers, the van driver was experienced and able to maintain control of his vehicle. Unfortunately, the young driver was not able to do so; his vehicle spun out of control on the toll road, ran up a concrete embankment supporting an overpass, and flipped several times before stopping and bursting into flames.

Illinois State Police and Fire Rescue from Winfield, IL, responded, but all they could do was extinguish the fire and recover two young male bodies. The body of the owner was rapidly identified via excellent dental records from the young man's orthodontist. Intraoral photos, a cephalogram, and panoramic X-ray all compared favorably with the postmortem dental examination; however, identification of the second victim was problematic. There were a number of discrepancies between the written records, the antemortem X-rays, and the postmortem findings. It was clear to the investigator that while human error in charting was a possibility, the more likely answer was overbilling of procedures to the young man's parent. Fortunately, it was possible to make a positive identification based on the objective dental X-ray evidence. Had no antemortem X-rays been available, the identification would have had to wait for fingerprint or DNA comparison. In this situation, a delay or exclusion based on the written dental record alone would have had serious ramifications because the young friends' faith stipulates burial within 24 hours. The investigator contacted the treating dentist in the process of the investigation and suggested that perhaps better record keeping was in order.

Radiographs, Odontogram, Dental Treatment Narrative



G34 The Case of the Missing Mandible

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After attending this presentation, attendees will be aware of how a missing mandible contributed to the resolution of three separate cases.

This presentation will impact the forensic science community by highlighting the importance of retaining records for as long as possible.

In 1986, a farmer in rural Dallas County, TX, was ploughing his field and came upon a decomposing human body. The farmer contacted the Dallas County Sheriff's Office to report the death and the Dallas County Medical Examiner's Office (MEO) was duly notified in turn. The medical examiner's investigators recovered the remains, but the decedent's mandible was not found. The decedent was examined at the morgue and the cause and manner of death were determined. The decedent was identified by comparison of antemortem and postmortem dental radiographs as Mr. A. The treating dentist was from Ohio and was subpoenaed to appear at the perpetrator's trial in Dallas County in order to "prove up" the dental records of the decedent. The perpetrator was tried and convicted. The treating dentist believes the original dental radiographs were retained by the MEO; however, the MEO states the radiographs were returned.

In 2010, in neighboring Denton County, TX, a death was reported to the Tarrant County Medical Examiner's District (the jurisdiction of the Tarrant County office also includes Denton, Parker, and Johnson Counties). The deceased was found seated on a farm combine in a remote area of his farm with an apparent self-inflicted gunshot wound to the head. The decedent was identified at the scene as Mr. B. by his ex-wife and also by his brother. The deceased had been despondent over the break-up of his family and financial woes as well. He was examined at the Tarrant County morgue and the cause and manner of his death were determined.

In 2011, the Tarrant County Medical Examiner's District was notified of a body found in a partially burned house in rural unincorporated Tarrant County. Upon investigation, the "body" was determined to be incomplete. In fact, the unidentified remains consisted of only a single human mandible found sitting incongruously in plain view on a kitchen counter. It was learned the house had belonged to Mr. B.; Tarrant County medical examiner's investigators found the cause of the fire had been ruled as arson — later investigation revealed Mr. B. had apparently set fire to his home in Tarrant County, then traveled to his farm in Denton County, where he committed suicide. The charred house was being cleaned and the fire damage was being repaired by the bank that had taken possession of the house prior to putting the house up for sale. The house had sat empty and unattended for approximately a year after the fire.

Attendees of this presentation will learn how these cases were intertwined and their eventual resolution. The case report underscores the utility of practitioners retaining dental records for as long as possible and raises the issue that, although the Identification (ID) was eventually made by DNA comparison, were the available dental records sufficient to have made an ID?

Dental ID, Odontology, Mandible



G35 Single Tooth Dental Identification Assisted by Digital Superimposition

*Amber D. Riley, MS**, 9855 Erma Road, #103, San Diego, CA 92131; *Anthony R. Cardoza, DDS*, 9530 Cuyamaca Street, #101, Santee, CA 92071; and *Raymond Johansen, DMD*, 601 E Arrellaga Street, Ste 202, Santa Barbara, CA 93103

After attending this presentation, attendees will better understand how specific utilization of Adobe® Photoshop® features may assist their ability to examine dental evidence forensically.

This presentation will impact the forensic science community by informing attendees that forensic dental comparison is an ideal instrument in death investigation because of the longevity and durability of the dental anatomy, even in scenarios of extreme heat, trauma, and decomposition.

In this case, there were numerous obstacles to performing a traditional, straightforward dental identification, not the least of which were the age of the antemortem dental records furnished for comparison, but even more significant to the investigation was the scarce amount of postmortem dental evidence that was provided by investigators for analysis, which consisted of only one tooth.

In November 2012, family members of a man reported missing found and turned in a single tooth from a site where previously skeletonized human remains and personal effects had been recovered in Imperial County, CA. Forensic dental consultants examined the tooth to determine: (1) if it was indeed a human tooth; and, (2) if it would be significantly unique for an expert opinion to identify or exclude the missing person.

There was discussion of using the tooth in an attempt to recover DNA from the pulp tissue for comparison with known family sources; however, any collection procedures would destroy the tooth for further odontological evaluation and it was uncertain that useable tissue for DNA analysis could even be collected from the tooth. Therefore, it was agreed that the risk of destroying the very limited evidence was too high and a dental comparison was the first and best option to investigate identity.

A full series of dental radiographs dated August 5, 1996, were collected from the missing person's dentist and multiple radiographic and photographic images were taken of the single tooth at the Office of the Medical Examiner in San Diego, CA. It was determined that the tooth was a virgin, permanent lower right molar. Based on the size, anatomy, distal curvature, and lack of space between the roots, it was agreed that the tooth was most likely a third molar, tooth #32 in the Universal Numbering System, or tooth 48 in the **Fédération Dentaire Internationale** (FDI) World Dental Federation notation.

Both the antemortem and postmortem radiographs were digitized and uploaded into Adobe® Photoshop® to allow a digital superimposition of the radiographs at varying opacities from 10% to 100%. The software also allowed the tooth images to be realigned on a consistent, measured, and reproducible gradient as well as their size enlarged to examine and measure minute and intricate anatomical details from both antemortem and postmortem images, without distortion and with a reproducible history of any manipulations made to the original image.

The chief forensic dental consultant using digital superimposition, digital enhancement of anatomical landmarks, and the measuring tools available in Photoshop® determined that the postmortem single tooth evidence and the antemortem dental records of the missing person were consistent to the level of probable (more likely than not). The Chief Medical Examiner signed the case out as positive identification based on the supporting quantitative data provided in the consultant's report detailing the process used and the images and data upon which his opinion was based.

Digital, Dental, Identification



G36 Identification by Photograph: A Case Presentation

Barbara L. Needell, DMD, 5280 N University Drive, Lauderhill, FL 33351*

After attending this presentation, attendees will understand how to overlay and compare antemortem and postmortem photographs to make a positive dental identification.

This presentation will impact the forensic science community by demonstrating a method of dental identification when dental radiographs are not available or are insufficient.

Dental identifications are usually accomplished by comparing antemortem and postmortem radiographs. In the absence of this information, other methods of identification must be found: (1) non-dental, such as DNA or fingerprints; or, (2) other dental, such as written records or photographs. When making an identification by photograph, how to use the tools (e.g., Photoshop®) to make an accurate comparison must be understood.

In 1981, a young woman, Tina, went missing in south Florida. She had recently moved from Ft. Meyers, FL, to live with a man who promised her a modeling career. When her parents discovered this, they went to Hollywood and met with the man. With their approval, Tina remained with this man who was a photographer. One week later, Tina's sister notified Pembroke Pines police that Tina was missing and they had lost contact. In 1982, a skeleton was found in West Palm Beach, FL, the county just north of Broward County where Hollywood/Pembroke Pines is located. The skeleton was cataloged and, for 31 years, stored as unknown remains.

In April 2013, the missing person's case was reopened due to an inquiry by the victim's sister. Her son had alerted her that there were unknown remains in West Palm Beach which reminded him of his aunt's case. Tina's sister was able to provide several photographs showing dentition. Photographs of the skeleton were taken in similar positions to the antemortem pictures. Using Photoshop®, the pictures were adjusted to the size of the skeletal teeth. This was accomplished by using several points on the teeth and measuring the distances between them. The overlays were created using two techniques of the Photoshop® program. One used the opacity slide function; the other used the pencil drawing function to outline the teeth. Both were successful and the skeleton was positively identified as Tina.

This presentation will demonstrate methods used to overlay antemortem and postmortem photographs. This technique aids in dental identification in the absence or insufficiency of antemortem radiographs.

Dental, Photograph, Identification



G37 A Case for the Records — The Importance of Antemortem Records in Making a Positive Dental Identification

Randolph L. Mitchell, DMD, 47 William Street, Lyons, NY 14489*

After attending this presentation, attendees will better understand the need for patience as antemortem records come into the medical examiner's office. Attendees will be able to sort out what information is key to the identification and what information helps to support the identification, while not actually being exemplars.

This presentation will impact the forensic science community by providing attendees with an appreciation of the need for complete antemortem records in a dental identification. Attendees will also appreciate the need for good documentation of treatments performed on patients and the role these documents play in dental identification.

This presentation will use a case study to demonstrate how important each piece of information that is provided to the medical examiner is in clarifying what appeared to be a major inconsistency between the decedent's antemortem and postmortem records. The net result is that it is the combination of all of the antemortem records provided, though they came from different sources and at different times in the investigation, that helped to explain the inconsistency between the postmortem dental and radiographic examinations completed at the Medical Examiner's Office (MEO) and the digitally transmitted antemortem radiographs initially provided by the decedent's dentist, which helped to make a positive dental identification possible in this case.

An interesting part of both the antemortem and postmortem records in this case was a personal effect found with the remains that was very tempting to use as a part of the evidence in the identification. Despite the temptation to use this personal effect in the identification, the personal effect was an object, not unique to the decedent, nor an actual part of the remains. Therefore, the personal effect should not be used as an exemplar in the scientific identification process, but may possibly play a supporting role of adding consistency with the antemortem and postmortem records, although on its own it adds nothing to the actual identification.

This case verifies that antemortem information drives dental identification. Recordkeeping, whether written or radiographic, from the decedent's primary caregiver or from a one-time treatment by a specialist, is extremely important in helping to make a positive dental identification. All records should be conveyed to the MEO when the primary caregiver is presented with a subpoena *duces tecum* by the MEO.

Identification, Records, Concurrence



G38 Fire, Fraud, and Forensics: A Complex Case Study in Dental Identification

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The goals of this presentation are to create a better understanding of the importance of dental records in a multi-victim fatality event involving children and to explain the necessity for open, professional communication between the treating dentist and the forensic dentist for an expedient result to the identification process.

This presentation will impact the forensic science community by illustrating the need for effective communication and cooperation between professionals involved in forensic cases and by exposing some of the ethical challenges faced during attempts to definitively identify victims in high-profile, tragic events.

On February 23, 2012, a tragic house fire claimed the lives of five victims, four of which were children. Multiple techniques were required to identify the victims due to an absence of antemortem dental films for two of the children and the adult, and fraudulent dental treatment notes for three of the four children. The techniques included: (1) age estimation to differentiate two of the children who were under the age of five years; (2) the use of distinctive dental characteristics, antemortem photography, and exclusion criteria to identify the adult; and, (3) the use of a law enforcement agency to definitively identify that three of the children's antemortem dental treatment notes were fraudulent.

The case discussion includes: (1) antemortem records review; (2) identification of the child victim; (3) age estimation techniques/procedures; (4) communication between the treating dentist and the forensic dentist; (5) deciphering fraud in the dental record; (6) ethical considerations for reporting fraudulent dental/medical records; (7) working under close media scrutiny; and, (8) lessons for community out-reach and education.

The sequence of events unfolded as follows. Initially, the Middlesex County Medical Examiner's Office obtained incomplete dental records for three of the children, all of whom were treated by the same dentist. The incomplete records contained panoramic dental radiographs of two of the children taken 11 months prior to the fire. A second request for the complete antemortem dental records was then made. The dental autopsies were begun using the incomplete records. After reviewing the antemortem radiographs, two of the victims were positively identified; however, when the additional dental records were received prior to the completion of the postmortem examinations, discrepancies existed between the dental treatment notes and the postmortem findings. The dental treatment notes indicated that multiple restorations were placed on all of the patients under the dentist's care after the date(s) of the antemortem X-rays. In fact, the restorations were not present in the postmortem examinations of any of the victims.

Upon completing the postmortem examinations, attempts to contact the treating dentist via telephone were made. The sole purpose of directly contacting the dentist was to rectify the discrepancies between the dental treatment notes and postmortem examinations so that accurate identification of the victims could be made. The dentist did not answer any questions that were asked during the phone conversation that would clarify the antemortem records, nor did he return any additional phone calls made at the behest of the medical examiner. Due to the absence of a response from the dentist, the assistance of the Middlesex County Prosecutor's Office was requested. The investigation that was subsequently launched led to the arrest of the dentist. The delay in identification of the victims caused by the discrepancies between the dental records and the postmortem examinations led to a media storm.

Ultimately, the two older children were positively identified by X-ray comparison, the two younger children were differentiated via age estimation, and the adult victim was identified by exclusion criteria and known individualizing characteristics captured in antemortem photographs. The dentist pled guilty to three counts of fraud and one count of obstruction. He was convicted, sentenced to two years of probation, and levied fines. The dentist is currently being investigated by the New Jersey State Board and the Attorney General's Office for Medicaid fraud.

Dental Identification, Fraud, Forensics



G39 Recovery and Processing of Multiple Partial and Total Cranial Fresh Cadaver Heads Purportedly for Surgical Teaching

Taylor L. Gardner, BfSc, Ontario Forensic Pathology Service, 25 Morton Shulman Avenue, Toronto, ON M3M 0B1, CANADA; Yolanda Nerkowski, BA*, Ontario Forensic Pathology Service, 25 Morton Shulman Avenue, Toronto, ON M3M 0B1, CANADA; and Robert E. Wood, DDS, PhD*, Princess Margaret Hospital, 610 University Avenue, Toronto, ON M5G 2M9, CANADA*

The goals of this presentation are to: (1) make attendees aware of the presence in the community of fresh, non-embalmed human surgical specimens available on the private market; and, (2) detail an occurrence where 15 whole or partial human heads were turned into a coroner's service for disposal and how they were evaluated and documented prior to their disposal.

This presentation will impact the forensic science community by assisting attendees to: (1) recognize the importance of having a mass fatality plan in place with an emphasis on dental identification; (2) have an awareness of the procurement, selection, and suitability of human specimens used for teaching purposes; and, (3) be cognizant of unethical practices related to full-body donation, including informed consent and precious metal "harvesting."

Medicine and science benefit from the use of human specimens for teaching and research, from educating medical students to taphonomy lab studies. This presentation will impact the forensic science community by making members mindful of how procurement occurs, why specimens must be suitable for their intended use, and selective disclosure of information regarding the fate of a body when consent is given. An increased awareness and ability to recognize disrespectful practices will contribute to the reporting of, and investigation into, unsavory and perhaps unlawful interference involving human specimens, and guide its regulation and oversight.

The Office of the Chief Coroner (OCC) was asked for cremation certificates for 15 human heads. These were procured for a dental implant surgery course. Information was obtained that the supplier — from outside of Canada — may have been under investigation unrelated to these specimens. Therefore, the OCC decided to review and confirm specimen identity.

This situation was treated like a "miniature, controlled, multiple-fatality incident," providing organized specimen analysis and as a training exercise for morgue staff in multiple-fatality protocols. Through joint efforts of specialists including a forensic anthropologist, advanced practice pathology assistants, a forensic pathologist, and an odontologist, the specimens were photographed, examined, and documented including: CT scanning, dental radiography, and DNA sampling. A summary examination and report on all specimens were completed.

After the exercise, it was discovered that this raised more questions than answers. The majority of specimens were clearly not suitable for implant training, having one or more of the following problems: (1) being completely dentate with few sites for implantation; (2) advanced sinus pneumatization; (3) inadequate alveolar bone; and, (4) presence of grossly carious roots. This made these "wasted" specimens. Furthermore, there was little uniformity in sectioning and disarticulation of specimens. Subjects were received as either partial, half, or full-head specimens. The variety of dissections observed begs the question of whether those providing their consent for body donation were informed of the extent to which their body would be "altered" or whether their body would be exported. It was also observed that more than a third of the specimens exhibited some loss of coronal structure and cast restorations, as evidenced by residual crown-cementation material and in the absence of any trauma/caries.

Additionally, teeth with non-precious materials in these same specimens were present and unaltered. The removal of these restorations, presumably posthumously, may have been deliberate and it is possible that these restorations contained precious metals that could be sold profitably for scrap.

Positive identification was achieved for all specimens. The early years of acquiring human cadavers for medical education purposes is tarnished. As far back as the late 1700s in North America, medical schools required specimens. Laws permitted the use of a deceased individual for this purpose if they were a criminal, the body was unclaimed, or the individual had given prior consent — which was rare. This lack of supply generated a market for human cadavers and "resurrectionists" would retrieve the recently buried for profit. This unlawful practice continued for years. In Ontario currently, bequeathals of a body for education and research is regulated by the Trillium Gift of Life Network Act (2002) and the Human Tissue Donation Act. Provided that there is no objection by next-of-kin, an individual can give consent prior to their passing or next-of-kin have authority to consent. The act states that the "use of the body or



Odontology Section - 2015

for the removal and use of the specified part or parts” may be used for therapeutics, medical education, or scientific research. Although there is no age restriction, suitability is based on cause of death, illnesses, recent surgical procedures, and weight. Legally, the anatomy inspector must have documented the name, sex, age, birthplace, and last place of residence, as well as the name of the school and date where the body was delivered.

Anatomical Teaching Specimens, Dental Identification, Postmortem Interference



G40 Identifying the Edentulous

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After attending this presentation, attendees will learn information regarding a new method of identifying the edentulous which may assist in victim identification.

This presentation will impact the forensic science community by introducing a new method of identification based upon the maxillary sinus configuration/morphology assessed from panoramic radiographs.

Identifying the edentulous is challenging in many respects. In part, the absence of teeth critically diminishes the use of thousands of dental reference points used in standard dental identification. The trabecular patterns in the edentulous may also be too few or unique to be used in the identification process.^{1,2}

Results of a previous study suggest that radiographic identification of the edentulous has a high error rate and should be dual reported.³ Forensic practitioners are well aware that dental prostheses should be labeled but, practically speaking, this recommendation is not generally accepted by the dental profession nor is it practiced. Moreover, many edentulous either do not have prostheses, do not wear them, or remove them at night. The present study seeks to produce a reliable source of reference for comparing Antemortem (AM) to Postmortem (PM) panoramic radiographs of the maxillary sinuses.

The technique provides a mathematical assessment for comparing the curves on the maxillary sinus floor and walls of an AM panoramic radiograph to a PM panoramic radiograph. The mathematical formula that will represent the sinus configuration will use strategic points on the sinus. Even the relatively amorphous sinus can be represented by a group of functions that define the different curves of the sinuses. Once the mathematical functions of both AM and PM curves are known using the method presented, and comparing the percentage of similarity of curves and concordant points on both curves, an identification can be established or refuted.

To test the present hypothesis, 50 pairs of panoramic dental radiographs (Orthopantomogram (OPG)) were collected. Each pair were taken at least five years apart using the same panoramic device. The initial OPG dental radiograph was assigned as the AM dental radiograph, and the other OPG dental radiograph taken at least five years later was assigned as the PM dental radiograph. The set of OPG dental radiographs of each patient was calculated and compared using the method presented that evaluates the correlation between AM and PM sinus formulas.

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Forensic Odontology, Edentulous, Maxillary Sinuses



G41 Cougar Attack Fatality

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After attending this presentation, attendees will understand how a cougar attacks, injuries caused by such an attack, a comparison of the dentitions of two different-sized cougars to the patterned injuries on the victim, and dental and oral anatomical comparative anatomy of humans and cougars.

This presentation will impact the forensic science community by providing information on how a cougar attacks, the bitemark-patterned injuries made by cougar teeth, the dental anatomy of cougar teeth, and the postmortem anthropophagy patterns on the victim. A discussion will be included on the failure to initially capture the cougar, causing community concern that a killer cougar was on the loose, which resulted in the killing of additional wildlife, including a second cougar, a bear, and a javelina, as well as causing injury to a horse and its rider.

A cougar attacked and killed a man in a semi-rural area of southwest New Mexico in June 2008. An attempt to kill the cougar that was feeding on the remains failed and the cougar escaped until it was ensnared one week later and dispatched with a bullet. Necropsy was performed on the animal and a comparison of its dentition to the bite injuries was made along with that of a second cougar that was captured and killed. The multiple-patterned injuries to the head and neck and subsequent postmortem anthropophagy feeding patterns were examined and compared to the teeth of a large adult male and a smaller adult female cougar.

Cougars typically attack prey from the rear by surprise, leaping on the back of the prey, clamping down on the back of the neck with its cuspid teeth, and clutching around the animal's rib cage with its massive forepaws, sinking the claws into flesh to ensure there is no escape. This is when the prey falls and the cougar places its focus on the lateral and anterior aspect of the neck, suffocating the prey by crushing the trachea and/or transecting major neck vessels with its powerful bite. A cougar can also break the neck of its prey when it is on the animal's back by forcing the head back with its forepaws while continuing to clamp down on the back of the neck. As in this case, a cougar often carries or drags its kill to a secluded area to feed on the viscera, neck, shoulders or hindquarters, frequently leaving drag marks at kill sites. They frequently try to cover their kills with soil, vegetation, or snow. The viscera may be covered separately and "scrapes" or "scratches" composed of mounds of soil, grass, or leaves are often found around carcasses and trails.

Noteworthy intra-oral trauma and tooth discoloration to both cougars was evident from their ensnarement and struggle to self-extricate from the snares. The excised tongue of the cougar, including its long, keratinized filiform papillae, the deeply furrowed palatal rugae, and the teeth class characteristics will be visually compared to the human mouth and dentition.

Cougar, Attack, Animal Bite



G42 Estimating the Incidence of Dog Bite-Related Injuries in Australia From 1999 to 2012

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After attending this presentation, attendees will understand the extent to which dog bites are a public health and forensic problem in Australia.

This presentation will impact the forensic science community by explaining how injuries due to dog bites are a largely unrecognized and preventable public health problem in countries with a high level of dog ownership. Reviewing the incidence of dog bite-related injuries will provide evidence for the development of improved public policy and prompt further research in forensic analysis of bitemarks.

Background: Previous estimates for Australia, made for the period 2001 to 2003, indicate that 1,800-1,900 persons require hospital treatment each year for bites from dogs and, on average, one or two of them will die from their injuries. The most common victims of dog bites are children between the ages of zero and nine years. Recent data are lacking because no studies have been conducted since to estimate the annual incidence of hospitalization due to dog bites in Australia.

Goal: To estimate the incidence of dog bite-related injuries in Australia from 2003 to 2011.

Methodology: Summary data on hospitalizations due to dog bite-related injuries with a World Health Organization International Statistical Classification of Diseases and Related Health Problems, Tenth Revision, Australian Modification code w54.0 (external cause of injury code) in each state or territory of Australia during the 2003-2011 study period were collated. The data were sourced from the injury surveillance units or health department registry of each state and territory in Australia. Annual and age-specific incidence density were calculated using mid-year population estimates (Australian Bureau of Statistics), trends in annual rates were estimated using Poisson regression, and sampling variation (super populations approach) has been summarized using 95% confidence intervals.

Results: In Australia during the period 2003-2011, there were 21,905 dog bites recorded as having required hospital treatment. They occurred at an annual rate of 11.6 (95% CI 11.5, 11.9) per 100,000. Age-specific rates were 28.6 (95% CI 27.7, 29.6) per 100,000 among ages 0-4 years, 20.6 (95% CI 19.8, 21.4) per 100,000 among ages 5-9 years, 11.6 (95% CI 11.0, 12.2) per 100,000 among ages 10-14 years, and 9.5 (95% CI 9.4, 9.7) per 100,000 among those 15 years of age or older. Overall, the annual rates increased from 10.1 (95% CI 9.8, 10.4) per 100,000 at the start of the period (average 2003-2004) to 14.1 (95% CI 13.8, 14.5) per 100,000 at the end of the period (average 2010-2011 when 6,264 bites occurred). The increase in rates was statistically significant ($p < 0.001$) and occurred at an increasing rate ($p < 0.001$).

Conclusion: A significant increase in hospitalization rates resulting from dog bites occurred during 2003-2011. These more recent estimates indicate that hospital-treated dog bites are now occurring at the rate of 3,132 bites per year. This study establishes the extent of dog bites as a public health and a forensic problem, thereby instigating a thought process to develop advanced forensic techniques to identify unknown perpetrating dogs involved in dog bites.

Dog Bites, Bitemark Analysis, Incidence Rates



G43 Visualization of Histological and Physiological Criteria Used in Dental Methods of Age Assessment

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After attending this presentation, attendees will understand the interest in using, as rationally as possible, the criteria included in these age-assessment methods, especially as each image of comparison can more accurately target the score for each criterion.

This presentation will impact the forensic science community by demonstrating through clinical cases that the estimated age of a victim can vary by a few years depending on the benchmark used to evaluate the criteria. The pictorial catalog of comparison was developed with the contribution of optical microscopy and will make experts aware that it is easier and more accurate to compare a picture with other pictures than a picture with drawings or descriptive texts.

The goal of this work is to develop easier and more efficient applications to estimate the age of an adult victim at the time of death. The study mainly includes the improvement of the interpretation of histological and physiological criteria used in the age assessment methods currently existing. Of concern are six factors: (1) abrasion; (2) periodontal disease; (3) root transparency; (4) secondary dentine; (5) cementum apposition; and, (6) root resorption.

All criteria were studied using the same protocol. The height of periodontitis raises no problem of interpretation because it only depends on a measure of the whole tooth in its socket before extraction, if possible. On the contrary, after extraction and fine cutting of the tooth on its main axis in buccolingual on the mesial and distal sides, the other five criteria are the subject of observations. These latter lead to the choice of a representative image of a score.

Generally, the interpretation advocated by this study of the age assessment methods is based on a macroscopic observation. This study is original as it uses a microscope connected by a Universal Serial Bus (USB) connection to the computer; the display of the image is observed on a screen before its capture. These enlarged views allow easier and more reliable reading of the factors studied. This is especially the case for secondary dentin apposition, cementum apposition, and root resorption where the macroscopic interpretation is often difficult as it is not totally visible.

After having established the picture catalog of comparison of all the factors, the latter is tested by means of teeth extracted from patients of a known age. The evaluation of the imaging is made by calculating the age for the methods of estimation using some or all of the histological and physiological criteria.

Conclusion: To conclude this work, the procedure is applied to an assessment of the age of a real victim. The use of the picture catalog of comparison allows easier and more reliable reading of the factors studied, specifically the cementum apposition and root resorption; however, age assessment is more efficient.

Age Assessment, Optical Microscopy, Postmortem



G44 The London Atlas — A New Method for Dental Age Estimation

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The goal of this presentation is to present a new method of dental age estimation and evaluate how it performs in comparison to other methods.

This presentation will impact the forensic science community by presenting a new method which has an electronic version that will revolutionize dental age estimation.

The purpose of this research was to develop a comprehensive, validated, evidence-based, practical, user-friendly atlas of dental age estimation and compare its performance with two widely used atlases.

Diagrams representing ages from 28 weeks *in utero* to 23 years were developed in The London Atlas based on the radiographic appearance of tooth development in 528 individuals aged 2-23 years and 176 neonates using the median stage of tooth development for each tooth in each age category/chronological year.¹

Accuracy was determined by ageing skeletal remains/radiographs of 1,514 individuals (aged 32 weeks *in utero* to 23 years) using The London Atlas (LA), the Schour and Massler (SM) atlas, and the Ubelaker (Ub) atlas.¹⁻³ Estimated age was compared to real age. Bias, absolute mean difference, and proportion of individuals correctly assigned by age were calculated. Intra-observer variation (Kappa) was measured by re-assessment of 130 radiographs.

To test the application of The London Atlas, a questionnaire was used to validate its use. Ninety third-year dental students were divided randomly into three subgroups; the researcher's identity was unknown to the students. Each group used one of the three atlases to estimate the radiographic age of six individuals and complete the questionnaire.

Excellent reproducibility was observed for all three atlases (Kappa: LA 0.879; SM 0.838; and, Ub 0.857). LA showed no bias ($P=0.720$) and correctly estimated 53% of the cases. SM and Ub showed significant bias by consistently underestimating age ($P=0.026$ and $P=0.002$, respectively) with 35% and 36% correctly estimated for SM and Ub, respectively. The mean absolute difference for LA (0.72 years) was smaller than SM (1.15 years) and Ub (1.17 years).⁴ LA was preferred over the other two atlases in all quality measures tested (clarity, design, simplicity, and self-explanation)

In conclusion, The London Atlas represents a substantial improvement over existing atlases facilitating accurate age estimation from developing teeth. Development of interactive online and mobile app versions is complete.

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Age, Estimation, Dental



G45 Dental Age Estimation: The 18-Year Threshold — A Source of Error Explored

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The goal of this presentation is to demonstrate how the use of simple probability without age stratification can lead to errors in age assignment at the 18-year threshold. Attendees will learn that a subject in the 17.00 to 18.99 age bracket will as likely be assigned an age *under 18* as to be assigned an age *over 18*. A method for overcoming this difficulty will be explored.

This presentation will impact the forensic science community by explaining the rationale for this cause for concern and, with audience participation, by exploring a way of overcoming this rationale.

Introduction: Since the publication of the probability thresholds in the study of third molar development the process of age assessment for emerging adults has been greatly simplified.¹ This seminal paper did not fully take into account the phenomenon of “censoring” the Age at Attainment (AaA) of Stage H.² This has resulted in datasets for Stage H being top heavy. The consequence is that the mean AaA is erroneously elevated. This results in age assignments that are too harsh on subjects in the 17.500 to 18.499 age range. Hence children are unfairly assigned adult status.

Materials and Methods: The DARLInG data base comprising 2,986 UK Caucasian cases was used as the Reference Data Set. The validation set for this study comprised a balanced sample of Dental Panoramic Tomographs (DPTs) of 1,000 females and 1,000 males in the 16.00 to 25.99 age range. For each six month age span a convenience sample of 50 females and 50 males at each six month age interval were examined. These were derived from the radiographic archives of Guy's and St Thomas' National Health Service Trust. Only Dental Panoramic Tomographs with at least one third molar were used.

The stage of development is defined using the anatomical descriptions from 1973.³

The estimated age was compared to the chronological age and the accuracy of the age assignment designated as correct or incorrect to give simple counts. These simple counts were then used to calculate the probability of a subject of unknown age being correctly assigned to childhood or adulthood.

Results:

Age Band	<18 Correct		<18 Incorrect		>18 Correct		>18 Incorrect	
	n	%age	N	%age	n	%age	N	%age
17.5 to 17.99 f	35	70%	15	30%				
17.5 to 17.99 m	41	82%	9	18%				
18.00 to 18.499 f					24	48%	26	52%
18.00 to 18.499 m					25	50%	25	50%

Conclusion: There is a 50% risk (0.5 probability) that a child will be assigned adult status. This, ethically is unacceptable.

References:

- Mincer HH, Harris EH, Berryman HE, The ABFO study of third molar development and its use as an estimator of chronological age. *J For Sci.* 1993; 38(2): 379-390.
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Stage H Errors, Age at Attainment (AaA), Probability Values at 18



Odontology Section - 2015

G46 Immigration-Related, Dental Age-Estimation Cases for the Del Rio and Laredo Sectors of the United States-Mexico Border in Texas

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After attending this presentation, attendees will have a better understanding of the complexity of the illegal immigration problem as well as the nature and scope of immigration-related, dental age-estimation procedures performed by a forensic odontologist for a portion of the United States-Mexico border in Texas.

This presentation will impact the forensic science community by raising awareness of one aspect of the complex and continuing problems confronted by multiple entities including, but not limited to, immigration and law enforcement personnel as they attempt to manage various problems associated with illegal border crossings.

Persons who are apprehended by federal, state, or local agencies following illegal border crossings constitute a serious problem. This problem has existed for many years but has changed in recent history because of (or despite) increased efforts by federal and state governments. The United States government has dedicated vast resources into increasing border security over the past decades including adding more than 650 miles of border fences and adding 21,000+ border patrol personnel according to one study. That same study reports that more than \$18 billion was spent on border enforcement in 2012.

A report from the Council of Foreign Relations includes information indicating that the best outside estimate is that the United States government's apprehension rate has increased in recent years because of increased border security. Others report that the problems with the United States economy have more to do with the decrease in illegal border crossings. Most studies estimate that the government now intercedes in about half of all illegal border crossings from Mexico. Most also agree that the incentive for illegal immigration is not going to disappear in the foreseeable future.

The Border Security, Economic Opportunity, and Immigration Modernization Act (S.744), if signed into law, will establish a 90% "effectiveness rate" as a goal for each of nine separate sectors of the southern United States border.

The effectiveness rate is the percentage calculated by dividing the number of apprehensions and "turn backs" in a given sector during a fiscal year by the total number of illegal entries in that sector. The nine sectors from west to east are San Diego, El Centro, Yuma, Tucson, El Paso, Big Bend, Del Rio, Laredo, and Rio Grande Valley. This presentation includes discussion of dental age estimation cases for the Del Rio and Laredo Sectors only.

The management of illegal immigrants in all sectors includes the necessity of discriminating between adult and juvenile detainees. Juveniles and adults are detained separately and the juvenile facilities are perceived to be preferable to the adult. Consequently, detainees of all ages often claim to be "under 18." Eighteen is the current age of majority in all four states bordering Mexico. Absent definitive proof of actual chronological age, immigration agencies sometimes request age estimation services from forensic odontologists to help them differentiate likely adults from likely juveniles. The recent increase in illegal immigration levels of younger juveniles both accompanied and unaccompanied has generated some activity in the dental estimation of age for younger juveniles.

This presentation will describe the history, nature, and scope of the immigration-related age estimation activities of one forensic odontologist from 1998 through 2014 for two sectors of the United States-Mexico border in Texas.

Age Estimation, Odontology, Immigration



G47 Dental Age Estimation: Appropriate Censoring of Stage H

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The goal of this presentation is to draw attention to a widespread error in the process of age estimation at the 18-year threshold.

This presentation will impact the forensic science community by showing attendees how to appropriately censor Stage H.

Attendees will learn how to censor Stage H of 3rd molars and will become aware of the errors resulting from no-censoring of Stage H of third molars. Attendees will also learn that failure to censor stage H leads to unrealistic overestimates of the mean Age at Attainment (AaA) for Stage H of 3rd Molars.

Introduction: Since the publication of the probability thresholds in the American Board of Forensic Odontology study of third molar development, the process of age assessment for emerging adults has been simplified.¹ This seminal paper did not take account of “censoring” the AaA of Stage H.² This has resulted in datasets for Stage H being top-heavy. The consequence of this is that the mean AaA is erroneously elevated.

Materials and Methods: A review of the literature revealed 14 studies that used the eight Tooth Development Stages described in 1973.² The mean AaA designated as x-tds and the Standard Deviation designated as sd-tds for stage H were extracted from the data.

Uncensored and Censored* Data for Stage H — males						
Year	Ethnicity	x-tds (years)	sd — tds (years)	Minus 3 sd (years)	Plus 3 sd (years)	Range (years)
2003	Spanish	19.45	1.15	16.00	22.90	6.90
2005	Spanish	19.74	1.09	16.47	23.01	6.54
2014	United Kingdom Caucasian	19.98	2.09	13.71	26.25	12.54
2008	Italy Caucasian	20.02	1.46	15.64	24.40	8.76
2002	Hispanics United States	20.10	2.60	12.30	27.90	15.60
2007	Turkish	20.10	1.80	14.70	25.50	10.80
2009	Korean	21.10	1.20	17.50	24.70	7.20
2004	Spanish	21.86	2.47	14.45	29.27	14.82
2012	Northeast Malaysian	22.37	1.98	16.43	28.31	11.88
2004	German Caucasian	22.50	1.70	17.40	27.60	10.20
2004	Japanese	22.50	1.80	17.10	27.90	10.80
2004	South African	22.60	1.90	16.19	28.30	12.11
2010	South China (Han)	22.72	2.27	15.91	29.53	13.62
2010	First Nations (Canada)	23.20	2.80	14.80	31.60	16.80

The Table is ordered by the AaA (x-tds), smallest to largest. It is clear that some of the AaA values are far too high. The assessment is that this cut-off starts at 21 years — the Korean data.



Odontology Section - 2015

From this AaA value, there are increasing AaA which reaches 23.20 years for the First Nations of Canada. The consequences of these elevated AaA values for the First Nations study can be seen in the Minus 3sd and Plus 3sd columns where, for example, the lowest value is 14.80 years and the highest value is 31.60 years. This is clearly not tenable. This problem can be overcome by censoring the data.³ There are three possible methods for this: (1) sort the ages for TDSs with the accompanying TDS so when stage G no longer appears, the censor point can be identified; (2) create a small graph with the Cumulative Probability — this enables identification of the censor point; and, (3) create a data set censored at the upper stage by the largest value of Stage G — beyond that point all the ages are for Stage H only. The data is delimited to +/- 3sd. This is achieved by using a simple calculation within a worksheet and then to extract only the data that lies between these lower and upper values with a spreadsheet with calculated values, viz.: =IF(AND(A5>=D\$5,A5<=D\$6),A5,""). This will limit the data set to +/- 3 sd values. This incorporates 99.7% of the population data and censors the data appropriately. This results in realistic values for the AaA of Stage H.

References:

1. Mincer HH, Harris EH, Berryman HE, The ABFO study of third molar development and its use as an estimator of chronological age. *J For Sci.* 1993; 38(2): 379-390.
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Age Estimation, Censoring Stage H, Age at Attainment (AaA)



G48 Unique Dental Morphology With Concurrent Dental Developmental Anomalies: A Case Study Demonstrating the Utility of a Multidisciplinary Approach

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After attending this presentation, attendees will understand how the medical examiner, the forensic odontologist, and the forensic anthropologist collaborate to analyze a case involving a human calvarium.

This presentation will impact the forensic science community by presenting a case with unique dental morphology and by demonstrating the utility of dental radiography beyond antemortem/postmortem comparisons.

A human skull was discovered when a man was clearing some brush behind trash bins in southwest Florida. The medical examiner assumed jurisdiction of the skull and requested that the forensic odontologist conduct a postmortem dental examination in the absence of antemortem records. The medical examiner also requested that a forensic anthropologist perform an osteological examination to establish a biological profile and record individualizing characteristics that might be useful for personal identification.

Following guidelines and standards established by the American Board of Forensic Odontology, the initial dental examination included dental charting, digital photographs, radiographs, impressions, and a written report.¹ Oral examination revealed significant occlusal wear and the external morphology of the maxillary incisors exhibited shoveling, double shoveling, and lingual pits. Radiographs demonstrated that the maxillary right third molar had closed apices and multiple teeth exhibited developmental anomalies.

The anthropological examination performed at the C.A. Pound Human Identification Laboratory included both non-metric and FORDISC® metric analysis.² The remains were classified as an adult, Native American male. This finding and the taphonomic appearance of the remains suggested that the calvarium was that of a prehistoric Native American and, thus, of no medicolegal significance.

Teeth are useful in anthropologic research because of their preservability, observability, variability, and heritability.³ These traits are also the reasons why teeth are valuable in forensic dental cases; however, anthropologists and odontologists tend to view teeth from different perspectives. Anthropologists focus intently on the surface details of teeth while odontologists look inside the teeth with radiographs. The presence of shoveling, double shoveling, and interruption grooves in the incisors are morphologic patterns present in this case which have been documented by anthropologists to occur at higher frequencies among those of Native American ancestry. Dental radiographs documented that the maxillary right third molar was a taurodont and the maxillary incisors exhibited dens invaginitis with distinctly bifurcated anterior pulp chambers. The use of cone beam computed tomography enabled 3D visualization of the teeth. To determine whether the dental morphology and anomalies in this case represent rare incidental findings or whether there is a correlation between the observed dental morphology and anomalies will require additional scientific research.

This case demonstrates how, under the jurisdiction of the medical examiner, the forensic odontologist and the forensic anthropologist are able to contribute to the disposition of a forensic case. Additionally, this case presentation demonstrates how forensic odontologists use dental radiography beyond forensic identification.

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Forensic Odontologist, Forensic Anthropologist, Dental Radiography



Odontology Section - 2015

G49 A New 12-Month, University-Based United States Fellowship Program in Forensic Odontology

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After attending this presentation, attendees will better understand traditional forensic odontological education in the United States and how a new full-time odontology fellowship will offer a different model of training as related to curriculum design, student assessment techniques, and alignment/collaborations with other forensic-related institutions and agencies.

This presentation will impact the forensic science community by enhancing attendees' abilities to critique and develop unique and evolving educational programs in forensic odontology.

Currently, obtaining training and required qualifications in forensic odontology in the United States is gained through a relatively few program opportunities, none of which are full-time/continual in nature. During the 1970s, acquiring odontology skill sets was basically limited to two options: (1) identifying an experienced, credentialed, and willing forensic odontologist to serve as a mentor; or, (2) participation in the week-long forensic odontology course sponsored by the Armed Forces Institute of Pathology (AFIP).

The mentorship model was often hit or miss in that locating such a willing *and* experienced individual was often difficult. Also, most forensic organizations now require that applicants for full membership and/or credentialing submit a more formal and organized curriculum conducted by a recognized and credentialed faculty. The weight or validity of the training course is also increased based on the topics and the amount of hands-on experiences conducted by the faculty.

Unfortunately, the AFIP course is currently unavailable due to governmental budget restraints and has an uncertain future. The loss of this traditional opportunity has created concern among many regarding the long-term need to create a cadre of highly-trained and competent forensic odontologists for the future.

Recently, there has been modest growth in training opportunities through the development of other excellent forensic odontology courses, though many are held biennially. These are typically affiliated with a medical examiner's office or university-sponsored programs which confer credibility. These programs range from three to six continual days to a robust "fellowship" program consisting of eight to nine onsite sessions lasting three to four days over a 22-month period. These programs are continuously evaluated by the Odontology Section of the American Academy of Forensic Sciences to judge each course's value in achieving "full membership" status in the Academy.

Recently, an advisory committee was formed in the Department of General Dentistry at the University of Tennessee Medical Center to explore the possibility of creating a new forensic odontology training program and graduate curricula. The committee included the Chair of the Department of General Dentistry, two board-certified forensic odontologists, two board-certified forensic anthropologists, the Dean of the University of Tennessee Medical Center's Graduate School of Medicine, and the Chief Medical Examiner of the Knox County Regional Forensic Center.

Consequently, a new program in forensic odontology has been developed and includes all of the forensic scientists mentioned above as faculty. It is based within the newly created Division of Forensic Odontology/Human Identification in the Department of General Dentistry at the University of Tennessee Medical Center's Graduate School of Medicine. The program duration is 9 to 12 months of continual graduate training involving case-based didactic lectures, demonstrations and hands-on exercises in the areas of dental identification, disaster victim identification protocol, bitemark analysis, human age/sex/ancestry estimation, and jurisprudence. A significant amount of the practical component of the residents' experience involves real-time casework through a collaborative effort with the aforementioned Forensic Center which serves approximately 20 counties in East Tennessee. A cooperative research alliance has also been created with the University of Tennessee's National Forensic Academy's Outdoor Research Facility in Oak Ridge, TN.

The Fellows' foundational knowledge and specific skillsets will be continually assessed through literature review presentations, specific procedural competencies, high stakes hands-on examinations, and a continuing portfolio of the residents' actual forensic casework. A project of original odontological research with national forensic meeting presentation and eventual publication will complete the training.

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Odontology Section - 2015

The first Fellow matriculated on August 1, 2014.

Forensic Odontology, Odontology Training, Case-Based Training



G50 A Forensic Odontology Aspect of Disaster Victim Identification Across Members of the International Organization for Forensic Odonto-Stomatology

Shirley Miranda, MScD*, 3302 Gaston Avenue, #711, Public Health Sciences, Dallas, TX 75246

The goal of this presentation is to present protocols from members of the International Organization of Forensic Odonto-Stomatology (IOFOS) that will aid in the sharing and decipherment of new technologies, management of unique cases, improving methodologies, and assisting fellow nations in disaster management.

This presentation will impact the forensic science community by promoting efficient and reliable guidelines in the identification of human remains in order to achieve concise and effective results.

Discussion: When it comes to the forensic odontology aspect of Disaster Victim Identification (DVI), each country operates differently as per the political climate or individual background/organization. It is a well-established fact that the Interpol Standing Committee on DVI includes forensic odontology as part of their guidelines. Forensic odontologists play a key role in DVI. Interpol provides designated forms and protocols to aid odontologists as part of their DVI manual.¹ This results in quality assurance as recommended by the IOFOS.

Besides evidence collection, examination and recording, interpretation, reporting, and presentation, protocols are one of the key tasks of forensic odontologists involved during a DVI when tsunamis, plane crashes, terrorist attacks, etc. occur.²

A study of protocols from members of the IOFOS will aid in the sharing and decipherment of new technologies, management of unique cases, improved and revised methodologies, and assisting each other in disaster management. The advantage of sharing protocols would be to promote efficient and reliable guides in identifications of human remains. When a standardized operation procedure has been utilized and maintained, a more concise and effective result can be obtained.

Goal: The goal of this study is to research and evaluate policies, protocols, programs, and an associated documented system of the forensic odontology aspect of DVI from countries who are current members of the IOFOS.

Methods: Currently, the IOFOS consists of 31 member nations. All participating members were emailed with a brief description of the study and a request to share the odontology aspect of the DVI protocol that was currently being used.

Results: The forensic odontology protocols of all the members who willingly shared this information were evaluated for efficiencies and deficiencies that included similarities, differences, and technology among others.

Conclusion: Improving guidelines based on research and recommendations will enable a nation to be self-sufficient as well as help in the aid of other nations during their time of need with the utilization of shared manpower and technology.

References:

1. Sigrid I Kvaal, Collection of post mortem data: DVI protocols and quality assurance, *Forensic Science International* (Impact Factor: 2.31). 06/2006; 159 Suppl 1:S12-4.
2. M Rajshekara, M Tennanta, The Role of the Forensic Odontologist in Disaster Victim Identification: A Brief Review, *Malaysian J of Forensic Sciences* 5(1), 2014, 78-85.

Disaster Victim Identification, IOFOS, Forensic Odontology Protocols



Odontology Section - 2015

G51 Current Trends in Forensic Odontology Research Nearly 30 Years Later

Shirley Miranda, MScD*, 3302 Gaston Avenue, #711, Public Health Sciences, Dallas, TX 75246; and Jeffrey W. Shirah, DMD, 5000 Saddle Drive, Austin, TX 78727

The goal of this presentation is to augment previous forensic research and indicate areas of limitations as well as aid in the development and improvisation of research and training facilities, including those in dental and dental hygiene schools.

This presentation will impact the forensic science community by promoting discussion concerning areas of deficiency in various fields of forensic odontology which may expose the need for more in-depth and advanced research in order for this field to continue to be a respectable, justifiable, and reliable scientific process in the medicolegal system.

Forensic odontology involves a science that is interwoven with the medicolegal system. The American Board of Forensic Odontology (ABFO) by itself conducts annual workshops in dental identification, civil litigation, age estimation, and bitemark analysis.

Deoxyribonucleic Acid (DNA) analysis methods, which are basically DNA profile tests in forensic dentistry that offer a new perspective in human identification, are a current research trend that is gaining widespread recognition. These tests are known to be highly reliable and are well documented as acceptable legal proofs in the judicial system. Bitemark analysis is still a highly controversial topic but newer trends in research could indicate a favorable use of this analysis as a scientific and reliable method.

This research study is based on a similar study done by Katz and Cottone in 1988 where current trends in forensic odontology were researched over a seven-year period (1980-1987).¹

Objective: The goal of this study is to review research presented in forensic odontology from the annual meeting of the American Academy of Forensic Sciences (AAFS) in the past ten years. An update on how forensic odontology research has evolved, including the introduction and efficiency of DNA analysis in the last decade, will be identified and discussed. A study in these areas will augment previous research and indicate areas of limitation as well as aid in the development and improvisation of research and training facilities, including those in dental and dental hygiene schools.

Method: Abstracts from 2004-2014 presented during the annual meetings of the Odontology Section of the AAFS were reviewed to determine the direction of forensic odontology research. The categories were based on the type of presentation which included literature reviews, case reports, newer techniques, research, and education. The topics of the presentation will include mass disaster, bitemarks, human identification, child abuse, legal issues, photography, and other miscellaneous areas.

Conclusion: The premise of this study will be to ensure that areas of deficiency in various fields of forensic odontology may expose the need for more in-depth and advanced research in order for this field to continue to be a respectable, justifiable, and reliable scientific process in the medicolegal system. The information obtained in this study would benefit a range of professionals from forensic odontologists, medical examiners, detectives, profilers, emergency room personnel, and coroners to law enforcement officials and social services.

Reference:

1. Katz JO, Cottone JA., "The present direction of research in forensic odontology," *Journal of Forensic Sciences* 1988 Nov;33(6):1319-27.

Forensic Odontology, Research, AAFS Abstracts



Odontology Section - 2015

G52 Forensic Odontology Science: Applications in the Family of Forensics

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The goal of this presentation is to provide examples describing how the application of forensic dental science can prove useful in a variety of contexts.

This presentation will impact the forensic science community by illustrating that knowledge gained in forensic dental research can reach across disciplines and circumstances.

This presentation consists of a description of a dental standard-of-care case, a sting operation conducted by a private detective, an industrial product investigation, and technique development for a crime lab.

“The Paperclip Dentist”: The State University of New York (SUNY) Laboratory for Forensic Odontology Research (LFOR) was contacted by the Massachusetts District Attorney to assist in a standard-of-care case. Among other questionable practices, a dentist was suspected of placing posts made from paper clips as definitive restorations. The insurance company was billed for final post and core placement. The material used in this tooth was examined. Analysis of the materials recovered with Scanning Electron Microscopy and Energy Dispersive X-Ray Spectroscopy (SEM/EDS) revealed zinc-coated iron and not stainless steel. The analysis aided in arraignment and subsequent conviction of the dentist.

Pet Crematoria Sting Operation: A private detective made an inquiry about how the SUNY laboratory could assist in analysis of material from a sting operation he had conducted. He was investigating the practices of individual cremation of pets and had heard that the SUNY laboratory had performed research on cremated remains.

In order to conduct his investigation, the detective had prepared a number of specimens and submitted them to pet crematoriums. The specimens consisted of mechanical toy cats. All the mechanical parts were removed and the empty cavity stuffed with five pounds of hamburger meat. Thus, all that was given to the crematoria was cloth/artificial fur and hamburger. Several of the crematoriums returned tins containing ashes with material that appeared to be calcined bone. Of course, this would not be possible given the nature of the specimens. Laboratory analysis confirmed the nature of the returned ashes as calcined bone, which brought into question the practice and expense of pet cremation. Some months later, a surprise visit was made to the SUNY laboratory by a New York State licensing investigator to verify the analysis that had been performed. New York state law requires crematoria to be licensed.

Foreign Object in Foodstuff: A sample from a candy-making company was sent to the SUNY laboratory for analysis. The company had received a customer complaint regarding a foreign object in a caramel nut chew. Having constructed a database of physical and chemical properties of dental materials, which has proven useful in resolution of victim identification in a plane crash and other national and international cases, it was no surprise that the database came to the laboratory’s aid in this circumstance. SEM/EDS identified the material as tooth structure with an adhering restoration. The restoration was a composite resin filling that was able to be identified by brand. The foreign material came from the customer who was eating the candy, not from the manufacturer. The analysis and resulting reward will be illustrated.

Retrieval of Hidden Pistol Serial Numbers: Hi-Point polymer pistols are popular street guns; however, the manufacturer of the polymer pistol has acted responsibly in providing hidden serial numbers inside the polymer housing. This was done so that serial numbers could not be readily removed by filing; however, retrieval of the serial numbers poses the problem of destruction of evidence. The question was raised by the Erie County crime laboratory as to whether dental/medical X-ray imaging could resolve the serial numbers non-destructively. A portable dental X-ray generator and digital sensor, together with subsequent image processing, was used to successfully reveal the hidden numbers.

Beyond the usual practice of victim identification by dental methods, whether it is a criminal case, forensic technique development, civil practices, or quality assurance for a business, forensic dental science has provided a basis for resolution of a number of issues and continues to be vital in the family of forensic sciences.

Odontology, SEM/EDS, X-Ray



Odontology Section - 2015

G53 Application of Digital Laboratory and Clinical Dental Evidence

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After attending this presentation, attendees will be informed about a new and increasing source of dental evidence to assist in casework.

This presentation will impact the forensic science community by increasing awareness of antemortem digital data sources as well as by opening discourse on the associated concerns and potential issues.

Advances in technology have rapidly changed the way the dental profession diagnose and treat their patient population. Initially, advances in computer technology were directed toward practice management. In more recent times, these advances have been directed in the diagnostic and restorative disciplines of dental practice. Computer matching software as well as digital radiography have played a significant role in mass disasters and most medical examiners' offices. Now digital restorative dentistry, besides modifying how patients are treated and methods of practice, provides a new and unique application and valuable source of antemortem information.

In situations such as a mass disaster or the discovery of unknown remains, the location of quality antemortem dental records is a critical step in the identification process. Through the years, forensic dentists have been very innovative in their methods of locating useful dental records. When reaching roadblocks through the normal pathway of collecting evidence from dental care providers, other avenues have been followed. These have included non-traditional methods such as medical radiographs of the skull, photographs, insurance company data, and military records. Requirements or mandates for dentists to maintain records vary from state to state. In the United States, the time frame can range from no mandate to ten years. There are regions of Canada that mandate up to 30 years of retention. During the search for records, there are times that dead ends are met and records have been purged with no recovery possible. History has also shown that the disaster itself can destroy the antemortem evidence. This was true in the crash of Arrow Flight 1285 in Gander, Newfoundland, and during hurricane Katrina.

The advances in digital dentistry have added a whole new dimension and data source in antemortem record acquisition. Digital scans of the dentition for prosthetic fabrication, orthodontics, protective mouth guards, and other oral appliances have become commonplace in dental practices and will only increase in time. Digital scans have been used for study models, single crowns, bridges, complete full dentures, implant design and fabrication, and orthodontic applications. This digital data may be stored in the office of the provider or in the dental laboratory's database. Being able to follow leads to connect the dots and locate the data may be frustrating, but in the end may be very rewarding.

These records will provide restorative information along with anatomical variances. Through these records, dentist can acquire 3D dental casts or models as well as high-definition digital photos. 3D printers can fabricate dental casts from the stored data. These records can be useful to compare restorative patterns, tooth position shape and size, and other possible identifiers such as rugae patterns. Besides the anticipated problems associated with locating these records, concerns associated with data storage need to be considered. The potential for data to be purged based on limits of storage capacity is a real issue. These issues will need to be addressed and possibly require legislative action and mandates. Custodial issues as well as ability to access, manage, and utilize the data are other concerns.

Digital Dental Evidence, Technology, Antemortem Records



G54 Failure to Diagnose Oral Cancer Is Not Always Malpractice

Yolanda Nerkowski, BA, Ontario Forensic Pathology Service, 25 Morton Shulman Avenue, Toronto, ON M3M 0B1, CANADA; Taylor L. Gardner, BFSc, Ontario Forensic Pathology Service, 25 Morton Shulman Avenue, Toronto, ON M3M 0B1, CANADA; and Robert E. Wood, DDS, PhD, Princess Margaret Hospital, 610 University Avenue, Toronto, ON M5G 2M9, CANADA*

The goals of this presentation are to: (1) provide the attendee with information as to the reasons and length of delay in oral cancer diagnosis in a major tertiary cancer center, from the standpoint of both the patient and the practitioner; and, (2) relate, by way of a case report, what can go wrong when a cancer is missed at the very earliest stages and how this can lead to erroneous legal conclusions.

This presentation will impact the forensic science community by informing attendees that the incidence of oral cancer is increasing and the general principles of cancer treatment, across all disease sites, is that early diagnosis improves survival rates; however, in order for an oral cancer to be diagnosed, it must “declare itself” by being detectable. Additionally, both the patient and practitioner have responsibilities in the diagnostic process. Practitioners and experts should be aware of reasons for not detecting oral cancers and the regulatory and legal ramifications of not being cautious in assessing cases of apparent misdiagnosis.

A general principle of oral squamous cell cancer management is that early detection results in increased survival and decreased morbidity. Delay to diagnosis occurs in all stages of oral cancer but is more frequently seen in single patients, non-smokers, and those with Stage IV (advanced) disease. With the increase in cases of oropharyngeal cancers related to human papilloma virus rather than tobacco abuse, it is likely that delay in getting these patients diagnosed will continue to be a problem. In a review of an institution’s database containing more than 26,000 oncology patients, delay to diagnosis could be divided into two parts: (1) primary delay, where the patient delays presenting to a healthcare practitioner; and, (2) secondary delay, where this first point of contact does not immediately diagnose the condition.

This present case details a situation in which a general dental practitioner failed to diagnose an odontogenic carcinoma, providing the patient with a diagnosis of aphthous ulceration. The patient did not keep all follow-up appointments and ultimately the condition progressed. Once the condition “declared itself” and became clinically obvious to the general dentist, he took photographs, radiographs, and ensured that the patient was seen immediately by an oral surgeon who equally quickly referred the patient into the provincial cancer system.

The patient ultimately complained to the Dental Regulatory Authority (DRA) and the dentist, without benefit of counsel or expert assistance, pled guilty to “failure to diagnose.” After he realized a long suspension was imminent, he retained a lawyer and an expert. This became problematic for all parties involved.

By reviewing the records of the case, the general community standard in diagnosing the far more common squamous cell cancer of the oral cavity, and undertaking a prospective study of 100 patients, the defense posited that the practitioner had not committed an error in this particular case. At this study’s institution, in the past 30 years more than 14,000 head and neck cancers have been treated in the clinic. Of these, precisely two were odontogenic carcinomas. The prospective study revealed the median time for patient-related delay to diagnosis and practitioner-related delay to diagnosis was approximately 5 weeks and 13 weeks, respectively. Furthermore, the patient, who later became a patient (and one of the two cases with this condition at this study’s institution), had successful management that was identical to the treatment the patient would have received had there been a prompt diagnosis weeks earlier. In the interim, the general dentist, presumably assuming he was not being diligent, had started taking biopsies of many variants of normal — unwittingly alarming a large number of otherwise well people.

Many issues came to bear in the defense of this individual including factual errors the defendant had previously agreed were true that were later found to be untrue and errors uncovered by the defense team. Despite the prior plea arrangement, the DRA, whose conduct was very reasonable throughout the process, recognized the importance of these new issues as well as information brought to the table by the defense and agreed to a grossly diminished penalty that was accepted by the dentist.

While failure to diagnose is not encouraged, it is important that experts realize that cancers must be of a certain size and exhibit clinical features that sufficiently raise the index of suspicion of the treating person. Additionally, it is prudent to retain competent legal advice and obtain the input of an expert in the field prior to entering into plea arrangements with DRAs or anyone else.

Oral Cancer, Misdiagnosis, Malpractice



Odontology Section - 2015

G55 Forensic Odontology (FOd) in the Canadian Armed Forces: FOd Activities Historical Review and Structure

Genevieve D.S. Bussiere, DMD, 2513 Sparrow Court, NW, Edmonton, AB T5S 0G7, CANADA*

After attending this presentation, attendees will understand, from a military viewpoint, the historical Forensic Odontology Disaster Victim Identification (FOd DVI) operations as viewed and experienced by military forensic dentists and auxiliary staff. These experiences include both domestic and international deployments. This presentation will present the challenges encountered and techniques used to overcome these challenges and will also present an overview of the Canadian Armed Forces Royal Canadian Dental Corps' FOd standards, training, and response capacity in the context of the Geneva Convention, North Atlantic Treaty Organization Standardization Agreement (NATO STANAG) 2464, and American, British, Canadian, and Australian (ABCA) requirements. The organization and evolution of the Canadian Forces Forensic Odontology Response Team (CF FORT) will be outlined and discussed.

This presentation will impact the forensic science community by increasing its situational awareness of the Canadian Armed Forces' (CAF) FOd capabilities and contributions; CAF lessons learned may influence future DVI responses.

This presentation will be an information overview of the Canadian Armed Forces (CAF) Royal Canadian Dental Corps' FOd standards, training, and response capacity in the context of the Geneva Conventions, NATO STANAG 2464, and ABCA requirements. The organization and evolution of the CF FORT will be outlined and discussed. This presentation will explain the origins of the team's composition and establishment and the different elements that are taken into consideration in preparing the team members to respond to the unexpected, maintaining a 48-hour notice-to-move readiness level. Finally, partnerships and supporting professionals within the military and civilian community will be acknowledged.

Participants will gain a military perspective by being presented with historical FOd DVI operations as are permitted to be shared, viewed, and experienced by Canadian Armed Forces military dentists and auxiliary staff. These experiences include domestic operations in support of provincial coroners/medical examiners working in collaboration with the Royal Canadian Mounted Police (RCMP) (i.e., Swiss Air Flight 111 and First Air Flight 6560) and international deployments and operations (i.e., identification of fallen soldiers in Afghanistan and actual support to international operations in collaboration with the Canadian Department of Foreign Affairs, Trade, and Development and the RCMP). Discussion will touch on the uniqueness of each response from the coordinated adaptation, including their analysis of the working environment, political expectations, response delay afforded, and the expected team composition and restrictions despite recognized standards.

This presentation will present the challenges encountered during each FOd involvement and techniques used to address/overcome them. Solutions found to address issues such as dealing with policy expectations, the requirements for transport and maintenance of equipment, working conditions, credentialing, dental documentation-evidence handling, responders' readiness level, training, physical and mental health well-being, communication, safety, and the documentation of lessons learned will also be discussed.

The contribution and challenges faced by CF FORT to the ongoing efforts put forth by the Canadian Armed Forces' Department of History and Heritage with the identification of recently recovered remains of our fallen World War I, World War II, and Korean War military members will also be discussed.

This presentation will give an overview of future considerations with an emphasis on the need for continued military and civilian community collaboration in standing together, ready to provide professional and timely support to DVI operations in all environments.

Canadian Armed Forces, Forensic Odontology, CF FORT



Odontology Section - 2015

G56 Odontology Support for Humanitarian Events

James P. Fancher, DDS, PhD, 345 Blue Lane, PO Box 682, Martindale, TX 78655*

After attending this presentation, attendees will understand the role of the odontologist in supporting humanitarian events.

This presentation will impact the forensic science community by providing an analysis of the results of postmortem dental examinations for undocumented border crosser deaths in South Texas. The role of the odontologist as a team member with forensic anthropologists will be emphasized.

The traditional role of the odontologist in mass disaster and humanitarian events is to provide identifications of deceased individuals by comparing antemortem and postmortem dental records. In many humanitarian events, such as natural disasters and cases of genocide, standard postmortem dental records can be completed on recovered human remains, but antemortem dental records often either do not exist or are difficult to locate and collect. In spite of impediments to traditional identification methods, the odontologist can provide critical information that will help narrow the search among a group of missing and unidentified individuals or will possibly lead to positive identifications.

Documentation of individual dental traits, age estimation, dental disease status, restorative characteristics, cultural accoutrements, and oral/facial trauma can contribute to building a biological and cultural profile that will help narrow the search for an identity. The odontologist may help provide enough evidence to narrow the search for an identity, exclude a potential match from existing data bases, or complete a positive identification. Additionally, DNA evidence for identification may also be provided by sampling an entire tooth or its pulpal contents. The trained odontologist is the logical choice to select teeth and perform pulp extirpations for DNA analysis.

Cases from Operation Identification (OpID) are used to illustrate the role of the odontologist in work that focuses on identifying undocumented border crosser deaths in the Rio Grande Valley of South Texas. These cases are the complete set of cases examined at one forensic anthropology center (Texas State University). Nearly all cases examined have full or nearly full dentitions. Existing dental treatment in 28% of the cases suggest that dental records may exist. Dental age estimations have been completed for 38% of the cases, with all the cases in good agreement with other anthropology age estimation techniques. Cultural characteristics of anterior restorations have been recorded that contribute to the profile of 14% of the cases. Untreated caries and periodontitis affect the majority of cases in highly variable degrees. Antemortem facial traumas, including fractured nasal structures and other facial bones, have also been recorded.

In conclusion, this analysis of dental data from a group of unidentified remains shows that the odontologist has had an important role in building the biological and cultural profile of sample cases from OpID. All work has been completed with the odontologist being a member of a forensic anthropology team. Even though definitive identifications by dental methods may be few, the detailed dental profile is a positive contribution to the work of the identification team. The dental profiles have helped narrow the search among missing individuals and have excluded potential matches from the National Missing and Unidentified Persons System database. The traditional methods of identification may need to be redefined to include the contributions of methods that refine searches.

Odontology, Humanitarian Identification, Operation Identification



G57 Restoring Smiles for Victims of Domestic Violence

Helena Soomer Lincoln, DDS, PhD, 714 W 20th Avenue, Spokane, WA 99203*

After attending this presentation, attendees will understand the effectiveness by which victims of domestic violence can recover their smiles.

This presentation will impact the forensic science community by providing resources to victims of domestic violence on how to restore their smiles in the most effective and economical way possible.

This presentation demonstrates an immediate solution to victims' problems when teeth are broken and lost as a result of domestic violence or assault. Assault victims frequently present with facial injuries, including trauma to the oral cavity and teeth. Loss of teeth results in not only physical and emotional trauma, but loss of integrity and confidence as well. Confidence is closely tied to the ability to speak and smile, both of which are severely affected by damage and loss of teeth.

Initial patient examination includes review of chief complaint, history of present illness, and imaging studies (panoramic X-ray, clinical photos, etc.), followed by physical examination and establishing diagnosis. First aid is given if necessary.

The following is a description of how effectively dentists and on-site laboratory technicians can work together in restoring the smile of an assault victim in a matter of few hours.

Alginate impressions are taken, poured up, and bite registration is completed. If broken, unrestorable teeth are present, these are ground off of the model, and a clear surgical stent is fabricated, then set aside for surgery. The models are trimmed and articulated with bite verification while the patient is in the chair. The mold and shade of new denture teeth are selected chair-side for the patient. These newly selected denture teeth are now set in wax on the models and wax try-in completed verifying proper esthetics, phonetics, and function with the patient. After the patient and dentist approve the setup of the teeth, the waxed denture borders are warmed up in preparation for border molding. A final impression is then made of the border-molded, waxed denture. Next, the impression is poured up and the dentures are immediately flasked and processed with a hydrocolloid processing technique, then trimmed and polished for delivery.

At the same time, the patient is locally anesthetized with 2% lidocaine and 1:100,000 epinephrine using a painless injection technique for extractions of broken and unrestorable teeth, as well as an alveoloplasty procedure or any other required preprosthetic surgery; unrestorable teeth are then extracted. A surgical stent is used during alveoloplasty, sutures are placed, and dentures are placed immediately. Patients leave smiling, often crying from happiness and excitement, and with regained confidence!

The technique described here can be used as a removable partial denture, implant retained partial denture, or full denture fabrication. If dental trauma is less severe and teeth are restorable, operative dentistry or fixed prosthodontics should be used. The key is to provide an immediate solution for the victim in order to restore their confidence and their smile!

Violence, Abuse, Smile



G58 The Implications of a Guilty Verdict for an Innocent Defendant

John P. Demas, DDS, 8814 Fort Hamilton Parkway, Brooklyn, NY 11209*

After attending this presentation, attendees will better understand the difficulties facing an innocent defendant in establishing innocence after having been deemed guilty.

This presentation will impact the forensic science community by explaining how a guilty verdict changes the dynamic for a defendant and how difficult it is to reverse a wrongful conviction.

Under the United States criminal justice system, there is (in theory) a presumption of innocence of the accused. What citizens have been told is that they are innocent of the charge until proven guilty. If it has been determined that a case should proceed to trial, the prosecutor is tasked with proving guilt beyond a reasonable doubt; however, the duty of the prosecutor is to seek justice, not merely to convict. It is not (or at least it should not be) incumbent upon a person to prove his or her innocence.

The American legal system, based originally on English common law, is an adversarial system. As such, the parties to the case, the state, and the defendant rely upon their respective advocates, the prosecutor and the defense attorney, to put forth their case. The presentation of evidence is governed by sets of procedural rules as interpreted by the trial judge and some evidence may be proscribed either because of its provenance or because of procedure.

Should a jury return a guilty verdict for a defendant who is, in fact, innocent of the crime with which they have been charged, the path to the truth may be so encumbered that justice is never served. A guilty verdict changes the dynamic for a defendant who is now, in the eyes of the law, the guilty party. Reversing a wrongful conviction is a slow, difficult exercise.

The process is not a simple presentation of facts or recitation of testimony which will result in the exoneration of the innocent. In fact, exculpatory evidence may never be presented at trial and attempts to correct a wrong and present such evidence before the trier of fact might be opposed and thwarted at every turn, post conviction. Additionally, some members of the forensic community may believe that should they, as experts, be presented with additional facts or information regarding a case in which they have testified and should such facts cause them to change their opinion, that they can simply present such revised opinion to the bench, it will be admitted, and justice will be served. It is not so.

When seeking post-conviction relief on the grounds of either manifest injustice or absolute innocence, the burden of proof is now tasked to the convicted individual. He or she must argue that a due process violation occurred or, in states which allow post-trial relief on the basis of newly discovered evidence, that said evidence “makes a different result probable on retrial.”¹ Without considerable resources on the other side of the prison walls (in the form of finances and earnest and dogged legal representation), it is not unreasonable to suggest that this task might well be beyond the means of most convicted individuals.

Reference:

1. Fabricant, C., Innocence Project, Inc., Memo on litigating post conviction cases.

Guilty Verdict, Wrongful Conviction, Post-Conviction Relief



H1 An Autopsy Report: Death Secondary to a Widely Disseminated Invasive Scopulariopsis Infection

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After attending this presentation, attendees will be aware of this clinically rare fungus causing disseminated infection and death in an immunocompromised host. Attendees will know that apart from culture-based methods and morphology, there are additional molecular-based methods that are able to provide more exact identification of offending organisms. This presentation also provides an opportunity to add to the body of literature relating to this specific species.

This presentation will impact the forensic science community, in particular the general pathology community, by alerting them to the various options available to them for organism (in this case fungal) identification in cases of infectious death.

Introduction: *Microascus* species and their *Scopulariopsis* anamorphs are fungi common in the environment, but rarely associated with invasive disease. This report describes a case of disseminated infection caused by *Microascus cirrosus* and compares this case with those previously reported in the literature.

Case Report: A 46-year old woman developed graft versus host disease of the gastrointestinal tract and multiple episodes of bacterial sepsis following both autologous and allogeneic stem cell transplants for non-Hodgkin's lymphoma. The patient died after rapid onset respiratory failure. Postmortem examination showed multiple white-tan deposits associated with surrounding hemorrhage and tissue necrosis in the heart, right lung, bilateral kidneys, thyroid, lymph nodes, and brain. The histologic correlate to these gross findings was an invasive infection by a fungus with septate hyphae and associated with abscess formation and angioinvasion. Postmortem cultures from right lung tissues revealed a darkly pigmented *Scopulariopsis* species. Subsequent phenotypic analysis of the isolate cultured on cellulose agar demonstrated the characteristic ascocarp and ascospore morphology typical of *M. cirrosus*. Identification was confirmed by a 100% sequence similarity to the ex type strain of *M. cirrosus* UAMH 9389 using the D1D2 domain of the nuclear large subunit ribosomal DNA gene region.

Results: Thirty-five cases of invasive infection caused by *Microascus* (*Scopulariopsis*) species have been reported in the literature with three caused by *M. cirrosus*. The species in all three cases were identified by observation of the mature ascomata with two of the cases confirmed by sequencing of the LSU D1/D2 gene region. Similar to the present case, one of the prior cases was also characterized by the formation of fungal abscess in the tissues and angioinvasion in another case.

Conclusion: This report expands the conditions associated with invasive *Microascus* (*Scopulariopsis*) infection and provides an opportunity to describe methods that can be useful in species identification.

Scopulariopsis, Microascus, Fungal



Pathology/Biology Section - 2015

H2 Incidence and Distribution of Intracellular Fat in Cardiac Myocytes in Chronic Alcoholics

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The goal of this presentation is to show the results of research describing the incidence of a change that can be seen in the cardiac myocytes of chronic alcoholics: the accumulation of intracellular neutral lipids.

This presentation will impact the forensic science community by suggesting that the presence of intracellular lipids in cardiomyocytes could serve as histologic evidence for a cause of death in chronic alcoholics in the absence of other pathologic findings at autopsy.

Chronic alcohol intake in some patients produces a congestive type of cardiomyopathy. Pathologically, alcoholic cardiomyopathy is manifested as cardiomegaly and biventricular dilatation. Microscopic sections of severely affected hearts show fibrosis. This investigation describes the incidence of intracellular lipids, a change that can be seen even earlier in alcoholic heart disease.

The objective of this research was to determine whether there is a significant increase in lipid accumulation in postmortem samples of myocardium from chronic alcoholics. The case material used in this study was obtained from 26 hearts of patients who were known to have histories of chronic, excessive alcohol consumption. These patients were autopsied at the University of Maryland Medical Center, the Baltimore VA Medical Center, and the Office of the Chief Medical Examiner for the State of Maryland. Hearts obtained from 35 autopsy patients at the University of Maryland Medical Center with no history of alcohol abuse served as the random control population.

Samples were obtained from the myocardium at the time of autopsy from the posterior right ventricle, the left ventricle, and the intraventricular septum. A modified Oil-Red-O stain was used to determine intracellular lipid droplets. The subjective intensity of lipid deposition was graded in terms of an arbitrary scale 0 to 5+ (0=no lipid, 5+=total replacement of the myocyte by lipid). Electron microscopy was performed on selected cases to confirm the light microscopic observations.

Microscopic examination of Oil-Red-O-stained sections of heart muscle showed that 13 of the 26 alcoholic hearts chosen had increased amounts of lipid in the myocardium, predominantly in the right ventricle. In no case was alcoholic cardiomyopathy diagnosed on a clinical basis, although gross examination of the heart in a few cases indicated the possibility of the presence of this disease. In two alcoholic cases, lipid distribution was widespread. In both cases, the cause of death was not clear cut. In the non-alcoholic population, seven of the 35 hearts showed evidence of intracellular lipid deposition. Of these seven patients, six had documented histories of treatment with chemotherapeutic regimens, a known cause of intramyocardial fat. Statistical comparison between the two study populations was calculated by the Chi-square test and gave a significant value of p less than 0.05.

The findings in this study suggest that intracellular lipid deposition may reflect occult alcoholic cardiomyopathy of varying severity, especially in the absence of other known causes of intramyocardial fat, such as prolonged anemia and chemotherapy. The lipid deposition observed in this investigation appears to be a sub-lethal, covert insult to the myocardium which precedes or predisposes to the overt symptoms of alcoholic cardiomyopathy. The finding of lipid-laden cardiac myocytes may also suggest the possibility of sudden/toxic metabolic cardiac death in alcoholics where no other significant or lethal pathologic findings are documented at autopsy.

Cardiac Myocytes, Alcoholic Cardiomyopathy, Alcoholism



H3 Congenital Valsalva Sinus Aneurysm Causing Sudden Unexpected Death in a 20-Year-Old Woman

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After attending this presentation, attendees will understand the importance of considering an acquired or congenital cardiovascular disorder as an etiology in sudden and unexpected death.

This presentation will impact the forensic science community by increasing awareness of the possibility of congenital cardiac disorders presenting with a range of clinical symptoms as the cause of sudden and unexpected death.

This is a rare case in which a young woman with recently diagnosed Acute Myeloid Leukemia (AML) presents with a pericardial effusion and dies suddenly as a result of a cardiac tamponade secondary to a rupture of a congenital right Valsalva sinus aneurysm.

A 20-year-old woman with high-grade Myelodysplastic Syndrome (MDS) was admitted to the hospital due to increasing respiratory distress. Her MDS converted to AML. While being treated with chemotherapy, imaging studies revealed a significant pericardial effusion. Magnetic resonance imaging revealed a massive chronic lobulated pericardial effusion. Cardiac magnetic resonance imaging was performed and revealed a large sinus of Valsalva aneurysm involving the ostium of the right coronary artery. The pericardial effusion occupied one-third of the chest volume and was composed of septations extending from the epicardium to the pericardial wall.

At autopsy, opening of the thoracic cavity revealed a distended pericardial sac containing a 3.0cm thick, yellow-red, encircling layer of partially clotted blood. Both the pericardial sac and epicardial surface of the heart were firmly adhered to the partial clot. The weight of the heart, including the hemopericardium was 1,340gm. Examination of the heart and great vessels revealed a 3.8x2.7x2.5cm partially clotted blood-filled cavity extending 1.0cm below the right coronary artery ostium (the right Valsalva sinus), through the interventricular septum, and into the wall of the left ventricle. This cavity perforated into the right ventricle; however, the thick adherent hemopericardium made it difficult to find the exact perforation defect. This cavity was associated with a tortuous aneurysm which bulged into the right ventricle below the tricuspid valve and into the left ventricle. The right aortic cusp was torn midline from the base. Microscopy of the lesion revealed dense collagen without the aortic elastic layers, as well as erythrocytes, acute inflammatory cells, hemosiderin laden macrophages, and layers of fibrin deposits, consistent with an organizing thrombus and recent hemorrhage.

The majority of natural causes of sudden and unexpected death are due to a cardiovascular disorder. These disorders include arrhythmias, ischemic heart disease, aortic stenosis, pulmonary embolism, and a rupture of a myocardial infarct.^{1,2} Congenital cardiovascular malformations are rare and can cause a rupture that leads to a hemopericardium. One rare lesion is an aneurysm of the sinus of Valsalva.

Aneurysms of the Valsalva sinus can be acquired through trauma, degenerative disease, or endocarditis of the aortic valve.¹⁻³ Congenital aneurysms of the Valsalva sinus are rare, although more common than their acquired counterpart and may be associated with a ventricular septal defect.^{1,3,4} There is a 3:1 male predominance and the individual is usually asymptomatic until adulthood.^{1,4} This aneurysm results from separation of the aortic media and the aortic valve cusp. This separation is due to the variable thickness of the collagen creating discontinuity, which can dilate, expanding into the cardiac septum or chamber.⁴ More than two-thirds of these aneurysms occur in the right aortic sinus, while 25% and 8% involve the posterior and left aortic sinuses, respectively.^{1,4}

Approximately 75% of individuals with a congenital aortic sinus aneurysm experience complications, with rupture of the aneurysm being the major complication.¹ The mean age the aneurysm ruptures is 31 years.^{1,2,4} Aortic sinus ruptures can involve more than one coronary artery. Ruptures may be large and acute or small and progressive, adding to the diversity of the clinical presentation.³⁻⁵ Surgical repair is often curative, as patients usually die within a year if they don't receive treatment.⁴

The range of clinical symptoms makes aneurysms of the Valsalva sinus difficult to detect. Because of this aneurysm's rarity, it often doesn't make the list of differential diagnoses. An individual may not be aware of having this condition, increasing the chances of being engaged in everyday activities when the aneurysm ruptures. The elusive nature of the condition will most likely result in a sudden and unexpected death requiring the examination of a forensic pathologist.



Pathology/Biology Section - 2015

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Valsalva Sinus Aneurysm, Cardiac Tamponade, Hemopericardium



H4 Usability of Immunohistochemistry in Forensic Pathology

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After attending this presentation, attendees will better understand the usability of Immunohistochemistry (IHC) in forensic pathology.

This presentation will impact the forensic science community by providing information concerning how decomposition and the time between death and the autopsy affect the usability of diagnostic IHC in forensic samples.

IHC is an important diagnostic tool in surgical pathology but has limited use in forensic pathology. It is the general opinion that proteins degrade rapidly in devitalized tissues, decreasing the usefulness of IHC. The purpose of this study was to examine how the time since death affects the ability to carry out IHC analyses on forensic tissue samples.

Cases for this study were selected on the basis of the elapsed time from death to autopsy. The cases were allocated to one of the four groups (A to D) defined as: (1) Group A included cases autopsied shortly after death (one to three days) with no macroscopic signs of body decay; (2) Group B cases were autopsied three to seven days after death with signs of body decomposition such as green discoloration of the skin, marbling of the vasculature, swelling, gaseous distention, and skin slippage; (3) Group C cases were autopsied 7-14 days after death with marked swelling, black discoloration of the skin, moist and gas-ridden soft tissue, and some skeletonization and mummification of the bodies; and, (4) Group D were autopsied more than two weeks after death and the bodies typically displayed skeletonization and mummification. All the bodies (groups A-D) were found inside buildings, which in Denmark are normally heated to room temperature.

Formalin-fixed paraffin-embedded tissue samples were collected from 37 bodies and consisted of: (1) 37 samples of liver tissue — group A (10), B (10), C (8), and D (9); (2) 35 samples of lung tissue — group A (10), B (8), C (9), and D (8); (3) 16 samples of brain tissue — group A (10), B (3), and C (3); and, (4) 35 samples of muscle tissue — group A (10), B (7), C (10), and D (8). Tissue Microarrays (TMA) were constructed and IHCs were performed using an automated stainer.

The quality of TMAs and the degree of histologic changes following autolysis were evaluated using light microscopy of hematoxylin-eosin stained sections. The degree of autolysis of lung and liver samples were scored from grade 1 to grade 5 according to the degree of nuclear destruction: normal nucleus (grade 1); karyolysis and pyknosis (grade 2); karyorrhexis (grade 3); no nucleus visible (grade 4); and, no cell visible (grade 5).

The sections were stained with anti-KL1, anti-S100, anti-vimentin, and anti-CD45 antibodies, which are commonly used as a primary antibody panel in cancer diagnostic. The evaluating scoring protocol was positive (moderate or strong nuclear and cytoplasmic staining of more than 90% of cells) and negative (no staining or very weak staining in less than 10% of cells).

A good correlation between the postmortem interval and the degree of histological changes was found in all tissue samples (lung, liver, brain, and skeletal muscle). Strong positive staining of bile duct epithelium using anti-KL1 antibody was found in eight of ten samples (80%) of group A and no samples of group B-D. The staining of glial cells and myelin in brains with anti-S100 antibody was found in ten of ten samples (100%) of group A, three of three samples (100%) of group B, and three of three samples (100%) of group C. The staining of lymphocytes and kupffer cells with anti-CD45 antibodies was found in 36 of 38 (95%) samples of group A, 25 of 28 samples (89%) of group B, 21 of 30 samples (70%) of group C, and 15 of 25 samples (60%) of group D. The staining of endothelium with anti-vimentin antibodies was found in 36 of 40 samples (90%) of group A, 6 of 28 samples (21%) of group B, 1 of 32 samples (3%) of group C, and no positive staining in group D.

The data showed that IHC staining can be used, with some limitations, in forensic tissue samples. The IHC staining of bile duct epithelium in liver with anti-KL1 antibodies was positive in 80% of samples within three days after death, while the staining of lymphocytes with anti-CD45, myelin, and glia with anti-S100 antibodies, and endothelium with anti-vimentin antibodies were positive for an even longer time after death.

Validation, Immunohistochemistry, Tissue Decomposition



Pathology/Biology Section - 2015

H5 Death Due to a Congenital Vascular Anomaly of Pulmonary Hamartoma Type: Malpractice or Tragic Fatality?

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After attending this presentation, attendees will understand more about the relevant role of forensic investigation in medical liability evaluations.

This presentation will impact the forensic science community by serving as a case study in which it was possible to attribute a patient's death during a routine medical procedure to a rare disease rather than to the medical procedure as had been previously thought.

The goal of this study is to comprehensively characterize relevant forensic features, which may become important in resolving forensic cases.

In the legal medicine field, cases that seem straightforward can often hide rarities that can alter the results of the entire investigation. This can sometimes occur during malpractice investigations. In such circumstances, apart from the normal individual variables, the fatal outcome of a medical procedure may be due to a rare disease that is difficult to diagnose and whose evolution may be difficult to predict.

A case is reported of a 49-year-old man who died in a hospital due to a pulmonary hemorrhage during an instrumental examination (bronchoscopy) performed to define a previously identified mass in the right lung. The magistrate required an autopsy to evaluate whether the doctor who had performed the bronchoscopy was liable for malpractice.

Examination of the cadaver revealed a marked anatomical alteration of the right lung, which was much smaller in size and volume than the left lung. The entire parenchyma showed a denser consistency. Opening of the trachea and bronchi revealed the presence of many blood clots bilaterally. Moreover, in the proximal portion of the right bronchus, immediately before the bifurcation, a dense, congested capillary network was evident on the endoluminal surface. In addition, on the external surface between the superior and the medial right bronchi, a mass could be seen, not protruding into the lumen but with a rich vascularization, that was partially fused to the bronchial wall. Accurate macroscopic examination of the right lung (after fixation in formalin) demonstrated a thick tangle of vessels in the cricoid cartilage region, extending distally deep down to the level of the pericardial sac.

Histological examination demonstrated that the mass was not a tumor but a congenital vascular anomaly of the hamartoma type. This is a rare finding that, owing to its intrinsic morphologic-structural characteristics (tortuous vessels content, thin aneurysmatic walls), had lacerated simply in response to the rise in pressure induced by insertion of the bronchoscope in the bronchial tree, causing copious bleeding that led to the patient's death from asphyxia due to internal volume overload.

Before the cause of death had been determined, public opinion had unanimously attributed the death to medical error. A routine practice like bronchoscopy should not cause death; therefore, the doctor must have made a serious mistake. Fortunately, the autopsy not only demonstrated the cause of death but also revealed a rare congenital lung disease.

There is an increasing tendency to blame the doctor for medical failures; if this is not restrained, it will lead to "defensive" medicine, ultimately harming patients, overall.

This presentation will impact the forensic community by underlining the importance of forensic investigations and their correlations with anatomic-pathological findings.

Bronchoscopy, Malpractice, Defensive Medicine



Pathology/Biology Section - 2015

H6 Adult Epiglottitis: A Case Series Review in an Autopsy Population

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After attending this presentation, attendees will be informed of the incidence, presenting complaints, and postmortem and laboratory findings in cases of sudden death due to acute epiglottitis in adulthood. Attendees will also be aware of what the main differential diagnoses are and how to distinguish one from another based on history, autopsy findings, and laboratory studies.

This presentation will impact the forensic science community by bringing awareness to pathologists and forensic pathologists of how this disease has now shifted from being a disease of children to a disease of adults and that it can often present with sudden death. This presentation will hopefully make attendees aware of the characteristic history and presenting complaints in these types of deaths and will guide decisions in ordering specific postmortem ancillary tests in order to make the correct determination regarding the cause of death.

Objective: This study was conducted to gain insight into the incidence of deaths in adults from acute epiglottitis and characterize the population affected, presenting symptoms, gross and microscopic features, and to discuss the main differential diagnoses at autopsy.

Method: A retrospective review was performed on medicolegal autopsy cases within the province of Ontario, Canada, in which the cause of death was given as epiglottitis between the years 2001 and July 2014 inclusive. Demographic features, clinical history, gross and microscopic autopsy findings, and the results of laboratory investigations were described. In total, 11 cases were identified.

Results: The incidence of acute epiglottitis as a cause of death was 1 in every 8,000 cases, or just under one case per year. The mean age of decedents was 50 years with a male predominance. Three were smokers; the most commonly reported concomitant diseases were diabetes mellitus, hypertension, hyperlipidemia, and chronic obstructive pulmonary disease. The most common presenting symptoms were sore throat, dysphagia, and low-grade fever with duration of symptoms ranging from several minutes to three days. All decedents complained of sudden shortness of breath before collapsing, except for one unwitnessed case. Three decedents had initially been seen by a physician and discharged home on antibiotics with a diagnosis of Streptococcal upper respiratory tract infection, only to collapse shortly thereafter. Gross postmortem findings included hyperemia and edema of the epiglottis and aryepiglottic folds with occasional swelling of the glottis and airway narrowing. Microscopy showed vascular congestion, stromal edema, and acute inflammation with a few cases also positive for the presence of stromal hemorrhages, abscess formation, and vasculitis. Five cases had positive blood and/or tissue cultures with a variety of organisms identified; Streptococcus was the predominant genus and in no case was *Haemophilus influenzae* isolated. Results from immunological tests performed in some cases were non-contributory.

Conclusions: Sudden deaths from acute epiglottitis in adults may be more common than previously appreciated. It should be kept in mind by forensic pathologists and hospital pathologists in cases of sudden death in which the presenting symptoms are consistent with an upper respiratory tract infection followed by an episode of acute shortness of breath. This study suggests taking blood and tissue cultures in cases with such a history in addition to serum for immunological testing to assist in differentiating the diagnosis when little more than a swollen epiglottis is identified at autopsy.

Epiglottitis, Adults, Autopsy



H7 Fatal Ice Cream: A Rare Case of Food-Induced Anaphylactic Shock

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After attending this presentation, attendees will understand the importance of a complete methodological forensic approach in fatal cases suspected for anaphylaxis and the relevance of immunoserological investigation to detect IgE-specific response to allergens.

This presentation will impact the forensic science community by emphasizing the rarity of fatal food-induced anaphylaxis and the importance of carrying out an exhaustive immunohistochemical study with anti-tryptase antibodies and of measuring allergen-specific IgE in blood samples from the corpse to indicate sensitivity to certain allergens in order to obtain a reliable postmortem diagnosis of anaphylactic shock.

Food-Induced Anaphylaxis (FIA) is a serious allergic reaction that may rapidly cause death in otherwise healthy individuals. There is no universal agreement on its definition or criteria for diagnosis. According to international recommendation, a food-induced allergy is diagnosed when two or more of the following symptoms occur rapidly and acutely after exposure to a known allergen: acute onset of an illness (minutes to several hours) with involvement of the skin, mucosal tissue, or both (e.g., generalized hives, itching or flushing, swollen lips, tongue, or uvula, rhinorrhea, conjunctivitis); respiratory compromise (e.g., dyspnea, bronchospasm, stridor, hypoxia); and, cardiovascular compromise (e.g., hypotension, collapse). Food-induced allergy is generally sustained by an immunological mechanism which is often IgE-mediated or by alternative immune systems (such as other forms of antibodies, immune complexes, or T-sensitive lymphocytes). The epidemiology of food-induced allergy has been difficult to quantify, with estimates varying widely. Most surveys indicate that food-induced reactions account for 30% to 50% of anaphylaxis cases in North America, Europe, Asia, and Australia, and up to 81% of anaphylaxis cases in children; however, the precise incidence of fatal anaphylaxis cases due to people with food allergies remains unknown. Fatal food anaphylaxis is rare and represents only 0.1% of all cases. Although a wide range of foods have been reported as the cause of FIA, the most commonly implicated foods worldwide are peanuts, tree nuts, milk, eggs, sesame seeds, fish, and shellfish.

This presentation concerns a 16-year-old male who had a medical history of allergic asthma, celiac disease, and known food-induced allergies for fish, fresh milk, peanuts, hazelnuts, walnuts, apples, kiwis, and peaches. He immediately collapsed a few minutes after eating a Danish-style ice-cream sandwich. Advanced life support with intramuscular adrenaline and resuscitation maneuvers were unsuccessful. A complete postmortem examination was performed a few days after death. Gross examination was unremarkable, except for mild cerebral edema and white foam in the main bronchi. Lungs were normal in shape, increased in volume and weight, and exhibited small subplueral petechiae. Histological examination revealed polivisceral stasis, mild cerebral edema, and acute pulmonary edema mixed to acute pulmonary emphysema. Myocardial interstitial edema was also detected. An immunohistochemical technique was used to estimate the mast-cell population using the anti-tryptase antibody as a mast-cell specific marker. A great number of degranulating mast cells with extracellular tryptase-positive material were observed. The identification of positive CD3 (++) and CD8 (+) cells in the duodenal mucosa confirmed the diagnosis of celiac disease. Toxicological analysis on blood specimens was unremarkable. A serum concentration of mast cell tryptase from femoral blood was 41.4ug/l. Research of total and specific IgE for more common food allergens was performed showing high values for kiwi, wheat, peach, shell fish, and gluten. Wheat composition of the ice-cream sandwich was indicated as the cause of food-induced fatal anaphylaxis.

Food-Induced Allergy, Anaphylactic Shock, Allergen-Specific gE



Pathology/Biology Section - 2015

H8 Sudden Intrauterine Death Related to a Fork Bead Cord

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After attending this presentation, attendees will be aware of the possibility of a sudden intrauterine death related to a bead cord abnormality.

This presentation will impact the forensic science community by explaining the rarity of a particular case and the relevance of autopsy and histological findings which resulted in a certain diagnosis of death.

A 29-year-old woman went to the hospital because of the onset of abdominal pain with initial intermittent contractions at 37 +5 weeks of pregnancy. Early clinical-instrumental investigations consisting of a medical appointment and cardiotocographic detection were found to be normal. Taking into consideration her imminent delivery date (a Caesarean was scheduled two days later), the woman, with her doctor's agreement, chose to stay at the hospital. In the afternoon (approximately 15 hours after her emergency room admission), cardiotocographic detection was repeated and confirmed to be normal. At that time, the fetus was alive and did not show any signs of distress. Doctors medicated the woman with a pain reliever and antibiotics in order to avoid the risk of fetal infections (vaginal swab for Group B Strep positive at 36 weeks). After a peaceful night, in the morning the woman stated she had not perceived fetal movements. A new cardiotocographic study demonstrated no fetal heartbeat, which was further confirmed by ultrasound. A lifeless female fetus of regular body development was removed by Caesarean section the same morning, revealing that the fetus was immersed in an amniotic fluid described as "bloody."

The public attorney requested an autopsy and histological examinations in order to exclude any medical responsibility. The main autopsy findings included bloody material in the nasal and oral cavity and the esophagus, the presence of bloody fluid in the stomach, and the almost complete absence of blood in the main vessels and the fetal heart. In the context of a paramarginal umbilical cord, an abnormality of the cord itself consisting of an accessory umbilical vessel that originated in the placenta, the umbilical cord was inserted after 15cm of free passage into the amniotic cavity. This implant is called a "fork." At the point of accessory vessel insertion into the placenta, there was a laceration of the vessel wall. Subsequent histological examination confirmed the presence of a vital laceration into the vessel accessory "fork" wall, corresponding to its placental implantation, with extensive blood infiltration of surrounding tissues. The remainder of the placenta was unremarkable and consistent with the stage of intrauterine development.

The cause of death was hemorrhagic shock resulting from the tearing of the accessory umbilical vessel characterized by the rare anatomical anomaly known as "furcate insertion" (insertion fork) of the umbilical cord. In the literature, such an atypical anatomical variant is among the rarest existing (about 0.1%). The possibility of rupture of this vessel, which occurred in the present case, can be determined by fetal movements, uterine contractions, or the overlap of the two mechanisms. For this reason, the rupture of these vessels occurs more frequently (although extremely rarely) during labor. The vessel rupture results in a rapidly fatal hemorrhage for the fetus. There are no obvious signs detected clinically nor are symptoms experienced by the mother, as blood pours into the amniotic cavity. The anatomical anomaly is usually not diagnosed before birth because specific ultrasound analysis of the conformation of umbilical cord is not performed routinely. Detection may only occur with an evaluation of the insertion and presence of physiological venous and arterial vessels through an dedicated ultrasound or, even better, an amnioscopia. This study notes only one similar case reported in the literature.

Fork Bead Cord, Sudden Intrauterine Death, Bead Cord Malformation



Pathology/Biology Section - 2015

H9 Death as a Consequence of an Intestinal Obstruction Due to an Abnormal Congenital Band in a 4-Year-Old Child

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After attending this presentation, attendees will understand the necessity of a correct autopsy technique when rare pathologies are discovered.

This presentation will impact the forensic science community by demonstrating the relevance of a solid technical skill when atypical findings are present at autopsy.

A 4-year-old child was found dead inside his home without any apparent cause. Questioning of his parents revealed that he had experienced an ill-defined increasing abdominal pain, which had started three days before his death and was associated with a high fever (37.7°C) and vomiting. The abdominal pain was treated with enemas of lukewarm water containing glycerin, paracetamol, and peridon. He had previously been healthy. External examination of the body revealed no injuries.

An autopsy was performed. Upon opening of the abdominal cavity, copious amounts of transudate mixed with blood were revealed totaling approximately 2,000cc. The small bowel loops were markedly distended and, in some sections, purplish-brown in color, suggesting necrosis. Part of the intestine was entrapped and strangulated by a fibrous band connected to the mesentery at four locations. The multiple occlusions resulted in the development of two non-contiguous areas of necrosis, one between the ileum and the ileocecal valve and the other extending from the lower third of the sigmoid colon to include the rectum.

Histological examination of the fibrous band showed connective tissue, with areas of ischemic necrosis at the level of the intestinal mucosa. Toxicological tests were negative. Death was due to intestinal obstruction and infarction caused by a congenital band. The obstruction and infarction resulted in dehydration and circulatory collapse.

An Abnormal Congenital Band (ACB) is among the multiple conditions that may cause an intestinal obstruction. ACBs are extremely rare and often manifest as intestinal obstruction, especially during childhood.^{1,2} The precise incidence of ACBs remain unknown, although studies have reported percentages varying from 1% to 3%.³ The etiology of ACBs are obscure and their locations include known embryogenic remnants, including the vitelline arteries and veins, and the omphalomesenteric ducts.⁴⁻⁶ The omphalomesenteric duct is an embryonic structure connecting the primitive bowel with the vitelline sac that disappears between weeks five and nine of fetal life. Incomplete regression may cause several types of congenital abnormalities, including Meckel diverticulum and entero-cutaneous fistula due to the persistence of the vitelline duct.

ACBs may originate from mesenteric anomalies. On approximately the 28th day of intrauterine life, the dorsal and ventral mesenteries transiently divide the peritoneal cavity into right and left halves; however, the ventral mesentery soon disappears, except around the liver and in front of the stomach. As the intestines assume their final positions, their mesenteries are pressed against the posterior abdominal wall. An ACB may therefore be a remnant of ventral mesenterium that failed to resorb completely.

Four types of congenital peritoneal bands have been described. In type 1, the cecum, which lies abnormally in the right upper quadrant of the abdomen, has a band called Ladd's Band, which extends across the second and third parts of the duodenum to the paravertebral gutter. Duodenal obstruction may therefore result from compression by Ladd's Band and/or from midgut volvulus. Type 2 bands extend from the hepatic flexure of the colon across the second part of duodenum to the right paravertebral gutter, causing duodenal compression at that site. Type 3 bands are hypertrophied hepatoduodenal ligaments, which obstruct the duodenum at the junction of its first and second portions. Type 4 bands are dense fibrous bands that bind the distal portion of the third part of the duodenum to the paravertebral fascia, causing extrinsic obstruction and always being associated with an incompletely rotated duodenum.

The child described in this study demonstrated a rare cause of death from intestinal obstruction due to an ACB. The band observed at autopsy could be traced back to the remnants of the omphalo-mesenteric or vitelline duct, indicating that it was a type 4 band.

In conclusion, clinicians should be aware of this entity as ACB may cause death in children.

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Pathology/Biology Section - 2015

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Anomalous Congenital Band, Autopsy Technique, Natural Death



Pathology/Biology Section - 2015

H10 A Fatality Due to Type I Long QT Syndrome (LQTS) Associated With Electrolyte Abnormalities and Therapeutic Levels of Citalopram

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The goal of this presentation is to review the relationship between Type I LQTS, dysrhythmia, and risk factors in teenagers.

This presentation will impact the forensic science community by presenting a case report of a lethal arrhythmia in Type I LQTS associated with blood levels of citalopram in the therapeutic range.

The acquired or inherited condition known as LQTS, defined by prolongation of the QT interval (the time interval from the start of cardiac ventricular depolarization to the completion of repolarization >480msec), is due to interference with normal myocardial repolarization.¹ It may cause ventricular fibrillation, Torsades des Pointe (TdP), palpitations, syncope, seizures, and sudden death. Presentation occurs at any age but childhood or early adulthood is typical. Inherited LQTS is due to 13 genetic mutations in ionic channel genes (channelopathies), most of which are dominantly inherited. Three genes make up the majority of cases (Types 1, 2, and 3). Type 1 is caused by mutations in the voltage gated potassium channel gene *KCNQ1* and is associated with arrhythmias during exercise or emotional stress. The heart usually appears grossly and microscopically normal in younger patients. Diagnosis depends on the clinical history, electrocardiogram changes, laboratory results, and a review of the patient's medication list. Known risk factors for TdP in these patients are: hypokalemia, hypomagnesemia, hypocalcemia, female gender, age over 60 years, and medications that block the potassium channel function.^{2,3} One such drug is the serotonin selective reuptake inhibitor citalopram; however, the blood levels needed to produce this side effect are debated.^{4,7} Of note, the Food and Drug Administration recently published a warning about citalopram used in higher doses.⁸

This study describes an 18-year-old female who collapsed and was found to be in ventricular fibrillation by paramedics. Just prior to collapsing, she was emotionally upset due to an argument with her boyfriend. After resuscitation, she converted to a sinus rhythm but developed prolonged seizures and expired from anoxic encephalopathy five days later. An admission electrocardiogram showed a corrected QT interval (Bazett's formula) of 648msec and serum chemistries revealed hypokalemia (2.9mEq/L) and hypocalcemia (7.6mg/dL, corrected). The reason for her electrolyte abnormalities was not identified. She had been taking 20-30mg of citalopram per day for depression; there was no history of hearing loss or sudden cardiac death in the family. The autopsy was unremarkable and her heart appeared structurally normal (337 grams). A postmortem blood sample submitted to GeneDx for genetic testing revealed a disease-causing missense mutation in the C-terminal end of the *KCNQ1* (R594Q) gene, a cause of Type 1 LQTS.^{9,10} This R594Q variant occurs within the assembly portion of the *KCNQ1* protein. An admission blood sample analyzed by National Medical Services Laboratories had a citalopram level of 120ng/mL. Reported premortem therapeutic levels are in the 40-100ng/mL range. Reported incidental postmortem ranges for this drug vary but are slightly higher: 400ng/mL, 90-760ng/mL, a median of 300ng/mL, 90-1,300ng/mL.¹¹⁻¹⁵ Lethal levels for this drug are usually much higher.¹⁶ The cause of death was attributed to a cardiac arrhythmia associated with the LQTS and electrolyte abnormalities. The contribution made by citalopram toward the initial dysrhythmia remains speculative but given the FDA warning about citalopram and TdP at higher doses, it is difficult to dismiss its role entirely as some would suggest.^{7,17,18} The case raises the question: Should clinicians screen for LQTS with an electrocardiogram before starting females on citalopram?

In summary, this was a case of inheritable LQTS in a teenager with risk factors for the development of ventricular dysrhythmias, including hypokalemia, emotional stress, and female gender. She was also on a medication previously associated with development of TdP, although typically at higher doses than prescribed. What contribution it made is uncertain. This report adds to the literature on citalopram blood levels in cases associated with the inheritable LQTS.



Pathology/Biology Section - 2015

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Arrhythmia, Long QT Syndrome, Citalopram



H11 **A Case of Lethal Idiopathic Plasmacytic Lymphadenopathy With Polyclonal Hypergammaglobulinemia: A Medical Challenge for the Forensic Pathologist**

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After attending this presentation, attendees will understand that the pathophysiology of Idiopathic Plasmacytic Lymphadenopathy (IPL) is not elucidated sufficiently and further clinical, histological, and immunological studies will be required in the forensic field, especially when medical malpractice is suspected.

This presentation will impact the forensic science community by reporting an uncommon case of lethal IPL with polyclonal hypergammaglobulinemia and renal failure in a young woman in which other differential diagnoses such as collagen, autoimmune, and infectious diseases were ruled out when she was still alive.

IPL was first described by Mori et al. in the early 1980s as a new disease entity resembling the plasma cell type of Castleman's Disease (CD). Both have multicentric lymphadenopathy, prominent polyclonal hypergammaglobulinemia, elevated erythrocyte sedimentation rate, elevated serum interleukin-6 (IL6) concentration, bone marrow plasmacytosis, and various abnormal laboratory data. Nevertheless, CD usually has an aggressive and fatal outcome associated with infectious complications or malignant tumors. IPL has a clonal B-lymphocyte proliferation producing high levels of immunoglobulin and/or their chains which affect the entire body. The clinical features are polymorphic because of the involvement of several organs (skin, and pulmonary, digestive, and renal systems). IPL can be asymptomatic for a long time but can exhibit an unexpected and rapidly fatal course. The treatment of IPL has not yet been established, but the efficacy of corticosteroid treatment, anti-cancer chemotherapy, and monoclonal antibodies has been indicated. Even though IPL is reported in literature, it is rarely recognized clinically and is often first diagnosed after the patient's death as a medical examiner's case for the forensic pathologist, who has to include it in the differential diagnosis of other diseases when medical malpractice is suspected.

This study reports the case of a 40-year-old woman admitted to the emergency room with acute renal failure and hypergammaglobulinemia. She had previously had frequent otitis, pharyngitis, and a poorly defined rheumatic disease. In the last month, she received antibiotics for bronchitis. Multiple myeloma was first suspected. The patient rapidly developed back pain, pericardial effusion, generalized pruritic rash of the face and trunk, splenomegaly (with no liver and lymph node enlargement), and finally coma (after six days). Bone marrow biopsy showed polyclonal plasmacytosis. Multiple myeloma was ruled out as well as infection, collagenopathy, and coagulopathy; therefore, a hypergammopathy due to a proliferative hematological malignancy was suspected. Despite medical efforts, the woman expired nine days after admission. A medicolegal autopsy was performed because of the lack of a diagnosis. The external examination of the body was negative. The autopsy showed brain swelling, pulmonary congestion, pleural adhesions, and hepato-splenomegaly. Histological examination of lymph nodes showed diffuse infiltrates of plasma cell and lymphocytes with features of erythrophagocytosis. Immunohistochemical staining confirmed plasma cells (CD138+) with a prevalence of kappa-positive cells, B (CD20+) cells, and T (CD3+) cells. Kidneys showed advanced glomerulosclerosis containing similar infiltrates. Toxicological analyses were negative. The final cause of death was multiple organ failure due to IPL with polyclonal hypergammaglobulinemia and advanced renal failure. The rapid worsening of the patient's condition did not allow the physicians to make the correct diagnosis. This case was particularly notable for the renal complications resulting from glomerulosclerosis and interstitial infiltration. The exact mechanisms of renal damage in IPL have an unknown etiology. It is supposed that an overproduction of IL6 induces proliferation of mesangial cells and interstitial infiltration of plasma cells. The process can be increased by paracrine or autocrine IL-6. No systematic evaluation of treatment regimens is available because much remains to be discovered about IPL. Possible medical liability associated with failure to diagnose and treat IPL deserves discussion.

IPL, Hypergammaglobulinemia, Autopsy



Pathology/Biology Section - 2015

H12 Sudden Death Due to Drug-Induced Vasculitis

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After attending this presentation, attendees will understand that levamisole-induced vasculitis can affect the heart and lungs and can cause sudden death.

This presentation will impact the forensic science community by emphasizing the seriousness of levamisole vasculitis of the heart and lungs which can result in sudden death.

Introduction: Levamisole is a veterinary antihelminthic drug previously used in humans to treat nephritic syndrome and various autoimmune disorders. In 1999, levamisole was withdrawn for human use by the FDA due to its adverse effects including vasculitis. During the last decade, levamisole appeared as an adulterant in cocaine and various complications are reported in living individuals due to its vasculitis that affected the skin, kidneys, and skeletal muscles.

Case Presentation: A 49-year-old man was discovered dead during a welfare check. He was last spoken to by his mother three days earlier and his only complaint was that he felt cold. He was found at the bottom of the stairs, face down and unresponsive on the basement floor. The deceased was an unemployed laboratory worker who previously worked in a research laboratory and lost his job approximately four years earlier.

Autopsy revealed superficial blunt force trauma of the face and a localized subarachnoid hemorrhage of the right temporal lobe. Otherwise the autopsy gross findings demonstrated no significant evidence of a cause of death. Microscopy revealed eosinophilic vasculitis of the intraparenchymal vessels of the lungs with acute and organizing alveolar injury. The heart showed intramural eosinophilic vasculitis and perivasculitis.

Toxicology Results: Levamisole (2.8mcg /ml) present in the blood; no evidence of cocaine or cocaine metabolite was noted.

Discussion: Vasculitis is a known complication of levamisole use and has been reported to affect the skin, kidneys, and muscles of the lower extremities. Agranulocytosis, neutropenia, and arthralgia are also noted. The only case of death noted in the literature is a case of acute coronary syndrome following the use of levamisole-laced cocaine. In that case, the left anterior descending coronary artery was affected by the arteritis. In the present case, the intramural heart vessels were involved while the coronary arteries were unaffected. The lungs showed vasculitis of the medium-size vessels with predominantly an eosinophilic perivascular infiltrate accompanied by acute and organizing alveolar injury.

Vasculitis, Sudden Death, Levamisole



H13 Natural Death Caused by Catecholamine Toxicity Due to Pheochromocytoma in a Young Adult

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After attending this presentation, attendees will understand the gross and microscopic characteristics of pheochromocytoma as well as laboratory findings associated with catecholamine toxicity.

This presentation will impact the forensic science community by enumerating the findings of pheochromocytoma and catecholamine toxicity and validating the necessity of complete autopsy examinations with histological analysis and ancillary laboratory testing.

A 22-year-old female with a questionable history of drug abuse complained of general malaise as well as a headache. She went to sleep in an attempt to alleviate her symptoms. She was found unresponsive on the floor next to the bed and was not able to be resuscitated. Her medical history is significant for a recently detected adrenal mass, recurrent hypertensive episodes, and elevated plasma and urinary catecholamines and their metabolites. Her family medical history is significant for von Recklinghausen disease (neurofibromatosis type 1).

At autopsy, multiple small cutaneous neurofibromas and café-au-lait spots were observed, supportive of a history of von Recklinghausen disease. The heart exhibited cardiomegaly and concentric left ventricular hypertrophy. Gross inspection of the right adrenal gland demonstrated a 5.5x5.0x3.5cm soft, brown, well-circumscribed mass arising from the medulla. Cut section of the lesion revealed soft, pink-brown tissue with multiple areas of hemorrhage. Microscopic examination of the mass showed large cells (some markedly pleomorphic) with basophilic cytoplasm, round nuclei, and prominent nucleoli in a nested arrangement with a rich intervening vascular network. A special laboratory chemistry request was made from a reference laboratory for measurement of free serum catecholamines. Chemistry of the plasma showed markedly elevated fractionated and free catecholamines (four orders of magnitude higher than the upper limit of normal). The cause of death was determined to be the result of catecholamine toxicity due to a pheochromocytoma of the right adrenal gland.

Pheochromocytoma is a rare neuroendocrine tumor that is seen in about 0.1% to 0.6% of people with hypertension. Estimates show an annual incidence of pheochromocytoma of approximately 1/100,000 people. Of those tumors, only 10% are found to be secretory. Pheochromocytomas generally follow the “10% rule” where approximately 10% of tumors are bilateral, extra-adrenal, show metastasis, and are seen in the pediatric population, respectively. Hereditary pheochromocytomas are seen in syndromes such as multiple endocrine neoplasia, Sturge-Weber, von Hippel Lindau, and von Recklinghausen disease. Familial tumors are more likely to be bilateral, multicentric, and are seen at a younger age.

Grossly, pheochromocytomas range from small to large with the mean of 7cm and 200g. They can be yellow, brown, or red and cut section may show a lobulated appearance, hemorrhage, or necrosis. Microscopically, pheochromocytomas are large cells with abundant basophilic or amphophilic granular cytoplasm with intracytoplasmic hyaline globules. The nuclei are round or oval with prominent nucleoli. There are rare-to-no mitotic figures. The cells can be arranged in a Zellballen, trabecular, or solid form and have a rich vascular network. Metastases are the best criteria for determining malignancy. Other criteria include mitotic figures, abnormal mitotic figures, spindling of tumor cells, necrosis, large nests of cells, high cellularity, and periadrenal adipose tissue invasion. Capsular and vascular invasion can be seen with benign or malignant lesions. Synaptophysin and chromogranin, immunohistochemistry stains are positive in tumor cells. S-100 can be used to stain the sustentacular cells. Hyaline globules within the cell stain positive with periodic acid-Schiff and resist degradation with diastase.

This case illustrates the broad range of diagnoses forensic pathologists must consider in their practice. Vigilance is required to ensure that proper collection techniques are performed for ancillary testing. For this case, a purple top tube of blood was required for the measurement of serum catecholamines, which is not a part of the autopsy standard operating procedure.

Pheochromocytoma, Catecholamine Toxicity, Adrenal Gland



Pathology/Biology Section - 2015

H14 Multiple Thromboses in a Case of Neonatal Dehydration and Failure to Thrive

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After attending this presentation, attendees will be more familiar with the antemortem signs and postmortem findings that may mimic neglect or head trauma in breast-fed infants with hypernatremic dehydration and failure to thrive.

This presentation will impact the forensic science community by explaining the ways in which intracranial hemorrhage and the physical signs of possible dehydration may be mistaken by the prosecuting pathologist for blunt force trauma or child abuse and neglect. Early recognition and monitoring of this condition by medical professionals may prevent the occurrence of avoidable complications of hypernatremic dehydration, including death.

The following is a case of multiple thromboses in an infant with failure to thrive and dehydration. This case demonstrates findings of vascular thromboses in end organs mimicking trauma in an infant.

A 13-day-old White male was found at 2:45 a.m. crying alone in a bassinet and was experiencing difficulty breathing. No pillows or blankets were reported in the bassinet with the infant. After feeding, the mother noted skin color changes and took the infant to the hospital at 4:15 a.m. The emergency department records reported shortness of breath and cyanosis of the hands and feet, lethargy, dehydration, and a two-pound weight loss since birth. The infant was intubated, but normal oxygen saturation was not achieved. He was subsequently transported to a different hospital where he sustained cardiopulmonary arrest at 5:25 a.m. Advanced cardiac life support efforts were initiated and intraosseous access was obtained, followed by normal saline infusion. Resuscitation efforts continued for 45 minutes and the child was pronounced dead at 6:00 a.m. Notification of death was received by the coroner, who sent the body to the medical examiner's office for autopsy.

At autopsy, the infant was markedly thin and demonstrated "tenting" on traction of the skin. Body weight at autopsy was 5lbs, 4oz compared to 7lbs, 15oz at birth. The oral mucosa was dry and the periorbital fat was diminished, giving the eyes a sunken and prominent appearance. Internal examination revealed minimal subcutaneous and visceral fat. Extravasated blood was in the right nephric space. Dissection of the viscera revealed multiple thromboses involving the pulmonary arteries with a wedge infarct of the left lung, the right renal vein with deep congestion of the kidney, and dural sinus thrombosis with hemorrhagic leukomalacia. No trauma was identified on or within the body. Hemorrhage of the brain was non-traumatic, ruling out physical injury to the head. Vitreous humor was inadequate for electrolyte analysis. Postmortem toxicologic examination was negative for drugs and ethanol; postmortem blood cultures were non-contributory.

Findings in this case such as brain hemorrhage and skin tenting suggestive of dehydration may be mistaken for blunt force injury or child abuse/neglect; however, this pattern of severe dehydration, weight loss, apnea, and death cannot simply be attributed to neglect or failure to thrive. Breast-feeding associated hypernatremic dehydration has been described in at least 178 infants in the literature since 1979.¹ Interestingly, like the infant in this case, all of these infants were reportedly born at term without complication or abnormalities.² Complications of this condition may include acute renal failure, elevated liver enzymes, disseminated intravascular coagulation, cerebral edema, intracranial hemorrhage, cavernous sinus thrombosis, and bilateral iliac artery thrombosis.^{1,2}

Inadequate volume of vitreous humor precluded the evaluation of sodium and urea nitrogen concentration; however, the findings in this case show such a striking resemblance to specific reports of hypernatremic dehydration and associated complications of weight loss, cerebral edema, transverse sinus thrombosis, and death in infants around the second week of life that it is the most likely cause of death.^{3,4}

The incidence of neonatal hypernatremia in breast-fed infants has been reported to be as high as 4%; therefore, the need for recognition and monitoring by health professionals is paramount.¹ Early detection and monitoring for hypernatremia may prevent an occurrence of this rare complication; awareness by medical examiners may prevent erroneous assumptions of abuse or neglect.



Pathology/Biology Section - 2015

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Dehydration, Neonate, Thromboses



Pathology/Biology Section - 2015

H15 Sudden Childhood Death Due to Acute Bronchospasm

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The goal of this presentation is to present a case which serves as a rebuttal to the general misconception that bronchial asthma is seldom, if ever, a fatal disease.

This presentation will impact the forensic science community in terms of competence (ability) to include acute bronchial spasm secondary to asthma in the differential diagnosis for sudden natural death in a child who does not have a documented history of this disease. This case is an unusual presentation (sudden unexpected death) of asthma in a child with previously mild respiratory symptoms.

A 7-year-old African American female was sitting on the steps of the shallow end at a community pool when she was witnessed to clutch her chest and fall forward into the water. A lifeguard reportedly pulled her from the water after less than 30 seconds, at which time she was noted to have a pulse and was gasping for air. Cardiopulmonary Resuscitation (CPR) was initiated and the child was observed to vomit a small volume of water following the initial rescue breath. At this point, her pulse had noticeably weakened. Witnesses then described "seizure like" activity for 15-20 seconds after which she became pulseless and cyanotic. CPR was continued by emergency medical services upon arrival. The child was pronounced dead upon arrival at the local hospital. The coroner was contacted and the body was sent to the medical examiner's office for autopsy.

Review of medical records revealed a medical history negative for obstructive airway disease and positive for seasonal allergies only. Gross examination of the body revealed moderately congested lung parenchyma with no other pathologic abnormalities in any other organ system, including the heart. Microscopic findings of the lung showed an exuberant peribronchial/bronchiolar inflammatory infiltrate with a predominance of eosinophils, basement membrane thickening, mucous gland hyperplasia, and smooth muscle hypertrophy. Sections of lung also showed mucous plugging with abundant eosinophils. Given the intensity of bronchial/bronchiolar constrictive findings and eosinophilic infiltrate, the findings and cause of death are consistent with acute bronchospasm due to undiagnosed asthma. Multiple sections of myocardium, including the cardiac conduction system, were also examined and found to be unremarkable.

This death was unexpected due to the relatively mild or asymptomatic nature of her prior clinical picture. Prior to death, the subject did not carry the diagnosis of asthma and it is likely that her asthma was mistaken for seasonal allergy symptoms. This case represents an unusual presentation of asthma and its disease course. Within minutes of being presumably free of symptoms, the subject experienced cardiopulmonary arrest.

Asthma-related deaths usually result from a combination of physiological abnormalities involving respiratory failure with hypoxia, hypercapnia, and acidosis, right ventricular failure secondary to pulmonary hypertension, and/or pulmonary infection.¹ The mechanism for a hyperacute attack includes both bronchospasm and inflammation.² Histopathological findings in fatal asthma have been shown to range from global mucus filling of all airways to moderate involvement to virtually empty airways.¹ Postmortem examinations have revealed mucous plugging in up to 74% of cases.² Asthma mortality rates are particularly high among women, African Americans, and children aged 5-14 years.² This case serves as a rebuttal of the general misconception that bronchial asthma is seldom, if ever, a fatal disease.¹ Individuals suffering from a fulminant form of bronchial asthma are at potential risk for rapid progression from a relatively controlled state to death within minutes.¹ Even patients who are asymptomatic on a daily bases can have a fatal attack.² The possibility of idiosyncratic anaphylactic reaction cannot be ruled out, as patients with bronchial asthma may be more susceptible to anaphylaxis than the general population.¹

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Bronchospasm, Sudden Death, Child



H16 Rapid Unexpected Death in Late Pregnancy Due to Ruptured Iliac Artery Dissection

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The goal of this presentation is to introduce a case report and associated literature review which will provide the attendees with a better understanding of the incidence of arterial dissections during pregnancy and the postpartum period as well as the potential hemodynamic and hormonal causes of such dissections.

This presentation will impact the forensic science community by providing attendees with increased competence in identifying arterial dissections that may cause morbidity and mortality in pregnancy and the postpartum period. This presentation will also caution attendees to look for arterial dissections in cases of unexpected death in pregnancy and the postpartum period.

Arterial dissections associated with pregnancy and the postpartum period have been reported since before the Civil War. The most common sites are the aorta and the cerebral and splenic arteries. The etiology is unclear but likely due to a combination of hemodynamic and hormonal changes. A case of sudden death due to a ruptured iliac dissection in late pregnancy is presented.

A 32-year-old woman in the third trimester of pregnancy presented to the emergency department in hemorrhagic shock. A computerized tomography scan showed a retroperitoneal hemorrhage. While in the operating room, a linear transmural disruption was identified at the right iliac artery bifurcation. The patient expired during the procedure before vascular repair could be performed. An autopsy was performed and confirmed a right iliac artery dissection. Histological sections of the artery showed medial degenerative changes.

The causes and risk factors of pregnancy-associated arterial dissection are unclear. Traditional risk factors for arterial dissection include hypertension, atherosclerosis, pre-existing aneurysm, extreme physical exertion, and trauma. Systemic diseases can also predispose patients to arterial dissections. None of these risk factors were present in this patient. Rather, the majority of women with pregnancy-associated dissections were otherwise healthy, without a history of trauma, and with normal peripartum blood pressures.^{1,2}

It has previously been suggested that the physical pressure and hemodynamic stresses from the gravid uterus may contribute to dissections related to pregnancy.¹ This may be a contributing factor in iliac dissections; however, the majority of dissections occur above the diaphragm (aorta and cerebral arteries) and cannot be fully explained by this hypothesis.

Most dissections occur in the third trimester or the postpartum period and not during labor, suggesting that the stress and exertion associated with labor is not the cause of arterial dissections. In one study, there was no correlation between dissection and the length of the second stage of labor.² One case of aortic dissection occurred in a patient who underwent an elective cesarean section with no active laboring.³ These support the idea that the stresses of labor are not the cause of arterial dissections.

The most promising hypothesis involves hormonal changes that may contribute to alterations in the arterial walls, which increase the risk for aneurysms and dissection. The histologic changes described in dissecting arteries in pregnancy include fragmented reticulin fibers, alterations in the amount of acid mucopolysaccharides, loss of normal corrugation of elastic fibers, and smooth muscle hypertrophy.^{3,4} Comparable changes have been observed in pregnant women without clinical dissection, suggesting that these arterial alterations are due to hormonal changes in pregnancy.⁵

The mechanism for pregnancy-associated arterial dissections is unknown; however, this is a serious problem that can lead to significant morbidity and mortality if not properly diagnosed. Further research needs to be undertaken to determine other potential risk factors and to aid in early detection of such dissections.



Pathology/Biology Section - 2015

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Arterial Dissection, Pregnancy, Iliac Artery



Pathology/Biology Section - 2015

H17 Nutritional Child Abuse: A Case Report of Kwashiorkor and Vitamin D-Related Rickets

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After attending this presentation, attendees will use this case report to serve as a control in the assessment of rickets-related bone changes and fractures.

This presentation will impact the forensic science community by facilitating discussion about osseous injuries in malnourished children.

Osseous injuries are often found in cases of child abuse and can occur actively through trauma and/or in a passive way through neglect by means of malnutrition. The presence of bone fractures presents challenges for the forensic pathologist as consideration of a plausible counterexample(s) must be pursued to explain pathological findings at autopsy. A counterexample to pediatric bone fractures due to non-accidental trauma is "fragile bones" due to rickets, osteogenesis imperfecta, osteopetrosis, etc. This consideration was applied to a 28-month-old girl known to be below the 3rd percentile for weight who was fed a vegetarian diet limited to plants, breast milk, and dairy milk. Following a one-week history of low-grade fever, cough, and increased drowsiness, the toddler died in the mother's arm. Autopsy revealed: (1) classical rickets-related bone changes including a well-healed pathologic fracture of a rib, an increased thickness of the calvarium, delayed fontanelle closure, prominent nodular costochondral junctions of the ribs (rachitic rosary), chondral hypertrophy at metaphyses, genu varum, and parathyroid hyperplasia; and, (2) body weight and height below the 3rd percentile and non-specific changes in keeping with protein malnutrition including generalized edema, ascites, bilateral pleural effusions, and hepatic steatosis. Radiological and histological investigations of the skull, vertebra, sternum, ribs, and long and metatarsal bones supported the autopsy findings of rickets. Biochemistry investigations revealed an undetectable vitamin D, megaloblastic anemia, and hypoproteinemia. There was no evidence of competing causes (hepatobiliary, intestinal, pancreatic, renal disease, diabetes mellitus, or inborn errors of metabolism) to explain the autopsy findings. Other findings included chronic asthmatic bronchitis, chronic atopic dermatitis, and evidence of acute shock in the colon and brain. The cause of death was attributed to a combination of asthma and malnutrition.

A careful evaluation of the clinical history, scene, autopsy, and ancillary postmortem investigations (radiology and biochemistry) are necessary to diagnose severe vitamin D-related rickets and protein malnutrition. Following review of this presentation, attendees will gain an understanding of the histological and radiological changes in a toddler with severe vitamin D-deficiency rickets in the setting of protein malnutrition. Given the rarity of these constellations of findings, this single case may serve as a control in the assessment of rickets-related bone changes and fractures.

Vitamin D-Related Rickets, Kwashiorkor, Osseous Injuries



H18 Genetic and Histopathological Factors in Thrombo-Embolic Disease: Results of a Preliminary Experimental Study

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After attending this presentation, attendees will understand how a comprehensive approach to fatal thrombo-embolism that includes the analysis of the clinical history of the patients, the research for genetic factors, and the histological age determination of thromboemboli may result in a better definition of the disease.

This presentation will impact the forensic science community by demonstrating the importance of the prophylactic measures in Venous Thrombo-Embolism (VTE).

VTE is the third most common cardiovascular disease after myocardial infarction and stroke. The mortality rate is high and there are many deficiencies in the diagnosis, prophylaxis, and therapy. In this study, a retrospective analysis of the reports of 500 autopsies performed at the Institute of Forensic Medicine of Catanzaro between 2005 and 2014 was performed. Twenty-two cases in which pulmonary embolism was recorded as the cause of death were identified, consisting of 13 males (59%) and nine females (41%) with a median age of 56 years (range 14-93 years). All subjects were Caucasian except one Negroid subject. The clinical histories of the patients who died were analyzed, with the identification of the risk factors for VTE. In the case of hospitalized patients, the measures of prophylaxis and therapy were evaluated. The role of genetic risk factors in the disease, particularly Factor V Leiden (G1691A) mutation, which is the most common genetic factor of thrombophilia, was studied. The presence of the mutation was researched in DNA extracted from blood samples. Additionally, in order to obtain a histological age of the process, the histopathological features of thrombo-emboli were studied using histological sections stained by Hematoxylin-Eosin (H&E) and trichromic stains (Masson).

The results of the clinical and epidemiological data showed that 18 subjects (81.8%) had at least one risk factor for VTE, while in four patients (18.2%) no clinical data that could be related to an increased thromboembolic risk was identified. Seven subjects (31.8%) were more than 65 years old at the time of the event, seven (31.8%) had major trauma, and seven (31.8%) were surgical patients. An immobility period was recorded in five subjects (22.7%), four subjects (18.1%) were obese, and two (9%) were receiving antipsychotic drugs. Central catheterization was present in two subjects (9%) and pregnancy and postpartum status were present in an additional two patients (9%). Finally, one subject (4.5%) was suffering from chronic respiratory failure and one subject (4.5%) had limb paresis.

The retrospective analysis also revealed that 13 deaths (59%) occurred in a hospital setting while nine subjects (41%) died before having the opportunity to receive medical care. Among hospitalized patients, ten subjects (77%) were hospitalized for other diseases and suffered an episode of fatal thrombo-embolism during the hospital stay. Three patients (23%) arrived at the hospital presenting with symptoms of VTE and none received a correct diagnosis. Among hospitalized patients, eight subjects received prophylaxis with low molecular weight heparin, while two subjects, despite the risk, received no prophylactic treatment for VTE. In 12 out of 22 subjects (54.6%), it was not possible to find the thrombotic site. In the other cases, five (50%) were femoral thromboses, two (20%) inferior-caval, two (20%) iliac, and one (10%) saphenous. The genetic results showed that none of the patients carried a Factor V Leiden (G1691A) gene variant, not even the younger subjects or those who did not show any clinical data that could expose them to an increased risk. The histopathological analysis revealed the age of the thrombi. The study of the morphological features of the thrombo-embolic samples reported that eight samples (36.3%) were between one and three days of age, eight samples (36.3%) were between three and five days of age, and six samples (27.3%) were between four and 20 days of age.

The results of this study highlight the importance of early identification of risk factors and the setting of primary prophylaxis measures in order to prevent the onset of the thrombotic process, especially as the clinical diagnosis of VTE is notoriously inaccurate. Moreover, it is known that even the non-fatal cases of VTE can result in serious chronic complications such as post-thrombotic syndrome and chronic pulmonary hypertension. It is clear that an accurate prophylactic treatment would be useful not only to prevent death but also morbidity. The results of this study indicate that even if the prophylaxis is correctly performed according to the guidelines, death cannot be completely avoided. This observation poses a question concerning the validity of current treatment approaches, despite the ongoing research in the field of thrombo-prophylaxis.

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Pathology/Biology Section - 2015

The genetic factors were determined to be non-significant in determining the risk compared to the environmental risk factors. The results of histopathological investigations showed that it can be a very large temporal range in which death can be avoided; therefore, early identification is important in order to prevent the progression of the disease to the fatal episode. In the histological age determination of thromboembolism, immunohistochemical investigations were not essential in establishing the cellularity of thromboembolic formation and are very expensive compared to basic histological investigations. The difficulty in finding the embolic source may be an important limitation in the age determination of the thrombotic process as it prevents evaluation of the residual thrombus. In this context, a decisive role could be played by postmortem computed tomography angiography. This imaging diagnostic method may be very helpful in the visualization of thrombus and may facilitate its subsequent detection during the autopsy.

In conclusion, there is a great need to implement diagnostic and prophylactic measures in VTE. It is necessary to pay more attention to the pathology and the risk factors, similar to what occurs with other cardiovascular diseases such as myocardial infarction. An accurate analysis of the clinical history of the subjects can be very useful to clarify the etiology of the disease while genetic investigation is not necessary. The histological age determination is essential to answer a number of questions about the nature and the evolution of the VTE, making it possible to establish the casual relationship between the exposure to certain risk factors and the occurrence of thrombosis.

Fatal Thrombo-Embolic, Histopathological Factors, Genetic Investigations



H19 Sudden, Unexpected Death Due to Fatal Rupture of an Undiagnosed Aneurysm of the Splenic Artery: A Forensic Approach

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After attending this presentation, attendees will be informed about a case of unexpected sudden death due to undiagnosed aneurysm of the splenic artery.

This presentation will impact the forensic science community by stressing the importance of performing accurate clinical and necropsy examinations for all cases of ambiguous sudden death.

Aneurysms of the visceral arteries are neither a rare feature nor undescribed in the forensic literature. Nevertheless, this case of an undiagnosed aneurysm of the splenic artery is reported because of its peculiar findings and the resulting medicolegal implications. The splenic artery is the most common site of a visceral artery aneurysm. Various statements about the frequency of visceral and splenic artery aneurysms are available in medical literature. The overall frequency of visceral artery aneurysms described in the literature ranges from 0.1% to 2%, with most of the visceral artery aneurysms involving the splenic artery. The data is mainly gained from postmortem examinations. Aneurysms of the visceral arteries are diagnosed in increasing number due to the use of imaging diagnostics. These incidental findings are mostly asymptomatic. The clinical relevance of visceral artery aneurysms is extremely variable and to some extent is dependent on the location and size. Non-specific gastrointestinal disorders and recurrent pain are possible manifestations. For large aneurysms, a pulsating mass may be palpable. Visceral artery aneurysms can rupture into various organs and the abdominal cavity, resulting in recurrent gastrointestinal bleeding, hemobilia, and a surgical abdomen. Extensive hemorrhage and hypovolemic shock may arise. Aneurysms occur congenitally or may be acquired as a result of arteriosclerosis, fibromuscular dysplasia, inflammation, and trauma. Among surgeons, there has not yet been a unanimous view on the preferential operative approach. In those patients with an aneurysm characterized by a diameter larger than 2-4cm, surgical interventions should be employed because of the increasing risk of rupturing.

A 38-year-old woman suddenly died at her home, in Portoferraio (Elba's Island, Italy) of unknown clinical causes. The day before her death, she woke up with nausea and vomiting. She went to the General Practitioner (GP) reporting symptoms such as diarrhea, abdominal colic, and vomiting. The GP, after a physical examination of the abdomen, performed an intramuscular injection of an antispasmodic and prescribed a gastroprotective and antiemetic therapy. After a partial regression of symptoms, the woman felt a severe abdominal pain and went to the nearest emergency room for treatment. At the hospital, a myocardial infarction was ruled out after an **electrocardiogram** and blood sampling for troponin and CK-MB were performed. No positive results were found. While hospitalized, the woman underwent radiological tests of the abdomen and was then discharged.

She returned home and had a light dinner before retiring. In the morning, after screaming due to acute pain, she suddenly died. Physicians who were promptly called could only confirm occurrence of the death. It was evident that the woman did not use any drugs and did not suffer from any disease, with the exception of a backache and heartburn over the previous two days.

The autopsy was performed at Portoferraio's hospital. The external examination did not highlight any relevant findings other than evidence of resuscitation efforts by the medical staff. Internally, approximately 1,000ml of blood was found in the intraperitoneal cavity. Additionally, the internal examination revealed a voluminous retroperitoneal blood clot in the perisplenic region as well as signs of blood loss, including pallor of the organs. To complete the autopsy, the block formed by esophagus-stomach-duodenum-liver-pancreas, spleen, and aorta was formalin-fixed for a more accurate investigation. The subsequent dissection yielded a ruptured, sacculated aneurysm of the splenic artery measuring 14mm in diameter. The data obtained at the autopsy attributed the death to internal bleeding resulting from a rupture of the aneurysm of the splenic artery.

Conclusions: The present case is especially interesting because the unexpected death came after non-specific symptoms caused by hemorrhagic shock resulting from the rupture of a visceral artery aneurysm. This case demonstrates the relevance of a careful postmortem examination in order to define the cause of death and assess for any eventual medical malpractice due to failure of a timely and accurate diagnosis.

Sudden Death, Splenic Aneurysm, Medical Malpractice

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Pathology/Biology Section - 2015

H20 A Tremor in the Hand That Rocks the Cradle: Fatal Consequences of Postpartum Angiopathy

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After attending this presentation, attendees will better understand fatal outcomes of postpartum angiopathy, its variable presentation, differential diagnosis, and uncertainties of its incidence, pathogenesis, and treatment. Attendees will also be able to describe its importance as an often unrecognized cause of maternal death and the role of autopsy (especially neuropathological examination) in diagnosis.

This presentation will impact the forensic science community by fostering recognition and optimal examination of cases of postpartum angiopathy which is of benefit to family, clinicians, and public health.

Maternal mortality is of singular importance, usually scrutinized by public health authorities. Acute neurological symptoms occurring after a seemingly uncomplicated pregnancy and delivery are particularly alarming. A fatal intracerebral hemorrhage in an otherwise healthy postpartum woman is reported; the proximate cause of death was not recognized by clinicians and the case was almost not reported for definitive investigation.

A 26-year-old woman underwent uncomplicated term vaginal delivery and was discharged five days later. Two days later, she developed endometritis; following intravenous antibiotics, she was discharged after being prescribed oral antibiotics. On the 13th postpartum day, she complained of feeling “peculiar” and had two brief episodes of dysphasia. She later sustained a tonic-clonic seizure, recovered fully, and was transported to the hospital where a Computed Tomography (CT) scan of the head showed no acute changes. She was transferred to a tertiary center for monitoring and further investigation.

A chest X-ray, CT scan of the pelvis and abdomen, and abdominal/pelvic ultrasound were normal. A lumbar puncture was unsuccessful, but soon afterward she developed a severe headache. She vomited, became incoherent, and rapidly became comatose. A CT of the head showed a large hemorrhage within the left frontal lobe. Subsequent scans showed no structural cause of the hemorrhage. She deteriorated and met brain death criteria six days after initial neurological symptoms.

Autopsy revealed a healthy-appearing woman with features of medical intervention and critical illness. Degenerative endometrial changes consistent with her postpartum status were present. A probe-Patent Foramen Ovale (PFO) was noted. The brain showed a large hemorrhage apparently arising in the left striatum extending into the frontal white matter and rupturing into the ventricle (with acute hydrocephalus), with secondary features of edema (transcranial/tonsillar herniation, left-sided sinus thromboses, and pituitary infarction). There was no identifiable structural etiology. No choriocarcinoma or septic foci were identified. The vessels showed no vasculitis or other abnormalities.

Postpartum angiopathy (Call-Fleming syndrome) usually presents within two weeks following normal pregnancy and parturition. It presents with an abrupt (maximum intensity within minutes), severe global or occipital headache (described as a “thunderclap” or “the worst”) and/or focal, transient neurological signs. Definitive diagnosis consists of angiographic demonstration of multiple segmental narrowing of large- and medium-sized cerebral arteries. Vasospasm is transient and initial angiogram may be normal.

No incidence numbers are available, although fatal cases and small studies are widely reported. Multiple reports suggest under-recognition, “more common than appreciated,” with a spectrum of outcomes, including disability. It is usually a benign condition, often grouped with “benign angiopathies” (a heterogeneous group of reversible cerebral vasospastic conditions), although this would seem to neglect uniquely postpartum physiology with hormonally mediated susceptibility to vasospasm and labile hypertension. Admissions for pregnancy-related strokes have increased since the early 1900s.

Pathogenesis is unclear. Vasospasm as a mechanism is favored by the typically benign outcome. Overlap with eclampsia is hypothesized and the use of vasospastic drugs (ergonovine, sumatriptan, bromocriptine) has been implicated in some cases.

Pregnancy is associated with an increased risk of cerebral events (venous thrombosis, subarachnoid hemorrhage, intracerebral hemorrhage), coagulopathy, vasculitis, migraine, and mechanical complications due to epidural (spontaneous cerebrospinal fluid leak, meningitis), as well as eclampsia (25% developing postpartum). The differential diagnosis is large, but most (except for vasculitis) can be excluded by imaging and laboratory studies.

No definitive treatment is established. Favored strategies include calcium channel-blockers and steroids; others include immunosuppression, aspirin, MgSO₄, hypervolemic therapy, and endovascular procedures (calcium channel blockers, balloon angioplasty, stents).

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Pathology/Biology Section - 2015

Autopsy was helpful in this case in suggesting the etiology of this death, educating physicians, and excluding other causes, particularly given the PFO and potential for septic emboli.

Postpartum Angiopathy, Maternal Mortality, Postpartum Stroke



H21 Death Caused by Kawasaki Disease in Infants: Illustration of Three Cases and Literature Review

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The goals of this presentation are: (1) to help attendees understand the manner of presentation of Atypical Kawasaki Disease (AKD) causing unexpected death in infants; (2) to take into account the clinical history and pathological and radiological findings to support diagnostics of the cause of death; and, (3) to perform a literature review to improve knowledge of Kawasaki Disease (KD) with atypical presentation for the forensic pathology community.

This presentation will impact the forensic science community by serving as a key aspect of KD investigation as it can augment traditional means of diagnostics in a systematized format via interdisciplinary communication and collaboration. In relation to cardiac aneurysm causing death, the crucial role of postmortem Nuclear Magnetic Resonance (NMR) imaging will be discussed.

This study reports three cases of AKD causing unexpected death in infants. Clinical history as well as pathological and radiological findings are taken into account to support determination of the cause of death. A literature review was performed to convey knowledge of KD with atypical presentation to the forensic pathologist community.

KD is an acute systemic vasculitis of unknown cause. It affects children younger than ten years of age, mostly those under three years of age. KD before the age of three months is rare. Although no specific laboratory tests exist that can definitively identify KD, there are clinical and laboratory findings that guide diagnosis and treatment. The currently accepted clinical diagnostic criteria for KD include fever for at least five days with four of the following symptoms: polymorphous rash; conjunctivae injection; oral mucosal changes; cervical lymphadenitis; and/or, erythema, swelling, or desquamation of the hands and feet. Diagnostic criteria for KD in infants two years of age or younger may be missed in cases of AKD. Patients with only four of these principal signs/symptoms may be diagnosed as having KD if coronary aneurysm is recognized. Laboratory studies show leukocytosis, elevated erythrocyte sedimentation rate, positive C-reactive protein, and thrombocytosis. As cases of KD may be rarely found in forensic pathology observation and tend not to be an obvious cause of death, especially in atypical presentation, showing findings of several infant cases may positively impact knowledge of such pathology. Early diagnosis may avoid mortality or negative patient outcomes.

Case Illustration 1: A case of AKD with a lack of typical clinical signs and a rapidly fatal course in a two-month-old infant. One week before hospitalization, the infant demonstrated rhinitis, coughing without fever, and later conjunctival hyperemia and an allergic exanthem on the chest and arms. On admittance, laboratory tests highlighted leukocytosis, thrombocytosis, elevated sedimentation rate, and positive C-reactive protein. General conditions were poor for seven days until sudden death occurred. The autopsy confirmed that death was due to cardiac tamponade caused by a ruptured and inflamed aneurysm of the left anterior descending coronary artery.

Case Illustration 2: A three-month-old male baby, three days after the compulsory vaccination and antipyretic administration, gradually showed eyelid conjunctivae and lip erythema, a diffuse cutaneous exanthem, and pharyngo-tonsillitis. He exhibited episodes of staring which lasted for a few seconds. The patient was hospitalized in the pediatrics and neonatology division for suspected sepsis. Death occurred nine days after admission from sudden cardiac arrest that was unresponsive to any type of intensive care. Autopsy revealed that the cause of death was due to a ruptured, inflamed aneurysm in the anterior descending coronary artery, with subsequent cardiac tamponade. The anatomical-pathological and clinical outline suggested AKD.

Case Illustration 3: A seven-month-old male baby, apparently well nourished and without fever or exanthem, was unexpectedly found agonal in his bed by his parents. He died in an emergency room a few hours later in spite of aggressive resuscitation efforts. Postmortem imaging was performed during the autopsy, with evidence of an occlusive thrombus in a left coronary artery aneurysm. Laboratory findings were consistent with AKD. The crucial role of postmortem imaging is discussed here in order to improve diagnosis tools.



Pathology/Biology Section - 2015

Discussion: Aneurysms of the coronary vessels are rare in children and in isolated forms they are indicative of KD. In KD, aneurysms occur in 15%-30% of patients, usually after an acute phase of the disease. Resolution or regression of aneurysms is found in more than 50% of KD cases, whereas thrombosis or rupture rarely complicate the remaining cases. KD is lethal in 0.5%-2% of patients and death always takes place suddenly and unexpectedly. In the acute phase of disease, death is caused by complications of coronary endarteritis (thrombosis with myocardial infarction) and myocarditis (myocardial insufficiency and arrhythmias).

Kawasaki Disease, Death, Diagnoses



H22 Thoracic Injuries Due to Cardiopulmonary Resuscitation in an Infant: A Case Report

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After attending this presentation, attendees will have an increased awareness of the potential pitfalls involving resuscitation artifacts in infants and how to differentiate these injuries from trauma inflicted prior to resuscitation.

This presentation will impact the forensic science community by presenting a case of Cardiopulmonary Resuscitation (CPR) injuries in an infant. These injuries, such as rib fractures and visceral trauma, are rare findings at postmortem examination. Nevertheless, it is critical that these injuries be recognized and differentiated from trauma caused by fatal child abuse prior to resuscitation attempts.

A case of a 4-month-old infant who died suddenly and underwent prolonged CPR is reported. He was born at full term via caesarean section with no prenatal or perinatal complications. There was no history of child protective services involvement or domestic violence. The child was found at night in his crib, unresponsive and in the supine position over blankets and a shawl. He had no vital signs upon arrival by the paramedics, who immediately administered full resuscitation measures. At autopsy, there were no external signs of recent trauma. There were minimally hemorrhagic left lateral rib fractures involving ribs 2-6, with extra-osseous displacement of bone marrow and crush artifact. There was extensive subpleural hemorrhage of the lungs with a resultant left hemothorax. No other injuries were found, no disease process was evident, and no congenital anomalies were identified. Toxicological, microbiological, and biochemical studies were non-contributory. A full neuropathological examination by a neuropathologist showed no significant findings. The cause of death was undetermined.

Based on a clinicopathologic correlation, the thoracic injuries were best attributed to the prolonged CPR, rather than injuries that occurred prior to death. These resuscitation artifacts are well-documented and are commonly seen at the adult autopsy in cases when CPR was administered. These artifacts have been thought to be an unusual finding in infancy; however, emerging data has shown that CPR-related rib fractures can occur in children.¹⁻³ This case underscores the emerging data that indicates rib fractures and other injuries can occur in collapsed infants who are subjected to CPR.

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Resuscitation Injury, Infant, Child Abuse



Pathology/Biology Section - 2015

H23 What a Smell! Discovery of a Seeking Congeners Behavior of Blow Fly Larvae

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WITHDRAWN



Pathology/Biology Section - 2015

H24 Survival of Blow Fly Pupae After Submergence in Fresh, Salt, and Polluted Water

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After attending this presentation, attendees will understand that the pupal stage of a blow fly can add another capability to forensic entomology practice and add another dimension to determining the minimum Postmortem Interval (PMI) when insects are found on a floating corpse.

This presentation will impact the forensic science community by providing data that will be potentially useful in adding a new component to estimating the overall duration of the floating time of corpses in forensic investigations that can assist in determining the minimum time-since-death.

Blow flies (Diptera: *Calliphoridae*) are generally the first insects that colonize a corpse in the terrestrial environment. In an aquatic environment, a body proceeds through a series of sinking and floating phases (bloat) and it is during the float phase that calliphorids can colonize a body in the water. Calliphorids collected on a body found in an aquatic environment can help to determine how long the remains have been floating and this information can assist in determining the minimum time since death. At present, only incomplete experiments exist concerning the capacity of any calliphorid life-history stage to survive reduced oxygen tension and whether they can survive submersion in water. Many previous authors have alluded to the forensic utility of blow fly immatures when a body is submerged, but again it is all anecdotal evidence.

The goal of this research is to estimate the survival of *Calliphora vomitoria* L. pupae in sea water, fresh water, and polluted water. Polluted water was collected from the Po River in Turin, Italy. Waters used in this research were chemically analyzed (pH, hardness, salinity, Na, K, Ca, Mg, Cl, NO₂, NO₃, NH₄, PO₄, SO₄, NH₄, and HCO₃).

Pupae (N=30 for each treatment) were sampled at different stages of their development (24 hours, five days, and nine days following pupation) and placed underwater for a minimum time of one hour to a maximum time of ten days. Pupae were placed in an open-weave mesh bag and weighted down so they remained completely submerged. Pupae were then removed from the water and placed in a dry environment. The time required to eclosion and the survival rate was recorded and compared with non-submerged, control pupae (N=100). During the entire experiment, the environmental temperature was 23°C and the water temperature was 24°C.

Preliminary results to date demonstrate that the survival of pupae decreases with increasing time of submergence. In general, no pupae survived following five days of immersion. Lower survival also occurred among pupae submerged in seawater over equivalent time periods. The time required to eclosion for control pupae and pupae immersed for 24 hours is not statistically different.

This study provides data potentially useful in estimating the floating time of corpses in forensic investigations when pupae are found adhering to, or entangled in, the decaying flesh, hair, or clothes of a corpse that has become submerged after the larvae had developed and pupated. This research complements the study by Singh and Greenberg and adds vital details to the questions of floating time and, hence, the overall minimum PMI.¹

Reference:

1. Singh D, Greenberg B. Survival after submergence in the pupae of five species of blow flies (Diptera: *Calliphoridae*). *J Med Entomol.* 1994;31:757-9.

Calliphora Vomitoria, Pupae, Submergence



Pathology/Biology Section - 2015

H25 Postmortem Artifacts Caused by the Water Beetle *Rhantus Validus*, Sharp (Coleoptera: Dytiscidae) on a Corpse Found in a Pond in Región de La Araucanía, Chile

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After attending this presentation, attendees will understand the potential capabilities and limitations of forensic entomology practices in order to determine the nature of injuries found on a body when insects are found on a floating corpse.

This presentation will impact the forensic science community by providing information that will be potentially useful in adding a new component to determine the circumstance of death of a corpse found in an aquatic environment.

The estimation of the time since death, determination of the manner of death, identification of the individual, and the pathological evaluation of a body can be impeded by postmortem changes. More accurate diagnosis of these changes are a result of continued research in forensic entomology and case studies; however, at present there is scant literature concerning forensic entomology and decompositional processes in aquatic environments.

In the majority of cases a body placed in water progresses through a series of sinking and floating phases. During the float phases, necrophagous blow flies (Diptera: *Calliphoridae*) and flesh flies (Diptera: *Sarcophagidae*) can colonize a body in the water. Some other aquatic or semi-aquatic insects can use the submerged body as an eventual source of food or as a substrate to attach (e.g., Diptera: *Simuliidae*, *Chironomidae*), but in water there are no specialized carrion insects.

The case study presented involves a decomposed body found in an artificial pond in Región de La Araucanía, Chile, in April 2014. The body was in the early floating stage, six feet from the edge of the pond. The face and majority of the body were immersed in the water, while the ears, the posterior part of the head, and the shoulders were exposed to the surface. Adult water beetles *Rhantus validus* Sharp (Coleoptera: Dytiscidae) were found on the body, associated with injuries on the skin. Water beetles such as *R. validus* are semi-aquatic insects adapted to living in water both as larvae and adults. These species of water beetles carry an air bubble between their abdomens and elytra which provides an air supply and have hind legs adapted for swimming. They are predatory insects with short and sharp mandibles. When they bite, they immediately deliver digestive enzymes.

The water beetles were collected from the body at the crime scene and sent to an entomologist. No other species of arthropods associated the corpse were observed. Four water beetles were found behind the left ear of the body, crawling in an asymmetrical groove of approximately 30x10mm (length x width), while one water beetle was found crawling inside an oval hole (10mm diameter) with regular margins on the upper part of the chest. The entomologist was asked to help clarify the nature of these injuries, in particular, if they could be postmortem artifacts caused by the predatory activity of water beetles on the body.

Postmortem Artifacts, *Rhantus Validus*, Freshwater Environment



Pathology/Biology Section - 2015

H26 Biodiversity of Functional Genes: An Aid in Soil Provenance

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The goal of this presentation is to acquaint attendees with the use of DNA microbial profiling for discrimination/comparisons of soil samples. Also, attendees will gain insight on the use of functional markers to define microbial communities and how their use will strengthen the crime scene investigations involving soil as evidence.

This presentation will impact the forensic science community by laying the foundation to adopt the use of functional markers over universal markers as they represent the true microbial community profile and would be better for studying soil structure.

Soils are treated as important pieces of evidence in forensic investigations owing to their mineral and organic properties. Most often, soils are examined utilizing physical properties such as color, particle size, and mineral content; however, these techniques do not always result in successful categorization of the samples. Hence, laying the path to complementary approaches such as that proposed by Horswell et al. DNA profiling of soil bacterial communities using terminal restriction fragment length polymorphism can be employed for forensic comparison and discrimination of soils. This method is advantageous as it does not require a large sample size and the equipment is already owned by the forensic laboratories for human DNA analysis, making it more cost-effective.

Studies have demonstrated that microbial soil community structure can be determined from small soil samples across different locations and can be used for comparisons. Based on the existing ecological literature, it is suggested that variations exist in microbial community profiles across land management and vegetation types. Thus, microbial community profiling can potentially be employed as an intelligence tool for the providence of unknown soil samples collected from crime scenes.

Soil bacterial community profiles have been categorized using 16S rRNA hypervariable domains. These domains are effective in constructing a DNA profile that would enable discrimination between various soil samples; however, 16S rRNA profile alone is not adequate to establish the functional diversity of these microbes. Microbes play an important role in maintaining the stability of soils by cycling the biochemical nutrients such as carbon, phosphorus, nitrogen, sulfur, and iron. These processes are carried out by the enzymes produced by soil microorganisms. Thus, investigation of these functional enzymes can provide a better understanding of the function of the soils.

The objective of this study was to compare the functional diversity of microbial communities among different soil types: Lauderhill Dania-Pahokee (soil type 2) and Perrine-Biscayne-Pennsuco (soil type 4) of Miami-Dade County, FL.

DNA was extracted from samples (n=36) collected from one transect belonging to each soil type. Degenerate primers for *mcrA*, *cel48*, and *dsrA* were used to amplify the gene using polymerase chain reaction followed by cloning and sequencing. Sequences were analyzed by Basic Local Alignment Search Tool (BLAST) and subsequently aligned to construct a phylogenetic tree.

Comparison of sequences of the *mcrA* gene obtained from KNT and CS transect with sequences from other Florida studies resulted in close alignment with *Methanothermobacter thermautotrophicus*; however, many were not associated with any known reference samples. The KNT- and CS-labeled sequences associated almost exclusively with either uncultured archaea or with the uncultured euryarchaeote from oligotrophic soils in the northern Everglades. While some of the clones from the two soil types examined in Miami-Dade County grouped together with a particular soil type, there was overlap in the clones from the different soil types. These overlaps for the gene *mcrA* are indicative of saturated anoxic soils which are also useful for discrimination of environmental ecosystems. Thus, the study of these overlaps is important as it can also provide useful information.

The assessment of phylogenetic and functional abilities of microbial communities will clarify the importance of microbes for soil function and hence define its structure. A well-characterized structure can thus be of extreme importance in soil provenance.

Functional Genes, Microbial Community Profiling, Soils



H27 The Application of Novel Next Generation Sequencing (NGS) Technology in Forensic DNA Research

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After attending this presentation, attendees will see the merits of Next Generation Sequencing (NGS) in forensic mitochondrial DNA typing and the advantages and disadvantages of different sequencing techniques.

This presentation will impact the forensic science community by shedding light on the discrepancy of mitochondrial DNA sequencing by Sanger's sequencing, ion-semiconductor sequencing, and sequencing-by-synthesis, which also paved the way for the application of NGS to forensic DNA typing.

In forensic medicine, mitochondrial DNA provides the evidence of maternal inheritance; the high variable regions (HV-1 and HV-2) in the D-loop are the most informative among all. The traditional Sanger's sequencing can generate high-quality mtDNA sequencing data; however, this can be a tedious task under the pressures of time limitation and human resources. In contrast, NGS offers more reads at the price of higher cost. Thus, this study compares the cons and pros of NGS with Sanger's sequencing.

Twenty-two anonymous cases were selected. Sample types included oral swabs for three cases, muscle for three cases, and bone for 16 cases. For Sanger's sequencing, HV-1 and HV-2 were amplified independently and the PCR products were purified and sequenced with 3130xL. For NGS, the DNA samples were amplified using two pairs of mtDNA-specific primers. The two amplicons covering the entire mitochondrial genome were ~8.5kb for each with some overlap and were subjected for library preparation. In brief, for ion-semiconductor sequencing, the amplicons were sheared to suitable size using a shearing enzyme, tagged with different barcodes to identify different cases, and amplified with emulsion Polymerase Chain Reaction (PCR) to provide adequate signal strength. A higher sequencing depth indicates a better data quality. Since the sequencing depth has coverage ranges of 1,500-folds, this indicated that the quality of the data was very high. For sequencing-by-synthesis, the amplicons were fragmented and tagged simultaneously by special transposase, labeled using different barcodes, and amplified with bridge-amplification in order to provide sufficient fluorescence signal. The sequencing depth maintained a coverage range of 2,000-folds, which shows that the data should be very accurate. Variants called from different platforms were compared, while 19 cases were determined by ion-semiconductor sequencing, 21 cases were determined by sequencing-by-synthesis, and all of their HV-1 and HV-2 regions were determined by Sanger's sequencing.

The polymorphisms of HV-1 and HV-2 regions include Single Nucleotide Polymorphism (SNP), indel, and heteroplasmy; most of the SNP could be identified by both NGS platforms. Moreover, both NGS platforms showed better sensitivity in detection of heteroplasmy and the determined heteroplasmic proportions were in concordance between the two platforms; however, in detecting indels, there were some discrepancies between them. For ion-semiconductor sequencing, the homopolymer emitted an accumulated proton signal, and thus the algorithm needed optimization, setting "hot spots" previously to approach the accurate length of homopolymer. For sequencing-by-synthesis, few adjustments were required for the estimated length of homopolymer. In some cases, the estimated lengths of sequencing-by-synthesis were one-base longer than the length determined by Sanger's sequencing. This is probably due to the conservative fashion of the determination of Sanger's sequencing.

The DNA quality should be a critical issue in the preparation of amplicons. In this report, the DNA samples from the bones were severely damaged. The DNA quantities extracted from the bones were poor and barely met the requirements of long-range PCR. NGS platforms brought resolutions for this challenge. For the whole mitochondrial genome, the company recommended different optimized primers for different platforms to amplify the genome, as well as shorter amplicons. Furthermore, both NGS techniques (ion semiconductor sequencing and sequencing-by-synthesis) suggest that transposase should be used in order to construct the library. This not only dramatically shortened the consumption of amplicons but also decreased the labor input. These improvements could make NGS more feasible in forensic DNA analysis.

Next Generation Sequencing, Mitochondrial DNA, Homopolymer



Pathology/Biology Section - 2015

H28 Contaminated Corpse Still Flavorful! A Review of Household Products' Effects

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WITHDRAWN



Pathology/Biology Section - 2015

H29 Genotyping of Used Drug Stamp Bags With Special Attention to the Minor Contributor

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After attending this presentation, attendees will better relate to the importance of evidence collecting, specifically DNA, of stamp bags found on-scene. Attendees will also learn of new and original changes to procedure to produce higher DNA yield.

This presentation will impact the forensic science community by showing that DNA can be obtained in sufficient quantities for a short tandem repeat profile from drug evidence at a crime scene and by providing several useful testing methods which vary in terms of time and expense.

Currently under review in the United States court system is the question of what penalty, for sentence and category of crime, should be applied to a distributor of illegal drugs in the event that those drugs were responsible for the death of a user. Touch DNA (tDNA) is the DNA that remains behind when an individual touches an object. It is very common for the tDNA to also be characterized as trace DNA, which is defined as being less than 100 picograms. It has been found that tDNA can be left behind on a number of items that a user might touch. A large quantity of drugs can normally be found wrapped up in magazines. Some research was done as to whether DNA could be obtained from these magazines; it has so far been determined to be improbable; however, since the drugs are distributed to users in smaller bags, known as “dime bags” or “stamp bags,” and these bags are normally made out of some waxy material, it was hypothesized that DNA could be obtained from these waxy, non-porous bags.

Research suggests that a detectable amount of DNA can in fact be obtained from drug stamp bags. The preliminary test, which had an average concentration of 1.47E-03 nanograms per milliliter, was conducted by using a double-swab method using PSS Select® Cotton Swabs on touched bags. The DNA was extracted via the Promega® DNA IQ™ System kit and analyzed on the rtPCR machine using the Life Technologies™ Quantifiler® Human DNA Quantification Kit provided by the laboratory. There was also a known DNA positive control along with a no DNA negative and a non-touched bag negative control. Genotyping was done with an ampFISTER® Identifiler® Plus PCR Amplification Kit on the 3130xl Genetic Analyzer.

It was hypothesized that a large amount of DNA was being lost due to the PSS Select® Cotton Swabs. In addition to the double-swab method, an enzyme was used to enhance the DNA yield. Also, sample bags were cut into pieces and a reworked PCI method was developed to more greatly enhance the DNA yield from the stamp bags.

This additional step provided samples that, when concentrated, produced viable DNA concentrations, some over the optimal 1ng for the ampFISTER® Identifiler® Plus PCR Amplification Kit protocol. Full profiles were found for several of the samples. This research is important because there are an increasing number of drug-related deaths and the person(s) making or delivering the product is left unpunished. If a full profile of the dealer or distributor can be determined, it is possible to target and reduce the places and sales of heroin and thus the amount of heroin overdoses per year.

DNA Yield, Drug, Stamp Bag



H30 Death Following Retrobulbar Injection of Desmopressin for the Treatment of Non-Arteritic Anterior Ischemic Optic Neuropathy: Which Implications Indicate This Off-Label Use?

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After attending this presentation, attendees will be aware of the possible clinical and legal implications following off-label use of corticosteroids and desmopressin for the treatment of Non-Arteritic Ischemic Optic Neuropathy (NAION).

This presentation will impact the forensic science community by illustrating a case of fatal acute myocardial infarction after retrobulbar injection of a synthetic replacement of vasopressin.

Since a standard treatment for NAION with proven efficacy is not available to date, most therapeutic approaches are empirical and include a wide range of agents presumed to act on thrombosis, on the blood vessels, on the disk edema, or presumed to have a neuroprotective effect. Among other proposed treatments, retrobulbar injection of corticosteroid and desmopressin represents an invasive approach, with potential for local and systemic complications, which does not appear to have been documented previously. The rationale of using desmopressin in the treatment of NAION is uncertain and perhaps a result of experimental findings demonstrating that desmopressin induces ciliary artery relaxation in dogs via V1-receptors through a mechanism which involves nitric oxide. In turn, this would enhance vascular permeability, thus facilitating the re-absorption of optic edema. A similar vasodilation effect can be attributed to corticosteroids.

A 60-year-old man, apparently healthy and with a negative history for cardiovascular disease, was hospitalized because of a unilateral sudden and painless severe visual loss (20/200) upon waking in the morning. The optic disc appeared hyperemic and edematous, with a focal severe swelling. Relative inferior altitudinal scotoma was present at visual field examination. The patient presented with an erythrocyte sedimentation rate 20mm/h and normal levels of plasma fibrinogen and C-reactive protein. The diagnosis of NAION was made. Two separate and immediately consecutive injections of betamethasone (2mg/0.5ml) and desmopressin (2mcg/0.5ml) were performed in the retrobulbar space. The administration of any preoperative medication or cardiovascular examination was not documented. In the patient's medical records, the total volume of the injections and the size of the needle used are not specified. The procedure was technically uncomplicated without any monitoring in progress during injections such as Electrocardiogram (ECG), peripheral oxygen saturation, or blood pressure measurement. Fifteen minutes later, the patient suddenly developed a cold sweat, dyspnea, thoracic pain, and severe hypotension. ST segment elevation acute myocardial infarction was diagnosed by ECG. Intensive care support was initiated; however, despite cardiopulmonary resuscitation, the patient died from irreversible cardio-respiratory arrest. At autopsy, the heart presented with a normal shape and weight (350g); coronary arteries showed significant atherosclerotic luminal narrowing. Histological investigation showed a stenotic atherosclerotic plaque (95%) complicated by culprit thrombosis of the left anterior descending artery, 2cm after its origin. Examination of the other organs was unremarkable, except for mild pulmonary edema and polyvisceral stasis. There was no evidence of increased orbital volume or sign of vagal compression secondary to retrobulbar hemorrhage.

Professional autonomy in the health care decision-making process renders the physician free to prescribe a drug for purposes other than that for which it has been approved, when the physician considers it both safe and effective according to his/her professional judgment.

The use of unlicensed and off-label medicines is a widely used medical practice, mainly in certain clinical settings. A physician's autonomy in healthcare decision-making represents an instrument to guarantee the progress and evolution of scientific knowledge while also practicing the most effective safeguards for health protection and promotion.

Though off-label prescription use is common and sometimes necessary for providing a pathway to clinical practice innovation, it presents significant risks. This practice may lack rigorous scientific scrutiny and there is little known about the degree of scientific evidence supporting it. According to the literature, a high percentage (approximately the 73% of off-label use) had little or no scientific confirmation. Unexpected adverse effects represent a possible risk. Since the off-label practice may expose patients to avoidable risks, it is mandatory for doctors to follow the lawful direction and ethical recommendation.



Pathology/Biology Section - 2015

This case is out of the ordinary for the forensic scientist because of the possible recognition of medical liability for personal injuries and murder when a patient experiences psychological and/or physical damage or dies as a consequence of the administration of an off label drug.

Desmopressin, NAION, Off-Label Prescription



Pathology/Biology Section - 2015

H31 Death Due to Butane and Propane Gas Inhalation in a Cell Block

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The goal of this presentation is to stress the importance of a correct methodology and multidisciplinary approach in cases where a chemical forensic expert is fundamental for case resolution.

This presentation will impact the forensic science community by illustrating the roles of the forensic toxicology and forensic pathology experts in cases of chemical intoxication by gases.

The case reported for this study occurred in a prison, where a cellmate found a 29-year-old convict unconscious on the bathroom floor of the cell. The cellmate, awoken by the strong smell of gas, rushed into the bathroom and found the man unconscious next to a gas camping stove that was still leaking gas. After resuscitation attempts, the man was declared dead and the body was moved to the morgue. Crime scene officers examined the prison cell. Two camping stoves were found, one in the bathroom and the other on the table of the cell. The deceased convict was a Latin American male, 185cm in height and 84.5kg in weight. External examination showed no evidence of injuries on the body; the presence of subepidermal petechial hemorrhages on the rear region of the chest was reported.

The autopsy revealed an intense passive congestion of the meningeal vessels. Nearly the entire surface of the right lung exhibited emphysematous bubbles, particularly in the inferior lobe. Examination of the heart identified a bilateral dilatation of ventricular cavities and focal myocardial fibrosis with pervious coronary vessels. Specimens of brain, lungs, heart, and coronary arteries were collected for histological analysis as well as specimens of liver, spleen, kidneys, and pancreas. Samples of heart blood, peripheral blood, gastric contents, urine, bile, and vitreous humor were collected during the autopsy and stored at -20°C for further toxicological examination. In addition, samples of brain, subepidermal fat tissue, liver, kidneys, heart and peripheral blood were collected in separate gas-tight containers. Air from the right and left bronchus and from the emphysematous bubbles of the right lung was collected using medical syringes. The gas-tight vials and the medical syringes were stored at -20°C for further toxicological examination. A routine microscopic histopathological study was performed. The lung sections showed marked congestion of alveolar septa and margination of the intralveolar contents with enlargement of alveolar spaces in the form of "blebs". The diffuse presence of subpleural bubbles, peripheral subpleural emphysema, and edema were observed. The heart sections showed stretched and wavy myocardial cells, consistent with dilatation of the ventricular chamber, as well as evidence of focal perivascular fibrosis. Intensive congestion of the liver and kidneys was observed. Toxicological analysis was performed on all specimens reported above. Screening tests performed with immunochemical technique both on urine and on blood detected the presence of benzodiazepines. No ethanol was detected in urine, peripheral, or heart blood. Propane and butane were identified and various concentrations of the two gases were measured by toxicological analysis in all specimens collected, as well as in the air collected from the bronchi and from the emphysematous bubbles of the right lung.

The analytical procedure based on gas chromatography/mass spectrometry analysis and the data obtained will be presented and thoroughly discussed both from the forensic pathology and the forensic toxicology point of view.

Butane and Propane Mixture, Camping Stove, Death in Custody



Pathology/Biology Section - 2015

H32 Effects of Different Killing and Storage Methods on Larval Length of the Blow Fly *Lucilia Sericata*

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The goals of this presentation are to provide attendees with information concerning a survey about the current state-of-the-art in sampling insects from a body and the crime scene and to illustrate a fast and easy way of sampling, killing, and storing entomological evidence.

This presentation will impact the forensic science community by explaining different killing and storage methods and their implications on minimum Postmortem Interval (PMI_{min}) estimation.

Forensic Entomology (FE) is an important tool to estimate the PMI_{min} by using the oldest insect life stage such as fly larvae collected at the crime scene and from the body itself during the autopsy. Sampling, killing, and storing methods are very important because they can influence survival and growth rate of living samples as well as influence the result of the morphometric examination of the dead specimens. There are several manuals on best practices in FE, leading to a certain amount of heterogeneity regarding the methods of sampling and storing insect evidence. Moreover, quite accurate recommendations exist which might confuse or even discourage the crime scene technician or pathologist and lead to unnecessary challenges at court when a report doesn't follow these recommendations. Interestingly, the scientific background for these recommendations and manuals is quite fragile as just a few studies examined so far note the effects of different killing and storage methods. The manuals should be: (1) as simple as possible; and, (2) based on scientific experiments which demonstrate the accuracy and validation of such recommendations. The present study deals with the killing and storage of the forensically relevant blow fly *Lucilia sericata*.

Three hundred newly hatched *L. sericata* larvae were supplied with ground beef *ad libitum* in an incubator at 20°C. For three days, 100 larvae were sampled from the food source every 24hrs and divided in two equal subsamples. The first subsample was killed by Hot (but not boiling) Water (HW) and stored in 75% ethanol. Half were stored at room temperature and the other half in a refrigerator at 6°C. The second subsample was killed by boiling in 75% ethanol and left in this killing solution (HE). Half of these were stored at room temperature and the other half in a refrigerator at 6°C. Lengths of all larvae were measured immediately after killing and every 24hrs until day four, then once more after seven days. This experiment was repeated two times with the same numbers. The data were captured in Excel® 2003 and analyzed with GraphPad® Prism® 5. The Kruskal Wallis Test showed no significant change in length of stored larvae, except for two treatments in the HW group which were stored under room temperature. While 24-hour-old larvae showed a significant decrease in length after four days of storage time (min 0.2cm, max 0.7cm, median 0.4cm vs. after day four min 0.15cm, max 0.68cm, median 0.33cm; p=0.0234), the 72-hour-old larvae revealed a slight significant increase in length after one week of storage (min 0.62cm, max 1.9cm, median 1.15cm vs. after day seven min 0.65cm, max 1.8cm, median 1.35cm; p=0.1157). Despite these significant differences from a statistical point of view, there are no differences which would lead to different age estimations of *L. sericata* as the observed variance in length appears to be negligible; however, the results suggest that it is possible to kill and store fly larvae directly in boiling 75% ethanol without using hot water beforehand. This simplifies the sampling and storing of fly evidence.

Forensic Entomology, Killing, Storage



H33 Optimization of a Method for the Extraction of DNA From Human Skeletal Remains

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After attending this presentation, attendees will better understand the challenges regarding the recovery of amplifiable DNA from bone and the development of a method for maximizing DNA yield from bone while reducing the coextraction of inhibitory substances. Additionally, attendees will learn how the use of multiplex Polymerase Chain Reaction (PCR) and current-generation sequencing technologies to amplify and sequence the entire mitochondrial genome can increase the discriminatory power of mitochondrial DNA (mtDNA).

This presentation will impact the forensic science community by illustrating how maximizing DNA recovery from challenging bone samples with an efficient extraction method will allow for amplification and sequencing of the entire mitochondrial genome with decreased labor and cost than Sanger-type sequencing methods.

It is often challenging to obtain Short Tandem Repeat (STR) profiles from DNA extracted from bone as a result of the low amounts of nuclear DNA present or due to DNA degradation as a result of prolonged environmental exposure. Although STR profiling is preferred due to its discriminatory power, mtDNA analysis is often utilized in these cases. The matrilineal inheritance and lack of genetic recombination enables use of mtDNA to trace maternal lineages, which is particularly relevant in forensic casework in the absence of reference material; however, these attributes also limit the discriminatory power of mtDNA analysis.

The forensic community currently focuses on the analysis of the non-coding control region of mtDNA. The control region contains two hypervariable regions (HV1 and HV2), where the majority of differences between individuals are found. Shared polymorphisms present within the human mitochondrial genome are used to define haplogroups or population lineages. In some instances, it is challenging to discriminate between individuals who share polymorphisms in their hypervariable regions. Expanding analysis of mtDNA beyond the HV region has been shown to increase resolution of common haplogroups that are not resolvable with analysis of the HV regions alone.¹ A multiplex PCR approach has been developed that enables amplification of the entire mitochondrial genome in nine PCR reactions. This, combined with current-generation sequencing technologies, will allow for rapid generation of whole genome sequence data. Sufficient quantities of amplifiable mtDNA must be obtained to utilize this method.

An efficient extraction protocol is required to maximize DNA yield from bone samples while minimizing the coextraction of PCR inhibitors naturally present in bone, such as calcium and collagen. DNA extraction from bone can be thought of in three discrete steps: demineralization, lysis, and purification. Determining which lysis buffer is most effective for bone tissue is a critical first step in optimizing a method for DNA extraction from bone.

Two cross sections (2.5cm x 2.5cm) of bone tissue were excised from a human femur. The tissue was pulverized using a SPEX® 6770 Freezer/Mill®. Bone powder (0.1g) was demineralized using a chelating Ethylenediaminetetraacetic acid (EDTA) solution to disrupt the structural matrix of bone.² Resulting sequestered divalent metal cations were then washed away, and the remaining cellular material was incubated overnight in one of three different lysis buffers (buffers A-C). Lysis buffers A and B were purchased from commercial suppliers. Lysis buffer C is commonly used in forensic casework and is prepared in the laboratory. Lysates were purified using the QIAamp® DNA Mini Kit. Purified extracts were quantified using a human mtDNA quantitative PCR assay.³

Preliminary data suggests that buffer A exhibits consistent performance and often yields higher DNA recovery from bone samples than the other buffers studied, although additional work is needed to further refine these results. Furthermore, sample preparation methods appear to impact DNA recovery. A significant increase in yield was observed when polycarbonate components were used rather than stainless steel components during pulverization. Future method development will include evaluation of different purification methods, specifically the use of commercially available silica spin columns and magnetic bead-based purification systems. A final protocol will then be used to extract DNA from weathered skeletal remains, which will better represent bone specimens encountered in forensic casework. Maximizing DNA recovery will make whole genome mtDNA analysis possible which will result in greater discriminatory power of mtDNA sequence analysis.



Pathology/Biology Section - 2015

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Bone, Extraction, mtDNA



Pathology/Biology Section - 2015

H34 Engaging Undergraduate Students in Forensic Entomology Research: Life History Studies of Three Necrophilous Beetles

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The goals of this presentation are to provide attendees with: (1) knowledge of the development times of two carrion beetle species, *Oiceoptoma inaequale* (F.) and *Necrodes surinamensis* (F.) (Coleoptera: Silphidae) at four constant temperatures; (2) instar determination of the hairy rove beetle, *Creophilus maxillosus* (L.) (Coleoptera: Staphylinidae); and, (3) the value of undergraduate research experience and mentorship in forensic entomology.

This presentation will impact the forensic science community by demonstrating the importance of understanding biological and life history data of common necrophilous beetles collected at a crime scene as well as by emphasizing the need for undergraduate research experience for all students interested in pursuing careers in forensic science.

Insects are poikilotherms (i.e., cold-blooded animals); thus, their growth and development rates are temperature-dependent. Furthermore, the summation of degree hours (thermal units) at a crime scene can be used to predict the overall energy budgets required to complete specific life stages of necrophilous insects present on human remains; however, entomologists must have access to published development data and the species-specific development thresholds for growth (min/max) in order to estimate postmortem intervals based on accumulated degree days. Development data are available for various forensically important fly species belonging to families such as Calliphoridae, Muscidae, and Sarcophagidae. There is limited-to-no controlled, laboratory-derived data available for predatory beetles commonly collected at decomposing remains (e.g., Histeridae, Silphidae, and Staphylinidae).

Watson-Horzelski determined the time of development and survivorship per life stage of the hairy rove beetle, *Creophilus maxillosus* (L.).¹ Following the same rearing protocol, development times were established for two carrion beetle species, *Oiceoptoma inaequale* (F.) and *Necrodes surinamensis* (F.) (Silphidae). Both species are common scavengers and predators of fly eggs and larvae associated with human cadavers and wildlife carcasses. Development studies were conducted at four constant temperatures (16°C, 20°C, 24°C, and 28°C) using a Caron Products® Insect Growth Chamber (Model 6025-1). All study specimens were maintained at 50% relative humidity and a 12:12 hour light:day photoperiod. All immature insects (egg, 1st, 2nd, and 3rd instar, and pupal life stages) were reared in separate containers and inspected twice daily (eggs) or daily (all remaining life stages) for survival, embryo development, time of molt, pupation, and adult emergence. Larval molts to progressive instars were determined by changes in cranial and pronotal width of the silphid larvae and confirmed by the presence of exuvia (cast skin) of the previous life stage.²

Identification of the correct life stage is critical in forensic entomology and for the estimation of time since death. Instar determination for the hairy rove beetle, another common predator of fly eggs and larvae, was performed according to protocols established by Watson and Carlton.² Study specimens of *C. maxillosus* were removed from Southeastern Louisiana University's Forensic Entomology Laboratory colony at known instar stages and preserved in 95% Ethanol (ETOH) (i.e., a total of 144, 153, and 208 1st, 2nd, and 3rd instars, respectively, were selected for the study). Morphological measurements included maximum cranial width, maximum pronotal width, and total body length (anterior margin of clypeus to tip of tenth abdominal segment). All measurements were performed under a Leica® MZ-16 stereomicroscope using digital calipers. The knowledge gained from both of these studies (morphological and development data) will enhance the ability to estimate time since death by providing additional life history information for three common predatory beetles present at the crime scene.

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Forensic Entomology, Postmortem Estimation, Beetles



Pathology/Biology Section - 2015

H35 Effects of Amitriptyline on the Development of *Megaselia Scalaris* (Diptera: Phoridae) and Implications on the Estimation of the Minimum Postmortem Interval

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After attending this presentation, attendees will have novel information about the effect of amitriptyline, a tricyclic antidepressant commonly used in cases of major depressive disorder with a high toxicity in cases of overdose, on the development of a forensically important indoor species, *M. scalaris*.

This presentation will impact the forensic science community by offering new data on entomotoxicology.

The study focuses on amitriptyline, a tricyclic antidepressant, never tested in Phoridae. The last data about effects of this molecule on forensically important flies, such as Sarcophagidae and Calliphoridae, were published in the 1990s.

In forensic entomology, the estimation of the age of the insects is used for the estimation of the minimum Postmortem Interval (mPMI). Insect development rate is mainly temperature dependent despite other parameters like photoperiod, overcrowding, and food availability. In addition, several studies demonstrated that drugs and other chemicals can affect the growth of larvae feeding on the dead body, leading to incorrect mPMI estimations.

Amitriptyline is a commonly used antidepressant in cases of major depressive disorder. It is a tricyclic molecule absorbed in the gastrointestinal tract and metabolized in the liver. This molecule shows a high toxicity in cases of overdose.

Studies on the effect of amitriptyline on insect development and accumulation/excretion have been performed in the 1990s on *Parasarcophaga ruficornis* (Diptera: Sarcophagidae) and on *Calliphora vicina* (Diptera: Calliphoridae) whereas no data are available for other taxa. The results of these studies demonstrated no effect of the molecule on the growth rate. During the same time period, amitriptyline and derivatives were isolated from empty puparia of *Megaselia scalaris* (Diptera: Phoridae) and from skin and fecal material of *Dermestes maculatus* (Coleoptera: Dermestidae) collected from a mummified body in New England.

The goal of this study was to investigate the effect of amitriptyline, often found in cadavers, on the development of *Megaselia scalaris*, a common species found on indoor cases both in Europe and in the United States. This species is very important for mPMI estimation in indoor cases, as observed in this study and reported in the specific literature.

Larvae of *M. scalaris* were reared on pork liver with four different concentrations of amitriptyline (0=control, 120, 240, 800ng/g). One hundred twenty larvae per each concentration were killed in hot water after 48 and 72 hours from the experiment beginning (eggs) and measured using a stereomicroscope equipped with a camera and microscope imaging software with an automatic calibration of the measurements. Pupa and wing measurements were also collected and analyzed.

Statistical tests (one-way and factorial Anova, Tukey post-hoc) were performed using microscope imaging statistical software, using 0.05 as significant level.

Statistically significant differences were observed in the larval size of the four treatments after 48hrs and 72hrs ($F_{3, 476}=62.59$ $p=0.000$; $F_{3, 476}=13.66$ $p=0.000$, respectively). The same result was obtained for the pupa length ($F_{3, 476}=12.42$ $p=0.000$).

The wing size, used in order to detect differences in size in the adults, shows statistically significant differences ($p=0.000$) with the control being smaller when compared to the specimens fed on food with different antidepressant concentrations.

Durations of the immature stage (larval and puparial stages) despite the size differences were not statistically different from the control at all the tested concentrations.

In conclusion, this experiment demonstrated that for *M. scalaris* amitriptyline has an effect on the larval size but not on the total immature developmental time, so the mPMI estimation can be affected if based on the larval size and not on the complete development.

Entomotoxicology, mPMI, Forensic Entomology



H36 Spatial and Temporal Variability in Soils — Their Importance for Intelligence and Forensic Application

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After attending this presentation, attendees will understand the potential of soil metagenomic profiling for determination of soil provenance. This presentation will illustrate the microbial patterns, specifically their spatial and temporal variability, in the soils while demonstrating to attendees the effectiveness of using microbial profiling compared to abiotic information for forensic applications.

This presentation will impact the forensic science community by demonstrating the potential of soil metagenomic profiling to assist in the identification of the soil's geographic location. This presentation will demonstrate the spatial and temporal variability of both biotic and abiotic data in soil, thereby illustrating the effectiveness of soil biotic data for use in forensic applications.

A vast array of information, both abiotic and biotic, is associated with soils. The current ecological hypothesis is that soil type (i.e., chemical/physical properties) is correlated to the community of microbes that inhabit that particular soil type.¹ Therefore, soil metagenomic profiling should produce a distinguishable biotic profile from a specific soil type from a particular geographic location and subsequent DNA and bioinformatics analyses of the soil community could provide a rapid method for soil provenance; however, the intrinsic spatio-temporal heterogeneity of soil also needs to be considered in the community analyses.² Microbial patterns as well as the spatial scale relationship between microbial community composition and environmental variables are largely unknown and it is important to understand these interrelationships for both ecological knowledge and for forensic applications.³ Microbial profiling effectiveness is dependent on the uniqueness among different habitat types, level of heterogeneity within a habitat, and stochastic processes in community over time.

In this study, bacteria, archaea, fungi, and plant universal DNA markers were polymerase chain reaction-amplified, separated by capillary electrophoresis and queried across six soil types in Miami-Dade County, FL, over two seasons (dry and wet; 2010-2011) and again four years later (2014). Abiotic information such as pH, inorganic/organic matter content, content, soil texture (% sand, % silt, % clay), and moisture content was obtained from the soil. Modeling approaches using geographic information systems were employed to study the soil processes and patterns by observing their spatial and temporal distribution using both abiotic and biotic information. The range of the various parameters across seasons (e.g., moisture content: dry=12.69-57.83%; wet=14.26-70.19%; pH: dry=7.15-7.94, wet=6.90-7.73) were correlated to the geographic location and soil type from which they were sampled. For the 2014 dry season, organic content ranged from 6.01%-38.31%, inorganic from 61.67%-93.99%, and carbon 3.01%-19.16%. The organic/inorganic and carbon data are pending for the 2014 wet season.

Soils should display limited temporal variability, in that soils should not change substantially over time, to be able to use pattern modeling for forensic or provenance applications. Bioinformatic algorithms (i.e., random forests and decision trees) were able to classify soils from a particular geographic location with >98% accuracy. The data from this study will add to that soil classification database and will be assessed for the ability to classify soils collected four years apart. These data then strongly verify the use of microbial profiling for provenance of soil.

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Microbial Profile, Soil Provenance, Temporal Variability



Pathology/Biology Section - 2015

H37 SIDS, SUID, and Sudden Death in the Young: Where Do We Stand Today?

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After attending this presentation, attendees will: (1) recognize factors from scene investigation, history, and autopsy which may help in identifying subsets of Sudden Unexpected (or Unexplained) Infant Death/Sudden Infant Death Syndrome (SUID/SIDS) in which the cause and manner of death can be given definitively; (2) understand subsets in which death certification contains uncertainty; and, (3) look forward to research and public health measures which may reduce the incidence of sudden unexpected death in infancy and increase understanding of the cause and manner in some infant deaths.

This presentation will impact the forensic science community by increasing understanding of the reasons behind the historical definition and changing evaluation of SIDS and SUID deaths. Attendees will look forward to future developments in understanding sudden death in the young and in certifying infant deaths.

This presentation briefly reviews the history of forensic medical opinion, and possible future developments, in the cause and manner of death in sudden unexpected deaths in infancy.

SIDS (“crib death”) has been under investigation for more than 40 years. During the decades of active research, numerous mechanisms of death have been proposed, many of which did not translate into clinical success in prevention. The most successful strategy, since its proposal by the American Academy of Pediatrics Task Force on Infant Sleep in 1992, has been supine positioning (“Back to Sleep”). Although the mechanism of underlying vulnerability in the triple-risk model is not understood, it is now universally agreed that babies are less likely to die suddenly and unpredictably in the first months of life if laid face up to sleep.

The advent of doll-scene reenactment provided startling evidence that some of the decrease in death rates was due to prevention of accidental suffocation in unsafe infant sleep conditions. As this was acknowledged in the forensic medical community, particularly after the Centers of Disease Control and Prevention (CDC) began to train death scene investigators in doll-scene reenactment, part of the ongoing decline in SIDS incidence was due to reclassification of many infant deaths as due to either asphyxia from accidental suffocation or SUID. SUID, often with the manner undetermined, is certified in infant deaths in which scene factors may play a role but the role cannot be determined definitively. This may include deaths during bed sharing with siblings, parents, and others; deaths in soft bedding which may result in suffocation; and deaths on sleep surfaces that are unsafe for small infants, such as couches, air mattresses, and beds intended for adults. The CDC now considers SIDS to be a subset of SUID.

Analysis of consecutive cases of SIDS/SUID examined over a two-year period in the Tidewater District branch of the Office of the Chief Medical Examiner reveals a continuum of cases ranging from unquestionable accidental suffocation, through suspected but unproven suffocation, to clear SIDS. Review of these cases suggests a considerable component of accidental suffocation in the majority of these infant deaths. Examination of the occurrence rate of SIDS/SUID in the different health districts of Virginia suggests that prenatal and postnatal training in safe sleep may have a significant effect on the incidence of SUID/SIDS.

Deaths in which parental training makes no difference may be those in which the mechanism of death is not related to suffocation. Despite research into brainstem receptors, ion channel abnormalities (some with long QT interval), defects in normal arousal systems, and different genetic alleles for metabolizing nicotine, the mechanism of death, if it is not accidental suffocation, is still not understood. The role of accidental suffocation, except in the most obvious deaths, remains unclear. At this time, often the best medical judgment is used to give parents whatever answers are available, without sufficient data to feel certain of the diagnosis.

The involvement of the CDC in the development of a Sudden Death in the Young database offers the possibility of separating out subsets of SUID, defined by testing, which would give definitive postmortem diagnoses. The database is still in its earliest stages and is still reliant on medical judgment in an area where judgment alone may not always be sufficient. As data accumulates and is analyzed, hopefully the future may see further decreases in deaths in infancy and more accurate certification of the deaths that do occur.

SIDS/SUID, Death Certification, Sudden Death in the Young



Pathology/Biology Section - 2015

H38 Deaths Due to Child Abuse: A Five-Year Review of Cases in the Cook County Medical Examiner's Office

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After attending this presentation, attendees will have a better understanding of the incidence and the significance of the injuries observed in fatal child abuse cases.

This presentation will impact the forensic science community by providing a review of the pattern of injuries in deaths due to child abuse.

Deliberately inflicted pediatric injuries are a significant issue in forensic pathology, with special regard to head injuries. Often questions arise regarding the plausibility of possible mechanisms of injury. This study reviewed case files from the Cook County Medical Examiner's Office from 2007 to 2012 to study the characteristics of homicides due to child abuse in children less than three years of age. The manner of death was determined to be homicide based on the autopsy findings and the investigation.

The cases were divided into the following age ranges: 0-11 months (group A); 12-23 months (group B); and, 24-35 months (group C). Data regarding age; race; sex; facial injuries; scalp injuries; presence and types of skull fractures; suture diastasis; extradural, intradural, subdural, and subarachnoid hemorrhages; cerebral edema; intracranial pressure; intracerebral findings; retinal and optic nerve hemorrhages; spinal cord findings; and extracranial injuries were collected using an electronic spreadsheet. Clinical details and information concerning the traumatic mechanism, where available, were also considered.

Fifty-one cases (25 females and 26 males) were identified. Most of the cases fell into the group A (26 cases; 51%), followed by group B (17 cases; 33%), and by group C (8 cases; 16%). Females were more numerous than males in group A (15 cases; 57%); in group B, males were more numerous (11 cases; 65%); in group C, children of both sexes were present in equal number.

External signs of impact to the head, that is to the face and/or scalp, were present in 42 cases (82%). Most of the examples of impact to the head consisted of bruises and abrasions of the skin and/or lacerations of the oral mucosa. Skull fractures were reported in 9 cases (18%), and showed a predominately linear appearance. Diastasis of the sutures was reported in 14 cases (27%). As might be expected, most of these examples were found in the youngest age range. Epidural hemorrhage was found in 10 cases (20%). Subdural hemorrhage was seen in 42 cases (82%). Subdural hemorrhage represented the most common intracranial pathology encountered, apart from cerebral edema, which was found in each case. Subdural hemorrhage was described as remote or recent, and/or with a thin film or layer of blood or a larger collection of blood. Subarachnoid hemorrhages were present in 27 cases (53%).

In 24 cases (47%), there were intracerebral findings, such as hemorrhages, contusions, herniations, and areas of infarction. In eight cases (16%), brain evaluation was affected by marked non-perfusion changes (so-called "respirator brain"). Retinal hemorrhages were a very common finding in head trauma (38 cases, 75%). Most were bilateral and sometimes involved multiple layers of the retina (preretinal, intraretinal, subretinal). Optic nerve hemorrhages were present in 37 cases (73%). Intravitreal hemorrhages and macular folds were rare. In 19 cases (37%) there were spinal cord injuries, such as hemorrhages, but no spinal fractures were observed.

This study reveals that in the cases surveyed, the majority of children who sustained abusive injuries fell into the 0-11 months of age range. Head injuries were the leading cause of death. Signs of impact to the head defined by the presence of skull fractures and/or bruises/abrasions to the head, often together with intracranial injuries, were present in 73% of the cases (19 out of 26) in group A, 94% (16 out of 17) of the cases in group B, and 88% (7 out of 8) of the cases in group C. As expected, epidural hemorrhages were rare and spinal fractures were not observed at all.

Death due to extracranial injuries was an uncommon finding and it occurred when internal organs were damaged. Among these, blunt force abdominal injuries were the most commonly encountered (10 cases; 19%).

These findings agree with other researchers who have found that head injuries are the leading cause of death in children less than three years of age. Moreover, in the cases reviewed for this study, retinal hemorrhages seemed to be strictly associated with head injuries.

Child Abuse, Head Injury, Homicide



H39 Reclassification of Sudden Infant Deaths in New Mexico

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After attending this presentation, attendees will understand the complexities surrounding the certification of unexplained infant deaths. Attendees will learn how changing classification schemes have impacted the downward trend of infant deaths after the introduction of the “Back to Sleep” campaign in New Mexico. Additionally, attendees will be able to apply knowledge gained to augment and standardize the practice of coding sudden infant deaths.

This presentation will impact the forensic science community by the presentation of a collaborative effort in reclassification of infant death in New Mexico and how this has impacted appropriate coding and standardization of these cases, particularly taking into account the diagnostic shift in manner of death. Standardization in coding necessitates that medical death investigators are familiar with the changing classification scheme and the implications for statistical tracking of infant death cases.

Background: The classification “Sudden Infant Death Syndrome” (SIDS) was coined to place sudden unexpected deaths of infants with no apparent cause of death into a category of exclusion to facilitate research. In practical use, the classification became a diagnosis, implying that SIDS was due to underlying disease processes; however, as scene investigation improved, forensic pathologists have realized that this is a far more heterogeneous group, consisting of cases of undiagnosed natural diseases, accidental suffocations associated with unsafe sleep environments, and covert homicides. Infant deaths certified as SIDS have decreased, in the decade surrounding the implementation of the American Academy of Pediatrics’ “Back to Sleep” Campaign introduced in 1994. As this decline represented a decrease in the number of accidental asphyxia deaths, it demonstrates that these deaths were erroneously being classified as SIDS. The other reason for the decline in SIDS death certifications is also obscured by a diagnostic shift from SIDS to “undetermined.”

Purpose: The intent of this study is to determine the extent of variation in certifying sudden unexpected infant deaths that exists among pathologists at a statewide medical examiner’s office and to investigate the changing trends of certifying these deaths in New Mexico.

Methods: A computerized query of all infant deaths less than one year of age between 2006 and 2011 yielded 134 cases originally certified as SIDS, Sudden Unexplained Infant Death (SUID), asphyxia, suffocation/strangulation, and undetermined. Scene, autopsy, and ancillary testing data were extracted from electronic and paper records and summarized into reports with accompanying scene and autopsy photographs when available. Nine forensic pathologists blinded to the original certification rendered a new cause/manner of death using current conventions. New diagnoses were compared to the original diagnoses as listed in the computerized records system and their corresponding ICD-9 coding by the Bureau of Vital Statistics using inter-observer agreement analysis (kappa statistics).

Results: The 134 study cases fell into three broad, original diagnosis categories: SIDS/SUID (66 cases), asphyxia/suffocation (23 cases), and undetermined (45 cases). Study population demographics demonstrated that 56.7% of the cases involved co-sleeping with an adult and 31% of cases were positioned prone to sleep.

When compared to original certification, a diagnosis of SIDS was not rendered by any pathologist in the study. All 66 cases originally coded as SIDS were classified to alternatives such as suffocation/strangulation or undetermined, with rare exceptions by three different pathologists involving natural deaths due to infectious causes or congenital anomalies. The pathologists reclassified more SIDS cases as undetermined (range 30-50 cases between pathologists) than as asphyxia/suffocation (range 14-34 cases between pathologists). Of the 23 cases originally called asphyxia/suffocation, most remained as such (range 8-21); however, some pathologists reclassified them as undetermined (range 2-15). The 45 undetermined cases largely remained undetermined (range 23-40), yet many were reclassified as asphyxia/suffocation (range 5-19), infectious (range 1-3), or as due to other causes (1).

Overall, poor agreement was observed between study pathologists and the original cause of death assigned to the infant deaths, with kappa statistics ranging from -0.15 to -0.01. The comparison of assigned manner to the originally assigned manner resulted in slightly better agreement, with kappa statistics falling in the “slight” to “fair” classifications of 0.08-0.18. When comparing certifications between reviewing pathologists only, moderate agreement was identified between the most experienced and the most junior pathologists, with a kappa of 0.46 and 0.42 for cause and 0.45 and 0.46 for manner.



Pathology/Biology Section - 2015

Conclusions: The practices of certifying SUIDs have shifted significantly over time, which may account for a large proportion of the decline in deaths certified as SIDS. Although poor inter-observer agreement existed between the original and reviewing pathologists in this study, no reviewing pathologist coded any study case as SIDS/SUID, accurately reflecting a diagnostic shift in the field. This study demonstrates the importance of consistency in the approach to certifying unexplained infant deaths.

Sudden Infant Death Syndrome, Sudden Unexplained Death, Classification



Pathology/Biology Section - 2015

H40 A Re-Examination of Patterns of Abuse in Children

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After attending this presentation, attendees will recognize the importance of anthropological skeletal analysis in pediatric cases and the recognition of fracture patterns associated with Non-Accidental Injuries (NAI) in children, including spiral fractures in long bones.

This presentation will impact the forensic science community by supporting the need for the combination of forensic pathology with trauma anthropologists during autopsy, expanding the definition of injuries associated with NAI, and reinforcing the need for a comprehensive understanding of bone biomechanics and the total trauma pattern.

The recent inclusion of in-house forensic anthropologists in medical examiners' and coroners' offices has evolved trauma anthropology. Trauma anthropologists use their training in human skeletal anatomy, biomechanics, and trauma interpretation in fresh autopsies to recognize and decipher the patterns associated with fracturing, allowing for a complete view of injury patterns. In pediatric cases, trauma anthropologists have revealed fracture patterns associated with NAI that may be overlooked in digital images and the forensic pediatric autopsy. Examination of the underlying bone by a trauma anthropologist is a key component to fully illustrating all injuries in pediatric cases. Fracture patterns associated with NAI include Classic Metaphyseal Lesions (CML), posterior rib fractures, complex skull fractures, and multiple fractures of varying ages.^{1,2} Spiral fractures are not accidental as some research suggests, rather they may be non-accidental patterns in all cases, and especially in non-weight bearing bones and when the child is not independently mobile.³

Biomechanical interpretations reveal how bone behaves as a material as well as to how bones behave in life; factors vital in understanding skeletal injuries.⁴ Spiral fractures specifically are a result of torsional forces acting in tension and shear on the movable (non-stable) end of the bone. Understanding biomechanics, fracture production, and directionality of fracturing allows for the anthropologist and the pathologist to understand events surrounding and leading to death.

A case of a four-month-old male is presented as an example of trauma anthropology contributions to autopsy findings. The case originally came to the medical examiner's office as an infant found unresponsive in his crib. The pathologist found multiple acute posterior rib fractures at autopsy and requested an anthropological examination of the injured ribs and all long bones to examine for CMLs. The anthropologist observed the previously noted posterior rib fractures, but also discovered undiagnosed injuries including sternal rib end fractures, evidence of healing rib trauma, and two acute spiral fractures to long bones. The latter injuries contributed to the pathologist's findings by providing a better understanding of a pattern of non-accidental inflicted trauma. The pair of spiral fractures in long bones represent severe and duplicated trauma to an infant and the healing rib fracture establishes a pattern of abuse. These findings are "enablers" for a child abuse investigation, scrutiny of caregivers, and eventual prosecution in cases of demonstrated abusive behavior. While everyone wants to believe the caregivers' story in a child death case, a total body trauma examination may substantiate or change an accidental death to a suspicious death or homicide. In this case, an immediate confrontation with caregivers culminated in a full confession of the mother, with specific accounts of the lethal event.

This presentation will discuss the biomechanics behind the creation of NAI and, in particular, spiral fractures and their omissions in literature as patterns associated with NAI in children. Obviously, the team approach between coroners, forensic pathologists, and trauma anthropologists is suggested in order to diagnose injuries that may be overlooked when a skeletal survey is not routinely conducted on infant deaths.



Pathology/Biology Section - 2015

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Trauma Anthropology, Spiral Fractures, Non-Accidental Injuries



Pathology/Biology Section - 2015

H41 Autopsied Cases of Suspected Child Abuse Showing Absence of Clinically Diagnosed Injuries

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After attending this presentation, attendees will understand that all that glitters is not gold when it comes to pediatrician-diagnosed child abuse.

This presentation will impact the forensic science community by exposing myths of pediatrician diagnoses of child abuse.

Unfortunately, child abuse cases occur, usually at the hands of the boyfriend of the child's mother. These cases are generally easily diagnosed and classified.

The forensic pathology cases investigated from 2010 to July 2014 total 420. The cases labeled as child, meaning newborn to age 12, number 34 or 8.1 % of the cases investigated. Of these, one was performed for the prosecution and 33 were for the defense. Of these 34 cases, ten were characterized by absent or trivial subgaleal hemorrhage, positive subarachnoid hemorrhage, positive subdural hemorrhage, and encephalomalacia. Two cases were diagnosed as having skull fractures in life, none of which were demonstrated at autopsy. Two cases were diagnosed as having "classic bucket handle metaphyseal fractures," none of which were demonstrated at autopsy. The remaining 24 cases revealed evidence of child battery including ruptured jejunum, multiple blunt impact injuries to the head and other areas, or non-accidental burn injuries.

Resuscitation by closed chest cardiac massage was first described by W.B. Kouwenhoven et al. in 1960. In 1964, Wilder reported on training ambulance personnel in the technique and reported no injuries. This was followed by legislative action which brought cardiopulmonary resuscitation to almost all areas of the United States by 1970.¹⁻³

In 1971, A.N Guthkelch wrote in the *British Medical Journal* that infants without evidence of subgaleal hemorrhage had subdural hemorrhage. Citing the work of Ommaya, he proposed that non-impact shaking could cause these injuries.^{4,5} As time has gone by, the three primary signs ("triad") of shaken baby syndrome have become entrenched in the medical literature and in pediatricians' training. The appearance of retinal hemorrhage, subdural hematoma, and encephalomalacia diagnosed in life has become the hallmark of criminal child abuse and has caused many caretakers and/or parents to be arrested and charged with a capital offense. In addition, Dr. Kleinman has written extensively and almost exclusively about the "classical metaphyseal fractures" seen in abused infants. Dr. Ayoub has written recently and criticized Dr. Kleiman's theories.^{6,7}

In this study, ten cases diagnosed in life and at autopsy are presented. Only one case had subgaleal hemorrhage and it was extremely small. Nine of the ten cases had no blunt impact injury to the skull as demonstrated by absent subgaleal hemorrhage. Two cases had clinically diagnosed classic metaphyseal fractures which were not demonstrated at autopsy. Two cases had clinically diagnosed skull fractures which were also not demonstrated at autopsy.

In spite of the negative autopsies, all ten deaths resulted in the prosecution of adult care takers, as abusive head trauma. Recommendations concerning proper autopsy techniques in suspected child abuse cases are presented. Suggestions concerning proper cause and manner of death statements are presented as well.

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Child Abuse, Infant, Autopsy

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Pathology/Biology Section - 2015

H42 Evaluation of Rib Fracture Injury Modes and Biomechanics in Abused Infants

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After attending this presentation, attendees will understand the biomechanical principles underlying fracture patterns in the infant rib cage. Non-accidental rib fractures in children have been studied for decades, but the forensic application and interpretation of bone injuries is unclear due to variation in: (1) research methodology; and, (2) fracture visualization techniques in the radiographic, medical, and anthropological literature.

This presentation will impact the forensic science community by contributing to the knowledge of the biomechanics, modes of failure, and the patterns of injury in the infant rib cage, and the potential for the application of this knowledge in the diagnosis of rib fractures in cases of child abuse. Rib fractures in infants are often difficult to detect through radiographic images, so increased forensic anthropological knowledge on the subject can help anticipate Non-Accidental Trauma (NAT).

Rib fractures are pathognomonic for abuse in young children.¹ Violent squeezing, shaking, punching, or combinations of assaults are reported to predictably create failures in the posterior, anterior, and lateral arcs of the rib cage. Often, anterior-posterior compression is offered as an explanation of fractures to the rib heads and costotransverse processes as well as the anterior costochondral areas of infant chests; however, lateral fracture production in the chest remains unclear in the literature.² Many researchers include lateral arc fractures as part of the same anterior/posterior bending failure associated with shaking and compressive injuries.

To address this discrepancy in the literature, fracture patterns were observed in ten NAT infant rib fractures. Rib fractures were recognized at autopsy, removed, and examined in a dry state with a stereomicroscope. Bone failures were assessed in terms of tension and compression.

Posterior and anterior arc fractures are by far the most observed; lateral arc fractures were rare, despite their common mention in the literature. Tension failure in the rib heads occur anteriorly as the rib neck is levered over the transverse process; failure first occurs in the area where the rib head is thin yet firmly attached to the vertebra body. The same bending motion may also create fractures in the area of the transverse process articulation. The latter fractures react like bending long bones diaphyses, where bone is highly resistant in compression due to thickened cortical bone, so failure occurs initially in tension on the internal rib surface.

Anterior rib fractures are a dissimilar reaction of the chest to posterior rib fractures. The cortical bone of the front rib is thin and trabecular bone is prominent. The structure is similar to bone metaphyses and epiphyses, where bone is weak in compression but capable of repeated trauma. Buckle fractures occur in front rib shafts and damages to costochondral junctions are most often observed in circumstances of anterior/posterior compression where flexible bone fails initially in compression.

The commonly described lateral arc failure scenario would suggest failure with compression internally and tension externally; however, if midshaft rib failures are more commonly associated with direct impacts, the biomechanics of failure should demonstrate the opposite direction of bending (i.e., an impact to lateral ribs would initially fail internally in tension). This lateral scenario appears to be a likely consequence in the non-accidental bone injury cases observed.

The recognition of tension/compression bone failure confirms the mode of bone bending and goes beyond spatial recognition and stereotypic explanations for rib fractures. Assessing skeletal injuries from a biomechanical perspective reveals specific relationships of bone injuries and conceivably facilitates the evolution of bone trauma interpretation as a scientific discipline. Therefore, fresh and healing fractures may reveal additional information concerning skeletal element failure. In the current study, lateral rib fractures appear to be rare and not a result of anterior-posterior squeezing.

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Infants, Child Abuse, Biomechanics of Rib Fractures



Pathology/Biology Section - 2015

H43 Rebleeding Into Subdural Neomembranes and the Myth of “Two in a Row” in Childhood

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After attending this presentation, attendees will gain an understanding of subdural neomembranes, their rebleeding tendency, and their relevance in unexplained deaths in childhood.

This presentation will impact the forensic science community by enhancing the knowledge of subdural neomembrane pathophysiology and providing an evidence-based assessment of the practical realities surrounding neomembranes in children, including the likelihood that the “two in a row” theory, or two accidental sublethal injuries, might explain a catastrophic neurologic event.

Whether small subdural collections or subdural neomembranes may predispose to rebleeding after minor head trauma is a point of debate in the criminal defense world.¹ Complicating the issue is the fact that chronic subdural collections by themselves in children have a significant likelihood of being caused by abuse, while rebleeding into chronic subdural collections is common in older age groups and may be encountered with trivial, or unrecognized, head injury. In effect, a traumatic lesion that is often non-accidental in children is used somewhat ironically as a means to lessen the culpability of potentially the same perpetrator who later inflicted an acute process that lead to catastrophic neurological collapse.

This issue is illustrated by the case of a 3-month-old boy who presented to the emergency department in a coma after falling off a couch onto a carpeted floor, per the father’s account. After initiation of resuscitative efforts, evaluation revealed acute subdural, subarachnoid, and intraventricular hemorrhages, bilateral retinal hemorrhages, and a healing right 11th rib fracture. The child was the product of a twin gestation; the twin was also found to have healing fractures. Despite resuscitative efforts, the infant continued to decline and expired after being declared brain dead. Autopsy examination revealed bilateral subdural and subarachnoid hemorrhages, bilateral retinal hemorrhages, a healing 11th rib fracture, diffuse cerebral edema with transient global ischemic necrosis, and a subdural neomembrane involving the falx cerebri. Minimal acute blood was associated with the neomembrane.

In this case, the accident as reported by the father was vigorously defended on the basis of the neomembrane, in effect indicating that the neomembrane facilitated the neurologic decline with trivial head trauma, consistent with a fall off a couch onto a carpeted surface.

In addition to this illustrative case, the pathophysiology of subdural neomembrane, subdural rebleeding, and accidental as well as non-accidental blunt trauma in children is reviewed, along with a review of the pediatric literature on acute and chronic subdural hemorrhage.

In conclusion, only rare cases of subdural neomembrane rebleeding are recorded in the literature, and no prospective data are available. Moreover, no cases of acute neurologic collapse due to rebleeding into a neomembrane have been described to date, suggesting that the “two in a row” theory does not occur in humans, as is often suggested at trial.

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Subdural Neomembrane, Rebleeding, Child Abuse



Pathology/Biology Section - 2015

H44 Intrauterine Liver Disease and Sudden Unexpected Infant Death: Causally-Related or Coincidence?

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After attending this presentation, attendees will understand the complexities of intrauterine liver diseases and specifically Gestational Alloimmune Liver Disease (GALD) in which transplacental maternal antibodies lead to fetal and neonatal liver disease with highly variable clinical and histopathologic features. Prior to recent understanding of the pathogenesis, liver transplantation was frequently required in order to save the lives of patients in whom the clinical diagnosis had been made antemortem.^{1,2} Attendees will be introduced to the forensic significance of this and related intrauterine liver disease as mimics of abusive injury and will understand the importance of making a correct diagnosis.

This presentation will impact the forensic science community by informing attendees of recent advances in the understanding of intrauterine and neonatal liver disease by discussion of three cases, all presenting as sudden unexpected infant death in which fathers were charged or convicted of murder. The presentation will emphasize the clinical features of the disease and its histopathologic features at autopsy. It will discuss the differential diagnosis of gestational alloimmune liver disease in the face of sudden unexpected death in infancy and its role as a natural disease mimicking inflicted trauma. Forensic pathologists will learn of simple and inexpensive ways of confirming or excluding the disease at autopsy in cases of sudden unexpected infant death.

Studies of abusive head injury by investigators in various disciplines over the past decade have confirmed the presence of numerous mimics of abusive head trauma. These mimics include natural disease as well as accidental injury. The differentiation of abusive trauma from its mimics is increasingly important in terms of the financial and human costs to society of the litigation and incarceration of the innocent.

Gestational liver disease has only been studied intensively for the last two decades and by relatively few institutions in the United States and Europe. At the present time, the understanding is incomplete and much basic research needs to be done in various areas.

This presentation will discuss the details of a series of three autopsy-confirmed cases of intrauterine liver disease which were accompanied by unexpected death under unusual circumstances in the first three months of life. Legal proceedings in two of the cases resulted in convictions with lengthy prison terms; the third resulted in acquittal. After reviewing the first of the three cases, the sensitivity to this diagnosis was heightened and two other cases were seen in the subsequent two-year period. In two of the three patients, terminal cardiorespiratory arrest had precluded any clinical investigation and in the third case the clinicians did not entertain the diagnosis due to its protean clinical presentation, resulting in minimal investigation prior to autopsy. In different published series, the clinical course of the disease has varied from benign or virtually asymptomatic to rapidly lethal. The associated liver findings have also been highly variable, ranging from little or no clinical evidence of liver disease with essentially normal liver histology through advanced liver disease with hepatic failure and/or cirrhosis. Biochemical testing (liver function tests) are also highly variable and normal transaminase levels are common. Likewise, blood ammonia levels are variable.

Abnormalities of iron metabolism appear to be the most common feature and prior to a decade ago was widely referred to generically as Neonatal Hemochromatosis (NH). The histological hallmark of NH was typically regarded as accumulation of storage iron outside the reticuloendothelial system and in epithelial cells in a variety of organs; however, reticuloendothelial iron may be seen in this disease and was the key initial finding in one of these three cases. The basic criterion for the diagnosis remains the presence of stainable iron in one or more of various epithelial tissues including liver, pancreas, thyroid, adrenal, and minor salivary glands.

In cases of sudden unexpected death in infancy, there is typically inadequate time for clinical investigation and therefore the diagnosis must be made on the basis of autopsy findings. These findings typically require an index of suspicion but are generally easily confirmed if appropriate iron staining is done. This presentation will recommend routine iron staining of liver, pancreas, thyroid, and adrenal medulla in all infants under six months of age. Even when the correct diagnosis is made, it may not be possible to define a specific linkage between the disease and death in any given case, making assessment of "reasonable doubt" difficult. This difficulty notwithstanding, it is important for the trier of fact to be given all of the information generated at the autopsy.



Pathology/Biology Section - 2015

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Liver Dease, Infant Death, Child Abuse



Pathology/Biology Section - 2015

H45 Fatal Aortoesophageal Fistulae Due to Foreign Body Ingestion in Young Children: Presentation of Two Cases

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After attending this presentation, attendees will understand how an aortoesophageal fistula may develop, the potential subtleties of clinical presentation, and will be aware of the hazards of ingesting coin batteries.

This presentation will impact the forensic science communities by increasing knowledge of the pathology of aortoesophageal fistulae and factors leading to their development. This knowledge may lead to greater public health awareness and prevention of deaths due to ingested objects, including coin batteries.

Foreign body ingestion is common in young children, with close to 80,000 cases reported to the American Association of Poison Control Centers' National Poison Data System in 2011. In the majority of cases, foreign body ingestion will not have an adverse outcome; however, it can lead to more serious consequences, including respiratory complications, esophageal erosions, or even an Aortoesophageal Fistula (AEF). In 2011, two deaths due to disc battery ingestion were reported in children under the age of five years.

At the Tarrant County Medical Examiner's Office in 2013 and 2014, two children less than two years of age with sudden onset of upper gastrointestinal hemorrhage and death were autopsied. Case 1 was a 14-month-old female in a store shopping cart who developed seizure-like activity and copious bleeding from the mouth prior to becoming unresponsive. At autopsy, two opposing round mid-esophageal ulcerations were present, with formation of an AEF. Blood was in the stomach and small bowel almost to the cecum. A foreign object was not identified. The child was evaluated by a pediatrician two weeks prior for vomiting and crying and was treated for bilateral otitis media. When she returned home, she was unable to swallow solid foods, though she could take liquids. This resolved within one week.

Case 2 was a 19-month-old male brought to an emergency room by a babysitter for blood coming from his mouth. A radiograph showed a foreign object in the stomach. The child died prior to transport to a tertiary care center. At autopsy, an esophageal ulceration with an AEF and a bronchoesophageal fistula were present, with blood and a large lithium coin battery present in the stomach. Three days prior to death, he was seen in an emergency room for a "barking" cough and was diagnosed with croup.

Esophageal foreign bodies as a group require early intervention because of their potential to cause complications, including erosion, periesophageal abscess, and fistula formation between the aorta and/or the tracheobronchial tree. Button or coin batteries are particularly hazardous due to their size and shape as they are easily swallowed and become lodged in the esophagus or airway. These batteries are used in many household items including flashlights, toys, watches, hearing aids, and remote controls. Lithium batteries generate current flow through saliva which hydrolyzes water, creating alkaline hydroxides that result in caustic tissue damage and rapid perforation. If untreated, AEF can lead to massive gastrointestinal hemorrhage with exsanguination, shock, and death as seen in both presented cases. A coin battery was found in the stomach in case 2. While no foreign object was found in case 1, the ulceration had characteristics similar to a round object. In retrospect, both children had prior symptoms of foreign body ingestion with partial obstruction that led to their evaluation by a physician; however, the diagnosis was not made. As evidenced by these cases, the presenting symptoms may be vague and easily confused with other common childhood illnesses. It is important for pediatric practitioners to be aware of the dangers of foreign body ingestion and the potential for formation of aortoesophageal fistulae, especially with the increased use of lithium coin batteries in household items.

Aortoesophageal Fistula, Foreign Body Ingestion, Coin Battery



Pathology/Biology Section - 2015

H46 Mortality Related to Falls From the Balcony of Children Younger Than 18 Years of Age

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After attending this presentation, attendees will understand the importance of the prevention of childhood deaths due to falls.

This presentation will impact the forensic science community by providing data related to childhood deaths due to falls from balconies.

In this presentation, 32 cases from the Istanbul Morgue Department's archive of victims less than 18 years old who fell from balconies were analyzed and compared to other reports in the literature for age, sex, cause of death, time of year, hospitalization period, autopsy findings, toxicology results, and relevant social responsibilities. Similar to developed countries that rank falls as the fourth-leading cause of death in children, falls are a major problem in Turkey.¹ Falls account for 5.9% of all childhood deaths.² Childhood falls are the source of 25%-34% of emergency responses in Unites States.³ One study conducted in Turkey found that 11.9% of all deaths occurring between the ages of zero to ten years are the result of a fall from a balcony, with the percentage increasing each year.^{4,5} In a study by Yayci et al. in Istanbul, falls ranked fourth and represented 15.5% of all trauma-related deaths reported during 2001-2005.⁶

This study retrospectively analyzed 34 autopsies performed during 2008-2012 in the Istanbul morgue in which a fall resulted in the death of a child less than 18 years of age. This analysis included each victim's age, sex, cause of death, time of year, hospitalization period, physical findings at autopsy, and toxicological facts. Eighteen cases (52.9%) of the victims were female and 16 (47.1%) were male. The youngest victim was 11 months old and the oldest was 17 years, 11 months old. The cause of death in 31 (91.2%) of the cases was extensive body trauma and in three cases (8.8%) death was reportedly due to blunt force head trauma. While there was no information about the height of the building where 22 (64.7%) of the victims had fallen, there was documentation that five of the victims had fallen from the 5th-floor balcony. The lowest height was the first floor and the highest was reported to be a 7th-floor balcony. Distribution according to time of year when the deaths occurred showed the highest rate of falls occurred in summer (n=15) and the lowest in winter (n=4). The maximum duration of hospitalization was reported to be 33 days and 22 (64.7%) of the cases had no record of hospitalization at all. Toxicological examination was insignificant in 20 (58.8%) of the cases while three (8.8%) cases were positive for therapeutic levels of medication; one (2.9%) case was positive for ethanol and one (2.9%) was positive for n-butane.

Many studies have concluded that the reason for an increase in fatalities due to falls during the summer months is because children use balconies more often during the warm season.⁷ Reports prepared by American Academy of Pediatrics Committee on Injury and Poisoning Prevention discuss in detail measures and recommendations on ways to reduce falls from windows, balconies, and rooftops.⁸ From a social perspective, to reduce the number of fall-related deaths, various public-awareness training programs should be prepared. Additionally, building-code regulations related to safety should be broadened and enforced.



Pathology/Biology Section - 2015

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Autopsy, Fall, Children



Pathology/Biology Section - 2015

H47 Shaken Baby Syndrome/Abusive Head Trauma: An Ongoing Debate Not Yet Resolved

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After attending this presentation, attendees will understand the main diagnostic criteria of shaken baby syndrome or abusive head trauma (the preferred term). Emphasis will also be placed on the ongoing challenge in everyday forensic practices when faced with ambiguous cases requiring a differential diagnosis between either an abusive or a resuscitative shaking of a baby.

This presentation will impact the forensic science community by promoting a multidisciplinary approach to clarify the pathophysiologic mechanism in explaining the nature of the injuries and establishing definitive signs to reach the most reliable diagnosis according to the clinical history and parental interview.

This study will present the case of a 5-month-old female baby who was found unconscious on the floor by her father after accidentally falling from the crib. The father immediately tried to resuscitate the baby by violently shaking her after grabbing her thorax and arms. The attempt to resuscitate failed and the man took his daughter to the Emergency Department (ED) where she arrived unconscious. On admission to the ED, the baby presented with an ecchymosis on the left hemi-thorax, an abrasion on the right arm, bilateral retinal hemorrhages, and subarachnoid hemorrhage involving the frontal lobes and around the tentorium. A medicolegal assessment was requested to establish if the case was suitable for referring the matter to the state prosecutor's office.

Shaken baby syndrome is a type of abusive or non-accidental head trauma that occurs when a child is violently shaken and therefore acceleration/deceleration/rotational forces are impressed on the victim's body, with or without impact. In the last decades, "shaken baby syndrome" has been one of the most used terms in pediatrics; however, the preferred term is now "Abusive Head Trauma" (AHT), which is defined as an injury to the skull or intracranial contents of an infant or young child (<5 years of age) due to inflicted blunt impact and/or violent shaking. Despite the wide range of clinical manifestations, AHT has classically been diagnosed by the presence of the so-called "triad," characterized by subdural hemorrhage, retinal hemorrhage, and neurological dysfunction. These three major clinical findings may be associated with various non-specific clinical signs such as skin lesions and bruising, bone injury mainly involving the ribs, and injuries to the neck muscles, the spine, or the spinal cord.

In the presented case, the medical history and interview were of concern to the physicians, especially as the father admitted shaking the baby in an attempt to resuscitate his daughter. Indeed, the clinical findings were suggestive of shaking. Moreover, innocent shaking related to resuscitation of an unconscious or semiconscious baby in an attempt to stop seizures, prevent suffocation, or waken a sleeping baby is reported in the literature.

Distinguishing between a real wrongdoing and an innocent action is often difficult from a clinical and forensic point of view. The main consequence of this differential diagnosis is represented by the decision to refer the matter to the state prosecutor. In any case, the "absolute proof" of abuse is not necessary to report an event to the state prosecutor's office. In the case presented, the severe neurological dysfunction sustained by the patient and the ambiguous anamnestic data, together with the referred accidental fall, justified reporting the episode to the state prosecutor's office.

Shaken Baby Syndrome, Abusive Head Trauma, Report to the State Prosecutor



H48 Do the Eyes Have It? Traumatically Induced Primary Optic Nerve Sheath Hemorrhage

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After attending this presentation, attendees will understand the possibility of the generation of optic nerve sheath hemorrhage without neck fracture, skull fracture, brain hemorrhage, or confluence with subdural hemorrhage.

This presentation will impact the forensic science community by demonstrating direct traumatic origin of optic nerve sheath hemorrhage without concomitant findings proposed by part of the forensic community as a prerequisite to the generation of such hemorrhage.

This presentation is based on a case study that provides essentially an experiment of nature. A 2-month-old infant was found unresponsive at his residence while under the sole care of his father. Death was pronounced at the scene without significant resuscitative efforts.

The infant presented postmortem without brain swelling and with soft fontanelles, but had optic nerve sheath hemorrhages and retinal hemorrhages as part of the anatomic findings. The calvarial plates were without fracture while dorsal calvarial sutures had soft tissue hemorrhage following the suture lines. There was right parietal subdural hematoma but no subdural hemorrhage contiguous with the optic nerves. There was subarachnoid blood staining but not in the proximal intracranial optic nerve sheaths. There was subdural blood about the cervical spinal cord but the neck on posterior dissection was without fracture.

An interview of the child's immediate caretaker first resulted in denial of knowledge about the origin of injury. Later, the caretaker admitted to an accidental drop of the child from shoulder height to the floor with intermediate impact of the child's head on a kitchen countertop two days prior to demise. The child was then less active than usual until found unresponsive on the day of death. The caretaker consented to further interrogation, including polygraph. He appeared truthful except upon questioning about shaking the child. When presented with the polygraphic findings, he then admitted to and described shaking the child vigorously when, after the fall, the child appeared in distress and he wanted to alleviate the symptoms to avoid discovery of injuries possibly incurred at the time of the fall.

This case does not solve the debate about impact versus shaking as the source of optic nerve sheath hemorrhages and retinal hemorrhages nor does it solve the issue of working backward from injury presentation to etiology of injury. The case does demonstrate that the optic nerve and retinal findings long considered associated with abusive child injury can, in fact, be the direct consequence of trauma. The finding of optic nerve sheath hemorrhage is not necessarily a secondary phenomenon related to resuscitation or the secondary effects of elevated intracranial pressure, but may be a primary finding.

Optic, Nerve, Hemorrhage



Pathology/Biology Section - 2015

H49 Case Report: *Institutional Experience With the Molecular Autopsy and Its Obstacles*

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After attending this presentation, attendees will better understand the ideal work-up in sudden unexplained toddler and young child deaths. Attendees will be more aware of the financial obstacles in place in the United States.

This presentation will impact the forensic science community by increasing awareness and opening a discussion about needed genetic testing in cases of sudden toddler and young child death.

According to the Center for Disease Control and Prevention Wonder database, sudden unexplained toddler and young child deaths, one to four and five to nine years old, respectively, account for 0.9/100,000 deaths per year (2011).¹ Like unexplained infant deaths, the cause for sudden unexplained toddler deaths can be elusive with no anatomic findings to explain the demise. "In fact, even after gross and histologic examination, at least 3% and perhaps as much as 53% of sudden deaths involving previously healthy children, adolescents, and young adults have no identifiable morphological abnormalities found at autopsy, remain unexplained, and are classified as autopsy-negative Sudden Unexplained Death (SUD)."² In cases of sudden toddler/child death, the ideal postmortem work-up would be similar to unexplained infant deaths and include a radiologic skeletal survey, bacterial and viral tissue cultures for microbiology, a full autopsy with detailed histological examination, review of medical and family history, and a thorough scene investigation. Because many cases have no findings whatsoever to explain the sudden death, blood and tissue samples should be retained for possible future studies, including genetic testing. The number of sudden unexplained toddler deaths in the United States due to genetic abnormalities is largely unknown. In general, medical examiners/coroners across the United States do not have easy access to molecular genetic testing due to financial constraints. The cost per positive diagnosis for long-QT syndrome alone can run up to \$45,000.³ As a result, opportunity to provide a definitive cause of death to the family is lost, as well as any chance of protecting other potentially at-risk individuals. As the molecular age of medicine advances, forensic pathology in the United States is slowly being left in the proverbial dust.

Within a few months' span, the Wake Forest Baptist Health (WFBH) department of pathology autopsy service autopsied two toddlers, ages two and four years of age, who had unexpected cardiopulmonary arrest and who were resuscitated and briefly placed on extracorporeal membrane oxygenation before being pronounced. Both toddlers had siblings; one had a twin. Full autopsies were performed but showed no clear grossly or histologically apparent etiology for their death except for a suggestion of possible cardiac fibroelastosis in one case. These two deaths were referred to the Pediatric Collaborative Care group at WFBH whose staff is composed of pediatricians in all subspecialties, medical geneticists, and other specialties as warranted for assessment. Circumstances and autopsy findings in both cases suggested a possible underlying genetic cause. This presentation highlights the current difficulties in obtaining funds in a cash-strapped medical examiner/coroner and hospital system and the various possibilities that may mitigate this problem.

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Molecular Autopsy, Sudden Unexplained Death, Genetic Testing



Pathology/Biology Section - 2015

H50 In Ballistics, Interpretations of Postmortem Multi-Slice Computed Tomography Are Often Simple ... But Not Always

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After attending this presentation, attendees will understand that even if the number of Postmortem Multi-Slice Computed Tomography (PMMSCT) explorations in ballistic contexts is high, some radiological aspects may be difficult in terms of interpretation. This presentation presents and discusses different surprising postmortem multi-slice computed tomography explorations which are didactical for radiologists and forensic pathologists. This presentation insists on the quality needed concerning the police inquiries and on the fact that postmortem multi-slice computed tomography explorations are a precious complementary exploration for the forensic pathologist, which cannot be interpreted without autopsy or external examination elements.

This presentation will impact the forensic science community by showing three different surprising PMMSCT explorations that are difficult in terms of interpretation for radiologists and forensic pathologists. Seeing the abnormal aspects is one step; giving medicolegal sense and interpretation to this aspect, with an integration of the radiological aspects in one coherent and intelligent forensic meaning, is another step. This second step, in some particular situations, may surely be the most complicated and most problematic.

PMMSCT is now worldwide developed. One of the most frequent indications of PMMSCT concerns gunshot trauma; however, even if the number of PMMSCT in ballistic contexts is high, some radiological aspects may be difficult in terms of interpretation. This presentation will discuss three different aspects of PMMSCT which are didactical for radiologists and forensic pathologists.

Case 1: The first case concerns an 88-year-old man found dead on the ground, close to the base of a crane in a construction area. Based on the police investigations, the main hypothesis was a suicide from a fall from height. The PMMSCT showed presence of multiple subcutaneous and intra-cranial metallic pellets in the left fronto-temporal area. The detection of a bone defect was difficult due to metallic artifacts; however, some radiological aspects, like the absence of a clear cutaneous defect and absence of peri-lesional hemorrhage, did not suggest acute or recent gunshot trauma. The medicolegal autopsy confirmed the different radiological aspects. Furthermore, a slight cutaneous scar was noted at the left fronto-temporal region. Consequently, the hypothesis of a previous suicide attempt was retained. The final cause of the death was a poly traumatism secondary to a fall from great height. This case illustrates an unexpected detection of stigmata of previous gunshot trauma in a context of a fall.

Case 2: The second case concerns a 77-year-old man found dead in a lake with an anterior abdominal gunshot cutaneous entrance wound. Based on the police investigations, the main hypothesis was a suicide by gunshot. The PMMSCT revealed the presence of multiple hyperdense foreign bodies of different sizes within the chest. This aspect was interpreted as intrathoracic metallic pellets associated with two bullets. The medicolegal autopsy revealed that the two bullets were metallic elements of a button of the jacket worn by the deceased and displaced within the body during the gunshot due to the button's interposition between the end of the rifle and the skin. The final cause of the death determined by the forensic pathologists was a suicidal abdominal gunshot. This case illustrates the importance of the external examination and the autopsy elements because in this case, it was very easy to misdiagnose metallic fragments of a button as bullets. The association of different types of oriented projectiles resulted in initially opining the manner of death to be homicide. Consequently, false positivity concerning this aspect may have important judiciary consequences.

Case 3: The third case concerns a 73-year-old man found dead at home. Based on the police investigations, the main hypothesis was a homicide secondary to a multiple blunt trauma using a hammer. The PMMSCT revealed the presence of multiple subcutaneous and hyperdense foreign bodies located at the right fronto-temporal area. This aspect was interpreted as metallic pellets. The police investigation at the deceased's home led to the discovery of an airgun with a box of numerous small metallic projectiles. The medicolegal autopsy confirmed those findings except for the description of one projectile in the left orbit. This case illustrates the unexpected detection of some metallic air-gun bullets in a context of blunt trauma with a hammer.



Pathology/Biology Section - 2015

These cases illustrate that sometimes interpretations of the postmortem explorations performed in ballistic trauma may be difficult, revealing some unexpected or atypical aspects. It also underlines that the interpretation of the PMMSCT necessitates collaboration between radiologists and forensic pathologists.

Postmortem Imaging, Gunshot, Pitfall



Pathology/Biology Section - 2015

H51 Flash Fire Victims and Carboxyhemoglobin Concentrations: A Report of Ten Simultaneous Flash Fire Fatalities With Autopsy Findings

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After attending this presentation, attendees will understand the diversity of Carboxyhemoglobin (COHb) concentration findings in victims who die in flash fires. Further learning objectives include: (1) dealing with mass-casualty incidents in small, resource-challenged rural jurisdictions; and, (2) the effect of safety standards, both in the United States and internationally, which influence bus- and large truck-accident fatalities and injuries.

This presentation will impact the forensic science community by the presenting autopsy data on the ten people who died in a conflagration resulting from a collision of a motor coach bus vs. a tractor-trailer, big-rig truck at highway speed.

Motor vehicle collisions have been a leading cause of death and injury in the United States for decades. Injuries and deaths are typically caused by physical trauma, thermal injuries, toxic gases, or a combination of the three. Ten people died in a fiery conflagration on a late afternoon in perfect weather resulting from a collision between a motor coach bus filled with high school seniors and a big-rig tractor-trailer in Glenn County, CA. Travelling at highway speed, the tractor-trailer inexplicably crossed from the southbound lanes across an unfenced, flat median strip without protective vegetation into the northbound oncoming traffic lanes on Interstate 5, a major highway traffic corridor in the western United States. The drivers of both the truck and the bus died as well as eight bus passengers. The collision resulted in both traumatic injuries and thermal injuries from the ensuing flash fire which was suppressed within minutes by the local volunteer fire department. Complete autopsies were performed under the jurisdiction of the Glenn County sheriff-coroner on the nine victims who died at the scene. A tenth victim, who briefly survived, succumbed to his injuries hours later after having been flown to the University of California Davis Medical Center in Sacramento, CA. An external examination without complete autopsy was performed on that victim at the Sacramento County coroner's office. Of the nine autopsied victims, two died from severe traumatic injuries alone without elevation of COHb concentration, three died from inhalation of products of combustion without elevation of COHb concentration and with moderate traumatic injuries, two died from inhalation of products of combustion without elevation of COHb concentration and without significant traumatic injuries, and only two died with measurable concentrations of COHb – the first with 18% COHb concentration and traumatic injuries, the second with COHb concentration of 45% plus a Cyanide (CN) concentration of 4.17µg/ml and without traumatic injuries. The diversity of these autopsy findings within a cohort of victims dying together over a narrow time period support the fact that COHb concentration cannot be used as the sole criterion that a person was alive in a fire. Death in a flash fire results from the effects of a panoply of toxic compounds present in a flash fire including superheated gases.

Selected mass-casualty incident management issues arising in a small, rural county including triage, fire suppression, victim identification, body part identification, and disposition are examined.

This presentation will further discuss fire and crash-related issues which would diminish or mitigate flash-fire vehicular fatalities, including escapement, time to incapacitation, on-board fire suppression systems, fire detection, fire protection of vehicle passenger-occupied areas and fuel tank/engine areas, and the epidemiology of fatal bus crashes. Current national and potentially global safety standards for motor coach busses and large trucks are reviewed. Regulatory agencies have a responsibility to ensure that established safety standards are continually updated, seizing upon opportunities to improve accident survivability to minimize injuries and deaths from motor vehicle collisions.

Flash Fire, Bus Crash, Carboxyhemoglobin



H52 A Case of Intrauterine Fetal Cranial Injury After Attempted Suicide During Pregnancy

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After attending this presentation, attendees will understand the importance of considering how relatively minor maternal trauma during pregnancy can lead to direct fetal injury *in utero*, especially during the second and third trimester.

This presentation will impact the forensic science community by showing the importance of manner-of-death reconstruction in unusual fetus injuries after maternal trauma.

Trauma in pregnancy is subdivided into three different types: blunt abdominal trauma; pelvic fractures; and, penetrating trauma. The effect of trauma on pregnancy depends on the gestational age of the fetus, the extent of disruption of normal uterine and/or fetal physiology, and type and severity of trauma. During the first trimester, minor trauma is not threatening to the pregnancy. During the second and third trimester, even relatively minor trauma can have significant adverse effects (e.g., maternal injury or death, shock, internal hemorrhage, intrauterine fetal demise, direct fetal injury, abruptio placenta, and uterine rupture). Blunt abdominal trauma associated with skull fractures and intracranial hemorrhage represents a rare event that complicates <1% of all pregnancies affected by trauma.

A case report of a 46-year-old woman who was 34 weeks pregnant when admitted to the Emergency Room (ER) after being found lying on the ground approximately 10 meters below an overpass. Witnesses noticed the woman driving fast along the overpass before being involved in a collision with the guardrail. Soon after the accident, she attempted suicide by jumping from the overpass guardrail. Police discovered the victim was concerned about her pregnancy and work.

She was admitted to the ER unconscious and obstetric/gynaecological personnel were consulted. Fetal heart tones were normal. A complete computed tomography scan showed fractures of five lumbar vertebrae, fractures of three ribs with pulmonary contusions, and a fracture of the left metatarsus with no cranial injury. After the mother was stabilized, the fetal heart tones were abnormal and, three hours after admission, the patient had an emergency caesarean section, delivering a male infant who was lifeless despite attempts at resuscitation.

No alcohol or drugs were found in the mother. After admitting to the suicide attempt, the mother was transferred to the psychiatric department where an unspecified episodic mood disorder was diagnosed. She recovered completely from the physical trauma.

Due to the unclear dynamics of the traumatic event, a medicolegal autopsy was performed on the baby. The external examination revealed a fully developed infant showing a deformity of the skull and left eyelid bruises. A massive subgaleal hemorrhage with a slippage of the left parietal bone below the right parietal bone was found. The brain showed a subarachnoid hemorrhage of the left temporal, parietal, and occipital lobes and of the right parietal and occipital lobes. Histological examination confirmed the brain injury and pulmonary signs of intubation. The cause of death was brain injury after maternal blunt abdominal trauma following a car accident and the fall from ten meters.

Direct fetal injury is significantly less common because the fetus is protected by the maternal body wall, uterus, and amniotic fluid. Fetal trauma usually occurs in late third trimester pregnancies when the amniotic fluid volume is low relative to the size of the fetus. Furthermore, the most common fetal trauma is head injury as the uterus is above the level of the pelvic rim and the head engages into the bony pelvis, possibly being compressed between the symphysis pubis and the sacral promontori.

The findings from this case confirmed that maternal “minor” trauma can be devastating for the fetus. Nevertheless, in any case of obstetric trauma, it is imperative to maintain the mother’s stability and survival since the survival of the fetus after a trauma depends on the mother’s condition with respect to oxygenation and hypovolemia.

Pregnancy, Blunt Abdominal Trauma, Fetal Trauma



Pathology/Biology Section - 2015

H53 **Skeletal Trauma Analysis in the Elderly: A Case Study on the Importance of a Contextual Approach**

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After attending this presentation, attendees will understand the advantages of using a contextual approach, as well as the value of forensic anthropological assessments, when interpreting patterns of trauma in potential elder abuse cases.

This presentation will impact the forensic science community by demonstrating the importance of considering age, medical history, and scene findings in interpreting the presence, timing, and cause of trauma by using a case study as an example. This presentation highlights the unique nature of the aging skeleton during forensic analyses. The elder skeleton presents different patterns of peri-mortem and postmortem trauma compared to younger adults, making interpretations of elder abuse difficult. In addition, this presentation will also underscore the importance of viewing all of the skeletal elements present, not just those in question.

With increasing age, the adult skeleton becomes weak and prone to fractures, especially in adults with osteoporosis, making injury interpretation difficult. Multiple studies have investigated common injury sites and mechanisms in elderly individuals; however, research concerning the timing of injury is lacking. Being able to distinguish accidental skeletal trauma from abuse-inflicted trauma, as well as postmortem damage, is significant in investigating possible cases of elder abuse and will impact criminal prosecutions.

In 2010, the mummified remains of a 93-year-old woman were found within her home in a sitting position on the couch, slumped over to her left side. She was completely clothed in winter clothing and covered by blankets, consistent with the season during which she died. The decedent's son failed to notify authorities of his mother's death and continued to cash her Social Security checks in the months following. Local authorities requested an autopsy due to the suspicious nature of the death and monetary gain by the son. During autopsy, peri-mortem fractures of the right ribs were observed by the medical examiner and a request was made for a forensic anthropological trauma analysis of the ribs. Initially, the medical examiner was only going to submit the ribs in question for trauma analysis. This could have resulted in an incorrect determination of the cause/manner of death. An incorrect conclusion of inflicted trauma could have resulted in inappropriate criminal charges.

Osteological examination revealed multiple bilateral antemortem rib fractures in different stages of healing as well as peri-mortem fractures. The decedent also displayed significant kyphosis and compression fractures throughout the vertebrae and advanced osteoporosis, as determined through radiographs of the proximal femur. In addition, she also had a left femoral head replacement consistent with common treatment for hip fractures in elderly individuals suffering from osteoporosis. A review of the decedent's medical history confirmed the diagnosis of osteoporosis and also revealed she had Chronic Obstructive Pulmonary Disease (COPD). COPD causes excessive coughing and, in someone with advanced osteoporosis, has been found to produce rib fractures.

Of particular concern in this case are the rib fractures. In juveniles, rib fractures in different stages of healing are consistent with abuse; however, with the decreased strength of bones in the elderly, their susceptibility to accidental injury, and decreased bone healing making existing fractures prone to re-fracture, the incidence of rib fractures in various stages of healing may not indicate abuse.

Along with the individual's medical history, examination of the scene photos was critical in making a final determination that the peri-mortem fractures observed on the left ribs were not due to inflicted trauma, but the result of the body weight exerting tension on the already fragile ribs. By taking into account aging factors, the scene, and existing medical conditions, the skeletal injuries were found not to be consistent with abuse.

Elder Abuse, Rib Fractures, Scene Evidence



Pathology/Biology Section - 2015

H54 Hydrogen Sulfide Fatality From a Domestic Sink Drain Exposure

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After attending this presentation, attendees will be aware of the potential lethal effects of sewer gas in the home setting. Attendees will learn of the potential hazards of hydrogen sulfide exposure, autopsy findings, and toxicology sampling and results.

This presentation will impact the forensic science community by stressing the importance of scene investigation, autopsy findings, and toxicology results in order to determine the cause of death in hydrogen sulfide exposure from sewer gas in a home death.

Often referred to as “pit gas” or sewer gas, hydrogen sulfide is a highly toxic gas. It is naturally produced by decaying organic material in the absence of oxygen and is a byproduct of many industrial processes. Its toxicity most often occurs in occupational settings such as petroleum refineries, commercial fishing holds, and pools of sewage sludge or liquid manure. Fatalities often involve exposure to high concentrations of hydrogen sulfide (>150ppm).

Hydrogen sulfide concentration as low as 0.03ppm can be easily detected by its characteristic rotten egg odor. Respiratory tract irritations occur between 50-100ppm. Olfactory nerve paralysis, causing a loss of ability to smell the characteristic odor, occurs between 100-150ppm. Pulmonary edema occurs within 300-500ppm; levels of 600-800ppm are promptly fatal.

Clinical presentation of hydrogen sulfide toxicity includes headache, nausea, and vomiting. High-dose exposure may result in unconsciousness, seizure, and coma. Massive exposure can cause cardiovascular and respiratory failure leading to death. Diagnosis of hydrogen sulfide poisoning is generally made on the basis of history and clinical presentation. Laboratory diagnosis is helpful and is made through measurement of sulfide, thiosulfate, and sulfhemoglobin concentrations.

A 44-year-old White female with a past history of asthma was attempting to unclog a drain under a kitchen sink in her residence. She was discovered by her roommate unconscious on the kitchen floor with her head inside the cabinet under the sink. The drain pipes and drain trap had been removed by the subject and a solution known as “Liquid Fire” had been poured in the drain. Her roommate called 911 and the police and fire department arrived at the scene. The responding officer described the horrific sewer gas smell coming from the house. The subject was immediately transported to a local hospital where she was pronounced deceased.

An autopsy revealed a dusky gray-green discoloration to the gray matter of the cerebral hemispheres due to sulfur compounds. Histologically, the cortical neurons exhibited focal early hypoxic changes. The lungs exhibited moderate pulmonary edema and focal bronchioles with mucus plugs consistent with a history of asthma.

Toxicological analyses in hydrogen sulfide poisonings include measurement of sulfide and thiosulfate in various body fluids and tissues. Thiosulfate, in urine, is considered better than sulfide in the detection of sulfide exposure. Thiosulfate concentrations reported in fatalities range from 2.8-72.6mcg/mL. Sulfhemoglobin is another marker for hydrogen sulfide exposure. In unexposed subjects, the concentration of sulfhemoglobin is <1% in blood. In this case, the urine thiosulfate concentration was 15.5mcg/mL and the blood sulfhemoglobin concentration was 6.3%. The drug screen was positive for cannabinoids and methamphetamine (378mg/mL).

Based on the circumstances surrounding the death and the findings at autopsy, the subject of this case report died as a result of hydrogen sulfide intoxication with methamphetamine abuse and asthma as contributing factors. The manner of death was accidental.

Pathology, Sewer Gas, Hydrogen Sulfide



Pathology/Biology Section - 2015

H55 A Complex Bullet Path: Entrance in the Head, Exit Through a Body Orifice, and Re-Entrance in the Arm

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After attending this presentation, attendees will have a better appreciation of unusual bullet paths in forensic practice, illustrated by a re-entrance wound in the arm, coming from a shot in the head which then exited through a natural body orifice.

This presentation will impact the forensic science community by providing an original example of a complex suicide following a homicide, with multiple gunshot wounds in the head, a re-entrance wound in the arm, and no visible exit wound.

Homicide-suicide is a particular form of interpersonal violence; it refers to the act of homicide followed by suicide of the perpetrator. Homicides followed by suicide patterns differ from both homicides and suicides internationally. In France, a homicide-suicide does not frequently result in a charge or trial. In this case, the findings at the crime scene initially led to a double-homicide suspicion due to the presence of multiple gunshot wounds.

A 58-year-old man, his wife, and their dog were found dead at home. He was lying on his bed in a supine position with three gunshot entrance wounds, one on the forehead, one next to the left temporal point, and one in the right arm. An unregistered .22 LR handgun (next to the bed, on his right side) and multiple fresh bloodstains were found in the bedroom. The wife was lying in a semi-supine position on the floor of their living room in a fresh pool of blood with a gunshot entrance wound on the vertex and no exit wound. No medical history was documented for either of them. Their dog also had a gunshot wound at the top of the head with no exit wound.

A radiological examination and autopsy of the man identified two bullets, one next to the C4 vertebra and one in the right arm. Three bullet trajectories were observed. The forehead wound had a close-range firing aspect, with soot depositions and a star-shaped laceration. The bullet's trajectory was through the frontal bone, the ethmoid, and the pharynx. The path from the left temporal entrance wound was through the frontal bone, the cribriform plate of the ethmoid, and the nasal septum. The second bullet was found in the subcutaneous area of the right arm. The three cutaneous wounds were confirmed as gunshot entrance wounds by a pathologist's examination. No other bullet was found at the crime scene or in the corpse. The entrance wound in the left arm was considered to be a re-entrance wound coming from the left temporal trajectory through a natural body orifice: the nostril. In this hypothesis, the decedent would have had his right arm wrapped around his face while he was shooting himself with his left hand. The toxicological analysis for all of the standard samples was negative. After complete investigation, the manner of death was classified as suicide for the man and homicide for the woman.

This case shows the importance of an open mind during the interpretation of bullet wounds paths, especially in complex cases such as this one. Multiple entrance wounds in the head and other locations might be self-inflicted, even if they appear to be suspected homicide.

Gunshot Wounds, Forensic Medicine, Homicide Suicide



Pathology/Biology Section - 2015

H56 Autoerotic Accidental Death by Self-Inflicted Asphyxia by Body-Wrapping in a Plastic Curtain

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After attending this presentation, attendees will understand an atypical method of autoerotic death by self-infliction due to body-wrapping in a plastic curtain.

This presentation will impact the forensic science community by providing data concerning an atypical method of self-inflicted asphyxia and the multidisciplinary approach to identify the causes and time-of-death.

Autoerotic accidental deaths are deaths occurring during solitary sexual rituals used to enhance sexual excitement. The majority of these fatalities involve the use of some type of apparatus that can enhance the sexual stimulation of the person, causing unintentional death. Hanging or self-inflicted suffocation are the most common forms of autoerotic accidental death.

In many cases, the diagnosis of autoerotic accidental deaths has been controversial and some cases have been classified as suicides with unusual features, rather than life-threatening accidents caused by sexual practices. The vast majority of autoerotic deaths are due to autoerotic asphyxia by hanging or by plastic bags (70% to 80%) and/or chemical substances (10% to 30%), but in approximately 5% to 10% of cases, atypical methods of autoerotic deaths are encountered (e.g., electrocution, overdressing/body-wrapping, foreign body insertion, and atypical methods of asphyxia).

In the present case, a 39-year-old man was found dead in his own apartment during the month of June, 2014, in Turin in northwest Italy. The door of the apartment was locked from the inside and no signs of forced entry were observed. The corpse was found on a bed, completely wrapped in a plastic curtain (typically used for showers). Both ends of the curtain were tied together, one end from the outside and the other end from the inside where the head was located. The curtain was laterally heat-sealed together using a hair straightener that was found on the floor close to a large number of empty beer bottles.

The body inside the sealed plastic curtain was found naked and in a supine position with the arms raised above the head. A plastic wrap was repeatedly rolled around his abdomen and three pieces of adhesive tape were tied to his penis. The body was highly decayed with body fluids spread over the bed and on the floor. Associated with this decomposition were blow fly (*Calliphora vicina* Robineau-Desvoidy, Diptera: Calliphoridae) larvae and pupae both inside and outside the sealed plastic curtain.

The death was considered as an autoerotic accidental death by self-inflicted asphyxia and not a suicide, even though it was determined that the subject was suffering some depression after he lost his job. In the context of this situation, the minimum time since death was established using the insects found on the body and in the surrounding area.

Asphyxia, Autoerotic, Body-Wrapping



Pathology/Biology Section - 2015

H57 The Utility of CT Imaging in Determining Cause of Death in a Case of Iatrogenic Barotrauma

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After attending this presentation, attendees will understand how postmortem Computed Tomography (CT) imaging: (1) can be used in detecting air within the body cavities, tissues, and vessels; (2) can add valuable pre-dissection information; and, (3) is useful in guiding postmortem dissection.

This presentation will impact the forensic science community by bringing to light the value of CT imaging as an adjunct to postmortem examination and by bringing attention to certain postmortem cases which would benefit from having pre-autopsy imaging.

Advanced postmortem imaging is increasingly being utilized in the field of forensic pathology with many centers worldwide currently incorporating Postmortem Computed Tomography (PMCT) into their daily practice. One of the myriad advantages of PMCT imaging is its ability to detect air in body cavities, tissues, organs, and blood vessels which can be challenging to appreciate during routine dissection. CT scan provides nearly 100% sensitivity and specificity for the diagnosis of pneumothorax. Tension pneumothorax and other iatrogenic barotraumas such as air emboli and subcutaneous emphysema are known complications of mechanical ventilation. A case of iatrogenic barotraumas is reported, demonstrating the advantages of PMCT imaging in assisting with determining the cause and mechanism of death.

A 21-year-old man with Duchenne Muscular Dystrophy (DMD) was admitted to the hospital and treated for pneumonia. His pneumonia progressed to respiratory failure and, after several unsuccessful attempts at intubation, he lost vital signs. A postmortem examination was conducted to determine the cause of death. Prior to the autopsy, a full body CT scan was performed which revealed a large right-sided tension pneumothorax with left mediastinal shift, a completely collapsed right lung, subcutaneous emphysema, and air emboli within the heart and cerebral vasculature. At autopsy, the body was that of a young adult male with obvious atrophy of the upper and lower limbs. The imaging findings allowed for modification of the postmortem dissection technique in order to demonstrate air within the body cavities. A needle thoracostomy expressed a large volume of air from the right pleural space. The lungs showed bilateral collapse with acute bronchopneumonia and the heart showed DMD-associated cardiomyopathy histologically.

Barotrauma is an uncommon but well-recognized complication of mechanical ventilation; when it occurs, the mortality rate can be significantly high. This case highlights some of the advantages of PMCT as an adjunct to dissection, particularly for visualization and documentation of suspected barotrauma. In conjunction with dissection, PMCT added valuable pre-dissection information and assisted with the completion of a thorough, reviewable forensic autopsy. The advantages of PMCT in detecting air can be applied to several other case types including some SCUBA-related fatalities and sharp force injuries to the chest and neck.

CT Imaging, Postmortem, Barotrauma



H58 Fatal Pedestrian-Vehicle Encounters: Is Pedestrian Blood-Alcohol Concentration a Significant Factor?

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After attending this presentation, attendees will better understand the importance of measuring the Blood Alcohol Concentration (BAC) in forensic autopsy settings of fatal pedestrian-vehicle encounters, according to a one-year study from a group in São Paulo, Brazil.

This presentation will impact the forensic science community by providing scientific evidence to justify and recommend a simple procedure broadly available within forensic institutes that may have considerable medicolegal implications, especially to the driver of the vehicle involved in the incident.

Introduction: External causes of injury are the third-leading cause of death in Brazil and fourth in São Paulo State. Among these, traffic accidents are the second in prevalence in the country and the leading cause in the state.¹ Nearly one-third of all traffic accident deaths involve pedestrians.² In Brazilian society, strict laws prohibit drivers from having any blood alcohol levels whatsoever; however, the BAC of a pedestrian who is struck is of no concern.³

Objective: The goal of this study was to analyze the prevalence of alcohol intoxication in fatalities that involve a pedestrian struck by a motor vehicle according to official data.

Methods: This transversal study collected data from 2005 from the Instituto Médico Legal do Estado de São Paulo and the Companhia de Engenharia de Tráfego de São Paulo concerning pedestrian deaths. The variables of interest were age, sex, time of accident, cause of death, and blood alcohol level. Measures of dispersion and variability were used to analyze the data obtained.

Results: Four hundred five deaths occurred as a result of persons being run over by vehicles in 2005 in São Paulo city. Pedestrians were between 4 and 93 years of age with a median age of 48 years; the standard deviation was 19.3 years and the variability coefficient was 40%. The relationship between the age and the number of deaths represented a normal distribution with highest incidence between 45 and 50 years of age. In 69% of the cases, men were involved and in 27.5% of the cases, the victims were elderly people (≥ 60 years old). The most frequent causes of death were multiple trauma and traumatic brain injury, each responsible for 35% of the deaths. Half of all cases involved people between 35 and 60 years of age with 42.9% having alcohol in their blood. Of the elderly people, 14.5% had alcohol in their blood. When considering the cases between 40 and 50 years of age, most (52%) had some level of alcohol in their blood. In the 10- to 20-year-old group, 13% had alcohol in their blood.

Discussion: The high rates of BAC observed in this study characterize a public health issue. Alcohol consumption may have directly reduced the ability of the pedestrian to visualize, comprehend, and respond to risks. While many studies have emphasized the role of alcohol consumption regarding the driver, most of them fail to shed light on how pedestrians could also play a role or help to avoid these mishaps. This study recognized an active role of the pedestrian in a substantial portion of fatal vehicle-pedestrian encounters, which is important from two different perspectives. First, it emphasizes the importance of collecting samples for BAC measurement in the forensic setting for pedestrians struck by a vehicle. Second, it underlines the need for educational policies specific for drinking in pedestrian population of all ages.

Conclusion: A significant portion (35%) of the people who were run over had some level of alcohol in their blood; this rate was even higher in the 35- to 60-year-old population (42.3%). It is not possible, therefore, to rule out alcohol use of the pedestrian, which will have medicolegal implications in a possible judgment. Coroners should suspect alcohol abuse and request BAC measurement in all ages so their report can support an equitable judgment. Public health policies focusing on the alcohol-consuming pedestrian population are needed.



Pathology/Biology Section - 2015

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Blood Alcohol Concentration, Pedestrian, Run Over



Pathology/Biology Section - 2015

H59 When Life Makes No Sense — Suicide by More Than 150 Stab and Incised Wounds With Atypical Features

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After attending this presentation, attendees will understand the difficulties in distinguishing between homicide and suicide when atypical sharp-force fatalities occur.

This presentation will impact the forensic science community by demonstrating the relevance of a concrete methodological approach when the circumstances surrounding death are not clear. In these cases, a complete crime scene investigation should be always performed, followed by an autopsy examination and laboratory analyses.

Deaths due to sharp force injuries are less common than other mechanisms of violent death. The most common manner of death associated with sharp force trauma is homicide, followed by suicide, while accidental fatalities are relatively rare. When a sharp force fatality with atypical features occurs, identifying the correct manner of death can be challenging for the forensic pathologists. At the crime scene, a large amount of blood is usually found due to the extensive blood loss from the skin injuries. Several features to distinguish between homicide and suicide are described in literature. The absence of the weapon, the presence of defensive wounds, and normally worn clothes are suggestive of homicide. The weapon at the crime scene and near the body, hesitation marks, and undressing are commonly related to suicide. Sometimes, it is possible for homicidal cases to have injuries that are indistinguishable from hesitation marks.

A case of atypical sharp force suicidal death is presented. A 32-year-old White male was found unresponsive in the living room of his flat, in a supine position. A pool of blood surrounded the body, showing no signs of sliding or splashing. The postmortem changes were consistent with a time of death of approximately six hours before the discovery of the corpse. Clothes were worn only on the lower limbs. On the body surfaces, multiple, widespread sharp force injuries were observed. Near the right hand, a kitchen knife was present. During the examination of the house, multiple bottles of psychoactive drugs were found. According to the declaration of the victim's parents, he had a history of bipolar disorder but no previous attempts of suicide were reported. No suicide note was found.

More than 150 injuries, widespread stab and incised wounds, were present, involving different body regions. Most of the wounds were incised wounds. On the back, more than 20 incised wounds were present, even on the back of the neck. At the internal examination, the six deepest wounds that penetrated the body were identified. On the right supraclavicular region, one lesion went deep inside the soft tissue of the neck and involved the external jugular vein. Upon a layered *in situ* dissection of the anterior neck structures, a hemorrhage in the soft tissue surrounding the affected vessel was observed. On the left hemithorax, one lesion penetrated the thoracic cavity and perforated a rib, but no injuries to the internal organs were present. On the abdomen, four lesions penetrated the abdominal cavity and two of these injured the small intestine and the right common iliac artery. Toxicological analyses were negative for alcohol, medical drugs, and drugs of abuse. A 3D reconstruction of the dynamic was performed using the 3D rendering software Poser Debut® on a MacOSX® computer.

Despite the scene circumstances and several aspects that were consistent with a suicidal death, the presented case combined several atypical aspects: the number of injuries — the total number was more than 150 considering cases of repeated incised wounds to the skin; the site of some wounds was not common (e.g., lesions observed on the back of the neck and the torso); there was a perforation of one rib by the knife; the toxicological analyses were negative, suggesting that the victim was in a state of manic psychosis characterized by dysphoria and paranoia and was determined to kill himself, despite being able to feel pain from the self-inflicted injuries.

Suicides involving a large number of sharp force injuries have been described; however, when atypical features are present, distinguishing between homicide and suicide can be difficult. In such cases, the evaluation of all available data is necessary to find the correct manner of death.

Suicide, Stab Wounds, Incised Wounds

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Pathology/Biology Section - 2015

H60 Burned Human Remains: The Importance of a Multidisciplinary Approach

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After attending this presentation, attendees will understand the complexity and critical issues related to forensic activity in cases of charred remains and will learn how to proceed with these types of cases.

This presentation will impact the forensic science community by illustrating an effective methodological approach to forensic activity and the identification of charred corpses. The recovery of burned skeletal remains may in fact represent an interesting, but sometimes difficult, challenge for forensic evaluation, given the frequently serious damage of the bodies. In such cases, it is essential to ascertain if the victim was exposed to fire before or after death. The forensic pathologist is called upon to determine the cause and manner of death and to discern if it was a case of homicide, suicide, or an accident. Another forensic need arising in such circumstances, both for the discovery of isolated bodies and in mass disasters, is the identification of the bodies, which is required for judicial reasons as well as ethical or religious causes. By developing a case report, the problems and strengths of the forensic activities carried out will be identified, thus providing useful information for the solution of fire-related deaths.

In December 2013, a group of forensic experts (two pathologists, an odontologist, a geneticist, and a toxicologist) were appointed by a judge in Prato, Italy, to identify seven victims recovered after a textile factory fire and to establish the cause of death.

In four cases, the toxicological investigations revealed very high levels of carbon monoxide ranging between 88.05% and 95.77%. Two people died from cyanide intoxication (with concentrations between 5.17mcg/ml and 8.85mcg/ml). In one case, there was a synergistic effect of the two substances (carbon monoxide and cyanide). The autopsy showed indicators of exposure to fire before death, with no traumatic injuries that could suggest a different cause of death.

The identification proceeded in accordance with the International Criminal Police Organization (INTERPOL) Antemortem (AM) and Postmortem (PM) protocols; in every case, one of the primary identifiers was satisfied. Given the high resistance of teeth to high temperatures, odontological examinations provided relevant data and genetic investigations confirmed all identities. Secondary means of identification, including personal descriptions, medical findings and devices, body piercing, clothing, and jewelry also proved to be very useful in correlating possible identities.

The primary difficulties were encountered in gathering AM information, due both to the language barrier (most of the relatives spoke only Chinese) and especially due to the lack of medical and dental information about the victims. The AM phase therefore required careful investigation by the forensic experts to overcome these problems and obtain data to compare with the examination on the remains.

Another critical issue, often causing mistakes in the identification process and in dynamics reconstruction, was the designation of the remains during the different forensic and investigation phases (recovery of the bodies, arrival at the morgue, then at the Forensic Science Department in Florence, Italy).

The contribution of each expert in charge was essential in solving the forensic issues, showing the multidisciplinary and integrated approach in every step of the activity (from the site inspection to the final report) as key to the solution of fire-related deaths.

Charred Corpses, Identification, Multidisciplinary Approach



Pathology/Biology Section - 2015

H61 A Retrospective Study of Drowning Cases in Tarrant and Adjacent Counties From 2008 Through 2013

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After attending this presentation, attendees will gain an increased knowledge and awareness of the prevalence of drowning and the most common risk factors associated with its occurrence.

This presentation will impact the forensic science community by increasing awareness of the fact that drowning is an all-too-familiar event in society and in the majority of cases is preventable. As many as 8,000 cases are reported annually in the United States. Education and heightened awareness is crucial to stemming this deadly tide. As with most dangers, prevention and avoidance of risk factors are the most reliable strategies. This presentation attempts to identify the most glaring risk factors and thereby enhance education on drowning prevention.

Drowning as a cause of death is a substantial concern among many communities, coastal or otherwise. It ranks among the leading causes of mortality among all age groups, especially in the pediatric population. In the United States alone, drowning accounts for 6,000-8,000 deaths annually. Therefore, it is imperative to be aware of the most common risks and circumstances involved in drowning. In this study, data was collected from case files of drowning incidents that passed through a medical examiner's office over a six-year period in order to determine the following questions: What sub-populations are most at risk for drowning? Where do drowning incidents most commonly occur? What are the risk factors and circumstances that prevented the victims from being extricated from danger? The data (n=234) indicated a predominance of male victims. In addition, an analysis of victims by age interval revealed a bimodal distribution, with toddlers and collegiate-age individuals being the most susceptible. With regard to location, swimming pools and lakes were the most common scenes of drowning. Identification of the lakes was performed to determine the sites where drowning occurred most frequently. One lake, Lake Lewisville, accounted for just over 25% of all recorded lake drownings in the area. Similarly, swimming pools were categorized as being private or public, and in-ground or above-ground. An overwhelming majority of the pools were determined to be private (82%) and in-ground (68%). Swimming pools also accounted for two-thirds of all pediatric drowning events. In the same vein, sufficient adult supervision was lacking in 90% of all pediatric drowning cases and barriers to pool entry were inadequate in 72%. In adult drowning incidents, alcohol use was found to be the most frequent risk factor present. Lastly, the phenomenon of "dry" drowning, in which the airways and lungs remain essentially free of the drowning medium, was considered and a novel set of criteria was generated to classify such cases. Of drowning events, 8% were identified as "dry" drownings but this sampling of individuals did not exhibit any discernible pattern with regard to demographics or circumstances.

Overall, this study corroborates and expands upon present knowledge and understanding of the epidemiology of drowning. Recognition of the most susceptible victims as well as the most likely circumstances surrounding drowning is invaluable to the formulation of strategies and the distribution of education to prevent drowning in the future.

Drowning, Epidemiology, Forensic Pathology



H62 Hemorrhagic Death With Particular Postmortem Computed Tomography Findings Associated With Decompression Illness: A Case Report

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The goal of this presentation is to describe an unusual case of fatal intraperitoneal hemorrhage with Decompression Illness (DCI).

This presentation will impact the forensic science community by illustrating particular postmortem computed tomography findings associated with DCI.

DCI is characterized by a wide range of symptoms caused by decompression of the body and includes two syndromes: Arterial Gas Embolism (AGE) and Decompression Sickness (DCS). In AGE, alveolar gas (secondary to pulmonary barotrauma) or venous gas emboli (via a cardiac shunt or the pulmonary vessels) enters the arterial circulation. DCS is caused by *in situ* bubble formation from inert gas dissolved within body tissues. Clinical manifestations range from trivial to fatal.¹

This report describes a case involving a diver in his mid-fifties who was working at a depth of 5m below the sea surface. About ten minutes after he started working, there was an explosive sound, and he returned to the sea surface rapidly. He was conscious at that time, but said that he could not breathe and collapsed shortly thereafter. He was transferred to a hospital and underwent cardiopulmonary resuscitation, but he could not be resuscitated. A doctor in the emergency room identified air bubbles within the blood during blood sample collection.

Postmortem Computed Tomography (CT) revealed massive hemorrhagic ascites and a large amount of gas within the heart chambers and blood vessels of the brain, liver, and groin region. In particular, the heart appeared to be expanded with gas. A radiologist stated that all of the gas was not likely to have been produced by putrefaction.

An autopsy was performed approximately 18 hours after the patient's death. No antemortem injuries were apparent on the surface of his body. The autopsy revealed traumatic rupture of the mesentery and approximately 2,600ml of blood with soft blood clots in the abdominal cavity. Only 5ml of blood was present in the heart, and the liver and kidneys were ischemic. No postmortem putrefaction changes were visible. Visualization of gas bubbles within the circulating blood was impossible because too little blood remained within the blood vessels. No bubbles were present in the hemorrhagic ascites. The decedent weighed 77kg and such massive hemorrhage was undoubtedly fatal. Therefore, the autopsy report concluded that the cause of death was attributable to intraperitoneal hemorrhage caused by traumatic rupture of the mesentery.

The characteristic finding in this case was a large amount of gas in the cardiovascular system, which was detected on postmortem CT. According to the literature, AGE can occur after ascent from a depth as shallow as 1.0m to 1.5m, and the minimum diving depth after which venous gas emboli can form is 3.6m, whereas DCS is uncommon at depths of <10m.^{1,2} In the present case, some of the gas may have formed gradually after death and it was impossible to determine the extent to which the gas contributed to the patient's death. Therefore, it was concluded that the gas had formed secondary to DCI, but the influence of the gas on the patient's death was not clear.

Reports of DCI with such particular CT findings as described in this case are rare.

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Decompression Illness, Postmortem Computed Tomography, Gas Embolism



Pathology/Biology Section - 2015

H63 A Bizarre Way to Kill the Ex-Wife: Two Knives Buried in the Chest, Two Additional Knives, and a Screwdriver Implanted in the Thorax

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After attending this presentation, attendees will better understand lesional pattern caused by a combination of sharp and blunt weapons used in domestic violence. This presentation also highlights the importance of a complete autopsy in order to accurately determine the cause and manner of death.

This presentation will impact the forensic science community by applying an *in situ*, step-by-step, dissection technique in order to preserve, observe, and evaluate penetrating injuries. Furthermore, emphasis is given to the fact that the internal examination often adds relevant and unexpected data, for example, to what was thought to be a clarified and undoubted homicide. This case should also alert the forensic science community to the different forms of domestic violence — sometimes brutal and bizarre and often unexpected.

The body of a 47-year-old woman, divorced and living with her son, was found inside their new house with multiple cuts and stab wounds. This was following one of her ex-husband's occasional visits, which was intended to assist her in house-hold work. Two knives and a screwdriver were found *in situ*, penetrating the front of the chest.

Due to the multiplicity of the injuries and the *in situ* position of the weapons, a different dissection technique was adopted in order to preserve and interpret each path, particularly the stab wounds. The neck incision was made in such a way that the original injuries were preserved and the internal examination of the thorax was performed while the weapons, visible externally, were still *in situ* for as long as possible.

During the autopsy, the external examination showed multiple injuries of different types (cut/stab wounds, blunt and sharp-blunt) in the neck and thorax which revealed extreme violence. The neck injuries were extensive and difficult to describe because of the massive destruction of superficial and deep anatomical structures. Internally, it was confirmed that the thorax was penetrated by the screwdriver and the two knife blades which were completely inside the thoracic cavity. Surprisingly, when the thorax was opened, there were two more knives inside, one with a serrated blade and a clean edge and one embedded in the left lung.

All the injuries were assessed, the paths of the blades were described, and a comparison between the injuries and the shapes of blades was made in order to determine how those injuries were inflicted.

This case highlights how a different approach to the autopsy technique would enhance the interpretation of injury patterns, especially when multiple weapons are used in combination. It is clear that, despite the importance of the information collected in the external examination, the opening of the corpse provided unexpected findings even regarding the number of weapons. It is interesting to note that even though multiple weapons were used in this case, none of them penetrated the heart and the cause of death was determined as hemorrhagic shock due to a combination of multiple sharp and blunt trauma.

Multiple Knives, Screwdriver and Knives In Situ, Stab and Sharp Blunt Injuries



Pathology/Biology Section - 2015

H64 Homicide or Suicide? A Case Report of an Atypical Hanging

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After attending this presentation, attendees will understand the distinction between suicide and homicide in cases of hanging. This presentation describes a case of hanging in which the crime scene was previously tampered with by the victim with the intent to simulate an execution in order to collect life insurance compensation. Characteristics of injuries, circumstances (for example, the victim's psychiatric disorders), and data collected from a detailed investigation of the scene as well as a complete postmortem examination including a toxicological analysis generally represent the major diagnostic criteria to determine the correct diagnosis.

This presentation will impact the forensic science community by outlining a complete methodological approach to interpretations of the summary of evidence that can contribute to the correct differential diagnosis between hanging or execution. A complete crime scene investigation has to be followed by external examination as well as a complete autopsy with the histological study of skin specimens to determine the validity of hanging marks. Radiological investigations should be also performed.

Case Report: A 44-year-old man was found lifeless hanging from a rope wrapped around his neck and anchored on the balcony railing of his office. The forensic pathologist was alerted by the prosecuting officer and a detailed crime scene investigation was performed. The office was in clear state of disarray. There were four overturned chairs and many documents scattered on the floor simulating the scene of a violent crime. Despite all of this, a chair was found next to the railing on the balcony where the rope was anchored. No suicide note was found. Cadaveric temperature was 36°C, rigor mortis was absent, and non-fixed hypostasis was observed on face, upper thorax, and hands. There were no relevant marks of external injuries. Excoriations and purple discoloration were present along the entire course of the ligature mark of the neck. Sub-conjunctival hemorrhagic petechiae were also recorded. Police officers related that the man phoned his girlfriend and alerted her that he was meeting someone concerning some job-related trouble and that he was worried about his safety because of this appointment. The prosecutor expressly requested a complete autopsy to determine the cause and manner of death.

Two days later, a postmortem computed tomography scan was performed. It excluded bone and visceral injuries and no traces of violence on the corpse were detected. The autopsy was unremarkable except for a mild hemorrhagic infiltration of the left side of neck associated with mild muscle fiber rupture. Mild cerebral and pulmonary edema with white foam in the main bronchi were also described. Pulmonary edema, congestion, and focal compensatory emphysema were recorded at microscopic analysis. Toxicological analyses on blood and urine were unremarkable.

The microscopic observation of the skin specimens from hanging marks demonstrated intra-epidermal erythrocytes and musculature alteration as "Zenker's necrosis." In addition, immunohistochemical investigation of skin samples was performed utilizing antibodies anti-tryptase, IL15, and CD15 which confirmed the vitality of reactions of the ligature mark.

The crime scene findings (a chair next to the railing was found), the absence of any external injuries, and the presence of vitality reactions in the ligature mark resulted in the conclusion of a suicide mimicking a homicide as the manner of death. Hanging remains one of the most commonly used methods for suicide worldwide. It may pose difficult challenges to forensic pathologists and disguising a suicide as a homicide is uncommon. In this case, the crime scene was tampered with in order to stage the death as a homicide. The police investigations revealed that the man subscribed to a life insurance policy that was valid in case of homicide. Furthermore, after a detailed investigation, a receipt issued for the purchase of the rope was discovered at the home of the victim, confirming the hypothesis of suicide.

Hanging, Altered Crime Scene, Homicide



Pathology/Biology Section - 2015

H65 Impact of Postmortem Computed Tomography on the Evaluation of Strangulation Deaths

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The goal of this presentation is to aid forensic pathologists in understanding the utility of using Postmortem Computed Tomography (PMCT) as an additional tool in the evaluation of strangulation victims. Increasing reliance on technology has costs. Attendees will develop an understanding of the added benefits and inherent weaknesses of PMCT for strangulation deaths so they can determine the instances in which the use of this modality is appropriate.

This presentation will impact the forensic science community by assisting forensic pathologists in determining the settings in which PMCT is beneficial for the analysis of strangulation deaths.

Purpose: The value of PMCT was evaluated to augment autopsy in the evaluation of strangulation fatalities. The use of advanced radiologic imaging modalities such as PMCT may enhance the ability to detect injuries which may not be seen at autopsy.

Methods: This study combined a meta-analysis of strangulation (ligature and manual) deaths from other institutions with a cohort of similar deaths from New Mexico. A PubMed® literature search identified 30 studies which described autopsy findings in 576 strangulation deaths (50% ligature, 30% manual, and 20% combined or other) and two studies which described autopsy and CT findings in six strangulation deaths (33% ligature, 67% manual). In addition, 13 strangulation deaths were identified that underwent both autopsy and PMCT (46% ligature, 38% manual, and 16% other). The cases with autopsy only were compared with the cases of autopsy + PMCT for the presence of laryngochoyoid fracture and soft tissue hemorrhage.

Results: Fractures were detected in 53% of autopsy-only cases and only 26% of autopsy + PMCT cases. The detection rates of hyoid bone and cricoid cartilage fractures in autopsy-only and autopsy + PMCT cases were not significantly different: 27% and 11% ($p=0.11$) and 11% and 11% ($p=0.93$), respectively; however, the two cricoid cartilage fractures identified in the autopsy + PMCT cohort were only detected via PMCT. PMCT identified all hyoid fractures seen at autopsy, 2/3 thyroid fractures seen at autopsy, and 6/6 hemorrhages also identified at autopsy. There was a significantly lower incidence of thyroid cartilage fractures in the autopsy + PMCT cohort (45%) when compared to the autopsy-only cohort (21%), with a p value of 0.04. There was no significant difference between detection rates of hemorrhage.

Conclusions: No significant differences were identified between the findings described in autopsy-only strangulation deaths compared to autopsy + PMCT strangulation deaths, with the exception of the lower incidence in thyroid cartilage fractures in the autopsy + PMCT cohort. It is likely that the distribution of injuries in the autopsy-only and autopsy + PMCT groups were unequal due to the small sample size of the autopsy + PMCT group. While the frequency of cricoid cartilage fractures were similar between the cohorts, the only two cricoid fractures detected in the autopsy + PMCT group were discovered by PMCT. PMCT may have a role in detecting these subtle fractures. Additionally, the results indicate that PMCT, in most cases, is equally able to detect injuries in strangulation deaths as autopsy and may be used in place of autopsy in certain settings such as cases of accidental or suicidal strangulations.

Strangulation, Postmortem Computed Tomography, Autopsy



Pathology/Biology Section - 2015

H66 Cardiac Arrest During Police Restraint in a Man With Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT): Frightened to Death?

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After attending this presentation, attendees will be able to better ascribe the cause of death in these controversial cases and will understand the role of the molecular autopsy in cases of sudden death.

This presentation will impact the forensic science community by highlighting the value of the molecular autopsy and by revealing how an evolving knowledge base can lead to reinterpretation of existing genetic data.

Instances of sudden and unexpected death while in police custody remain complex and controversial cases in forensic pathology, providing unique diagnostic challenges. In general, the circumstances of these cases have resulted in two major theories to account for these deaths: “excited delirium syndrome” and positional (restraint) asphyxia; however, some cases that are not easily explained by one of these theories may be best explained by a theory from another emergent area in forensic pathology, non-structural genetic heart disease. One such case, a sudden arrhythmic death during struggle/restraint, is presented.

A 45-year-old man with developmental delay was walking outdoors, when, in a tragic case of mistaken identity, he was identified as a suspect by police officers, who attempted to take him into custody. He resisted this arrest violently. He was taken to the ground and restrained in a face-down position, from which he moved his extremities and pushed his chest off the ground. He was subsequently observed to be cyanotic and non-responsive. He was placed into a recovery position and resuscitation attempts were started. Paramedics found him to be asystolic. He was transported to the hospital and pronounced dead. The interaction with police was too brief to account for an asphyxial death. There was no history of a delirious state.

At autopsy, there were minor external blunt force injuries and small hemorrhages within the neck, arms, and back. The heart showed mild cardiomegaly with concentric left ventricular hypertrophy and sub-occlusive atherosclerotic luminal stenosis. Toxicological testing was negative for common drugs, including cocaine and its metabolites. Postmortem molecular testing demonstrated this man to be heterozygous for a catecholaminergic polymorphic ventricular tachycardia (CPVT) associated mutation (Phe189Leu) in the Calsequestrin 2 (CASQ2) gene. This mutation was initially classified as class II mutation (unknown significance), which would be expected to cause disease in a heterozygous state. Subsequently, the mutation was reclassified as a class I mutation (deleterious) that may cause disease in a heterozygous state. The cause of death was cardiac arrhythmia precipitated by struggle/restraint in a man with CPVT. This case illustrates the difficulty assigning a scientific cause of death in rare and controversial cases, the value of the molecular autopsy in identifying disease causing mutations, and how an evolving knowledge base can lead to reinterpretation of genetic data.

CPVT, Police Restraint, Molecular Autopsy



H67 **Atypical Self-Strangulation Through a Sphygmomanometer: An Uncommon Suicide Method**

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The goal of this presentation is to share the exceptional nature of the presented case which suggests taking sphygmomanometers into consideration as possible tools to perform self-strangulation. At the same time, it emphasizes the importance of the management of psychiatric patients and patient, whether psychiatric or not, with cognitive disorders; indeed, particular caution is required in order to keep them at a safe distance from objects that although apparently harmless, can become lethal.

This presentation will impact the forensic science community by presenting an unusual form of suicide performed by a young Italian man with a standard mercury sphygmomanometer, a manner of death previously not described in the reviewed literature.

A forensic approach by means of scene investigation, circumstantial data collection, autopsy, and toxicological investigation led to the conclusion that the cause of death was a mechanical asphyxia, ascribed to self-strangulation by an atypical item. The analysis of blood and urine excluded the presence of alcohol, amphetamine-methamphetamine, MDMA, barbiturates, THC, cocaine, opiates, methadone, and benzodiazepines.

Italy's National Institute of Statistics data for the period 1993-2009 suggest that hanging and other forms of asphyxia are the most common methods of suicide among men (52.1%), while for women asphyxia only represents the second cause of suicide (33.4%), with falls from height being the first (35.1%). Moreover, these official data also show that suicides most often occur in people over 45 years of age.

World Health Organization data confirms that suicide among young people is rare; however, in recent years, the trend has been increasing, especially in developing countries, with globalization-related changes in work practices and stress suggested as the possible causes of identity loss.

In the presented case, a 16-year-old male was discovered lifeless and seated on his bed by his mother (a nurse). The victim had suffered from Attention-Deficit/Hyperactivity Disorder Syndrome and was supported by personal teachers. He had a solitary and depressed character and had recently spent considerable time visiting satanic websites.

One day, after coming home from school while his parents were temporarily absent, he stole his mother's sphygmomanometer and strangled himself.

A thorough analysis of the literature reveals several cases of strangulation mainly related to accidents or homicides (e.g., deaths from life-threatening sexual practices; a peculiar case of a thief trapped between automatic sliding doors during a bungled attempt to enter a supermarket during the night; a victim of the well-known Isadora Duncan syndrome; strangulation by a scarf caught in the moving wheel spokes of a vehicle; choking by a rope passed around the victim's wrists, ankles, and throat (as a typical Mafia homicide); and so on). Other researchers describe uncommon suicidal strangulation methods (e.g., nylon rope around the neck and tied to the right ankle to prevent the rope from untying; a walking stick utilized with a tourniquet effect).

The use of a sphygmomanometer for this purpose is even rarer as it requires the repetition of the action to inflate the cuff around the neck with a progressive asphyxial mechanism and the onset of symptoms connected to this action.

Uncommon Suicide, Self-Strangulation, Sphygmomanometer



H68 The Importance of Postmortem Computed Tomography in a Gunshot Wound-Related Death: A Case Report

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After attending this presentation, attendees will have a better understanding of the usefulness of Postmortem Computed Tomography (PMCT) in a gunshot wound-related death.

This presentation will impact the forensic science community by demonstrating the accuracy of PMCT in the depiction of the wound track, discerning between entrance and exit wounds, and showing the bone's fracture and the internal course of the bone fragments.

PMCT is especially useful in gunshot wound cases, allowing an easier location and retrieval of the bullet and/or its fragments inside the body that may help in identifying the ammunition and the weapon type utilized; however, PMCT permits a detailed preliminary 3D documentation of the ballistic effects, allowing one to obtain essential information such as: accurate depiction of the wound track; discerning between entrance and exit wounds; and, demonstrating a bone's fracture and its fragments' course inside the body. Of these, the study of the bullet course inside the body is of extreme importance for the reconstruction of the event and to understand the positions of perpetrator and victim, with special regard to homicide cases involving fatal gunshot wounds.

A case of gunshot wounds in which PMCT scans were performed is presented. The case concerns a 73-year-old White male found unresponsive in his truck. Multiple gunshot wounds were observed on his body, affecting the upper arms, the neck, and the head.

A preliminary PMCT scan was performed before the postmortem examination. The following features were described in the CT report on the soft tissues of oropharynx: just in front of C2 vertebral body, a foreign conical-shaped metallic body was present (2x1.3cm) referable to a bullet; on the mandibular body, a burst fracture and a dislocation of teeth were present; on the left mandibular branch, multiple fractures were present; on the right maxillary bone, a displaced fracture was present that led to a dislocation of the alveolar arch; on the atlas vertebra, a fracture of the right anterior arch and a fracture of the right lateral mass were present; a misalignment between the atlanto-epistropheal joint and the C3 vertebral body was present; and, at the external examination of the body, 18 gunshot wounds were observed. Despite the high number of gunshot wounds, only six bullets were fired, suggesting the presence of multiple re-entry wounds. At the postmortem examination, the wound paths were able to be clarified.

In this case, the PMCT found all the wound paths and their direction inside the body. In particular, five wound paths (of which 4 blind paths), were identified with multiple re-entry wounds. In all cases, the wound courses traveled left to right, front to back and upwards. This study found the PMCT extremely accurate in the localization of the bullet; however, due to the PMCT, the misalignment between the atlanto-epistropheal joint, difficult to visualize using the standard autopsy examination, was found.

According to these findings, the dynamics of the event were reconstructed. The subject was sitting in the cockpit of his truck, while the offender was standing at a distance of >50cm from the left door (driver's seat side). The offender started firing multiple times, aiming at the head of the victim. The victim tried to protect himself by performing a right-side rotation and putting his left arm, forearm, and hand to the face, like a shield. The suggested scenario justifies the large number of gunshot wound found on the body, despite only six shots being fired.

In conclusion, the reported case emphasizes the importance and the advantages of the PMCT in forensic science and ballistics. The multislice scans together with the 3D rendering allow easy localization of bullets and their fragments inside the body in order to identify and reconstruct the wound paths, with special regard to the injury to the soft tissues, the parenchymal organs, and the bones; however, this technique is of great help in the identification of injuries located in body regions which are difficult to locate by dissection, such as the posterior neck region, the soft tissues of the face, and the cranial bases. The biggest disadvantage of this technique is the inability to recover the bullet or its fragments from the body by use of this technique alone.

Virtopsy, Gunshot Injuries, 3D Reconstruction



Pathology/Biology Section - 2015

H69 Terminal Performance of Zombie-Killing Bullets and Polymer-Tipped Bullets

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The goal of this presentation is to illustrate the terminal effects of polymer-tipped handgun bullets on the human body and the counter-effects of the body on the tipped bullet.

This presentation will impact the forensic science community by illustrating the terminal ballistics/performance of polymer-tipped handgun bullets that were recovered during autopsy. This presentation will illustrate the need for awareness of tipped bullets and collaboration between the forensic pathologist and the firearm examiner when dealing with cases of polymer-tipped bullets.

Introduction: Polymer-tipped rifle bullets have been around since the mid-1980s. They were first developed by Nosler® to provide streamlined shape with the expansion of a hollow-point bullet. Polymer-tipped handgun bullets, which are a more recent invention developed in the mid-2000s, are being encountered more by forensic professionals.

Polymer-tipped handgun bullets are designed to expand reliably and not clog like standard hollow-point bullets when fired through heavy clothing. The polymer tip/insert is also designed to feed reliably in semi-automatic pistols. The bullets are advertised to deliver controlled expansion with large deep-wound cavities over a wide range of velocities.

Materials and Methods: The autopsy and scene pictures were reviewed for two individuals that were shot with Hornady® polymer-tipped bullets. The first individual was a 24-year-old male who weighed 215 pounds and measured 74.5 inches. He was shot twice with Hornady® Zombie-Max™ ammunition in the trunk. The second individual was a 27-year-old male who weighed 148 pounds and measured 68 inches. He was shot once in the chest with Hornady® Critical Defense® ammunition.

Results: A slightly deformed non-expanded 45 caliber Hornady® Zombie-Max™ bullet with a green polymer tip was recovered from the left thigh. The bullet entered the right lower back and injured the skin, subcutaneous tissues, and muscles of the right buttock. The bullet lacerated the urinary bladder and fractured the pubic ramus. A deformed and fully expanded 45-caliber Hornady® Zombie-Max™ bullet was recovered from the chest muscles. The bullet entered the left back and injured the skin, subcutaneous tissues, and muscles of the back. The bullet lacerated the left lung and heart and fractured the left 9th rib.

A slightly deformed, non-expanded 40-caliber Hornady® Critical Defense® bullet with a red polymer tip was recovered from the soft tissues of the anterior chest. The bullet entered the left lateral chest and injured the skin, subcutaneous tissues, and muscles of the chest. The bullet lacerated the heart and fractured the left 3rd and 5th ribs.

Conclusions: Two of the three Hornady® polymer-tipped bullets did not perform as advertised. The 40-caliber Hornady® Critical Defense® bullet passed through a thick hooded sweatshirt and two thin T-shirts before impacting and fracturing two ribs. The 45-caliber Hornady® Zombie-Max™ bullet passed through denim jeans and cotton boxers before impacting and fracturing the pubic ramus. The counter-effect of human bones on the bullets caused crimping of the bullet tip, thereby entrapping the polymer insert, resulting in minimal expansion of the bullets.

Ballistics, Polymer-Tipped Bullets, Zombie



Pathology/Biology Section - 2015

H70 Savage Murder: Mutilation and Dismemberment — Why, When, and Where?

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The goal of this presentation is to illustrate an unusual case of homicide with dismemberment and to highlight some of the challenges of investigating and examining such cases.

After attending this presentation, attendees will have a better appreciation for the difficulties in evaluating homicides with mutilating injuries, as well as offer insight into the possible racial motivations which underlie such gruesome crimes. Attendees will also gain a greater comprehension of how the examination/investigation can be confounded by the different environmental conditions in which body parts may be found, which may result in postmortem artifact and may obscure the lesions associated to the immediate cause of death.

Case Report: On February 14, 2011, the mother of a 23-year-old African American man reported her son missing after his co-worker called her, worried that her son had not shown up for work for two days, which was unusual. Initial investigation revealed that the son was last seen with a White female in a bar three days prior and left to go to her home later that night. An interview with this White female revealed that her half-brother, who resided with her, came home and found the man with her in bed. The half-brother physically removed the man from the bed and told her that he was going to take him home. The suspect's best friend informed police that the suspect contacted him later the following day and asked him come to his house to help him. Upon arrival at the home, the best friend learned that the suspect had killed a man and already dismembered the body and placed the body parts into two separate plastic bags. The best friend further related that he helped to dump the body at the bottom of a steep drop over a hillside away from the residence area, where the trunk and upper and lower limbs were taken out of the bags and buried. The head was thrown away randomly to an unknown location by the suspect. At the time of discovery of the body parts, the trunk and all four extremities were well preserved. The head was found 24 feet away from the rest of the body parts and showed a significant amount of animal activity. A total of six dismembered body parts were found: (1) head, decapitated at the 3rd and 4th cervical intervertebral disc; (2) trunk with attached proximal upper and lower extremities; (3, 4) distal segments of the upper limbs severed at the lower third of the humerus; and, (5, 6) distal segments of the lower limbs severed at the distal third of the femur. The skin and soft tissue margins of the body segments were somewhat regular. The bone sections of limbs showed coarse striations and break-away spurs indicating the dismemberment tool was a saw.

The autopsy also demonstrated that the decedent sustained at least 72 sharp force injuries. The incised wounds seen on both the right and left hands were most consistent with defensive-type injuries. The stab wounds in the neck transected the left common carotid artery and perforated the trachea with extensive hemorrhage in the adjacent soft tissues which, taken together, were considered as the antemortem lethal injuries leading to the death. The rest of the incised and/or stab wounds were distributed in a clustered fashion involving the head and neck, right upper back, and left upper and mid back with relatively less or no hemorrhage in the adjacent soft tissues. Postmortem toxicology studies show ethanol: 0.140% in bile and 0.219% in urine.

Police found the blood-stained seats from suspect's car, the decedent's clothing, and blood-stained gloves in the basement of suspect's mother's house. DNA testing matched these blood stains to the victim's and the DNA in the gloves matched those of the suspect.

Discussion: Dismemberment is a relatively rare method of body disposal. Criminal dismemberment, with regard to all legal autopsies, has an average frequency of 1:500. Most cases involve the "traditional" tools for severing the body apart, such as a saw, axe, or knife. The features of the margin sections of the soft tissues and bones indicate that the suspect cut the skin and soft tissue with a sharp knife, then separated the bones with a saw. In the literature, most dismemberment is performed at the site of homicide. In this case, the killing was in suspect's car and the dismemberment was performed at the suspect's mother's house. The postmortem artifacts or decomposition related to environmental exposure sometimes obscure the physical integrity of the injuries or, in some instances, make the pattern and severity of injuries impossible to determine. The victim's head in this case was separately thrown in an open, outdoor environment. The result of this was that the soft tissue of the left side of the face and the neck, including the skin, muscle, tongue, and hyoid bone, was partially consumed by animals. The entrance of the lethal stab wound on the anterior neck was not clearly present at the autopsy but careful dissection and examination of the wound path and adjacent tissues still allowed the examiner to make the right determination. The suspect subsequently confessed that he attacked the victim in his vehicle with a stab through victim's anterior neck. At that time, the victim was intoxicated with ethanol and defended himself, but inefficiently.

Dismemberment, Sharp Force Injury, Postmortem Artifact

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Pathology/Biology Section - 2015

H71 The New Italian Law on Femicide: Was There a Real Reduction of Cases of Femicide? The Foggia (Apulia, Italy) Scenario

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After attending this presentation, attendees will better understand the evolution of the Italian scenario of femicide after the enactment of a new Italian law.

This presentation will impact the forensic science community by presenting information about the introduction of new Italian legislation and its effects on the reduction of cases of femicide, as reported by the Italian Ministry of the Interior; however, in Foggia, an increase of femicides was observed, resulting in many discordant opinions on the matter.

The word femicide is very recent. In 1992, Diana Russell, with the term “femicide,” defined it as extreme violence against a woman by a man because she is a woman, while in the 1990s, several terms were used to describe the murder of a woman that occurred within a relationship or cohabitation, including spousal homicide and family-related homicide. Statistics show that when a woman is killed, the perpetrator is often a man who has been intimately involved with her. A total of 2,412 femicides were recorded in Italy between 2000 and February 2014. Of these, 1,698 were committed inside the victim’s house or during relationships and 1,059 were committed by the husband, intimate partner, or ex-boyfriend. In 2013, a woman was murdered every three days in Italy and femicide represented 35.33% of all homicides. Due to the severity of this issue, in Italy a law was enacted in 2013 (Law n° 119, October 15th 2013). This law concerns new rules to fight gender-based violence in order to prevent femicide and protect victims, all within the framework outlined by the Convention of Istanbul (2011), the first legally binding international instrument on preventing and combating violence against women (defined as a violation of human rights and a form of discrimination against women and shall mean all acts of gender-based violence that could result in physical, sexual, psychological, or economic harm or suffering to women, including threats of such acts, coercion, or arbitrary deprivation of liberty, whether occurring in public or in private life) and domestic violence (violence that occurs within the family or domestic unit or between former or current spouses or partners, whether or not the perpetrator shares or has shared the same residence with the victim). According to the data of the central management of the Italian Criminal Police (State Police), in the first two months of 2014 compared to the same period in 2013, a decrease in cases of femicides was noted. In fact, in January/February 2013, femicides represented 29.41% of all homicides, compared with 22.4% of cases in January/February 2014; however, only the complete data of the year 2014 provide an insight into whether the law has achieved the desired results.

The goal of this study is to review the autopsy cases performed at the Department of Forensic Pathology, University of Foggia, Italy. Cases of femicide were selected and categorized according to the emotional relationship between the victim and the killer and on the basis of manner of death. The cases of femicide from 2001 to 2014 at the Department of Forensic Science of the University of Foggia were analyzed. An analysis of 1,281 autopsies performed in the department is presented with the goal of selecting which and how many of these were murders that had female victims, evaluating the mechanism of death, and the history of sentimental relationship between the victim and the offender. Of the 136 homicides in Foggia, 38 were femicides committed by the husband (18), intimate partner (15), ex-boyfriend (2), son (1), cousin (1), and uncle (1). Of the 38 femicides, 14 presented with blunt injuries, eight with stab wounds (1 with decapitation), 7 with gunshot wounds, 5 from asphyxial mechanism (1 with drowning after being linked to the hands and feet and sealed by scotch tape on the mouth, 2 by suffocation, 2 by strangulation), and 4 combined gunshot and blunt injuries.

Unfortunately, the data seems to confirm the doubts about the effectiveness of the law on femicide as they show an increase. In the case series, femicides represented 27.56% of all homicides before the enactment of the law and 40% of all homicides in the months after the law came into effect. In conclusion, the data continue to show a worrying increase in femicides.

Femicide, Homicide, Italian Law



Pathology/Biology Section - 2015

H72 Lethal Drug Intoxication of a Bank Robber: The Role of Physical Restraint

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The goal of this presentation is to discuss controversies that often arise concerning deaths that occur under physical restraint, with disputes due to increasing public concern on the recourse of violence by police officers and to the byzantine definitions of self-defense among countries' legal frameworks.

This presentation will impact the forensic science community by stressing the prominent role of a thorough synopsis of the results from testimony, scene inspection, and a complete autopsy – with radiological and toxicological analysis – when dealing with deaths that occur during physical restraint.

Choke holds, also known as “*shime waza*” in the sport of judo, are widely used by police officers to subdue violent suspects. It was once believed to be a safe and harmless way of controlling and subduing violent suspects; however, recently, multiple case reports and reviews have emphasized the possible lethal effect of neck compression during physical restraint.

Conversely, cocaine is a major cause (36%) among undetermined deaths occurring during physical restraint or custody. Cocaine is a powerful sympathomimetic agent; its role in the sympathetic sensitization of the myocardium has been widely discussed. Cocaine increases myocardial inotropy, heart rate, and blood pressure in a dose-dependent manner, thus increasing myocardial oxygen demand.

Some case series report deaths that occurred during choke holds on subjects whose blood samples were positive for cocaine; however, in these reports, cocaine blood levels are not quantified.

A case of an attempted bank robbery is presented. The perpetrator came into the bank wearing a baseball cap and sunglasses holding an electronic device, claiming it was a bomb. One of the bank clerks and a customer immediately realized that the electronic device was a power transformer and attempted to push the robber outside the bank. During the struggle, the robber pulled a screwdriver out of his pocket; he was tripped and pulled down. The robber was then immobilized on the floor, in prone posture, with the bank clerk pressing the assailant's thorax and an arm locking the neck, while the bank customer was blocking the robber's arms. The immobilization lasted approximately five minutes, per witness testimony. During this period, the robber's behavior escalated; he was agitated, shouting blasphemies, threatening bystanders, and suddenly showed disorganized and inconsistent thought processes and disturbances in speech. When police officers arrived at the scene, the robber was quiet. He was then handcuffed, but while moving him in a supine posture, the robber's face was described as cyanotic. Resuscitation attempts were then started but proved to be ineffective.

Scene inspection revealed bloody fluid on the ground, arising from the mouth and nostrils. Rectal temperature, measured two hours after death, was slightly increased from that expected. Conjunctival petechiae, mouth ulcers, and a small abrasion on the nose were found at external examination, with minor bruises on the arms and legs.

At autopsy, a meticulous dissection of the neck was performed, since choke holding of the robber's neck was reported by witnesses. Minor hemorrhagic infiltration of the soft tissue surrounding the left lesser horn of the hyoid bone was identified, without any fractures. A computed tomography scan with 3D reconstruction confirmed the macroscopic examination results. The lungs were congested and edematous, with foamy fluid in the airways. Heart dissection showed plaques on the right coronary artery (30%) and circumflex artery (50%). Histology revealed contraction bands in the myocardium.

Toxicological analysis was performed on blood, urine, and vitreous humor; gas chromatography/mass spectrometry assessed lethal blood levels of cocaine, benzoylecgonine, ecgonine-methyl-ester, and cocaethylene. Urine and vitreous humor were negative. Cocaine consumption occurred within an hour prior to the attempted bank robbery.

No evidence was found of significant trauma or a natural pathological cause which could independently justify the death. Data resulting from witness testimony, autopsy, and ancillary postmortem analysis allowed the determination of cocaine intoxication as the cause of death; the manner was declared as accidental. The mechanism of death involved a terminal arrhythmia, most likely due to sympathetic sensitization of the myocardium by cocaine and the stressful effects of the attempted bank robbery and the subsequent struggle. It can be speculated that the pre-existing coronary artery disease played a role in the establishment of the arrhythmia and in the failure of the resuscitation attempts.

Drug Intoxication, Physical Restraint, Choke Hold

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H73 The Forensic Science Investigations in Recent Cases of Victim's Cannibalism: Reality or Fiction? A Case of Matricide and Review of Literature

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After attending this presentation, attendees will be able to describe the impact of forensic science in cases of cannibalism.

This presentation will impact the forensic science community by demonstrating the crucial role of a multidisciplinary approach in a case of matricide in order to demonstrate that cannibalism occurred.

Introduction: Cannibalism is one of the most contentious topics in anthropology and there are many historical reports of cannibalism.^{1,2} The figure of the cannibal as an icon of primitivism took form in the encounter between Europe and the Americas and became a defining feature of the colonial experience in the New World.³ In mythology, religion, and literature, there are many examples of cannibalism that have been passed down over the centuries which do not strike us as shocking as long as they remain fixed in a symbolic context. There is not just one, but many forms of cannibalism.⁴ Today cannibalism is very rare. In a review of the literature, there are no studies which reported cases of parricide and cannibalism. Many studies have analyzed reasons for parricide; some studies have revealed that the parricide may increase with the presence of mental disorders or for the absence of an adequate treatment in subjects with psychiatric disorders.^{5,6} The attackers are usually suffering from schizophrenia with symptoms of active psychosis at the time of the crime.⁷⁻¹³ Less well known are the reasons that bring a murderer to cannibalism of his victim. In particular, in this study, there was cannibalism in the case of matricide.

Case Report: A case of a young man suffering from schizophrenia who killed his mother is reported. The matricide occurred through the use of a single weapon with sharp serrated edges. The body of the victim was found in her apartment. The victim was on the bathroom floor with evident dismemberment of the body. In the kitchen, some small portions of human tissue were found in the pots on the stove, presumably to be eaten. Portions of these human remains were boiled, while other portions were roasted. The technique of bloodstain pattern analysis was employed to detect the presence of small air bubbles in the blood stains imprinted on bathroom tile as by repeated and violent blows against the surface above the victim while still alive. The portions of human tissue found in the kitchen belonged to the victim. Moreover, feces belonging to the murderer was found in the toilet. There were larvae on the stool. Genetic analysis of the larval content showed the presence of the genetic material of the victim. This confirmed that the portions of human tissue found in the kitchen had been eaten by the murderer and therefore it has been scientifically proven that cannibalism had occurred. An autopsy with histopathological and postmortem computed tomography investigations were performed. The analysis of bony evidence revealed the presence of a "Y"-shaped incision from a blade with a serrated edge. In this case report, the examination of the injuries inflicted on the bones of the head allowed a determination that the trauma occurred when the subject was still alive. The histopathological examination of cranial bone showed the presence of edema with intraosseous hematic effusion, while the investigation of other dismembered body parts did not show this histopathological data.

Conclusions: The forensic investigation allowed the determination of the cause and manner of death. In this case, as in the cases of patricide, a correlation with psychiatric disorders was noted, in particular with schizophrenia, in the choice of victim's cannibalism. Therefore, this rare case demonstrated how the multidisciplinary approach in the evaluation of the findings at the crime scene was crucial in obtaining a scientifically proven result.



Pathology/Biology Section - 2015

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Forensic Science, Cannibalism, Matricide



H74 Death Following Intravenous Administration of Sucralphate

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After attending this presentation, attendees will understand how sucralphate — a drug used orally to prevent gastric ulcerations — can cause severe complications when administered intravenously and how to identify intravascular presence.

This presentation will impact the forensic science community by presenting a case report which serves as an example of the importance of applying unconventional analyses to answer a particular question in routine postmortem casework.

Sucralphate is a drug used for prevention of gastric and duodenal ulcers as well as for prevention of stress ulcerations of the gastric mucosa, particularly in patients under intensive care treatment for burns, multitrauma, neurosurgery, and more. It is administered orally, typically via a gastric tube, and will exert its effect by binding to the mucosa, where it stimulates factors that enhance the gastric defense barrier, thereby protecting against ulcerations. The many aluminum groups on this large sugar molecule are supposed to be crucial for the cytoprotective effect, similar to other antacids. Aluminium sucralphate has been shown not to enter the blood circulation to any significant amount upon oral administration.

An 83-year-old man underwent surgery for aortic valvular stenosis. After the surgery, bleeding from the gastric tube was noticed. Gastroscopy revealed erosive gastritis and it was decided he should receive tranexamic acid and omeprazol as well as four units of erythrocyte concentrate. In addition, aluminium sucralphate (brand name Andapsin®) was ordered in a dose of 2g (10mL)x3. His condition remained stable until the second day, when he suddenly developed bradycardia and experienced shortness of breath. He was taken to the intensive care unit, where he lost consciousness. Pericardiocentesis revealed no blood in the pericardial sac. Shortly thereafter, he became pulseless and stopped breathing. Resuscitation was unsuccessful. Later, a nurse who was new at the clinic and not experienced with all routines, reported that she was unsure if she had administered sucralphate into the gastric tube or into the central venous catheter just before the patient developed bradycardia.

The case was reported to the police because of suspicion of malpractice and a forensic autopsy was performed. Gross macroscopic findings included heart enlargement and heavy lungs, but no blood in the thoracic or abdominal cavities. Samples were taken for toxicology, including a sample from the left jugular vein/superior vena cava at the position of the intravenous catheter. Analysis for sucralphate was requested. The forensic toxicology laboratory explained that this drug would not be possible to identify by their screening method and that no known method existed for verification analysis of such a large molecule as aluminium sucralphate phosphate. Given the large number of aluminium groups present on the drug molecule, analysis for aluminium was considered a possibility since no or very little amount of this drug should be expected to be found in the blood if the drug was given orally. The department of inorganic chemistry at Umea University, Sweden, was contacted and agreed to analyze aluminium levels in the samples. To this end, both femoral and jugular blood samples were submitted from this patient, as well as blood samples from an additional two patients who later died at the same intensive care unit, although by known causes. One of them had also been administered sucralphate. An empty test tube was also sent for analysis. An accredited method for analysis of aluminium using graphite-oven atomic absorption spectroscopy was performed. The results are shown in the table below.

Case	Al conc (mcg/L)	Sucralphate dose
Present case, jugular blood	8800	2g x 3
Present case, femoral blood	2100	2g x 3
Control patient #1	30	1g x 4
Control patient #2	10	No
Empty test tube	2	No

Microscopy included immunohistochemistry with an anti-fibrinogen antibody, 1:500, and with Vulcan Red as a chromogen. Widespread, intravascular, well-developed networks of fibrin, capturing large numbers of polymorphonuclear cells, typical of extensive micro-embolism, were seen in samples from the lungs and kidneys.

Conclusion: The intravascular microthrombi are an expected finding upon binding of sucralphate to fibrinogen, promoting its aggregation. The extreme increase in aluminum levels in the blood of the patient along with the presence of microthrombi strongly supported the notion that sucralphate indeed had been given intravenously.

Antacid, Intravascular Coagulation, Malpractice

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H75 Alibi Verification Using Diatoms

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After attending this presentation, attendees will understand the use of diatoms in a forensic case to verify an alibi.

This presentation will impact the forensic science community by utilizing evidence that was previously disregarded but in actual fact contributed to solving a case.

The corpse of a young girl was found on the shore of Bracciano Lake (Rome, Italy) one early morning in November, 2012. The corpse was identified as a girl who disappeared the day before, after she spent a night out with her boyfriend. The relationship between them was known to be turbulent and the boy, when questioned about the events of the night of the girl's disappearance, admitted to having an argument with her. Furthermore, he told the investigators that because of the argument he let the girl out of the car late that night, not far from the lake and a few kilometers from her house. When questioned about the time between letting her out of the car and the discovery of the corpse, he stated that he was at home and had never been at the lake that night.

The autopsy noted there was no sign of violence associated with the body. She had not been sexually abused and the results of the toxicological analyses were negative. The death was found to be as a consequence of natural causes but the results were still controversial. Due to the circumstances surrounding the death, a further investigation was requested to clarify the events of that night. In particular, an investigation was conducted to verify if the boy had been at the lake that night.

The resulting investigation was an analysis of the boy's clothing to determine the presence of diatoms and to identify if the diatom assemblage of the lake could be matched both to his clothes and to the place where the corpse was found.

Diatoms are unicellular organisms that are present in almost all natural aquatic environments such as seas, lakes, rivers, streams, and even puddles of rain water, but are generally absent in tap water. Many species are habitat specific. Diatoms have a hardened silica structure that is resistant to chemical treatments and have morphological features that can be used for species identification. Diatoms can be found in clothing, proving a possible contact with a particular water type in which a specific diatom community is present.

All the garments belonging to the boy underwent a diatom test and samples of both tap water of the house and the water of the lake were used as reference. The glass bottles used to collect the water were sterile to avoid the presence of extraneous diatoms, while each garment collected was placed in a separate bag to avoid possible diatom transfers between items. Formalin was added to both of the reference waters and after settling for 48 hours, the supernatant was discarded. The pellet was first treated with H₂O₂ and then with 10% HCl. This was followed by three washing steps: (1) a 2g-sample of each of the boy's clothes were rinsed in 70% ethanol; (2) each sample was placed in a 50ml plastic tube with 50ml ethanol solution (70% in water) and left in a rotator for 48 hours; and, (3) the cloth was removed and after settling for 48 hours, the supernatant was discarded.¹ The pellet underwent the same treatment as the reference waters.

Samples were mounted with a high refractive index medium and were analyzed with an optical microscope. The species composition was determined from both a subsample from the lake, tap water, and clothing pellet and the species of diatoms were compared and recorded. A large number of diatoms were found in the water samples collected from the Bracciano Lake, while only a single diatom cross-matched with the sample of tap water. Furthermore, diatoms extracted from a number of pieces of clothing belonging to the boy were a positive match with the diatoms collected from Bracciano Lake.

An interesting discovery during this investigation was the identification of the outfit that the boy was wearing the night of girl's disappearance, which happened to be the clothing that was matched to the diatom test.

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Diatoms, Alibi, Clothing

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H76 Sudden Death After Methylprednisolone Sodium Hemisuccinate Injection: A Rare Case of Anaphylaxis

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After attending this presentation, attendees will understand a fatal case of anaphylactic shock due to methylprednisolone sodium hemisuccinate in a 19-year-old male, affected by optical neuritis, in treatment with endovenous administration of methylprednisolone sodium hemisuccinate.

This presentation will impact the forensic science community by examining a case that, because of the rarity of anaphylactic shock due to methylprednisolone, underlines the usefulness of the laboratory tryptase enzyme immunoassay, such as a complete immunohistochemical study of all specimens, on blood samples to obtain a correct etiopathogenetic definition in cases of anaphylaxis.

The diagnosis of death from anaphylactic shock is difficult and often misunderstood because it is uncommon. Although anaphylaxis is not a frequent adverse effect of corticosteroid treatment, its diagnosis is very important because of the widespread use of corticosteroids that, due to their efficacy as anti-inflammatory and immunosuppressive agents, are widely used in the treatment of a large number of diseases, such as asthma, allergies, and autoimmune and neoplastic diseases. Adverse reactions due to corticosteroid hypersensitivity, including anaphylaxis (first described in 1974), angioedema, urticaria, generalized cutaneous eruption, and severe bronchospasm occur only in 0.3% of cases involving systemic administration. Corticosteroids probably act as haptens because of their low molecular mass.

The following case concerns a sudden death due to methylprednisolone sodium hemisuccinate administration.

Case Report: A 19-year-old healthy male suddenly presented with a decrease in visual acuity more evident in the right eye. After several clinical and instrumental checks including fundus examination, optical computed tomography, and ocular electrophysiology, a right eye retrobulbar optical neuritis was diagnosed. The patient was started on a treatment with 25mg prednisone tablets. After nine days, the patient showed hyperglycemia (glucose concentration of 379mg/dl) associated with the presence of ketone bodies in the urine. Due to this condition, he was prescribed first metformin and then subcutaneous insulin therapy. After five days, the boy was admitted to the emergency department for the persistence of hyperglycemia (400mg/dl). A diagnosis of diabetic ketoacidosis was made and intravenous insulin therapy and hydration with saline and bicarbonate infusion were administered. The following day, he suspended infusion therapy and started subcutaneous insulin therapy. After ten days, because of the worsening vision, the boy started a three-day endovenous administration of Solu-Medrol® in 250cc saline drip with a slow drip of two hours duration. On the second day, after the daily administration, the patient showed cough and spontaneous urinary loss associated with seizures, stupor, and falling to the ground with subsequent cardiopulmonary arrest. The patient died, despite cardiopulmonary resuscitation efforts. An autopsy was performed 24 hours after death. External examination was unremarkable; internal examination showed cerebral edema with moderate swelling of cerebellar tonsils and bilateral pulmonary edema. Histological and immunohistochemical (CD4, CD15, CD68, CD20, CD3, CD8, and CD45) investigation of brain specimens revealed a diffuse cerebral edema in association with bilateral optical neuritis and chiasmal optic neuritis. Lung samples showed subpleural and interstitial hemorrhage, intra-alveolar and diffuse interstitial edema, and acute stasis; other organs were unremarkable.

The laboratory tryptase enzyme immunoassay on blood revealed a tryptase concentration of 136.50mcg/L (n.v. 0.00-15.00mcg/L), greater than the cut-off value of 45µg/l for the diagnosis of anaphylactic shock. The death was attributed to anaphylactic shock due to methylprednisolone sodium hemisuccinate, most probably due to its excipient.

Corticosteroid, Anaphylaxis, Methylprednisolone



H77 **Body Packers and Body Stuffers: The Role of Cardiac Oxidative Stress on Myocardial Damage**

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The goal of this presentation is to elucidate the mechanisms of acute cardiac toxicity for very high blood concentrations of cocaine such as those found in dead body packers and body stuffers. Presented are seven cases of death from cocaine overdose in the absence of other psychotropic substances; the results of the immunohistochemical and biochemical analysis used to study the role of oxidative stress in the myocardial damage.

This presentation will impact the forensic science community by emphasizing the problem of acute myocardial damage and the necessity of understanding the mechanisms of acute cocaine cardiotoxicity in order to explore a clinical-translational approach to this problem.

The “body-stuffer,” usually small-time drug user or dealer, is a person who, when intercepted by authorities, ingests the drug coarsely packed in pouches made from scraps of polyethylene, cellophane, aluminum foil, or paper. These packages can easily open, causing acute poisoning syndromes.

Case 1: A 29-year-old woman in police custody was referred for a general disease. She was admitted to the emergency department but a fatal cardio-respiratory arrest occurred suddenly. No foreign material was detected from a total-body computed tomography scan. The autopsy revealed a single, open plastic bag in the gastric lumen and several deep ulcers of the gastric mucosa. Cocaine and benzoylecgonine were detected in the blood (1.72mcg/ml and 4.31mcg/ml, respectively) and in the urine (5.74mcg/ml and 43.23mcg/ml, respectively).

Case 2: A 32-year-old man was found in cardiopulmonary arrest in an apartment. During the autopsy, ten single plastic bags were found in the intestinal lumen and necrosis of the intestinal wall was detected. The concentration of cocaine in urine was 147mcg/ml.

Case 3: A 34-year-old man was found in cardiopulmonary arrest in an apartment and a spoon with white powder was found next to him. The autopsy revealed a single plastic bag in the esophagus. High levels of cocaine and benzoylecgonine in his blood (23.76mcg/ml and 6.57mcg/ml, respectively) and in urine (230.42mcg/ml and 737.27mcg/ml, respectively) were detected.

Case 4: A 30-year-old man was found in a car. A single opened plastic bag in the gastric lumen was found at the autopsy. High levels of cocaine in his urine and blood were found (42.29mcg/ml and 3.00mcg/ml, respectively) and the concentration of benzoylecgonine in his blood and in urine were 11.09mcg/ml and 92.50mcg/ml, respectively.

Case 5: A 20-year-old female ingested a plastic bag with cocaine and after two hours developed convulsions and respiratory failure. The autopsy revealed a single plastic bag in the gastric lumen. Cocaine and benzoylecgonine in her blood (7.03mcg/ml and 10.67mcg/ml, respectively) and urine (816.92mcg/ml and 540.17mcg/ml, respectively) were detected.

Case 6: A 35-year-old man was found in his car. An autopsy was performed and a broken plastic sachet was found in the intestinal lumen. The levels of cocaine in his urine and in blood were 11.41mcg/ml and 0.10mcg/ml, respectively, while the levels of benzoylecgonine were 1,609.26 mcg/ml in his urine and 8.87mcg/ml in his blood.



Pathology/Biology Section - 2015

Case 7: A 17-year-old man ingested a plastic bag with cocaine to avoid arrest. During the autopsy, the package was found in the gastric lumen. In his blood, the following concentrations, 98.1mcg/ml of cocaine and 86.1mcg/ml of benzoylecgonine, were detected; in his urine, their concentrations were 10mcg/ml and 4.2mcg/ml, respectively. At the autopsy, cerebral and pulmonary edema, slight heart hypertrophy, and multiple bluish areas in the ventricular wall were described. The histological investigations confirmed the macroscopic findings and revealed diffuse foci of contraction band necrosis. An immunohistochemical study with Ab anti IL-1 β , IL-6, TNF- α , IL-8, b1 adrenergic receptors, NF-kB, Bcl-2, and apoptosis was performed. The biochemical examination revealed an alteration of antioxidant systems, the reduction of the GSH/GSSG ratio and a significant increase of MDA.

Body Stuffer, Acute Cocaine Cardiotoxicity, Cardiac Oxidative Stress



H78 Crystallins Aging Process and Related Post-Translational Modifications (PTMs) in Determination of Human Age

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After attending this presentation, attendees will learn that forensic chemists can use the techniques presented here to identify the age of unknown deceased persons by using state-of-the-art technology.

This presentation will impact the forensic science community by presenting a method for determining human age by Post-Translational Modifications (PTMs) in lens crystallins as a novel forensic method for fast and potentially accurate age determination of unknown deceased persons.

The increase in the proportion of D-isomer of Aspartic Acid (Asp) relative to the natural occurring L-isomer (i.e., racemization) has been widely used in archaeology and geochemistry as a tool for dating; however, its application in forensic science is has been limited. Among the methods available to identify the age of the deceased is the analysis of the D/L-Asp ratio in human teeth; however, this can take a long time due to the fact that the samples must be dissolve. In comparison, the human eye lens is relatively easy and quick to analyze and could be used as a complementary technique to already existing methods. Among the applications of this dating method is the age determination of unknown casualties of mass disasters.

Human lens protein crystallins have shown extraordinary potential as biological clocks; over the human lifetime, the lens accumulates these proteins with no turnover. This key characteristic indicates that in healthy people most PTMs of crystallins are mainly due to aging. In this study, several important PTMs will be investigated, including deamidation, racemization, and aggregation (disulfide bond formation). Racemization primarily occurs in aspartic acid, while deamidation is one of the most frequently occurring PTMs of asparagine and glutamine. Moreover, disulfide bond formation leads to the formation of water insoluble proteins resulting in protein precipitation and cataracts.

At this stage, this study has used carp eye lens as a model to develop techniques for detecting eye protein PTMs. The developed method for crystallins analysis will be then applied to human crystallins in collaboration with George Washington University Medical School.

To extract the crystallins, decapsulated lenses were homogenized and dissolved in buffer. For deamidation and aggregation studies, proteins were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and desirable bands were cut, reduced, alkylated, and digested by different enzymes including trypsin. The digested solution was analyzed using nano-liquid chromatography with tandem mass spectrometry.

The presented method for determining human age by PTMs in lens crystallins gives forensic scientists a method for fast and potentially accurate age determination of unknown deceased persons.

Dating Human, Eye Lens, Proteomics



H79 **Hydrophilic Polymer Embolization: A Common Incidental Finding at Medical Autopsy**

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After attending this presentation, attendees will: (1) understand what hydrophilic polymer is and how and why it is used in the medical community; (2) be able to identify hydrophilic polymer emboli and the associated tissue reactions on histology slides; and, (3) become familiar with the potential medicolegal implications of hydrophilic polymer embolization.

This presentation will impact the forensic science community by providing education and improving competence in identifying a common, but only recently described, autopsy finding.

Background: Hydrophilic polymers are substances that are commonly applied to the surface of vascular catheterization devices in order to reduce friction and decrease vascular injury related to cannulation. These polymers have the potential to detach and travel within the bloodstream, causing hydrophilic polymer embolization. These basophilic, often coiled, and focally granular emboli are commonly detected in the lungs at autopsy in patients who have undergone cannulation with large-bore vascular catheters prior to death. Hydrophilic polymer embolization is most commonly an incidental finding but associated complications have been reported.

Design: A retrospective selective autopsy review was conducted at a tertiary care center to identify cases of hydrophilic polymer embolization in the lungs during a 20-month period (November 2012 to June 2014). Autopsy cases were selected for review based on likelihood of detecting emboli; only cases involving large-bore vascular catheterization (central venous catheter, Peripherally Inserted Central Catheter (PICC) line), Extracorporeal Membrane Oxygenation (ECMO), angiography, etc.) prior to death were included for review. Original hematoxylin- and eosin-stained slides of lung tissue were examined for the presence of hydrophilic polymer emboli. The total number of slides containing hydrophilic polymer emboli, number of emboli per slide, and tissue reaction to the polymer were also noted. These histological findings were subsequently correlated with clinical information obtained prior to death.

Results: A total of 33 autopsy cases were reviewed (ages: 2.5 months to 78 years; 19 males). In all cases, the decedents had at least one medical procedure involving large-bore venous catheterization prior to death. Of these, nine cases (27%; ages: 2.5 months to 72 years; four male) showed histologic evidence of hydrophilic polymer emboli within the lung tissue. Six of these cases (67%) demonstrated unilateral lung involvement; one case (11%) demonstrated emboli within two lobes of the same lung. In two cases (22%), three lung lobes showed histologic evidence of hydrophilic polymer embolization. Two cases (22%) demonstrated no evidence of tissue reaction to the polymer. Five cases (56%) revealed macrophages and/or giant cells surrounding the polymer; however, in two cases (22%), a more significant reaction was identified, including macrophages and/or giant cells, vascular intimal proliferation, and in one case (11%), thrombus. On medical record review, none of the nine cases were associated with any morbidity or mortality directly linked to hydrophilic polymer embolization.

Conclusion: Hydrophilic polymer embolization, particularly in the lungs, is a common finding at autopsy in patients that have undergone large-bore vascular catheterization prior to death. This finding is largely incidental; however, histologic evidence of tissue reaction can be seen in many cases and rare cases of associated morbidity and mortality have been reported. As such, polymer emboli represent a newly-recognized iatrogenic complication with potential medicolegal implications.

Hydrophilic Polymer, Embolization, Catheterization



Pathology/Biology Section - 2015

H80 Fatalities Associated with Aldicarb

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After attending this presentation, attendees will better understand the rodenticide aldicarb, including its intended uses, toxic effects, and because of its availability in certain communities, its use as a suicide modality.

This presentation will impact the forensic science community by providing information on aldicarb, which may be easily obtained and used illegally in households for pest control within the United States. Aldicarb has been intentionally ingested and poses a risk for accidental overdose in adults and children due to improper packaging and its accessibility to small children. Aldicarb is often admixed with food and placed on floors and cupboards for rats and cockroaches to consume.

Aldicarb is a rodenticide, insecticide, and nematicide. It is a systemic carbamate that is a potent cholinesterase inhibitor.¹ Symptoms of aldicarb intoxication include miosis, nausea, vomiting, diarrhea, diaphoresis, headache, and blurred vision.² The mechanism of death is often respiratory depression and pulmonary edema.

The poison is produced in Latin American countries, such as the Dominican Republic and Mexico. Aldicarb is still widely used as a commercial pesticide in farming. The use of aldicarb as a pesticide in households is illegal in the United States, as directed by the Environmental Protection Agency. In communities with large Hispanic populations such as New York City, aldicarb is often brought from countries of origin by families and vendors because of its desired efficacy as a rodenticide.³

A review of the toxicology database from the Office of Chief Medical Examiner City of New York identified two fatalities where aldicarb was detected. In both cases, aldicarb directly contributed to the cause of death.

Case 1: A 60-year-old man was found dead in his bed at home. The decedent had been depressed, expressed suicidal ideation, and had been abusing alcohol. Initial investigation revealed a coffee mug containing granules on the bedside table. Further investigation of the scene by the investigator and a family member revealed an envelope labeled “Tres Pasito,” along with several empty vodka bottles, which were found in a closet. A foam cone was described by family. At autopsy, several granules were noted on the face, chest, and in the gastric contents. Advanced atherosclerotic cardiovascular disease was also noted at autopsy.

Case 2: A 44-year-old woman was taken to the emergency department, having been found unresponsive at home. The decedent had a history of depression with suicidal ideation. The family reported to the treating physician that an open package of rat poison they referred to as “Tres Pasito” was found near the woman. Despite approximately one day of resuscitative measures, the patient did not survive. At autopsy, innumerable tan flakes and granules were found in the esophagus, stomach, and small intestine. Liver necrosis, acute tubular necrosis, pulmonary edema, and acute diffuse anoxic ischemic encephalopathy were diagnosed clinically and confirmed by autopsy.

In both autopsies, toxicological analysis of the postmortem blood, urine, and gastric contents by liquid chromatography revealed the presence of aldicarb. The cause of death was acute aldicarb intoxication and the manner of death in both cases was suicide. Both decedents had intentionally ingested the poison.

Aldicarb is usually not included in routine toxicology panels. It therefore may easily be overlooked by a forensic pathologist or a clinician. A thorough scene investigation and detailed history from the family, including questions regarding depression and past suicidal intent, may provide answers that raise the level of suspicion to warrant further and more specific toxicologic analyses.

Clinically, the presenting anticholinergic symptoms should always remind a treating physician to include aldicarb in the differential diagnosis, as there are many communities in the United States where aldicarb is easily purchased at the neighborhood market.

References:

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3. Poisonings Associated with Illegal Use of Aldicarb as a Rodenticide – New York City, 1194-1997. *Morbidity and Mortality Weekly Report*, CDC. 1997, 46 (41): 961-963.

Suicide, Aldicarb, Toxicology



Pathology/Biology Section - 2015

H81 An Extremely Enjoyable Rave Party Resulting in a Fatal Sleep: The Reliability of Postmortem Computed Tomography in a Case of Crush Asphyxia

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After attending this presentation, attendees will understand the importance of Postmortem Computed Tomography (PMCT) in cases of crush asphyxia, with special regard given to the presence of gas (pneumothorax and subcutaneous emphysema) in corpses in a good state of preservation.

This presentation will impact the forensic science community by showing how PMCT allows forensic pathologists to clarify the causes of death and to verify the presence of vital signs even before the traditional autopsy.

The use of PMCT is growing in the field of forensic pathology as a supplement to the traditional autopsy because it is an objective and non-invasive approach. In particular, CT is an extremely reliable diagnostic instrument in several important areas of forensic pathology, such as identification of fractures, foreign bodies, and gas.

The corpse of a 28-year-old man was found under a car. According to the statement of the car's owner, in the early morning, he started his car but the vehicle did not move easily. Therefore, he exited the vehicle and found an unresponsive man lying on the ground under the car. Rescuers jacked up the car and extracted the corpse. According to the circumstantial evidence, the victim had been at a rave party the night before, where he had been heavily drinking.

A preliminary PMCT examination was performed five hours after the death. The following features were described on the CT report: atlo-axial dislocation, bilateral sternoclavicular joint dislocation, and multiple vertebral and rib fractures. On the upper part of the thoracic cavity, an anterior bilateral pneumothorax was present together with diffuse subcutaneous emphysema of the upper anterior trunk.

At the external examination, an ecchymotic mask was observed. Multiple abrasions and lacerations were irregularly widespread over the entire body surface. On the left side of the left nipple, a circular-shaped imprint abrasion was observed. Palpation of the skin over the anterior chest showed evidence of crepitus. The specific autopsy test for pneumothorax was performed, showing a negative result. Rib fractures were confirmed, surrounded by hemorrhage of the soft tissues. Lacerations of the parietal pleura, multiple tears of the lungs, and a left hemopneumothorax were found.

Toxicological analyses showed a concentration of ethanol of 351mg/dL in the peripheral blood and 387mg/dL in the urine. Immunohistochemistry analyses on the left hemithorax imprint abrasion were performed, showing a positive reaction to glycophorin.

In this case, compression of the chest interfered with the respiratory movements. Moreover, it led to severe injuries of the chest wall, resulting in bilateral rib fractures and tears of the lungs with hemopneumothorax. The air then moved into the subcutaneous tissues of the thorax due to a pressure gradient. Since a subject needs to be alive to realize this gradient, pneumothorax and subcutaneous emphysema are vital signs.

The well-timed PMCT examination allowed identification of these vital signs before the traditional autopsy. According to these findings, a specific autopsy technique to identify pneumothorax was performed with negative results, probably due to the small amount of air in the pleural cavities. Thus, PMCT can be considered as the most sensitive method for the identification of body gas, with special regard to small size pneumothorax. Moreover, the circular abrasion located on the left thorax was analyzed by immunohistochemistry, showing a positive reaction to glycophorin. Hemorrhages in the soft tissue surrounding the rib fractures were observed, confirming that the subject was still alive before being run over by the car.

In this case, PMCT was useful to clarify the mechanism of death and to identify signs of vitality before the autopsy examination. Since the autopsy test for pneumothorax identification was negative, without postmortem CT the presence of pneumothorax would definitely have been unrecognized.



Pathology/Biology Section - 2015

The main limitations of PMCT in case of pneumothorax and subcutaneous emphysema are the preservation status of the corpse and the employment of cardiopulmonary resuscitation procedures. A corpse in a good state of preservation, together with detailed circumstantial evidence and crime scene investigation, could allow the forensic pathologist to clarify the cause and mechanisms of death using only PMCT.

Postmortem Computed Tomography, Pneumothorax, Crush Asphyxia



Pathology/Biology Section - 2015

H82 3D Body Surface Documentation in Forensic Pathology

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After attending this presentation, attendees will understand the cost and benefit of employing innovative 3D body surface documentation techniques in the course of forensic postmortem investigation; they will comprehend technological and methodological advances that allow documenting physical evidence in 3D in a rapid, affordable, yet photorealistic and accurate manner.

This presentation will impact the forensic science community by presenting results that advance currently available documentation techniques employed in the course of routine forensic postmortem examinations.

For the last few years, several projects have invested in establishing image-guided postmortem examination in the framework of forensic pathology — utilizing either medical imaging technologies (Computed Tomography (CT), magnetic resonance imaging) or optical surface scanning. Cross-sectional and surface data are both stand-alone techniques which bring a number of benefits but also a few shortcomings into postmortem examinations. While CT-based images have the capacity to generate a 3D model of any internal structure, under the condition that the contrast between two neighboring tissues or structures is sufficient, they provide little or no information about surface coloring (bruises, lesions), spots, or subtle morphological interferences. Optical surface models, in contrast, lack information on what is going on beneath the surface, but feature high-resolution geometry and surface color information.

Several 3D surface technologies, in particular photogrammetry and optical and laser scanning, have recently advanced into affordable, flexible, and accurate techniques involving a reasonably long learning curve; however, forensic postmortem investigation as performed on a daily basis has not benefited from their full potentials and the currently utilized methods are far behind what 3D surface documentation can provide. Conventionally, 2D photography presents the gold standard utilized throughout the entire process of postmortem examination, documenting the pre-autopsy state of preservation of the corpse, the presence of unique somatic traits or perishable findings, and injuries and/or pathological changes; however, photography discards surface depth — information highly valuable in terms of damage assessment or trait uniqueness.

In this study, two approaches to 3D external body documentation were tested — traditional digital camera-based photogrammetry combined with commercial photo-scan software and stereophotogrammetry-based scanner VECTRA H1®, a novelty product among portable hand-held surface scanners. In order to conduct the study, two forensic cases admitted for postmortem examination at the Department of Forensic Medicine, Hradec Králové, Czech Republic, were selected. A 63-year-old male, who died of traumatic, self-inflicted injuries (suicide by hanging) and a 63-year-old male diagnosed with heart failure. Both cases were photographed in 360° manner with a digital camera mounted on a tripod. Altogether, 35 to 70 images per case were taken, corresponding to 20 to 40 minutes of capturing time. Approximately the same time was required to document the body surface with the hand-held scanner, where up to 120 scans were taken.

The optical surface scanner proved itself to be a useful tool for being able to document small-to-large areas of the body surface. As not being specifically designed to scan objects on a larger scale, post-processing requires rather time-consuming manual image alignment; however, the device produced high-resolution 3D images, comparable in quality to any professional digital camera. The photogrammetry also provided photorealistic records of body surface capable of capturing, for instance, ligature marks, tattoos, and skin lesions in high quality. Moreover, the utilized software was able to create the whole body 3D surface mesh automatically; however, both methods failed when the surface was covered with body hair or reflective, moist areas were being documented.

In conclusion, both methods produce realistic, actual-size, or easy-to-calibrate 3D surface models useable as an advanced method of postmortem documentation and were easily fit to be the subject of further examinations, such as 3D mesh comparison or indirect measurement (e.g., body measurements, angles of penetrations). Digital 3D data can be easily archived, transported, and shared between laboratories; they provide a real-time access for re-examination of physical evidence. Furthermore, no requirements for calibration or operating skills make them ideal to be easily integrated into a daily workflow.

3D Body Scanning, Image-Guided Examination, Photogrammetry



Pathology/Biology Section - 2015

H83 Postmortem Changes on Computed Tomography (CT) Can Assist With the Diagnosis of Biliary Tract Disease

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After attending this presentation, attendees will gain an appreciation for some of the differences in postmortem imaging and clinical diagnostic imaging; specifically, the impact of postmortem artifacts and changes on diagnosis and forensic examination.

This presentation will impact the forensic science community by illustrating a technology-based example of forensic theory — namely that, in some circumstances, the changes that occur and progress after death (as visualized on postmortem Computed Tomography (CT)) can be the key to stepping backward to determine how and why some people die.

Postmortem changes such as hypostasis in the lungs, hematocrit effect in the dural sinuses, and air collections caused by gas-forming organisms can be distracting to pathologists and radiologists assessing Postmortem Computed Tomography (PMCT) images. Much like postmortem artifacts on gross examination, these findings can obscure, mimic, or be intensified by real pathology. It is important for forensic pathologists to know the difference and be able to draw inference from these findings in order to reinforce their conclusions.

Reported are two cases of sudden unexpected death where biliary tract disease was suspected by localized gas collections following PMCT and confirmed at autopsy. The first involved a 70-year-old woman with a short history of bilious vomiting with sudden collapse. In another case, a 55-year-old man was found dead sitting upright in his bathtub. At the time of discovery, he was slumped over, but his face was not submerged. For two days he had not been feeling well, but did not seek medical attention. In both cases, the postmortem interval to CT was less than ten hours.

Dramatic pneumobilia was noted on PMCT of both cases in the absence of putrefactive changes elsewhere. The first case also showed an obstructive pattern in the small bowel with dilatation and air-fluid levels and, on further examination, a large obstructing ovoid structure was seen in the jejunum. Gallstone ileus was confirmed at autopsy with a fistula between the gallbladder and duodenum. Pneumobilia is attributed to gas from the small bowel gaining access to the biliary tract. In addition to pneumobilia, the second case showed fat stranding in the vicinity of the gall bladder bed, but no other significant findings. The common bile duct was dilated and ascending cholangitis was detected by histology. Pneumobilia in this case is attributed to fermenting processes of gas-forming organisms in the biliary tract.

These cases illustrate the fact that not all postmortem changes seen on CT imaging should be discounted. Occasionally, regional differences in postmortem changes or artifacts can be leveraged to guide specialized dissection and sampling at autopsy. For this reason, postmortem changes must be assessed in the context of the clinical history, external examination, the postmortem interval, their extent and localization in the body, as well as the internal autopsy findings.

Postmortem CT, Forensic Pathology, Postmortem Artefacts



H84 Idiopathic Arterial Calcification of Infancy: A Case Report With Postmortem Computed Tomography (PMCT) and Histologic Findings

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After attending this presentation, attendees will understand the rare genetic disorder of Idiopathic Arterial Calcification of Infancy (IACI) and advanced radiographic, gross, and histologic findings.

This presentation will impact the forensic science community by describing the different pathological and clinical diagnostic modalities and by increasing awareness of this rare genetic disorder.

This presentation reports the Postmortem Computed Tomography (PMCT) and histologic findings of IACI, a rare genetic disorder with approximately 180 cases reported in the literature. IACI is characterized by extensive generalized arterial calcification and intimal proliferation of large- and medium-sized arteries. In 75% of cases, the affected infants are either homozygous or compound heterozygous for an Ectonucleotidepyrophosphatase/Phosphodiesterase 1 (ENPP1) gene mutation. ENPP1 is a cell surface enzyme that generates extracellular pyrophosphate, which regulates vascular smooth muscle differentiation and inhibits soft tissue calcification. The critical vascular stenosis and reduced vessel compliance in IACI causes severe heart failure with hydrops fetalis, hypertension, postnatal cardiomegaly, respiratory failure, and myocardial infarction. Most affected infants are diagnosed at autopsy. *In utero* diagnosis of IACI is based on the presence of dystrophic calcifications by fetal echocardiography. Although several cases of prolonged survival with persistent cardiovascular sequelae have been reported, the majority of affected infants will not survive beyond six months of age, especially when coronary arteries are involved. Although a curative treatment for IACI and medical management of cardiovascular effects has generally been unsuccessful, the use of prostaglandin E1 and bisphosphonate such as etidronate, a chelating agent, have been used to treat hypertension and to prevent and reduce further arterial calcification, respectively. In addition, spontaneous remission of the disorder has been reported.

An infant was the first born to an 18-year-old Caucasian female at 37 weeks and 4 days gestation. The prenatal care, which started at 11 weeks gestation, was reportedly unremarkable. Family history was remarkable for three miscarriages involving her paternal half-brother, a neonatal death to a paternal aunt, and a maternal aunt with two miscarriages. The baby was born vaginally with some difficulty in extraction with left shoulder dystocia and asymmetric Moro reflex. The arm movement rapidly improved, but she still had asymmetric Moro reflex at the time of discharge two days after delivery. The post-discharge course was unremarkable with normal feeding and weight gain. Two days before her death, she was noticed to be more restless and fussy and appeared to be straining and stretching her legs, but her feeding was still normal. She was found unresponsive on the 20th day of her life.

As per routine protocol, a full-body PMCT was performed at the Office of the Chief Medical Examiner in Baltimore, which showed extensive bilateral calcification of the following arteries: carotid and its facial branches, subclavian, axillary, brachial, ulnar, radial, aorta, all coronaries, main pulmonary and distal branches, intercostal branches, mesenteric, splenic, renal, common iliac, internal and external iliac, femoral, and tibial. Additionally, significant cardiomegaly with dilated chambers was noted.

The gross examination showed a normally developed female infant, weighing 2,500 grams and approximately 52cm long. The review of organ systems was remarkable for hepatosplenomegaly, palpable calcifications throughout the vascular system, cardiomegaly (42 grams, normal predicted weight 24 grams), four chamber dilatation, and bilateral remote cerebellar infarcts.

Histologic sections of the cardiovascular system showed biventricular circumferential subendocardial acute infarction with wavy and hypereosinophilic fibers and acute inflammatory infiltrates, diffuse calcification of internal elastic laminae and significant luminal narrowing with intimal proliferation in all three coronary arteries, calcified intimal plaques, and calcification of internal elastic lamina with marked intimal proliferation. Microscopic sections of the lungs showed diffuse calcification of internal elastic laminae of large- and medium-sized pulmonary arteries and intimal proliferation. The review of other organ systems was unremarkable, except for calcifications of periadrenal and perilyngeal small vessels. The cause of death was certified as cardiac arrhythmia due to IACI.

Early screening and genetic counseling are recommended in future pregnancies of all affected families.

IACI, Postmortem CT, ENPP1



Pathology/Biology Section - 2015

H85 A Prospective Double-Blinded Comparison of Autopsy and Postmortem Computed Tomography (PMCT) for the Evaluation of Pediatric Trauma Deaths

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After attending this presentation, attendees will understand how and when PMCTs can supplement or supplant autopsy in pediatric trauma deaths and how the two procedures compare in recognizing injuries and determining cause of death in these cases.

This presentation will impact the forensic science community by offering an alternative or supplement to autopsies for specific types of pediatric deaths.

Given rapid advances in radiologic imaging technology, these modalities might offer an alternative to autopsy in certain types of deaths. In order to better understand when PMCT could be utilized to either replace or supplement traditional autopsies for children ages five years and younger dying of trauma, autopsy reports and PMCT findings from a prospective cohort of these deaths investigated by the New Mexico Office of the Medical Investigator between 2011 and 2013 were compared. Each decedent's autopsy report, completed by a pathologist blinded to the PMCT results, and full-body PMCT report, completed by pediatric radiologists blinded to autopsy results, were coded by a certified Abbreviated Injury Scale (AIS) coder. The results were entered into a shared electronic database (REDCap). Autopsy and PMCT results were reviewed in tandem for each case by a forensic pathologist and radiologist who were not involved in the original assessment of the case and each injury on each report was classified as being a match between autopsy and PMCT, missed and should have been seen (false negative, category 1), or outside the usual range of the alternative modality (category 2). Total numbers of injuries found per procedure, Maximum AIS (MAIS) score by region, cause of death as determined by each procedure, and numbers of "misses" were calculated and compared.

Fifty-five pediatric deaths with complete autopsies and PMCT scans were reviewed, with 64% (35/55) of the decedents male, a median age of one year, and an over-representation of American Indian decedents (15/55, 27%) compared to New Mexico's general population (10% American Indian). Forty-seven percent of deaths were in children under the age of one year. The most common cause of death was suffocation while sleeping (18/55, 32.7%), followed by motor vehicle crashes (12/55, 21.8%). Nine deaths (16%) were the result of homicidal violence. In most of the cases (82%), the cause of death was determined to be correct for both autopsy and PMCT. In nine cases (16.4%) the autopsy cause of death was deemed correct by the review panel and PMCT cause of death incorrect, with the converse being true in one case (1.8%). The calculated Injury Severity Score (ISS) was the same for autopsy and PMCT reports in 13 cases (24%), but was more severe when calculated from autopsy results in 33 (60%) of the reviewed cases. Autopsies revealed 335 total injuries, and PMCT detected 267 injuries, with 120 of these determined to be matches between modalities, leaving 482 unique injuries. Overall, 153 injuries seen on autopsy should have been seen on PMCT (31.7%) and 106 injuries (22%) seen on PMCT should have been seen on autopsy. An additional 37 injuries (7.7%) were determined to be outside of the scope of an autopsy and 54 injuries (11.2%) were outside the imaging capability of PMCT. The highest percentages of false negatives for PMCT were for external findings and head injuries (48% and 32%, respectively) and the highest occurrence of false negatives for autopsy were in the chest and extremities (35% and 23%, respectively). Comparing MAIS scores by region, the agreement between autopsy and PMCT ranged from fair (external, kappa=0.34) to almost perfect (extremities, kappa=0.98). MAIS scores for other regions demonstrated moderate to substantial agreement.



Pathology/Biology Section - 2015

While neither modality is perfect, both autopsy and PMCT can be successfully used to correctly determine the cause of death in pediatric trauma cases. Consequently, PMCT can supplant autopsy in certain cases; however, the most detailed identification of injuries in fatal pediatric trauma comes from combining autopsy with PMCT and would be the ideal approach for pediatric homicides. False negatives will be fewer with the use of autopsy for head injuries, while PMCT improves the opportunity to identify chest and extremity injuries. In most cases, the severity of injuries by region agreed well between procedures.

Pediatric Injuries, Autopsy, Computed Tomography



H86 Micro-Computed Tomography (Micro-CT) Features of Laryngeal Fractures in a Case of Fatal Manual Strangulation

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After attending this presentation, attendees will be aware of a new application of micro-Computed Tomography (CT) for dealing with the identification of the signs of pressure of the neck in cases of subtle manual strangulation.

This presentation will impact the forensic science community by suggesting a novel approach for the identification of laryngeal fractures by means of micro-CT analysis.

It is widely accepted that there are no specific or fully determinate signs of asphyxia. In cases of subtle fatal neck compression, postmortem findings on external examination of a body as well as on autopsy may vary considerably depending on the type of violent trauma, the strength of the resistance exerted by the victim, and on the intensity and duration of neck compression. Based on the detection of a combination of morphological hallmarks such as external injuries to the skin, hyoid or laryngeal fractures, hemorrhages of the overlying soft tissues, and subconjunctival petechiae, the diagnosis of homicidal neck compression is generally accepted.

A case is presented of a 45-year-old schizophrenic patient found on the floor of the bedroom of a psychiatric ward in cardiopulmonary arrest. The patient was successfully resuscitated; nevertheless, brain death was declared after two days in a vegetative state. The roommate of the deceased, who was also schizophrenic, stated that “the voice of God told me to kill my roommate” and death occurred after a prolonged scuffle. The body showed only vague signs of violence at external inspection (i.e., a subtle bruise on the forehead and a minute scratch on the left arm).

At autopsy, the identification of subepicardial petechiae and moderate pulmonary emphysema were not useful for the diagnosis of asphyxial death because these signs might be related to resuscitative maneuvers with prolonged artificial ventilation. Since focal hemorrhagic infiltration of the tracheal mucosa and the superior horns of the thyroid cartilage were observed, a CT scan of the entire larynx was performed, with the goal of identifying laryngeal fractures. Moreover, both superior horns of the thyroid cartilage were analyzed thorough a micro-CT, a radiological technique for tissue analysis with a spatial resolution of a few microns. The 3D reconstruction allowed for identification of multiple cartilage fragments of the larynx. Consequently, the cause of death was identified as asphyxia due to manual strangulation.

Considering that micro-CT analysis is able to generate images with a high level of detail compared to conventional radiological techniques, in cases of subtle fatal neck compression this type of investigation could be useful for the morphological identification of comminuted bilateral fractures. This may be particularly useful in cases involving children and young adults where fractures or cartilage damages may be minimal or absent as a consequence of the elasticity of the ligaments around the larynx and hyoid bone as well as the early stage of ossification.

Manual Strangulation, Laryngeal Fracture, Micro-CT



Pathology/Biology Section - 2015

H87 Challenging the Role of Autopsy — Results of a Multicenter Study to Validate Multi-Phase Postmortem CT-Angiography (MPMCTA)

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After attending this presentation, attendees will better understand Postmortem Computed Tomography-Angiography (PMCTA), in particular the recently developed method of MPMCTA, applied by an international group of dedicated scientists in a large-scale multicentric study. Strengths and weaknesses of the method will be highlighted in comparison to autopsy and indications for performing MPMCTA will be proposed.

This presentation will impact the forensic science community by demonstrating the potential of the applied MPMCTA protocol to enhance postmortem evaluation of the cause of death. It provides a solid database to define indications for MPMCTA, conventional autopsy, or a combination of both. Results of previous studies are confirmed, indicating that in a variety of case categories, autopsy should no longer be considered the gold standard for postmortem diagnostics, but rather the combination of autopsy with contrast-enhanced postmortem imaging techniques like MPMCTA.

Purpose: Recently developed PMCTA greatly enhances postmortem diagnostics due to its ability to reliably discover even discrete vascular pathologies. While different technical approaches are pursued across the world, the need for validation and standardization of the method increases in order to facilitate its transition into forensic routine. With this goal, an international working group has performed a prospective multicenter study to validate previously published MPMCTA, define its indications, and evaluate its advantages and limitations, especially compared to conventional autopsy.

Method and Materials: Five hundred cases were included in this prospective multicenter study. All cases received MPMCTA followed by conventional autopsy. All CT images were read by a team of one forensic pathologist and one radiologist, both experienced in forensic imaging and blinded to autopsy results. All findings were recorded for each method and categorized by anatomical structure (bone, parenchyma, soft tissue, vascular) and importance for the forensic case (essential, useful, not important).

Results: The majority of findings were visualized with both techniques. MPMCTA was superior to autopsy at identifying skeletal and vascular lesions, where it detected a number of lesions essential to the forensic case which were not seen at autopsy. Conventional autopsy provided better information about essential soft tissue lesions and allowed distinguishing postmortem vs. antemortem vascular occlusions. Best results were obtained when combining both techniques.

Conclusion: Both MPMCTA and autopsy are able to detect potentially essential lesions not detected by the respective other method. This opens the path to defining indications for either one or the other method, or a combination. Combining both techniques increases the overall quality of postmortem diagnosis and in many cases augments diagnostic confidence regarding the cause of death. The results of this study provide researchers and practitioners with a solid data base and will help promote the transition of MPMCTA into daily routine for clinical and forensic pathologists.

Multi-Phase Postmortem CT Angiography, Forensic Radiology, TWGPAM



Pathology/Biology Section - 2015

H88 Diagnostic Values of Postmortem Computed Tomography (PMCT) and Multi-Phase Postmortem CT-Angiography (MPMCTA) in Blunt Trauma Death

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After attending this presentation, attendees will better understand PMCT and MPMCTA in forensic practice for the diagnosis of blunt trauma.

This presentation will impact the forensic science community by explaining how PMCT and MPMCTA are effective tools in blunt trauma death diagnosis and how these techniques provide accurate data that conventional autopsy cannot obtain regarding the reconstruction of bony fractures and air detection.

Background: In the last decade, postmortem imaging techniques have gained a remarkable acceptance in the forensic field practices. They proved themselves as objective, non-invasive diagnostic tools for both external and internal body injuries. This is especially true in blunt trauma cases which are the most common injuries faced in forensic field practice. In this study, the sensitivity and specificity and accuracy of PMCT scan and MPMCTA were examined and compared with the conventional autopsy in diagnosing the major findings and the cause of death in blunt trauma cases to determine the advantages and disadvantages and to outline a framework for their ideal application in forensic cases.

Methods: This is a prospective study. Data was obtained from 50 decedents presented to the legal institute of Hamburg University with death allegedly due to blunt trauma. Each case underwent a whole body examination by CT and the MPMCTA was performed only for 15 cases. The resultant radiological findings are validated by conventional autopsy. Both autopsy and radiological findings are divided by body regions into the head and neck, thorax, abdomen and pelvis, extremities, and vascular system. They are then compared and correlated to the most common traumatic patho-anatomic findings, including soft tissue injuries (contusions and lacerations), hemorrhage, fractures, presence of free air in body cavities, and air emboli, in addition to the detection of the cause of death.

Results: The preliminary results show the low sensitivity and specificity of PMCT in detection of soft tissue injuries compared to MPMCTA and conventional autopsy, which almost have the same results. Both PMCT and MPMCTA are superior to autopsy in detection, configuring, and reconstruction of bony fractures and in detection and localization of air emboli and free air; however, the detection of hemorrhage is relatively dependent on the blood amount and the location of the bleeding source. Despite the presence of contrast-related artifacts, the MPMCTA has a higher sensitivity and specificity in identifying the source of the bleeding compared to the PMCT and conventional autopsy.

Conclusion: Together, PMCT and MPMCTA are effective tools in blunt trauma death diagnoses and are able to provide accurate data that the conventional autopsy cannot, regarding the reconstruction of bony fracture and air detection; however, with the observed contrast artifacts in MPMCTA and the low ability of PMCT alone in detection of the soft tissue injuries, the chance of these imaging techniques replacing the conventional autopsy is decreased.

PMCT, MPMCTA, Blunt Trauma



Pathology/Biology Section - 2015

H89 **Thanatology of the Vascular System and Its Influence on Postmortem Computed Tomography (PMCT) and Multi-Phase Postmortem CT-Angiography (MPMCTA)**

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After attending this presentation, attendees will understand the complexity of blood distribution in postmortem imaging, especially due to collapsed vessels and the presence of gas in the vascular system. To avoid misinterpretation of PMCT images, knowledge of thanatological changes is important.

The goal of this presentation is to describe postmortem changes of the vascular system by investigating blood distribution, gas presence, and collapsed vessels on PMCT and their influence on MPMCTA.

Postmortem changes of the human body are broadly described in thanatology; however, there is little knowledge about the influence of postmortem reactions on the vascular system. Radiological non-invasive methods have opened new perspectives in exploring the inside of bodies and can contribute to the understanding of thanatology.

MPMCTA allows the detailed visualization and investigation of the vascular system. By applying this standardized technique, the quality of the postmortem exam can be significantly increased, especially concerning the detection of vascular lesions such as stenosis, malformation, or laceration of vessels; however, the presence of remaining blood can create artifacts that have to be recognized as such because they can imitate vital embolism or vascular occlusion.

In a first study, the postmortem MDCT data of 118 human bodies was studied. Cases with internal/external bleeding or corporal lesion allowing contamination with external air were excluded. Major vessels and heart cavities were systematically explored. Presence of gas was semi-quantitatively assessed by a trained radiologist.

Collapsed veins were observed in 61.9% of cases (CI95% 52.5 to 70.6), and arteries in 33.1% (CI95% 24.7 to 42.3). Vessels most often affected were for arteries: common iliac (16.1%), abdominal aorta (15.3%), and external iliac (13.6%); and, for veins: infra-renal vena cava (45.8%), common iliac (22.0%), renal (16.9%), external iliac (16.1%), and supra-renal vena cava (13.6%). Cerebral a. and v., coronary a., and subclavian v. were not affected. The presence of collapsed vessels was associated to a minor degree of alteration. Concerning the presence of gas, it has been observed that arteries and veins follow the same pattern of appearance of gas for the quantity and location.

A recently performed study describing artifacts due to remaining blood was carried out in 54 cases of MPMCTA. Essentially, this study has shown that artifacts visible in the vascular system during MPMCTA always appear in the same localizations. The main artifacts observed were first, filling defects of the cardiac cavities and pulmonary arteries and second, contrast agent layering in the coronary arteries and the right atrium. Thanks to this reproducibility and stability, it is possible to recognize those phenomena as artifacts and guarantee a proper radiological interpretation of the images.

By comparing the results of the two studies, the thanatological changes of the vascular system are the origin of the artifacts that can be recognized in PMCT and especially in MPMCTA. In order to prove this hypothesis, a future study is necessary superimposing the results of PMCT and MPMCTA on the same cases.

In postmortem imaging, collapsed vessels and gas in the vascular system are common and knowledge of thanatological changes is important in order to avoid misinterpretation. Due to postmortem changes, blood distribution is complex. These thanatological changes also influence MPMCTA; the remaining blood distribution is the reason for the presence of artifacts in the vascular system after angiography.

Thanatology/Postmortem Changes, Vascular System, PMCT



Pathology/Biology Section - 2015

H90 Detection of Pulmonary Fat Embolism in Cases With Postmortem CT-Angiography (PMCTA) — Preliminary Results

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After attending this presentation, attendees will understand the repercussions of the realization of a PMCTA with an oily contrast liquid, particularly in cases where a pulmonary fat embolism is suspected, and a method that could be able to diagnose pulmonary fat embolism before the realization of the PMCTA.

This presentation will impact the forensic science community by serving as a key to determine the indication of a PMCTA with oily contrast liquid in the cases where a pulmonary fat embolism is suspected and could be the cause of death, if a method to diagnose this pathology is not available.

Introduction: Pulmonary fat embolism can be a cause of death in cases with trauma, during orthopedic surgery, and also in non-traumatic conditions, such as burns, pancreatitis, fatty liver, or sickle cell disease. As PMCTA becomes more widespread, it is important to determine how it affects the diagnosis of pulmonary fat embolism.

Purpose: The purposes of this study were to determine if the oily contrast liquid used in PMCTA induces artifactual pulmonary fat embolism, if such artifacts differ from non-artifactual (original) pulmonary fat embolism, and if pulmonary fat embolism can be detected and graded before PMCTA.

Material and Methods: Data acquisition for this prospective study was performed between November 2013 and December 2014. Consecutive cases of adults who received PMCTA followed by autopsy were included in this study. Cases were excluded if the state of alteration was too advanced. Pulmonary biopsies of each lung were taken before and after the PMCTA as were fragments of each lung with a twin-edged knife during the autopsy. The samples were examined under the microscope without fixation or staining and after an Oil-Red O staining. Pulmonary fat embolism was graded according to Falci et al.

Results: Original pulmonary fat embolism was diagnosed in seven cases out of 23 on biopsies performed before PMCTA, all having presented traumatic events before death or rib fractures due to resuscitation attempts. As expected, structures with the aspect of pulmonary fat embolism were present in almost all cases (21 cases out of 23) after PMCTA. The microscopic aspect of original and PMCTA-induced pulmonary fat embolism was identical. Grading of the pulmonary fat embolism according to Falci et al. depended on the quality of the biopsies.

Conclusions: PMCTA with oily contrast liquid induces artifactual pulmonary fat embolism that cannot be visually differentiated from antemortem pulmonary fat embolism; however, antemortem pulmonary fat embolism can be diagnosed with biopsies performed before PMCTA. In order to assure the diagnosis and correct grading of pulmonary fat embolism, the quality of the biopsy should be checked before PMCTA with oily contrast is performed. If it is impossible to obtain biopsies of good quality, the indication of a PMCTA must be discussed in cases where pulmonary fat embolism is suspected, particularly if it is a potential cause of death.

Pulmonary Fat Embolism, Postmortem CT Angiography, Diagnosis



Pathology/Biology Section - 2015

H91 Limitations of Multi-Phase Postmortem CT-Angiography in Cases of Fat Embolism: The Goal of Immunohistochemistry to Distinguish Between Artificial and Vital Fat Embolism

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After attending this presentation, attendees will understand how a new technique has been developed for the Multi-Phase Postmortem CT-Angiography (MPMCTA) that allows the visualization of the vascular tree of the body via the use of an lipophilic contrast agent, Angiofil®; however, the contrast agent diffusion in the tissue has to be considered in terms of histological differential diagnosis in certain important pathophysiological events such as the embolism and, in particular, the fat embolism. The goal of this presentation is to underline the usefulness of the immunohistochemical approach in these cases to make a correct histological diagnosis between artifact due to the use of Angiofil® and true pathology such as fat embolism.

This presentation will impact the forensic science community by presenting the immunohistochemical study as the only means to distinguish between vital fat embolism and Angiofil® artifacts, lending certainly to the diagnosis of fat embolism as cause of death also in cases in which MPMCTA was performed. In this way, this technique can also be usefully performed in traumatic deaths and in all cases of suspicious fat embolisms.

In the context of forensic imaging, MPMCTA has developed as a new technique that allows the visualization of the vascular tree of the body. This is a standardized technique that consists in the performance of a native CT-scan (without contrast-agent injection), followed by three angiographic phases (arterial, venous, and dynamic phase). The angiographic phases consist of the perfusion of the vascular system with an oily contrast-agent mixed with paraffin oil through the accesses in the femoral artery and vein. This allows complete opacification of the vascular system, showing very small vascular lesions or abnormalities as exact sources of bleeding. The oily liquid Angiofil® is a contrast agent consisting of iodized linseed oil. To perform whole-body CTA, Angiofil® is mixed with paraffin oil. A mixture of 6% is determined as adequate to obtain an excellent opacification of the whole vascular system. This lipophilic contrast agent, Angiofil®, is easy to handle and highly radio-opaque. Because of its lipophilic abilities, it is retained intravascularly, hence it facilitates virtual segmentation of vessels and yields an enduring signal which is advantageous during repetitive investigations. As described in the literature, the oily liquids enter the venous system by passing through small arteriovenous shunts, while they arrest in the capillary microcirculation. This leads to embolization of lipophilic agent contrast in capillaries or small precapillary vessels and in this manner the Angiofil® can itself cause an "artificial" fat embolism.

The search for fat embolisms is often important because it may help determine the cause of death and it is also an important vital sign in cases of trauma. Fat embolism refers to the presence of fat droplets within the peripheral and lung microcirculation while fat embolism syndrome is a clinically relevant syndrome caused by the distribution of fat in the systemic vascular tree and characterized by clear signs and symptoms.

The study concerned 22 autopsies (MPMCTA was not performed prior) in which the fat embolism was pointed out as the cause of death and compared these with a control group of 22 autopsies in which the MPMCTA was performed prior to autopsy and in which any suspicious fat embolism was excluded and the death was related to other causes. A complete immunohistochemical study of all organs of the two groups was also performed. The immunohistochemical panel used to reveal fat embolism on the brain, lung, and kidney samples included anti-fibrinogen and anti-CD61 antibodies. The immunohistochemistry performed on the 22 cases of fat embolism revealed platelet aggregates (anti-CD61 antibody positive reaction) at the edge of fat globules and an adsorbed fibrinogen (anti-fibrinogen antibody positive reaction) layer to the interface of fat globules. In contrast, in the control group (in which the Angiofil® was injected) the results of the immunohistochemical investigations were negative for reaction of fibrinogen and platelets around the empty spaces in the blood vessels, thus providing evidence of an artificial fat embolism.

Fat Embolism, Multi-Phase Postmortem CT Angiography, Immunohistochemical Study



Pathology/Biology Section - 2015

H92 Implications of Postmortem Magnetic Resonance Imaging in Sudden Cardiac Death

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After attending this presentation, attendees will understand the importance of the execution of a postmortem cardiac Magnetic Resonance Imaging (MRI) for the detection of cardiac alterations and as a guide for histological collection in the early stage of Arrhythmogenic Right Ventricular Cardiomyopathy/Dysplasia (ARVC/D).

This presentation will impact the forensic science community by showing that the interpretation of postmortem radiology, especially in the cardiovascular field, can help the examiner in performing the autopsy.

ARVC/D is characterized by fibro-fatty replacement of the Right Ventricular (RV) myocardium and by ventricular arrhythmias as the main clinical manifestation. It represents an important cause of sudden death in young people. A genetic inheritance can be demonstrated in at least 40%-50% of the cases.

A case of a fatal arrhythmia that occurred at home in a 25-year-old man due to an early phase of ARVC/D is reported. An autopsy was carried out in order to determine the cause of death. The execution of a cardiac MRI — performed before the examination of the heart — was crucial to point out two areas of fatty infiltration not evidenced macroscopically. Histological examination of the specimens collected under the guidance of imaging was suggestive for typical alterations of ARVC/D whereas no signs of infiltration were observed in other cardiac specimens.

Case Report: A 25-year-old male student suddenly collapsed in his apartment before sleeping at night. The event was witnessed by his girlfriend. Cardiopulmonary resuscitation initiated in a few minutes by an emergency physician was unsuccessful. The family denied any previous pathology. Family history was negative for sudden death or cardiomyopathy. A complete postmortem examination was performed 24 hours after death. External examination was insignificant. At internal examination, the pericardium was opened anteriorly revealing the enclosed heart. The great arteries were transected 3cm above the aortic and pulmonary valves, the pulmonary veins and the superior and inferior vena cava, and the heart was removed. It was normal in size and weighed 360g. Other organs were unremarkable. Blood and a full-thickness block of myocardium were retained (frozen) pending onward referral for genetic testing. After routine autopsy, the heart was suspended and fixated at room temperature with 10% buffered formaldehyde solution. After 24 hours of fixation, a cardiac MRI was performed using a 1.5T clinical scanner using a brain bird-cage multichannel coil. MRI protocol included a whole heart 3D-SSFP acquisition with the following parameters: slice thickness 1mm (interpolated to 0.5mm), NEX 1, FOV 19cm, matrix 512x512, 60° flip angle, TR/TE equal to 8.4/4.1. FSE images with the following parameters were also acquired: slice thickness 2mm, NEX 1, FOV 32cm, matrix 256x256, 90° flip angle, TR/TE equal to 840/8.1. In 3D-SSFP images, two focal areas of suspected fat infiltration were detected in the sub-tricuspidal region and in the lateral free-wall. These regions with suspected fat infiltration were also evidenced in FSE images. The heart underwent macroscopic examination: coronary vessels and main branches, examined by multiple cross sections, were normal; no atherosclerotic lesions were detected; RV wall thickness was 0.4cm. The investigation of the two areas clearly highlighted by MRI revealed small foci of fat infiltration without clear pathological significance. Full-thickness blocks of myocardium were removed for histological examination from anterior, lateral, and posterior left ventricular free wall, from ventricular septum, from RV outflow tract, and from both atria, according to the recommended guidelines for best practice. Furthermore, other samples were obtained directly from the areas indicated by the MRI (as being suggestive for fat infiltration). Specimens were embedded in paraffin and routinely processed.

The histological examination of “MRI guided” cardiac specimens, stained with haematoxylin-eosin and Masson, revealed fibrous and fatty tissue replacement that had swept inwards from the epicardial aspect to the endocardial aspect of the ventricle chamber. Myocardial disarray, lymphocytic infiltrates, and foci of contraction band necrosis were also observed. No remarkable alterations were detected in all other cardiac specimens. Postmortem toxicological analyses were negative for alcohol, drugs, and common toxicants. The cause of death was attributed to ARVC/D.

Sudden Death, Histopathology, Cardiac MRI

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Pathology/Biology Section - 2015

H93 Visualization of Myocardial Infarction by Postmortem Single-Organ Coronary Computed Tomography: A Feasibility Study

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After attending this presentation, attendees will understand that radiological examination is generally considered a good complement for conventional autopsy; nevertheless, it was thought to have limited application in cardiovascular pathology.

This presentation will impact the forensic science community by demonstrating the main diagnostic findings in postmortem multi-detector coronary artery computed tomography in cases of sudden death in adults.

Introduction: Postmortem imaging is increasingly used in the forensic field in cases of natural death related to cardiovascular diseases, which represent the most common causes of death in developed countries. Whereas radiological examination is generally considered a good complement for conventional autopsy, it was thought to have limited application in cardiovascular pathology. The goal of this study was to investigate the role of postmortem multi-detector coronary artery Computed Tomography (CT) in cases of sudden death in adults.

Materials and Methods: Eleven patients that were revealed by standard autopsy procedures to be negative for macroscopic extra cardiac lethal findings were selected for the study. Later, isolated single organ Postmortem Computed Tomography Coronarography (PMCTA) using an iodinated non-ionic contrast medium was conducted in these same individuals. After computed tomography examination, all isolated hearts were carried to the forensic pathologist to undergo a conventional histology assessment.

Results: In seven of the 11 cadavers, a final diagnosis of myocardial infarction was made after a complete autopsy and histology procedures. In six of the 11 cases, PMCTA scanning of the isolated hearts confirmed the autopsy findings and showed the presence and localization of occlusions or severe stenosis as well as the extension of the myocardial hypoxic area by the extravasation of contrast medium.

Conclusion: Isolated single-organ PMCTA could be considered a valid and useful tool in combination with traditional autopsy investigation (macroscopic sections and histology) for identifying the cause of death by recognizing the presence and degree of coronary artery disease and myocardial infarction area visualization.

Postmortem TC, Myocardial Infarction, Sudden Cardiac Death



Pathology/Biology Section - 2015

H94 “Virtopsy” Utility on Mummified Corpses: Two Italian Iconic Cold Cases

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After attending this presentation, attendees will understand the usefulness of Computed Tomography (CT) scan analysis, especially in very fragile corpses such as mummified corpses, through the presentation of two iconic Italian cold cases.

This presentation will impact the forensic science community by explaining whether or not spiral CT scans were important in finding out cause, time, and manner of death of bodies found in different circumstances and conditions, all of which were involved in famous media and/or Italian judicial events.

In the field of forensic medicine, the use of diagnostic imaging such as pre-autopsy CT represents an essential preliminary accessory examination for postmortem forensic assessment. Imaging techniques present the possibility of realizing a “virtual autopsy,” called “virtopsy”. Moreover the lack of the suffix “auto” underlines the objectiveness of this method that results in non-invasive, 3D, and sometimes the only possible evaluation on mummified, extensively decomposed or pre-skeletonized bodies where it is impossible to perform angio-CT scan or Magnetic Resonance Imaging (MRI). A CT scan examination cannot be considered an alternative to conventional autopsy, but rather a first useful tool in unusual cases, such as the cases described here. The goal of this study was to evaluate the role of a previous CT scan analysis performed in the forensic examination of extensively decomposed corpses. A CT scan was used in all the corpses deemed “precious,” which will certainly be at the center of a case status. Experience dictates that virtopsy should be routinely performed on precious bodies involved in complex cases, to crystallize them in “as is” condition before they undergo forensic examination, where they will be necessarily destroyed. The CT-scan will guarantee objective data available during the entire time of a trial and beyond.

Case 1: The mummified corpses of two young brothers were found in a pit of a large abandoned house in the downtown Gravina in southern Italy two years after their disappearance. At the time of the discovery, their father was in preventive detention as he was suspected of having beaten his sons to death and hidden their bodies. A CT-scan performed on the brothers prior to autopsy revealed multiple fractures of the pelvis and inferior limbs related to a fall. The subsequent autopsy and the histological examination confirmed the bone lesions previously detected and also discovered a soft peri-fracture haemorrhage residual confirming the “vitality” of these injuries. The cause of death of both children was identified as severe trauma due to falling from a height. The lack of any kind of possible voluntarily inflicted injury allowed the father be blame-free. The CT-scan examination was essential for localizing the injuries without modifying the related bone connections and was a useful guide for those who performed the forensic autopsy.

Case 2: The mummified and partially skeletonized corpse of a girl was found in the loft of a church 17 years after her disappearance. A boy belonging to a very influential family was suspected of the girl’s murder; his family and the church itself attempted to hide and confuse the truth. The CT-scan and 3D reconstruction of the girl’s remains described the interruption of the inferior cortical layer on the posterior arch of a number of ribs. During the autopsy, it was possible to identify the exact pattern of lesions as being sharp and cut injuries identified by the CT-scan results. Virtopsy alone could not replace the forensic autopsy which made it possible to detect the exact nature, number, and direction of the lesions’ patterns and manner.

Virtopsy, CT Scan, Mummified Corpses



Pathology/Biology Section - 2015

H95 A Scream From the Past: A Multidisciplinary Approach in a Concealment of a Mummified Corpse

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After attending this presentation, attendees will understand the necessity of a multidisciplinary approach in solving difficult forensic cases such as mummified bodies.

This presentation will impact the forensic science community by showing the methodological approach in cases in which mummified bodies are found.

Mummification is a drying of the tissues in place of liquefying putrefaction. During mummification, water evaporates from tissues, preventing bacterial putrefaction. Mummification commonly occurs in a dry environment, preferably with a moving air current; this is usually, but not exclusively, a warm place with a specific degree of environmental oxygenation. More recently, the discovery of a mummified body in a domestic environment is rare and is generally due to the subject's social isolation while alive.

An 83-year-old white male was found mummified and sealed with tape, covered by two plastic bags, and walled within a masonry ledge built inside his bedroom. The mummification process occurred in an environment characterized by the rare process of the *Anaerobiasis mummies*.

In this case, during the police investigation of an apartment in the suburbs of Rome, a silicon-sealed door on the 2nd floor was found. Upon opening the door, a messy bedroom with a silicon-sealed window was discovered. The floor was dirty, covered with white-colored dust and rubble. In one corner of the room, an abnormal protrusion in the wall was observed. Once the ledge was demolished, a dead body in a semi-supine position with the back on the floor and the legs on the wall was found inside. Two plastic bags covered the body. Once the plastic bags were removed, the body was entirely wrapped in brown adhesive tape. No microfauna near the body were observed. The apartment owner said the body was that of his father who died two years previously of cardiovascular disease. After the father's death, the son concealed the corpse in order to obtain his annuity retirement benefits.

Upon external examination, the skin showed a dry appearance with a yellow to brown color and a leathery consistency. No traumatic injuries were present on the body surfaces. Dewatering mold spores were observed on the body surface, especially on the feet.

A postmortem computed tomography scan was performed before the traditional autopsy. The 3D rendering of the Digital Imaging and Communications in Medicine (DICOM) images using the open source software Osirix® on a MacOSX® computer was produced and revealed no traumatic injuries. At the postmortem examination of the heart, significant atherosclerosis involving left anterior descending and the left circumflex coronary arteries was present. Toxicological analyses on the organ samples were negative for drugs and alcohol. At the microscopic examination of the skin of the hand, forearms, and legs, no injuries or other anomalies were observed. At the microscopic examination of the heart, strongly decomposed tissue with interstitial fibrosis and wavy fibers was observed, consistent with an acute ischemic injury. The cause of death was due to atherosclerotic cardiovascular disease and the manner of death was pronounced as natural.

A natural mummification process usually requires 6 to 12 months in adult subjects to be completed, and an environment with hot, dry, moving air is required. In this case, the body was completely mummified despite these environmental conditions not being present. As the body was completely wrapped in tape and walled in, an anaerobic environment could be inferred. Aufderheide underlines how exclusion of air would enhance preservation due to the inhibition of the putrefactive processes. At the same time, a casing that covers the corpse protects it from insects and scavengers.



Pathology/Biology Section - 2015

When a mummified body is found, the manner of death could be related to accident, suicide, homicide, and/or concealment of a corpse. On the one hand, forensic pathologists must know that a complete mummification process can occur in special environmental conditions, different from what is described in literature. On the other hand, when a mummified body is found, a multidisciplinary approach is required in order to reach the correct diagnosis. Thanks to the data concerning the crime scene, the autopsy findings, the radiological examination before the autopsy, and the histopathological and toxicological analyses, the resolution of a case can be possible even many years after the death of the subject.

Mummification, Concealment, Multidisciplinary Approach



Pathology/Biology Section - 2015

H96 Jay Dix Memorial Bonus Day

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After attending this presentation, attendees will understand how and why deaths related to the previously specified topics occur. Attendees will learn a systematic approach to the evaluation of such deaths that can easily be implemented in their daily practices.

This presentation will impact the forensic science community by presenting a comprehensive review of what causes and contributes to deaths related to the previously specific topics. Attendees will be able to systematically evaluate deaths in which the previously specified topics may have played a role that they encounter in their daily practices.

A proper medicolegal death investigation is a multidisciplinary process that often involves non-medical personnel as well as medical professionals. This annual series of lectures is intended to provide the non-forensic pathologist forensic scientist a comprehensive basic review of selected topics in forensic pathology in order to increase familiarity, understanding, and enhance inter-discipline communication.

This year's lecturers will discuss: the investigation of sharp force trauma; infant deaths; deaths temporally related to apprehension by law enforcement personnel; deaths related to sports and recreation; and, asphyxial deaths.

Case 1: Sharp force injuries are one of the major categories of mechanical injury. They result from the mechanical division of tissues by sharp or pointed objects. Sharp force injuries include stab wounds, cuts (incised wounds), and chop wounds; the latter being caused by relatively heavy edged objects such as a machete or axe. Multiple mechanisms play a role in injury and deaths involving sharp force injuries. Understanding and evaluating injuries and deaths in which sharp force injuries may have played a role requires basic knowledge of injuries caused by sharp forces and how to distinguish them from other types of trauma, recognition of patterned injuries, and recognition of injury patterns (e.g., defensive wounds, "hesitation marks"). This lecture will provide a comprehensive review of these issues.

Case 2: The death of an apparently healthy infant is a devastating event for the infant's survivors and is accorded significant attention by society. Infant death may be caused by a wide variety of diseases and injuries, involve a variety of mechanisms, and can be natural, accidental, or homicidal. External and/or internal evidence of disease or injury may be lacking. Accurate recognition of the cause, mechanism, and manner of death has important implications for the survivors, other interested investigative and health agencies, and society in general. Recognition of factors involved in sudden unexpected infant deaths can help in enhancing the safety of other family members and serve as a basis for formulating death prevention strategies. This lecture will discuss the investigation and interpretation of findings in sudden unexpected deaths involving infants.

Case 3: There are multiple causes, mechanisms, and contributory factors that can play a role in deaths that are temporally related to custody. The custody process can be divided into several stages — pre-custody, pre-incarceration, and incarceration. Particular diseases and injuries tend to occur and/or become manifest during each of these stages. This lecture will systematically review what diseases and injuries cause/contribute to death in the phases of custody related to apprehension and arrest, how they affect physiology and anatomy, when they are typically operative, and how they are manifest. Recognizing what occurs during the various stages of custody allows a systematic approach to assessing deaths that occur during the custody process. This lecture will review the conceptual and practical aspects of understanding and investigating deaths that are temporally related to the apprehension/arrest phases of custody.

Case 4: There are multiple causes, mechanisms, and contributory factors that can play a role in deaths that are temporally related to participating in and, occasionally, while being a spectator at sporting or other recreational activities. Understanding these deaths requires understanding of the physical requirements to perform particular activities, susceptibility of particular diseases to stresses associated with particular activities, effects of various chemical and/or biological agents that may be taken to enhance performance, and physical injuries associated with particular recreational activities. This lecture will provide a comprehensive review of these issues in the context of investigating deaths that occur in relation to sports/recreational events. Understanding factors that are involved in deaths occurring in these circumstances also helps in instituting appropriate safety measures to protect participants and spectators.



Pathology/Biology Section - 2015

Case 5: Human life requires the uptake and utilization of oxygen along with the release of metabolic waste. Failure of these processes leads to asphyxia. There are numerous entities — mechanical and chemical — that can cause asphyxia through a variety of mechanisms, present in a wide range of scenarios, and that can be associated with a broad range of physical findings. Proper evaluation of these deaths requires knowledge of the various entities that can cause asphyxia, mechanisms through which these agents affect physiological function, scenarios under which these deaths occur, and factors that contribute to these deaths. This lecture will comprehensively discuss the investigation of death in which asphyxia may have played a role.

Trauma, Infant Death, Asphyxial Deaths



Pathology/Biology Section - 2015

H97 Ecology of Decomposition: A Chemical and Biological Profile of the Maggot Mass

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After attending this presentation, attendees will understand the chemical and biological factors that influence maggot mass ecology and its potential use as a forensic tool.

This presentation will impact the forensic science community by exploring a new microenvironment and establishing a more accurate and reliable method to estimate the postmortem interval based on possibly predictable characteristics of maggot masses.

Carion decomposition is largely attributed to microorganism and insect activity. These two pathways of nutrient renewal have traditionally been considered as separate mechanisms of decomposition and studied as such; however, recent research has shown that interactions between insects and microorganisms culminate in the formation of maggot masses. Yet maggot masses associated with carcass decomposition harbor a microenvironment that has not been fully analyzed. In this study, a preliminary chemical and bacterial profile of the maggot mass is provided using a swine model (*Sus scrofa domestica*). More specifically, this is the first comprehensive view into the bacterial microbiome of the maggot mass through sequencing the V4 region of the 16S rRNA gene. To provide further description of the microbiome, culture techniques were utilized to establish what constituents of this community can be easily observed in the laboratory. In doing so, the goal is to understand the ecology of the maggot mass so that it can be used to its full forensic value.

Three swine carcasses were decomposed in a tropical savanna ecosystem in Palolo Valley, Oahu, HI. Swine were killed via electrocution and placed at the site one hour postmortem. Microbial samples were collected when the maggot masses became established (74h postmortem). The maggot mass was swabbed twice daily from 74h-128h postmortem for sequencing and culture. Simultaneously, the skin was swabbed near the maggot masses for culture. Additionally, the pH, oxidation-reduction potential, and the temperature of the maggot masses were measured. Swabs for culture were immediately transferred to the laboratory and streaked onto standard nutrient agar and incubated at 22°C for isolation. Once isolated, bacterial samples were identified via Matrix-Assisted Laser Desorption/Ionization-Time of Flight (MALDI-TOF). Swabs for sequencing were transferred to the laboratory and stored at -20°C until the survey was complete, then sequenced.

The maggot masses exhibited obvious chemical trends. From 74h to 104h postmortem the pH became more alkaline (6.4±0.5 to 7.4±0.2). At 104h to 122h postmortem, the pH became more acidic, decreasing from 7.4±0.2 to 6.7±0.1. During the final day of the study, the pH regained alkalinity and rose to 7.6±0.1. Oxidation-reduction potential increased from -283milliVolts (mV) to -78mV indicating a highly reducing environment. The temperature remained relatively constant, averaging at 35.6°C within the maggot masses and generally higher than the environmental temperature averaging at 26.8°C. Microbial data will be presented but a diverse bacterial community dominated by Proteobacteria (specifically Gammaproteobacteria), Firmicutes, and Bacteroidetes is anticipated. This community is expected to change over time as decomposition progresses, putrefactive bacteria migrate, insects oviposit on the carcass, and resources become depleted.

The results show that the chemistry of the maggot mass changes during decomposition. The shift in pH is similar to previous studies and probably occurs when macromolecules are broken down into smaller amino acid constituents. As those amino acids were further broken down into inorganic compounds such as ammonia and incorporated into the environment, the maggot masses regained alkalinity, increasing the pH. This noticeable trend in the maggot mass pH has great potential as a forensic tool as a postmortem interval estimator. The oxidation-reduction potential indicated a highly reducing environment that corresponds with sulfur reduction. From this measurement, the presence of sulfur-reducing bacteria is expected, possibly verifying the assumption that phylum Proteobacteria is a part of this maggot mass community. Finally, the maggot mass temperature being higher than environmental temperature indicates that the bacterial community might include thermotrophic taxa. These data provide novel insight into the ecology of the bacterial community. More tests, specifically in different seasons and climates, are needed to verify if these trends are consistent and predictable.

Microbiome, Postmortem Microbiology, Taphonomy



Pathology/Biology Section - 2015

H98 ForenSeek: A New Tool for Forensic Entomology

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WITHDRAWN



Pathology/Biology Section - 2015

H99 Factors Related to Body Temperature From Scene to Autopsy: Implications for Forensic Entomology

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After attending this presentation, attendees will better understand the temperature variation experienced by insect larvae during death investigations. These temperature variations represent an underestimated source of variation that occurs during transport and storage of the body prior to autopsy. This information is important to the calculation of the heat energy units (Accumulated Degree Hours (ADH)) that the developing insects use to complete their developmental milestones. These milestones are used by forensic entomologists to determine insect age, Time Of Colonization (TOC), and ultimately to generate an estimate of minimum Postmortem Interval (PMI_{min}). Heat energy as measured by temperature over a lower developmental threshold can be used by the insect toward growth and developmental progression. Hence, knowledge regarding the temperature history of the developing insect larvae is critical to accurate TOC estimation.

This presentation will impact the forensic community by improving accuracy in the calculation of TOC by more accurately accounting for the temperatures experienced by the developing larvae prior to collection. One assumption often made during the calculation of ADH is that when the body is stored in the morgue cooler, the temperature is below the 50.0°F/10.0°C lower developmental threshold assumed for most flies. Thus, no growth is assumed during cooler storage; however, the data to be presented will show that there is not only an extended cooling period for the body before it drops below the threshold temperature in the cooler, but that under some circumstances the body may never drop below the lower developmental threshold for the insect larvae.

Small temperature sensors were placed with bodies during transport and storage to investigate a variety of cases involving various stages of decomposition, insect activity, scene temperatures, scene location, nearest local weather station temperature, and decedent characteristics (e.g., weight, age, primary cause of death). These factors were used to develop a preliminary regression equation to analyze the importance of these factors on the amount of time that a body may stay above 50.0°F/10.0°C before insects can be collected during autopsy. These data are regularly collected by the medical examiner's office and hold potential to be used to adjust for transport and storage temperatures during casework. Forensic entomologists are often sent specimens collected during or after autopsy from which to estimate a TOC. A better understanding of the factors affecting body cooling and temperatures when insect growth might occur during transport and storage will aid in improving the accuracy of TOC and PMI_{min} estimates.

Time of Colonization, Minimum Postmortem Interval, Accumulated Degree Hours



Pathology/Biology Section - 2015

H100 Should I Stay or Should I Go? Study of Thermic Strategies of Calliphoridae Larvae

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WITHDRAWN



Pathology/Biology Section - 2015

H101 Aging Blow Fly Pupae Using Hyperspectral Imaging: Another Tool in the Forensic Toolbox

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After attending this presentation, attendees will understand the effectiveness of hyperspectral imaging in aging pupae and the advantages of this technology for entomological determination of the minimum Postmortem Interval (minPMI).

This presentation will impact the forensic science community by demonstrating the potential of hyperspectral imaging as a non-invasive and reliable technology for the accurate estimation of pupal age in forensic investigations.

Forensic entomology has been a useful tool for crime scene investigators for the best part of a century in the western world. There have been numerous cases where entomology has played a crucial role in helping to work out the details of a crime or unattended death. Estimating minPMI is still one of the most fundamental questions following a death and the application of the developmental rates of insects associated with a corpse and time frames of insect succession onto decomposing remains is a common basis for such calculations in legal situations. Blow flies are the predominant taxa used to indicate minPMI as they are among the first insects colonizing remains after death. The developmental duration of blow flies and other forensically relevant insects is strongly driven by temperature; and, specimen age is determined using reference data detailing temperature-dependent developmental time frames for specific life stages encompassing egg, larval instars, pupation, and eclosion. Problematically, there are almost no established methods which allow precise estimation of the age of a specimen beyond identification of the start and end of the life stage collected. Thus, where the duration between stages is lengthy, for instance between pupal formation and adult fly eclosion, considerable error can be introduced to the minPMI estimate. Only limited external morphological indicators of the puparia are identifiable externally and there exists a preference within many legal systems for non-invasive techniques whereby evidence remains unchanged and available for review and/or supplemental analysis.

Hyperspectral imaging was employed to discriminate between subtle differences in the reflectance characteristics of pupae. Conventional imaging and spectroscopy are integrated within hyperspectral imaging systems to obtain both spatial and spectral information from an object. Hyperspectral imaging is a promising alternative technology in the field of forensic entomology as it is non-destructive, non-invasive, suitable for both live and preserved specimens, portable (not restricted to laboratory), rapid, and comparatively cheap. As such, hyperspectral imaging is fast emerging as a valuable tool in forensic investigations with a wealth of untapped potential. Reflectance-based methodologies have been used to successfully analyze a wide range of biological phenomena in arthropods (e.g., vision in honey bees and orb-webbing spiders, courtship and territorial displays among fiddler crabs). Furthermore, hyperspectral imaging has been used to identify species (e.g., tobacco budworms and corn earworms) and to age species (e.g., midges). At present, no study has reported on the use of hyperspectral imaging as a tool in forensic entomology. This work developed a predictive model for determining pupal age for two blow fly species, *Calliphora dubia* and *Chrysomya ruffifacies* (Diptera: *Calliphoridae*) at two developmental temperatures (24°C and 30°C). This was correlated with the morphological changes occurring during pupal metamorphosis. Furthermore, hyperspectral imaging was able to distinguish between different aged pupae that appear similar to the human eye. The potential of hyperspectral imaging analysis in forensic case work is extensive and will be discussed.

Hyperspectral Imaging, Blow Flies, Aging



H102 Diurnal Oviposition of Blow Flies: Does Time of Day Influence the Likelihood or Magnitude of Oviposition?

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After attending this presentation, attendees will better understand the impact that the time of day has on blow fly (Diptera: Calliphoridae) oviposition (egg laying).

This presentation will impact the forensic science community by making attendees aware that any information regarding blow fly oviposition is critical for accurate Postmortem Interval (PMI) estimations; this research provides important insights into diurnal blow fly oviposition and the abiotic factors that may be influencing it.

Many studies have been conducted on the nocturnal oviposition behavior of blow flies and most authors conclude that it does not occur. Forensic entomologists know that blow flies lay eggs diurnally, but according to this research, there have been no studies documenting differences in hourly diurnal oviposition in blow flies. Studies of other insects show two daily oviposition peaks and researchers are interested in determining whether or not blow flies exhibit a similar behavior.

This presentation has important implications for forensic science. If this research finds a diurnal time period during which oviposition does not occur, it will impact forensic entomologist's PMI estimations. The PMI is the time period between death and corpse discovery and entomologists provide an estimation of this interval based on insect activity. A long delay in oviposition during the morning hours could result in entomologist's needing to push PMI estimations back to the previous day.

This study seeks to document any differences in the likelihood or magnitude of oviposition by blow flies in relation to hours after sunrise. Research is being conducted in Valparaiso, IN, during the spring, summer, and fall months. Research commenced in June 2014 and will continue for multiple field seasons.

Chicken liver (17.5 grams) was put into an aluminum foil bowl and placed inside a foam container with vermiculite in the bottom. Three replicates were exposed to colonization every daylight hour starting one hour after sunrise and ending at the sunset hour. The liver was removed from refrigeration two hours before field exposure. The temperature of the liver prior to field placement was approximately 23°C. After one hour of field exposure, liver was removed from the field and checked for the presence of eggs. Any egg masses were weighed using an analytical balance. Eggs were reared to third larval instar maggots for identification. Light readings were taken hourly and weather data (temperature, humidity, and wind speed) were downloaded from Valparaiso University's weather station.

The preliminary results of four trials show no oviposition in the morning hours. Oviposition has been found between the hours of 1:15 p.m. and 6:15 p.m. and has occurred most often at light values between 4,000-8,000 lux. This is significantly lower than the highest light values of the day, which were around 100,000 lux. Oviposition has happened at temperatures ranging 21°C-26°C. One run yielded no oviposition, but temperatures did not get above 20°C. The warm weather flies *Phormia regina* (Meigen), *Lucilia coeruleiviridis* (Macquart), and *Lucilia sericata* (Meigen) have been identified in this study and the unseasonably cool summer in Valparaiso could be influencing their activity. Oviposition has been found after lux values peak for the day and within three hours of the highest temperature. Researchers will continue to examine light, temperature, and humidity to see whether fluctuations in these variables correlate with oviposition events.

These early results indicate a strong preference for oviposition in the afternoon hours. Blow flies were observed on the bait one hour after sunrise, but did not oviposit for seven hours after their initial activity. Any information regarding blow fly oviposition is critical for accurate PMI estimations and this research provides important insights into diurnal blow fly oviposition and the abiotic factors that may be influencing it.

Blow Fly, Diurnal Oviposition, Forensic Entomology



Pathology/Biology Section - 2015

H103 The First Use of Postmortem Microbiomes in Human Death Investigations

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After attending this presentation, attendees will understand that in recent years several research groups have recognized the potential use of postmortem microbiomes in forensic investigations. Several of these studies have demonstrated that the process of describing changes in microbial communities during the decomposition process of vertebrate remains has the potential for use in estimating the minimum Postmortem Interval (PMI_{min}) range. Specifically, these studies have employed metagenomic techniques, such as Illumina® MiSeq® and 454 pyrosequencing, to identify microorganisms present throughout the decomposition of model organisms (e.g., swine and mouse carcasses) or donated human remains placed in facilities dedicated to cadaver decomposition research; however, there has yet to be a study to survey the postmortem microbiome associated with human cadavers from real-world cases and evaluate the potential use of these bacterial communities in death scene investigations.

This presentation will impact the forensic science community by providing the first baseline database of the human postmortem bacterial communities found on human cadavers during routine death investigations of a medical examiner's office. Determination of the PMI_{min} range is a critical measure following events such as homicide or unwitnessed deaths, and resolving the precise window of time and location of both the decedent and witness(es) is essential for excluding or including witness accounts and for defining the circumstances of death. Microorganisms are ubiquitous in the environment and associated with humans both antemortem and postmortem and are often overlooked and underutilized biological indicators of circumstances and length of time since death. Little is known about this postmortem microbiology and biodiversity in human cadavers, particularly the microbial succession of indigenous microflora residing on or in the human body throughout decomposition; however, recent work suggests that bacterial communities are quite dynamic during the postmortem interval on model organisms. While there are several research groups developing predictive models based on the changes in microbial communities to estimate PMI_{min} ranges on these surrogates for human cadavers (e.g., swine and mouse), none to date have applied these statistical approaches to PMI_{min} estimates for human remains; the first step toward developing models to be used in human death investigations is to establish a baseline database of known bacterial taxa found on human cadavers resulting from various manners of death and in differing progressions of the decomposition process. The goal of this presentation is to describe the human bacterial communities on different areas of cadavers in relation to manner of death and autopsy-estimated PMIs from human remains discovered in a major metropolitan city — Detroit, MI. Here, the first baseline database of the postmortem microbiome developed from human remains investigated by a medical examiner's office is presented.

Bacterial samples were collected from human remains received into the Wayne County Medical Examiner's Office in Detroit, MI; each cadaver represented different circumstances of death and progression of decomposition. Individual DNA-free sterile cotton-tipped swabs were used to aseptically collect individual bacterial communities from six areas: the external auditory canal, nose, mouth, umbilicus, rectum, and the trabecular space between the inner and outer tables of the occipital bone. DNA extractions were performed according to the manufacturer's instructions using the Invitrogen™ PureLink® Genomic DNA Mini Kit. DNA was quantified using a Qubit® 2.0. All bacterial DNA samples were sequenced using Illumina® MiSeq® (2x250bp paired-end). Library construction and sequencing of the 16S rRNA V4 gene region was performed by the Michigan State University Genomics Core Facility using a modified version of the protocol adapted for the Illumina® HiSeq® 2000 and MiSeq®. Samples were collected from 50 cadavers representing four manners of death: homicide, suicide, accident, and natural. There were distinct bacterial community assemblages found on individuals based on body region and related to manner of death. Additionally, there was increased variation of bacterial community composition within an individual (across all sampled body regions) compared to communities among individuals.

This project greatly expands on earlier studies of the postmortem microbiome by partnering with a medical examiner's office to characterize bacterial communities associated with a large number of human cadavers during routine death investigations. These data offer a transformative way to rectify common practical issues associated with studying human decomposition while moving the science of postmortem microbial communities in a direction that addresses the importance of replication and real-world cases.

Postmortem Microbiome, Medical Examiners, PMI Estimates



Pathology/Biology Section - 2015

H104 Cadavers Show Distinctive Thanatomicrobiome Signatures

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After attending this presentation, attendees will understand the challenges/rewards of sampling the microbiome in human cadaver samples and its potential usefulness for determining the Postmortem Interval (PMI).

This presentation will impact the forensic science community by providing detailed information regarding how to sample the microbiome in order to understand how microorganisms colonize internal organs after human host death.

One of the challenges in forensic science is estimating PMI accurately. PMI is defined as the elapsed time-since-death and it is used in civil and criminal investigations to prove or disprove testimonial evidence (e.g., insurance fraud and homicides, respectively). Although there are many methods to determine PMI, most are susceptible to a range of errors and biases due to an overall poor understanding of how human bodies decompose.

Recent advances in DNA sequencing technologies have resulted in a paradigm shift in the understanding of the microbiome in healthy and diseased humans. For instance, it is now known that 90% of the total cells in a human body are microorganisms; however, what happens to these cells when a human dies is not known. The working hypothesis is that microorganisms involved in decomposing human bodies (i.e., thanato-, Greek definition of death, microbiome) provide an accurate clock for determining the PMI.

The objectives of this study were to survey the thanatomicrobiome of internal organs (the spleen, liver, brain, heart) and blood in human cadavers using two DNA sampling methods to determine which one was optimal for future studies.

The thanatomicrobiome were sampled from human cadavers with PMIs ranging from 20 to 240 hours. Amplifying the 16S rRNA genes and sequencing the amplicons from organ tissues and the blood of five cadavers was used to determine the effectiveness of the DNA sampling methods. Pair comparisons revealed that the conventional DNA extraction method (bead-beating in phenol/chloroform/ bead-beating followed by ethanol precipitation) yielded more 16S rRNA amplicons (28 of 30 amplicons) than the second method (repeated cycles of heating/cooling followed by centrifugation to remove cellular debris) (19 of 30 amplicons). Shannon diversity index of the 16S rRNA genes revealed no significant difference by extraction method. DNA sequencing of 19 organ tissue and blood samples yielded a total of 599,268 reads with an average of 31,540 reads per sample (~500bp).

Ordination plots and hierarchical clustering of the annotated data revealed that, in general, the thanatomicrobiome was highly similar among organ tissues from the same cadaver but very different among the cadavers, possibly due to differences in the elapsed time-since-death and/or environmental factors.

Thanatomicrobiome, Cadaver, 16S rRNA



Pathology/Biology Section - 2015

H105 DNA Extraction and Sample Storage Considerations for Using Metagenomic Sequencing Approaches to Evaluate Soil Microbial Communities Associated With Human Death Scenes

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After attending this presentation, attendees will understand how to collect bacterial evidence from human cadaver-associated soils using optimized DNA extraction methods, specifically testing the use of commercially available DNA extraction kits and the efficacy of short-term sample storage on the quality of genomic DNA to be used in downstream molecular applications (e.g., metagenomics).

This presentation will impact the forensic science community and practitioners interested in using bacterial communities found in soils associated with human decomposition by comparing five methods of DNA extraction. Soil, particularly cadaver-soil, provides crucial physical and forensic evidence that may be used to link a criminal to a crime scene, identify an unknown corpse, or determine if a body has been moved from its original burial location. The sensitivity and precision in Polymerase Chain Reaction (PCR) amplification of the extracted DNA is paramount for forensic lab protocols; however, complex organic and inorganic substances in soil samples make standardizing methodologies for DNA extraction difficult. Recent studies suggest there are important variations in methods that provide optimal extraction of high-quality genomic DNA for downstream molecular applications, such as PCR and next generation sequencing.

The goals of this study were to evaluate soil extraction and storage methods to determine an effective, relatively rapid, and standardized method for obtaining ample concentrations of high-quality DNA. Five DNA extraction methods were compared to assess the yield and purity of DNA from soil collected beneath five body zones of replicate human cadavers. Furthermore, the efficacy of short-term DNA storage at 6°C and in an RNA stabilization solution on DNA yields was compared between two commercial extraction kits using non-cadaver-associated soils.

The cadaver-soil samples were obtained from cadavers placed at the outdoor research facility Freeman Ranch located at the Forensic Anthropology Research Facility (FARF) at the Forensic Anthropology Center at Texas State (FACTS) University in San Marcos, TX. Samples were collected from the soil located under and juxtaposed to five body zones: the cranium, the right and left foot, and the right and the left olecranon. Four commercial soil DNA extraction kits and one lab-optimized DNA extraction method were used to extract genomic DNA (in duplicate) from each of the cadaver-soil samples. A spectrophotometer for optical density measurements was used to assess DNA yields (ng/μl) and quality; specifically, the purity of DNA extractions were evaluated by measuring the ratio of Ultraviolet (UV) absorbance at 260/280nm and 260/230nm for protein and humic acid contamination, respectively. Following quantification of DNA, each sample was tested for the presence of the desired 16S rRNA gene regions using targeted amplification by PCR. The results of the DNA extraction method comparison study revealed DNA yields of 50-107ng/μl with the purity of samples ranging from 1.59-1.93 (260/280nm) and 0.75-1.82 (260/230nm). As an additional comparison, DNA was extracted from non-cadaver soil that was preserved using either cold storage (6°C) or an RNA stabilization solution for 20 days. Non-cadaver-soil samples stored in an RNA stabilization solution had a greater mean DNA yield compared to soil samples stored at 6°C for both commercial extraction kits.

This research provides new information and specific parameters by which cadaver-soil microbial DNA can be evaluated and is important for downstream molecular applications, which could be used to improve the estimates of postmortem timelines. These results further aid in formulating standardization protocols for forensic laboratories to achieve reproducible DNA results.

Necrobiome, Soil Bacteria, Standardized Protocols



Pathology/Biology Section - 2015

H106 Does Carcass Mass Influence the Structure of Grave Soil Microbial Communities?

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After attending this presentation, attendees will understand that decomposing carcasses of contrasting mass can have similar effects on the changes in the structure of postmortem microbial communities.

This presentation will impact the forensic science community by illustrating that changes in grave soil microbial communities associated with swine carcasses in a field setting are similar to those observed with other mammals, including humans, as well as those conducted in controlled laboratory settings.

Postmortem microbial communities are crucial and dynamic contributors to the decomposition of a corpse. The activity of these decomposer microorganisms drives many postmortem changes, such as bloating and ethanol production. The development of soil microorganisms as physical evidence requires answers to several fundamental questions about the relationships between corpses, decomposition, and microbial communities. Yet one variable has received little experimental attention: how does the corpse mass influence the structure of postmortem microbial communities?

To investigate the effect of corpse mass on the structure of postmortem microbial communities, gravesoils associated with decomposing swine (*Sus scrofa domesticus*) carcasses in a pasture near Mead, NE, were collected in the summer from one to 15 days postmortem. 16S rRNA amplicons and 18S rRNA amplicons were sequenced to characterize the bacterial and archaeal communities (100 basepair reads) and eukaryote communities (~120 basepair reads), respectively.

The decomposition of all carcasses resulted in a significant change in gravesoil bacterial and archaeal communities. These differences were characterized by a decrease in acidotrophic bacteria (Acidobacteria) and basal soil-dwelling bacteria (Planctomycetes, Verrucomicrobia), which coincided with increases in the abundance of Proteobacteria, particularly Gammaproteobacteria. Significant differences were also observed between control soil eukaryote communities and those associated with post-rupture carcasses (days 9 and 15), with the exception of the 1kg neonate carcasses, which may be due to day 15 samples failing to sequence for these samples. As seen in decomposition studies both in laboratory and field settings, Rhabditidae nematodes bloomed after rupture in the other gravesoil and completely dominated microbial eukaryotic communities at day 15.

It is concluded that regardless of carcass mass, decomposition has a significant effect on soil microbial communities, although this needs to be confirmed for microbial eukaryotic communities associated with 1kg neonates. It is recommended that the decomposition of corpses greater than 50kg should be investigated in detail to determine if trends discovered in this study's data set extend to larger decomposing subjects (i.e., are corpses greater than 50kg associated with different gravesoil microbial communities?).

The current findings are similar to those of other recent investigations into the postmortem microbiome. It is becoming clear that a predictable succession-like change in microbial communities occurs to decomposition. Importantly, this study demonstrates that carcass mass has little overall effect on the decomposer microbial community, which is similar to previous studies into the release of bioavailable chemicals into gravesoils. This research has important implications for forensic science because it suggests that a microbial clock for estimating the postmortem interval may be robust to mass of a decomposing carcass.

Postmortem Microbiology, Forensic Taphonomy, Decomposition



Pathology/Biology Section - 2015

H107 Entomotoxicology: The Past and Where to Go Next

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After attending this presentation, attendees will be aware of what research has been conducted within the field of entomotoxicology, the downside of this research, and the direction the research needs to take in the future.

This presentation will impact the forensic science community by taking a good look at the research that has been conducted thus far, the implications it gives, and how a thorough field study may change these implications.

Entomotoxicology focuses on the secession trends of blow flies and/or developmental effects and toxicology levels of the maggots feeding on the tissues. The current research within the field of entomotoxicology has been done in laboratory settings on maggots fed on treated liver or other food sources. The small amount of research accomplished with injecting pigs or rabbits with controlled substances prior to death still only focused on feeding the liver to maggots in a laboratory setting.

Laboratory research can give an idea of the possibilities of entomotoxicology, but has a few downsides. Research conducted by feeding the larvae an artificial diet does not take into account any metabolism of the controlled substances by the human prior to death.¹ Even though studies performed on tissue from deceased humans can account for metabolism of the controlled substances, it does not take into account the real-life patterns of oviposition of Diptera: *Calliphoridae* and variation of toxicology levels within the human body. Blow flies normally start oviposition at the natural orifices of the body, primarily the head region. Provided the controlled substances reach the brain, ingestion of the tissue by the maggots may affect their growth and development. The current research has focused on specific tissues like the liver. With the exception of wounds in the abdomen, the liver is not a tissue that is fed upon by maggots initially.

Field studies are vital to gaining a more complete understanding of how and if controlled substances are affecting the growth and development of blow fly larvae. Examination of toxicology levels of muscle tissue, along with major organs, will allow for a better understanding of how far into decomposition and feeding of the maggots before toxicology levels will be detected within blow fly larvae.

This presentation will examine the research that has been conducted and discuss the direction research needs to take.

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Entomotoxicology, Entomology, Toxicology



Pathology/Biology Section - 2015

H108 Aggregation of Diptera Calliphoridae Larvae: A Keystone Behavior in Forensic Entomology?

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WITHDRAWN



Pathology/Biology Section - 2015

H109 Validating an Equation to Estimate Postmortem Interval on Oahu, Hawaii

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After attending this presentation, attendees will understand a proposed universal formula for estimating Postmortem Interval (PMI), how the formula was used at actual human death scenes on Oahu, HI, and how the formula was inconsistent in providing accurate estimates of PMI.

This presentation will impact the forensic science community by providing key data from actual death scenes in a tropical climate in the pursuit of an accurate universal formula to estimate PMI.

In medicolegal death investigation, PMI can be one of the key elements of an investigation. Establishing PMI may help to identify a deceased person and corroborate witness statements. There are currently several methods to estimate PMI, including livor mortis, potassium concentration in the vitreous humor, and forensic entomology, but none of these methods both accurately calculate PMI and are universally and conveniently applied. To improve the ability to estimate PMI, a recently developed equation was implemented at ten indoor death scenes on Oahu, HI, under the jurisdiction of the City & County of Honolulu Department of the Medical Examiner.¹ The hypothesis that this equation will not be accurate in a tropical climate on Oahu because it was developed in a temperate climate on the United States mainland was tested.

In the current experiment, temperature and relative humidity data were collected at each death scene and these values, along with an estimate of soft tissue mass loss, were used to generate an estimate of PMI (days). These estimates were compared to available information about the dates that the decedents were last known alive, in order to determine the accuracy of the formula. Mean values of temperature, relative humidity, and soft tissue mass loss were compared using a *t*-test.

For five of the ten cases (50%), the estimated PMI was consistent with the known PMI. All of these cases had a known PMI of five days or less and an estimated soft tissue mass loss of 15% or less. In contrast, all of the cases in which the estimated PMI was inaccurate had known intervals ranging from four to 16 days. In four of these five cases, the estimated soft tissue mass loss was 20% or greater. Mean temperature ($P=0.82$) and relative humidity ($P=0.44$) were not significantly different between the two groups of cases (accurate vs. inaccurate); however, death scenes with accurate PMI estimates were associated with significantly ($P<0.05$) less soft tissue mass loss than death scenes with inaccurate estimates.

The current data reveal that this equation can be accurate in cases with relatively little decomposition. Like many other methods to estimate PMI, the accuracy of this equation decreased as PMI increased. The reason for this is possibly related to climate; the equation was developed in a temperate climate while the current experiment was conducted in a tropical climate. It is probable that bodies follow a different decomposition pattern in these two climates, particularly during the cooler months of the year. Although the measurements of temperature and relative humidity were instrumentally objective using a datalogger, estimates of soft tissue mass loss were more difficult due to their subjective nature. Although this equation was accurate in only 50% of these cases, it is believed that PMI estimates can be improved through the development of a standardized system to estimate soft tissue mass loss as well as an increased number of data points.

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Decomposition, Medicolegal Death Inquiry, Forensic Taphonomy



Pathology/Biology Section - 2015

H110 Development of PowerQuant™ System: A New Robust Human and Male-Specific DNA Quantification System Which Monitors DNA Integrity

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After attending this presentation, attendees will better understand a new robust and sensitive DNA quantification system for determining total human and male DNA content in a casework sample. Also, attendees will learn about assess DNA integrity using the PowerQuant™ System and its utility in optimized casework sample processing for downstream Short Tandem Repeat (STR) applications.

This presentation will impact the forensic science community by serving as an information tool for learning about a robust and sensitive quantitative Polymerase Chain Reaction (qPCR) assay for human DNA detection and the use of quality indices for optimized sample processing in challenging casework samples.

Current qPCR-based human DNA-specific quantification systems allow for quantification of the amount of amplifiable human and male DNA present in a sample and whether autosomal or Y-chromosomal Short Tandem Repeats (Y-STRs) are likely to be more informative based on the auto/Y quantification ratio. While this information is useful, casework samples present additional challenges such as low quantity and quality (degraded DNA and/or presence of inhibitors). Any new qPCR quantification system should therefore be sensitive, robust to inhibitors, and able to provide information on the integrity of the human DNA sample in addition to the standard human and male DNA quantification results. Due to the robust performance in the presence of inhibitors of current commercial STR systems, it is also important that any new quantification system exhibits comparable tolerance to inhibitors. To address this need, the PowerQuant™ System has been developed. This is a five-color, four-target probe based qPCR assay that simultaneously quantifies the total amount of amplifiable human DNA and human male DNA in a single assay. The multicopy targets used allow for detection down to 0.1pg/ul DNA concentrations with minimal variation in auto/Y ratios across single-source male samples. This assay also includes a new larger degradation amplicon derived from a separate region of the sample autosomal quantification target that may be used to monitor the integrity of a DNA sample. As larger amplicons tend to be more sensitive to inhibition, the internal positive control has been designed to have a similar response to inhibitors as the degradation amplicon, thereby minimizing the potential for falsely flagging inhibited samples as being degraded.

Data will be presented demonstrating sensitivity, consistency of auto/Y ratios in male DNA samples, resistance to inhibitors, ability to detect DNA degradation, species specificity, and male specificity at various ratios of male-to-female DNA.

qPCR, Degraded DNA, Inhibitor



H111 In Situ Detection of Latent DNA Using Nucleic Acid Binding Dyes and an Alternative Light Source

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After attending this presentation, attendees will gain an appreciation of a method to detect latent DNA using nucleic acid binding dyes and its application in forensic science.

This presentation will impact the forensic science community by improving the collection of latent evidence by developing a presumptive test that detects DNA present at crime scenes, thus allowing a more targeted approach to swabbing for Short Tandem Repeat (STR) analysis.

DNA is deposited onto a surface by touch, yet few means have been developed for its *in situ* detection. Collecting touch DNA-type samples can be difficult as the DNA is not directly targeted, leading to many samples containing no DNA that leads to a waste of expensive reagents and kits. A range of dyes are available that bind to DNA at high specificity and the use of these dyes to detect latent DNA on various substrates and as a biological stain for forensic evidence is reported here. Six common nucleic acid-binding dyes were selected due to their increase in fluorescence in the presence of double-stranded DNA and their effectiveness in detecting latent DNA on surfaces was determined.

It has been suggested that epithelial cells are sloughed off the skin surface and can be transferred onto various substrates by touch, known as “touch DNA.”¹ It has been postulated that these cells are keratinized and lack nuclei; the DNA present on the surface is either present as a free molecule (cell-free DNA) or within a cell membrane transferred from DNA-rich sources, for example, touching the eyes, nose, and mouth.²⁻⁵ DNA contributed by any biological source of DNA, such as saliva, blood, and from touch, that cannot be seen by the naked eye refers to latent DNA. Currently, there are presumptive tests for saliva (phadebas which detects alpha-amylase), for semen (acid phosphatase, an enzyme reaction), and for blood (luminol which reacts with iron found in hemoglobin); however, none of these presumptive tests detect the latent DNA present within these biological samples.^{6,7} Currently, there are no presumptive tests for touch DNA, either.

For the detection of latent fingerprints, there are many techniques available such as powder dusting, cyanoacrylate fuming, silver nitrate, ninhydrin, indandione, and many others; however the more sensitive the technique the less applicable it is to the crime scene; items need to be taken to the laboratory for testing and often have a long reaction time.⁷ The methods applicable for crime scene testing, such as powder dusting, are not highly sensitive.⁸ Current methods for fingerprint enhancement generally work by interacting with amino acids; there are currently no methods that enhance fingerprints by detecting the DNA present.

In this experiment, common biological samples were stained with six selected dyes (GelGreen™, GelRed™, RedSafe™, SYBR® Green I, Diamond™ Dye, and EvaGreen™) to look at what was fluorescing in the samples such as hair, saliva, skin, and blood. The dyes were also applied to surfaces where latent DNA was present in fingerprints and as cell-free extracted DNA to determine the sensitivity of the dyes. The dye/DNA complex was detected using an alternative light source, the Polilight® (PL500), with an excitation wavelength of 490nm and emission through a 530nm or a 555nm interference filter.

Diamond™ dye, commonly used for gel staining, was found to be one of the more sensitive dyes and also had a longer lasting fluorescent signal that could still be detected after a month of applying the dye. This is advantageous because, if forensic evidence needs to be re-examined, then no additional dye is required to view the DNA present.

In conclusion, this study provides evidence that latent DNA can be detected with the use of nucleic acid binding dyes on substrate surfaces such as glass as a quick and sensitive method for detection. Furthermore, this research may be used to aid in development of a presumptive test for detecting latent DNA at crime scenes.



Pathology/Biology Section - 2015

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Latent DNA, Binding Dyes, Fingerprintmarks



Pathology/Biology Section - 2015

H112 Comparison of QIAcube® Differential to Manual Differential Extraction When Purified Using the QIAamp® DNA Blood Mini Kit

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The goal of this presentation is to provide a comparison of an automated differential wash protocol utilizing the QIAcube® and the QIAamp® DNA Blood Mini Kit protocol and a manual differential extraction procedure. These preemptive studies were needed to complete an internal validation at the Washoe County Sheriff's Office to ensure reliability of these systems and techniques for casework. Prior to placing a new method into service in a crime laboratory setting, accredited laboratories must perform internal validations according to Standard 8 of the Federal Bureau of Investigation (FBI) Quality Assurance Standards to verify that developmentally validated methods work reliably and robustly.

This presentation will impact the forensic science community by introducing studies performed to internally validate the QIAcube® automated differential wash protocol prior to purification using the QIAamp® DNA Blood Mini Kit protocol on the QIAcube®, as well as educating laboratory staff interested in exploring automated differential extraction techniques. Furthermore, the combination of the QIAcube® differential wash protocol with the QIAamp® DNA Blood Mini Kit protocol on the QIAcube® will highlight difficulties encountered when compared to other purification protocols more commonly coupled with the QIAcube® differential wash protocol.

In a 2008-2012 survey performed by the United States Department of Justice, an average of 237,868 victims reported being sexually assaulted each year, which calculates to an occurrence approximately every two minutes. Although only about half of all sexual assaults are reported, a great deal of time and effort goes into processing evidence from these cases due to the potential of samples containing female-male mixtures on which differential extractions must be performed. In forensic casework, a differential extraction is a method that incorporates the combination of phase separation with differential centrifugation to isolate sperm cells from other cell types in order to generate two distinct profiles of the victim and the assailant. Traditionally, differential extractions have been performed manually, requiring an analyst to undergo repeated pipetting and multiple centrifugation steps. Due to the hands-on nature of the approach, the quality and consistency of the separations tend to be variable from analyst to analyst. A combination of the number of sexual assault cases reported along with the time required to analyze samples from these cases has caused backlogs to become commonplace among many crime laboratories across the country. Bringing automated differential extraction procedure online would benefit analysts by not only reducing the backlog of the laboratory but also by streamlining the workflow of a lengthy process.

This study focused on determining the utility of QIAGEN's® QIAcube® for differential extraction of samples and compared it to the manual method currently being used by the Washoe County Sheriff's Office. The QIAcube®, introduced in 2007, was originally designed to extract nucleic acids and proteins and, therefore, capable of centrifuging, vortexing, pipetting, and extracting a supernatant from a pelleted sample. This study evaluated the QIAcube's® abilities, using a custom protocol, to perform differential separations on up to 12 mock sexual assault samples at a time. Experiments included a buffer study comparing three potential buffers incorporated into the lysis mixtures; a sensitivity and reproducibility study based on a 1:3 semen dilution series, with and without female epithelial cells present; a mixture study utilizing mixed female epithelial cells and semen; a cross contamination study using mixed female blood and semen; as well as a matrix and mock evidence study consisting of a mixture of female epithelial cells and semen pipetted onto different substrates along with various proficiency test samples. All studies were performed by a graduate student using a combination of four QIAcubes®. For comparison, the sensitivity and reproducibility studies were also performed by an experienced analyst. There was no sign of cross-contamination between samples, even though the tubes remain open all at the same time in the instrument. Interestingly, the manual method consistently yielded DNA concentrations approximately twice as high as the QIAcube® for the sperm fraction. Extensive troubleshooting was performed to include the use of different reagents and temperatures as well as a variety of protocol variations. In conclusion, the Washoe County Sheriff's Office will not be utilizing the QIAcube® to perform differential extractions unless future modifications of the standard protocols result in higher male yields.

Differential Extraction, QIAcube®, Troubleshooting



Pathology/Biology Section - 2015

H113 Detection of Genetic Variations in Cardiac Channelopathies Using Ion Torrent™ Next Generation Sequencing in a Cohort of Autopsy-Negative Sudden Unexplained Deaths

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After attending this presentation, attendees will better understand the considerable interest in next generation sequencing technology and the manner in which it performs a thorough genetic analysis of arrhythmogenic disorders in postmortem investigations.

This presentation will impact the forensic science community by demonstrating the implementation of a new strategy using the Ion Torrent™ Personal Genome Machine® (PGM™) System that allows the simultaneous study of the major arrhythmogenic genes in a quick and cost-efficient manner.

Background: Genetic testing for cardiac channelopathies in Sudden Unexplained Deaths (SUDs) has developed substantially over the last years. The Next-Generation Sequencing (NGS) technology provides an unprecedented opportunity to screen genetic variation underlying the arrhythmogenic genes in a short period of time at low cost. The goal of this study is to develop a strategy of systematic postmortem mutation detection on the major genes implicated in cardiac channelopathies (long QT syndrome, short QT syndrome, Brugada syndrome, and catecholaminergic polymorphic ventricular tachycardia) in order to identify the possible cause of death and to develop prevention measures for relatives.

Materials and Methods: NGS workflow based on an AmpliSeq™ panel was designed for sequencing 23 targeted genes on the Ion Torrent™ PGM™ Sequencer. The molecular analyses focused on 16 SUD cases of young people (under 35 years of age), autopsied at the Institute of Legal Medicine of Strasbourg over a period of five years. In all cases, the cause of death could not be determined after a rigorously autopsy associated with histopathological and toxicological analyses according to the guidelines of the Association for European Cardiovascular Pathology. DNA was extracted from fresh frozen tissue (heart and liver).

Results: An average of 150 variants were identified per sample; however, after the prioritization using a novel scoring program (VaRank), the number of putative variants, secondarily confirmed by sequencing, was reduced significantly. In a case of SUD that occurred during a psychiatric hospitalization, a heterozygous substitution on the *Ank2* gene, previously described as an ankyrin-B mutation associated with cardiac dysfunction, was successfully identified. Moreover, in a case of SUD that occurred during an attraction in an amusement park, a heterozygous substitution on the *Ryr2* gene, not previously described, was successfully identified, which might be damaging according to the bioinformatics prediction. Some interpretation problems encountered due to the multiplicity of identified variants were illustrated and the necessity to correlate the genetic results to the clinical data in order to identify the possible cause of death was highlighted.

Conclusion: This study illustrates that the NGS approach based on AmpliSeq™ libraries and Ion Torrent™ PGM™ sequencing may be an efficient approach integrated to the postmortem examination.

Next Generation Sequencing, Ion Torrent™, Sudden Cardiac Death



Pathology/Biology Section - 2015

H114 Evaluation of the Ion Torrent™ PGM™ for Use With Low-Copy and Degraded Whole Mitochondrial Genome

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After attending this presentation, attendees will understand the applications of next generation sequencing in forensic DNA analysis and, more specifically, its use with whole mitochondrial genome sequencing and human identification.

This presentation will impact the forensic science community by determining the threshold of sensitivity of the technology in the realm of human identification and to showcase the performance of Next Generation Sequencing (NGS) systems on compromised forensic samples.

The field of forensic DNA analysis, since the beginning of DNA fingerprinting assays, has continued to grow alongside newer and more sensitive technologies in the past decades. The use of NGS has found its niche among disease diagnosis/research, genetic research, genomic studies, and is beginning to delve into the world of forensics; however, this technology has yet to find its permanent place inside the forensic science toolbox as the community tends to adhere to more tested and well-used technologies that have undergone rigorous forensic optimization.

A continuing area of research in forensics is the analysis of mitochondrial DNA (mtDNA) for use with degraded or otherwise compromised samples where nuclear DNA is not present or is too damaged for Short Tandem Repeat (STR) analysis. Though the copy number may vary by tissue type, typically mtDNA is approximately 500 times more abundant than nuclear DNA; this overwhelming majority of mtDNA along with its circular form may allow for some copies to remain undamaged or whole, unlike nuclear DNA.

In many cases, the only evidence recoverable from a crime scene may be teeth, bones, or a shaft of hair, none of which may have useable nuclear DNA to generate an STR profile. Typically, the HV1 and HV2 control regions of the mitochondrial genome (mtGenome) are analyzed for human identification purposes, yet the HV1/2 regions have a lower power of discrimination compared to traditional STR methods; however, the entire mtGenome holds much more data than the HV1/2 regions alone and could offer increased intelligence in human identification. The prospect of sequencing the whole genome offers a potentially higher power of discrimination, especially when databases become more robust and more studies are performed to better understand variation within the mtGenome.

Recently, the Research, Development, Test, & Evaluation (RDT&E) laboratory at the University of North Texas-Health Science Center has developed a high-throughput protocol for mtGenome sequencing. This study evaluates the performance of the Ion Torrent™ PGM™ with whole mitochondrial DNA sequencing under three phases. The initial phase tested the mtGenome sequencing protocol at the Defense Forensic Science Center with pristine reference samples. The second phase tested the method under more stressful conditions with low-copy samples. A dilution series of DNA concentrations used in similar studies was used to determine the threshold at which “useable” analytical data can be obtained for forensic human identification purposes with the Ion Torrent™ PGM™.¹ The final phase tested the system with degraded samples that are representative of real-world evidentiary samples. Samples were enzymatically degraded to generate varying lengths of fragmented DNA. Since the mtDNA sequencing protocol begins with a long-PCR reaction requiring longer, intact DNA, the method needed to be altered. A custom RNA-capture reaction was used to preferentially enrich for mtDNA within the degraded sample in place of PCR.²

The combination of data from all three phases of this study will allow for a greater understanding of the application of this instrument and system for human identification. By testing the performance of the Ion Torrent™ PGM™ protocol with low-copy and degraded samples, forensic laboratories can begin to develop methods by which this technology can be incorporated into the field and provide the most useful data possible.

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Pathology/Biology Section - 2015

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Next Generation Sequencing, Mitochondrial, Whole-Genome Sequencing



Pathology/Biology Section - 2015

H115 Identification of Non-Synonymous SNPs in Archaeological Hair Protein: Calculation of Measures of Identity and Biogeographic Background

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After attending this presentation, attendees will understand how proteomic datasets can be used to obtain forensic and genetic information such as quantifiable measures of identity and biogeographic background.

This presentation will impact the forensic science community by highlighting novel methods that extend the amount of genetic information which can be obtained forensically and anthropologically in the absence of usable DNA, either due to contamination or degradation.

Methodology has been developed to extract identifying genetic information from proteomic datasets. DNA typing has revolutionized forensic practice and jurisprudence; however, DNA often is degraded due to biological, chemical, or environmental factors. Protein is considerably more stable and more abundant than DNA and persists in the environment for a longer period. Protein also contains genetic information in its primary structure, the result of non-synonymous SNPs (nsSNPs) that manifest as Single Amino-Acid Polymorphisms (SAPs). These SAPs-containing peptides are accessible to shotgun tandem mass spectrometry. This study has identified nsSNP-containing peptides from 35 alleles in 26 genes expressed in the forensically informative hair shaft proteome. Complex proteomic datasets from trypsin digests of the hair shafts of 54 validated European American individuals were obtained for this study. Peptides corresponding to nsSNPs expressed in this protein population were identified and collated for each individual. The combined probability of each individual nsSNP profile was calculated using genotypic frequencies of each allelic combination in the European population (1,000 Genomes Project) and the “product-rule.” The power of genetic discrimination ranged from 1 in 1,002 to 1 in 9,000. The average power of discrimination was 1 in 280. The power of discrimination increased as a function of proteomic dataset quality ($r^2=0.624$, $n=58$, $p<0.0001$). When the power of discrimination is calculated using genotypic frequencies from the African population, increased powers of discrimination are achieved. This is consistent with a decreased likelihood that the samples originate from an African origin. Relative likelihood measurements of European compared to African genetic origin range from 1 to 780 with an average of 50, a median of 18, and a standard deviation of 116. ($n=64$). Direct validation of the imputed status of each nsSNP allele was achieved with Sanger sequencing. A total of 430 genotype determinations were made from the proteomic data and 426 assignments were confirmed (specificity=99.1%, FPR=0.93%). The overall sensitivity was 31%. Framework has been established for the use of proteomic datasets as a source of identifying genetic information, allowing measures of identity and biogeographic background to be made from forensic or anthropological protein sources, including bone, teeth, preserved soft tissue, and trace evidence such as fingerprints.

Hair, Proteomics, Bioarchaeology



Pathology/Biology Section - 2015

H116 A Multiplex PCR Assay for Simultaneous Analysis of 13 Rapidly Mutating Y-STRs

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After attending this presentation, attendees will better understand the new tool that can be used in forensic DNA analysis for casework samples, specifically for sexual assault cases and population study.

This presentation will impact the forensic science community by adding a useful new tool for forensic DNA analysis.

Y-chromosome Short Tandem Repeat (Y-STR) profiling has been broadly applied in forensic casework in sexual assault cases where male/female or male/male mixtures are expected and also for population studies, genealogical research, and kinship analysis. Recently, rapidly mutating Y-STRs were described. These loci are expected to help with investigating inbred populations and also differentiating closely related males. A multiplex panel has been developed comprised of 13 Rapidly Mutating Y-STRs (RM-Yplex) that can be amplified simultaneously. The multiplex will aid investigating the human genetic structure of United Arab Emirates populations and would also be used to investigate unresolved forensic cases in the Department of Forensic Sciences and Criminology at Dubai Police.

Thirteen simultaneously amplified markers included in multiplex are: DYF387S1, DYF399S1, DYF403S1ab, DYF404S1, DYS449, DYS518, DYS526I/II, DYS547, DYS570, DYS576, DYS612, DYS626, and DYS627. Four primer sets for DYF387S1, DYS570, DYS576, and DYS612 loci have been redesigned to accommodate the loci within the multiplex using 5-dye chemistry. An allelic ladder was developed and sequenced using alleles found in United Arab Emirates populations. A developmental validation was conducted for the RM-Yplex assay to investigate the robustness of the multiplex assay including sensitivity, specificity, male/male mixtures, and male/female mixtures studies. A sensitivity study resulted in a sensitivity of the assay in amplifying as low as 62.5pg of male DNA template. In the specificity study, full male profile was detected using 1ng of male DNA template in the presence of 2,000 ng of female DNA template. A stability study using three different common inhibitors encountered in DNA profiling analysis including hematin, humic acid, and tannic acid has been demonstrated. RM-Yplex has shown great stability by resisting a concentration of 100ng/ μ l from each of hematin and humic acid, whereas it has shown more stability with tannic acid by resisting as much as 200ng/ μ l of such inhibitor. In the male/male mixture study, a complete unique minor profile has been identified successfully at 1:3 and 3:1 ratios. The RM-Yplex multiplex assay was used to amplify non-probative casework samples which gave significantly more probative information than normal autosomal amplification kits being used in DNA profiling analysis and currently used Y-STR Polymerase Chain Reaction (PCR) amplification kit results.

This study illustrates that RM-Yplex multiplex is extremely sensitive, does not exhibit cross-reactivity with female DNA, and successfully types male DNA in the presence of overwhelming amounts of female DNA. The assay was successful in typing various forensic casework samples. Thirteen RM Y-STR markers have been analyzed in 600 male samples from United Arab Emirates populations. Allelic frequencies, haplotype diversity, haplotype frequencies, and discrimination capacity were determined for the 13 RM Y-STRs. Mutations pattern analysis of the RM Y-STR loci in a typical United Arab Emirates family has been carried out and will be presented.

This research project funded by Dubai Police General Headquarters.

Rapidly Mutating, Y Chromosome, STR Multiplex



Pathology/Biology Section - 2015

H117 On Combining MicroRNA Analysis With DNA STR Profiling in a Single Stream Process

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The goal of this presentation is to explain the benefits of microRNA analysis in forensic samples in order to perform body fluid identification. Newly identified controls will be demonstrated using a novel approach, combining them with DNA profiling in a single reaction. It will emphasize the opportunities arising from co-extraction of microRNAs with genomic DNA. The presentation contains state-of-the-art research that is yet largely unpublished and will update attendees on some current developments in the upcoming field of microRNA research in forensic science.

This presentation will impact the forensic science community by expanding on a novel approach where microRNA analysis is combined with DNA Short Tandem Repeat (STR) profiling to identify a human and the tissue of origin, simultaneously. New markers and controls will be revealed to work using a novel capillary electrophoresis-based approach. The presented analysis method is fully compatible with current DNA profiling workflows and allows analysis of cold-case extracts. It has the potential of becoming a versatile and cost-effective method of learning more from biological samples.

MicroRNAs have a potential to be ideal forensic markers due to their small size (~22nt), high abundance per cell, and sensitive and specific Polymerase Chain Reaction (PCR) -based detection. Thousands of microRNAs are present in biological material and they are rich in information due to their tightly regulated and cell type-specific expression. Their advantageous properties increase the chances of successful analysis from challenged crime scene samples. In addition, it has been demonstrated previously that informative microRNA expression levels can be obtained from common DNA extracts without a change in protocol and will likely be present in cold-case extracts, too.

Following an earlier pilot project on a single stream process with the integration of microRNA analysis into a DNA profiling multiplex, progress on this line of research is now presented. The small nucleolar RNAs SNORD7, SNORD44, SNORD47, and the microRNA hsa-miR-93-5p have been identified as endogenous controls. These endogenous controls have been used real-time PCR experiments — in combination with results from other research groups — to determine a larger panel of microRNAs that allow differentiation between blood, saliva, vaginal material, and mixtures thereof.

With the markers identified, the transition has been made to analysis by capillary electrophoresis. Here the analysis of the endogenous controls using capillary electrophoresis on ABI's® 3130 genetic analyzer is presented and the effects of combining their analysis with genomic DNA human identification STR markers in a single reaction are explored. The endogenous control markers are reverse transcribed using a multiplex stem-loop reverse transcription, followed by multiplex PCR with labeled primers for the cDNA and genomic DNA markers simultaneously. This approach was demonstrated before, when it was shown that blood and saliva can successfully be distinguished by amplifying hsa-miR-451a and hsa-miR-205 cDNA during DNA profiling. This will now be expanded with the newly identified endogenous controls. Future work will include the incorporation of the additional body fluid-specific markers, working toward a single reaction that can provide a DNA profile and body fluid identification on single source and mixed samples.

MicroRNA, Body Fluid Identification, Capillary Electrophoresis



Pathology/Biology Section - 2015

H118 Postmortem Bacterial Translocation: When Does it Happen?

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After attending this presentation, attendees will better understand how and when bacterial translocation occurs in a human corpse, which is one of the first microbial changes during decomposition.

This presentation will impact the forensic science community by serving as a key factor in the understanding of postmortem changes which may improve the Postmortem Interval (PMI) evaluation.

The role of human enzymes and exogenous and endogenous bacteria during the decomposition of an adult corpse is relatively poorly documented in the forensic literature. Bacterial translocation is the migration of viable bacteria from the gastrointestinal tract to extraintestinal sites, such as the blood. This phenomenon is well described for various diseases, but not from a taphonomic perspective. This study describes the epidemiology of microorganisms and bacterial translocation during human corpse decomposition.

Methods: A blood sample was taken from the subclavian area in corpses with short (less than 72 hours) and known PMI. Blood agar and enrichment cultures were performed under aerobic and anaerobic conditions, and bacterial isolation and identification were performed using phenotypic and biochemical methods and completed by the mass spectroscopic identification of anaerobic bacteria.

Results: Analyses were performed in 18 cases with a PMI between eight and 72 hours. More than 27 different bacterial strains were isolated from eight positive samples. All of the bacteria were anaerobic or facultative anaerobic bacteria, and most were known to belong to gut microbiota. No bacteria were detected from ten samples (<0.2 CFU/mL). The shortest PMI with a polymicrobial sample was four hours without including the refrigeration time (overall PMI of 27 hours). These experiments are still in process.

Discussion: As a bacterial system, a human cadaver selects microorganisms by its growth temperature. Bacterial growth is particularly influenced by anaerobic conditions rapidly after death. Proteolytic and gas-producing bacteria from the gut were identified when the PMI was short, despite the taking of samples far from the intestines. This study provides evidence that bacteria from the gut can be present and spread during the early stages of human decomposition. Under certain circumstances and depending on the cause of death, bacterial translocation could happen at different times, even during the agonal phase, following the same mechanisms as in people suffering from illnesses. A postmortem bacterial contamination also occurs later and participates in the cadaver's microbial community. Furthermore, data gained from this research may be used while interpreting results from postmortem genomic sequencing studies.

Taphonomy, Microbiology, Bacterial Translocation



Pathology/Biology Section - 2015

H119 Apoptosis in Brain Tissues: Antemortem and Postmortem Cellular Responses

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After attending this presentation, attendees will understand the intricate process of apoptosis at molecular level after death and will also gain insight into various signaling pathways and the proteins involved.

This presentation will impact the forensic science community by providing detailed information about the process of apoptosis at cellular and molecular levels postmortem and its prospective to be correlated to Postmortem Interval (PMI), which is a constant area of interest for forensic scientists.

Death is an unavoidable tragedy of life that everyone must face at some point. For forensic scientists, death is much more than just the loss of life. It is the opportunity to better understand how and perhaps even when the loss of life occurred. In terms of a criminal investigation, the determination of PMI may be the single most important task given to a forensic scientist. This determination allows the authorities to assess potential suspects. Over the years, technology has evolved to assist law enforcement agencies in the determination of PMI and although great strides have been made, there is still room for vast improvement. Cell signaling could be a key step in the right direction.

Apoptosis is commonly defined as an active, programmed, and normal physiological process of living organisms during embryogenesis and an indispensable part of eliminating damaged or unwanted cells. Although still being scrutinized, it is suggested that after the physical death of the person, cellular death, also known as apoptosis/necrosis, occurs. In simple terms, this means cellular death through apoptosis is correlated with the physical demise, which forms the rationale behind the present study.

This study will specifically target the cellular process of apoptosis related to the brain structure and function. Brain tissue has been the organ of choice for this study, with the hypothesis that being positioned far from a microbe-rich gut, decomposition will be slower in a postmortem brain. Apoptosis has two pathways that are primarily responsible to the trigger response known as the intrinsic and extrinsic pathway. The intrinsic pathway or mitochondrial pathway, as it is sometimes referred to, occurs when there are signs of cellular stress that can manifest themselves in various ways such as DNA damage or loss-of-survival factor. The second pathway, known as the extrinsic pathway, begins outside the cell, on the cellular surface. Pro-apoptotic receptors are triggered by ligands that initiate this process. Determination of the elapsed-time-since-death, more specifically PMI, is one of the biggest challenges in forensic science. After death, decomposition is activated by the process of autolysis causing cell damage, and finally directing toward cell death.

Ten brain tissues from cadaver and human healthy nerve cells from ATCC® were analyzed using human apoptotic Polymerase Chain Reaction (PCR) -array and 84 key genes were screened. The working hypothesis is: cell death is necessary for the decomposition of tissue to occur and therefore may be useful in determining the PMI. Research is underway to compare the expression profile of various pro- and anti-apoptotic proteins postmortem and antemortem from brain tissues using PCR arrays. Furthermore, the postmortem proteins expression pattern obtained will be correlated with PMI.

Apoptosis, Postmortem Interval, PCR Array



Pathology/Biology Section - 2015

H120 Marijuana Edible Consumption as a Contributing Factor in Death: Two Cases and Live Anecdotal Accounts

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After attending this presentation, attendees will have a better awareness of the manifestations of marijuana edibles as a possible contributing factor in various manners of death.

This presentation will impact the forensic science community by presenting the autopsy findings and circumstances surrounding two deaths associated with marijuana edible consumption in Denver, CO. In addition, anecdotal accounts from the State of Colorado Marijuana Enforcement Division will be discussed in an effort to further understand the characteristics of this phenomenon and potential outcomes.

Introduction: As of November 6, 2012, the State of Colorado approved Amendment 64 which included legislation governing commercial manufacturing of marijuana products and retail sales. As a result, there has been an increase in recreational marijuana consumption, including marijuana edibles. Popular marijuana edibles available for purchase include cookies, candy bars, rice crispy treats, peanut butter cups, gummy bears, and various pastries. Currently, there have been two deaths in Denver, CO, where marijuana edible consumption played a contributing role to a variable degree in the cause of death.

Case 1: A 19-year-old African male and his three friends recently traveled from Wyoming to Denver, CO, for spring break and were residing in a hotel room on the 4th floor. According to his friends, the decedent consumed marijuana cookies and soon thereafter exhibited hostile behavior (pulling items off the walls) and spoke erratically. The decedent's friends attempted to calm him down and were temporarily successful; however, the decedent then reportedly jumped out of bed, went outside the hotel room, jumped over the balcony railing, and landed on the interior atrium floor. The decedent was pronounced at the scene. Autopsy findings included multiple injuries compatible with a fall from height; toxicology was positive for Delta-9 THC in the chest cavity blood.

Case 2: A 44-year-old Caucasian female contacted 911 requesting assistance as her husband was acting strangely and reportedly having hallucinations after consuming marijuana candy. The decedent repeatedly requested assistance and asked for authorities to hurry up as her husband was retrieving a gun from the safe. The 911 dispatcher heard a loud scream followed by the sound of a gunshot. Autopsy findings included a contact-range gunshot wound to the woman's head. The decedent's toxicology results were negative. The decedent's husband was arrested and is currently awaiting trial.

Discussion: Given the recent legalization of recreational marijuana use in Colorado, the resulting clinical manifestations of marijuana edible consumption are more formally becoming further elucidated. In addition to Case 1 above, anecdotal reports of post-consumption clinical symptoms have been reported by living individuals to include paranoia, suicidal ideation, and hallucinations. The possibility of these clinical symptoms playing some role as a contributing factor in a death should be considered in the appropriate forensic setting.

Marijuana, Edible, THC



Pathology/Biology Section - 2015

H121 Poor Man's Methadone: A Case Report of Loperamide Toxicity

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After attending this presentation, attendees will understand the recreational abuse of the antidiarrheal drug, loperamide (Imodium®), its mechanism of action, and the typical findings in cases of loperamide toxicity.

This presentation will impact the forensic science community by discussing the abuse of loperamide, a commonly used over-the-counter antidiarrheal drug, in order to increase awareness in the forensic and medical communities of the possible role of loperamide in cases suspicious for opioid toxicity but that have no evidence of toxic concentrations of commonly abused opioids.

The case of a 19-year-old male with a history of drug abuse who was found dead at his residence, possibly after recently attending a party, is presented. Bottles for his medications (including cyclobenzaprine, benzonatate, and nabumetone) were found at the scene, along with bottles for medications prescribed to other people. No other illicit drugs or paraphernalia were noted at the scene.

At autopsy, the major significant finding was massive urinary retention, seen frequently in opioid toxicity, with the bladder containing at least 750 milliliters of urine. Initial routine toxicology testing revealed non-toxic concentrations of alprazolam and fluoxetine as well as marijuana metabolites. Based on the strong suspicion of a drug-related death, further analysis utilizing the Liquid Chromatography/Time-Of-Flight/Mass Spectrometry (LC/TOF/MS) was requested.

The LC/TOF/MS analysis identified an unusual set of split isotope peaks, consistent with chlorine, and a molecular formula (C₂₉H₃₃ClN₂O₂) suggestive of the chemical composition of loperamide. A comparative sample of loperamide from a drugstore was analyzed by LC/TOF/MS, resulting in a mass and retention time matching the compound in the decedent's sample. Confirmatory and quantitative testing by an outside reference laboratory using gas chromatography detected 63ng/mL of loperamide, more than six times the therapeutic peak concentration. Based on these results, the cause of death was determined to be "Toxic effects of loperamide with fluoxetine and alprazolam" and the death was classified as accidental.

Loperamide (Imodium®), a synthetic phenyl piperidine opioid, is available as an Over-The-Counter (OTC) antidiarrheal medication. Like other opioids, loperamide acts in the gastrointestinal tract at mu-opioid agonist receptors, the myenteric plexi, by slowing intestinal motility. The opiate effects in the central nervous system with other opiates, such as euphoria, analgesia, and respiratory depression, are avoided with loperamide because of its decreased central nervous system penetration. Because of its decreased central nervous system effects and low abuse potential compared to other opioid drugs, loperamide was removed from Schedule V classification and can currently be dispensed without a prescription.

Loperamide is a substrate of P-glycoprotein, which causes it to be actively pumped out of the central nervous system at the blood-brain barrier; however, when P-glycoprotein is inhibited, loperamide can cross the blood-brain barrier freely and exert central nervous system effects similar to more commonly used opiates. P-glycoprotein is inhibited by many substances, including protease inhibitors, quinines, and even high doses of loperamide. Although normally poorly absorbed at therapeutic doses, loperamide ingested in massive doses can circumvent the inhibitory mechanism, remain in the central nervous system, and cause central opiate effects.

According to various sources, recreational loperamide abusers describe taking massive doses (50-200 pills) of the standard over-the-counter 2 milligram formulation, resulting in a cumulative dose of 100 to 400 milligrams. This concentration is frequently used to obtain a euphoric high, but it can be used to avoid symptoms of opiate withdrawal; for this reason, loperamide is also known as "poor man's methadone."

Loperamide toxicity in children is frequently reported to poison control centers for accidental ingestion. Most cases of adult loperamide toxicity report gastrointestinal symptoms and drowsiness with infrequent fatal outcomes. Due to its unrestricted access and low cost, loperamide abuse may be more common than the medical community realizes. Loperamide could be relatively unnoticed at a scene investigation and the drug may go undetected with routine drug screening.

Loperamide, Toxicology, Opioid Abuse



Pathology/Biology Section - 2015

H122 A Fatal Case of Reye's Syndrome Associated With Pepto-Bismol®

Sasha Osbourne, MD, Institute of Forensic Sciences, 1885 Old Spanish Trail, Houston, TX 77054; and Stephen K. Wilson, MD, 1885 Old Spanish Trail, Houston, TX 77054*

After attending this presentation, attendees will be familiar with the etiology, pathogenesis, and potential fatal complications associated with Reye's syndrome.

This presentation will impact the forensic science community by informing attendees of how the current generation of both doctors and parents would benefit from reminders about the dangers associated with the use of all salicylate-containing compounds in children and teenagers.

A fatal case of a 7-year-old male who developed profound vomiting and loss of appetite three days after receiving an influenza A vaccination is presented. His mother gave him Pepto-Bismol® and Gatorade® as treatment and sent him to bed. He was found unresponsive less than 12 hours later and pronounced dead four minutes after arrival at the hospital. Autopsy findings included diffuse mixed micro- and macrovesicular steatosis of the liver, microvesicular steatosis in the renal tubule cells, and cerebral edema. A postmortem nasopharyngeal swab screening was positive for influenza A. Toxicology was positive for salicylate. Follow-up with the mother indicated that she was aware of the warnings against aspirin use in children and she maintained that she only gave her son Pepto-Bismol® and Gatorade®.

Reye's syndrome is a rare severe neurologic disorder consisting of a biphasic illness characterized by a viral infection followed by an acute onset of non-inflammatory encephalopathy and hepatic failure, with rapid progression to death if not properly treated. It most commonly affects children younger than 16 years of age who are treated with aspirin during certain viral infections, including varicella and influenza. The exact cause is unknown, but it is thought to result from mitochondrial impairment resulting from the actions of salicylate and its metabolites, hydroxyhippurate and gentisate. This impairment appears to be intensified during viral illnesses. The incidence of Reye's syndrome has drastically decreased since the 1980's public health campaign in the United States and Europe, which advised parents and healthcare workers of the dangers of using aspirin and other aspirin-containing products to treat children with chickenpox or influenza like illnesses. In 1986, the Food and Drug Administration mandated that all medications containing aspirin (salicylate) were required to include labels with warnings against use in children because of the potential to cause Reye's syndrome. As a result of these initiatives, the number of Reye's syndrome cases in the United States has declined from 555 cases in 1980 to approximately two cases per year currently.

This case represents the need for reiteration of the dangers of using less-familiar salicylate-containing compounds in children, especially since most young doctors and parents have had no experience with this syndrome, given its near eradication since the effective public health campaign of the 1980s.

Reye's Syndrome, Aspirin, Forensic Pathology



Pathology/Biology Section - 2015

H123 Comparison of the Concentrations of Morphine, Methadone, and Diazepam When Sampled From Cardiac, Subclavian, Femoral, and Popliteal Sites and From Clamped and Unclamped Subclavian and Femoral Vein Samples

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After attending this presentation, attendees will understand that the distance of a blood sample from the trunk has a significant impact on measured postmortem drug concentrations as does sampling technique, but to a lesser extent.

This presentation will impact the forensic science community by illustrating that popliteal blood yields lower drug concentrations than femoral blood and are probably more representative of antemortem drug concentrations. It seems that popliteal blood drug concentrations have not been previously described.

Thirty cases were sampled as follows: Right Subclavian Blood (RSCB) — dissection/clamp technique; Left Subclavian Blood (LSCB) — blindstick technique); intracardiac blood, Right Femoral Blood (RFB) — dissection/clamp technique); Left Femoral Blood (LFB) — blindstick technique); and, left and right popliteal blood. Cardiac blood was sampled in the right auricle after chest dissection. Bilateral popliteal blood was sampled after dissection and clamping of the popliteal vein because of its small caliber and localization in the popliteal fossa.

To assess Postmortem Redistribution (PMR) for each substance the mean concentrations ratios were calculated as follows: (cardiac)/(subclavian); (cardiac)/(femoral); (cardiac)/(popliteal); (subclavian)/(femoral); (subclavian)/(popliteal); and, (femoral)/(popliteal).

To compare sample techniques for each substance, mean subclavian and femoral concentrations were compared as follows: (right subclavian — dissection/clamp) — (left subclavian — blind stick) and (right femoral — dissection/clamp) — (left femoral — blind stick).

The results indicate that the popliteal sample site appears to be less subject to PMR as seen especially in the statistically significant concentrations mean ratios (femoral)/(popliteal): morphine (N=17, mean ratio=1.26, p=0.003); methadone (N=24, mean ratio=1.27, p<0.001); and, diazepam (N=14, mean ratio=1.53, p=0.012).

With regard to the differences between subclavian and femoral site sampling techniques, results show that subclavian morphine concentrations tend to be lower when drawn from a clamped subclavian vein, though this is not true for femoral sampling. Methadone and diazepam concentrations are lower when drawn from either clamped vein. For methadone, the difference is statistically significant in both femoral and subclavian sites. This means that clamping the subclavian and femoral veins and isolating them from heart blood will result in lower concentrations of drugs as shown in the in the following results: (1) subclavian blood: morphine: N=17, RSCB mean concentration=83.18, LSCB mean concentration=94.06, p=0.066; methadone: N = 24, RSCB mean concentration=1105.58, LSCB mean concentration=1370.83, p=0.0009; diazepam: N=14, RSCB mean concentration=435.21, LSCB mean concentration=503.14, p=0.079; and, (2) femoral blood: morphine: N=17, RFB mean concentration=59.47, LFB mean concentration=57.94, p=0.64; methadone: N=24, RFB mean concentration=823.42, LFB mean concentration=1042.83, p=0.037; diazepam: N=14, RFB mean concentration=416.36, LFB mean concentration=616.21, p=0.052.

This study is the first to describe popliteal blood concentrations of morphine, methadone, and diazepam and illustrates that sampling from this site results in drug concentrations lower than those in cardiac, femoral, and subclavian sampling. This means that popliteal blood is less prone to postmortem redistribution due to its distance from the trunk. This study also demonstrated that clamping a blood vessel before sampling tends to result in lower drug concentrations, even in central sites, but the results for morphine showed that there may be significant difference in the behavior of different drugs. For methadone, this concentration difference was statistically significant. It is likely that similar results with other drugs subject to postmortem redistribution will be obtained, but will require further sampling efforts.

Popliteal, Redistribution, Techniques



Pathology/Biology Section - 2015

H124 Electrocution Deaths of United States Service Members From 2003 to 2012

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After attending this presentation, attendees will be aware of the injuries and epidemiological characteristics of electrocution deaths in the United States military over a ten-year period.

This presentation will impact the forensic science community by documenting the incidence of electrocution deaths within the United States military and describing the types of injuries observed with different electrical sources.

Although uncommon, deaths due to electricity occur within the United States military. An understanding of the electrical source and injuries documented at autopsy are crucial in developing tactics, techniques, and procedures to prevent future occurrences.

Methods: A retrospective review was performed by querying the Armed Forces Medical Examiner Tracking System (AFMETS) for all United States Service Member deaths that occurred under the jurisdiction of the Armed Forces Medical Examiner from 2003 to 2012 for which the cause of death was certified as electrocution. For each individual case, the following information was obtained: decedent's demographics, circumstances surrounding the incident including electrical source, physical findings/injuries observed at autopsy, and manner of death certification.

Results: Of the more than 6,500 deaths under the jurisdiction of the Armed Forces Medical Examiner during the prescribed ten-year period, there were 26 deaths of United States Service Members due to electrocution. All of the decedents were males between 19 and 33 years of age. In 13 (50%) of the cases, the individual came in direct contact with a power line. Of these 13 cases, 5 (39%) of the decedents were on the ground, 4 (31%) of the decedents were in a vehicle, 2 (15%) of the decedents were on a rooftop, and 2 (15%) of the decedents were wearing a communication radio with an antenna that came in contact with the power lines. In 6 of the 26 cases (23%), the individual came in direct contact with an electrified source due to faulty wiring. In 4 of the 26 cases (15%), the individual was working on a power source. In the remaining three cases, an individual contacted a high-voltage junction box, the rebar the individual was guiding was electrified when the crane came in contact with power lines, and an individual placed an electrical device in a bathtub. Physical findings/injuries ranged from no injuries in an individual that was in a pool with faulty wiring to decapitation and partial amputation of an extremity in an individual that contacted a power line. The manner of death in 25 of the 26 cases (96%) was certified as accident and in the case of the electrical device in the bathtub, the manner of death was certified as suicide.

Conclusion: Deaths due to electrocution in the United States military are uncommon. For those that do occur, the majority of the cases involve either contact with power lines or contact with an electrified source due to faulty wiring. In order to prevent these injuries, it is crucial to develop tactics, techniques, and procedures to eliminate or reduce the hazard. Such strategies include ensuring the proper safety procedures are followed when in the vicinity of power lines or working with electrical equipment and developing mitigating devices such as the overhead wire mitigation kit that can be placed on vehicles that may encounter low-hanging wires.

Electrocution, United States Service Member, Accidents



Pathology/Biology Section - 2015

H125 All-Terrain Vehicle and Snowmobile-Related Deaths

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After attending this presentation, attendees will: (1) understand the demographics of snowmobile and All-Terrain Vehicle (ATV)-related deaths; (2) appreciate common patterns of injury and toxicology findings in snowmobile and ATV-related deaths; (3) understand the various mechanisms of death encountered in snowmobile- and ATV-related deaths, including blunt force trauma and asphyxia; and, (4) have a better appreciation for the unique aspects of investigating deaths due to snowmobile- and ATV-related activities.

This presentation will impact the forensic science community by increasing awareness of the fact that snowmobile- and ATV-related deaths represent an important category of motor vehicle deaths investigated by Medical Examiner/Coroners (ME/Cs). ME/Cs should be familiar with injury patterns and common circumstances surrounding snowmobile- and ATV-related deaths, which are distinct from each other and from other motor vehicle-related deaths. Toxicology testing and interpretation are also important components of a death investigation of snowmobile- and ATV-related deaths. Detailed examination of the demographic characteristics, injury patterns, toxicology findings, and circumstances preceding injury may be helpful for implementing safety recommendations to reduce risk of injury and death.

In this study, the characteristics of ATV- and snowmobile-related deaths that occurred in southeastern Minnesota between 1999 and 2013 are described. During this time period, there were 10 snowmobile-related deaths and 16 ATV-related deaths. All snowmobile-related deaths occurred between the months of December and April, and all ATV-related deaths occurred between April and November. The average age of all deaths was 35.8 years (37.4 years for snowmobile deaths and 31.8 years to ATV-related deaths) with an overall male-to-female ratio of 7.67 (10.0 for snowmobile deaths and 4.33 for ATV-related deaths). Survival of greater than one day occurred in approximately 40% of snowmobile- and ATV-related deaths. Toxicology testing on the day of initial injury was available in 18 of the 26 deaths and showed that 66.7% of snowmobile-related deaths and 16.7% of ATV-related deaths were associated with a blood ethanol concentration above 80mg/dL. The circumstances surrounding injury could be categorized into four patterns: (1) struck a stationary object; (2) struck a moving object; (3) roll-over/ejection; and, (4) other. The percentages for circumstances of snowmobile accidents were 30% (struck a stationary object), 50% (struck a moving object), 20% (roll-over/ejection), 0% (other), and for ATV accidents 31.3%, 12.5%, 25.0% and 31.3%, respectively. The primary blunt force injury compartment (head, torso, or non-blunt force) was determined for both snowmobile- and ATV-related deaths and found to be 70%, 30%, and 0% for snowmobile-related deaths, and 62.5%, 25.0%, and 12.5% for ATV-related deaths, respectively. The “other” category for ATV-related deaths included one drowning and one compressional asphyxial death.

Snowmobile, All-Terrain Vehicle, Motor Vehicle Deaths



Pathology/Biology Section - 2015

H126 Suicides in Manitoba, Canada — A Ten-Year Study From 2003 to 2012

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After attending this presentation, attendees will better understand suicides that occurred in Manitoba from 2003 to 2012, including the annual incidence of suicides, distribution by sex, locations of suicides across the province, and the presence of alcohol and drugs in confirmed suicide cases.

This presentation will impact the forensic science community by reinforcing the need to continue taking proactive measures and working with suicide prevention agencies and other health care institutions in an attempt to reduce/prevent suicidal deaths.

This was a retrospective study and involved reviewing the electronic case files of all deaths from January 1, 2003, to December 31, 2012, that were classified as suicides per this study's informational resources. The overall annual incidence of suicide was 14.28 per 100,000 population. This was higher than the national average (11.5 per 100,000).¹ The overall incidence per 100,000 population over the years ranged from 11.04 to 15.52.

There were more deaths among males (17.94 per 100,000) than in females (7.15 per 100,000). As well, the incidence of suicide was higher for rural areas (13.54 per 100,000) than for major cities (12.76 per 100,000). There are many First Nations communities throughout rural Manitoba and this could possibly explain why suicide statistics for the rural areas are higher.

From the identified cases of suicides within the ten-year study, the causes of death were found to be: hanging — 802 (50.2%); gunshot wounds — 245 (15.3%); drug overdose — 285 (17.8%); carbon monoxide poisoning — 66 (4.1%); and, other means — 200 (12.5%). The most common method of suicide was hanging. The low number of deaths due to firearms may be due to the low number of firearms in Canada. There were no deaths due to insecticide, pesticide, or weedicide poisoning during this ten-year period. This was due to the strict controls imposed on the use and distribution of these substances in Canada. In less-developed countries, the incidence of suicide due to insecticide poisoning is very high and is estimated to account for 30% of suicides worldwide.²

In 2012, there were 169 suicides; toxicology was performed in 158 of the cases (93%). Of those tested, drugs and/or alcohol were found in 135 cases. Death was due to drug overdose in 28 of these cases; however, only four cases had blood alcohol levels of greater than 80mg/100ml. In the remaining 107 cases, where death was due to causes other than drug overdose, 42 cases had blood alcohol levels of more than 80mg/100ml. Therefore, the prevalence of alcohol and drugs is common in cases of suicide in Manitoba; however, deaths actually due to alcohol and/or drugs are low.

In conclusion, although not all suicides are preventable, any effort taken to prevent even one death from occurring is worthwhile. Other jurisdictions are encouraged to compare their figures with the results of this study and to work with their local agencies and health care programs in suicide prevention.

References:

1. Navaneelan, T. Health at a glance. Suicide rates: an overview. *Statistics Canada*, 2012.
2. Gunnell, D., Eddleston, M., Phillips, M.R., and Konradsen, F. The global distribution of fatal pesticide self-poisoning: Systematic review. *BMC Public Health*, 2007, 7:357.

Suicide, Incidence, Manitoba



Pathology/Biology Section - 2015

H127 Epidemiology of Suicide in Denver

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The goal of this presentation is to provide data of epidemiologic significance regarding suicides in Denver, CO.

This presentation will impact the forensic science community by providing valuable information concerning an up-to-date and detailed analysis of suicide deaths in a large United States jurisdiction over a 14-year period.

This presentation consists of a retrospective analysis of all deaths categorized with a manner of suicide which occurred in the city and county of Denver, CO, from January 1, 2000, through April 8, 2014. Suicide is defined as death caused by self-directed injurious behavior with any intent to die as a result of the behavior. Suicide is the second-leading cause of death among children, teenagers, and young adults in Colorado, second only to motor vehicle traffic-related accidents. It is the leading cause of death by injury for Coloradoans ages 35-74 years old (Colorado Violent Death Reporting System, 2007). Colorado has one of the highest suicide rates in the country. Of note, the suicide rate in Denver is approximately equal to the overall state rate.

It is believed this is the first epidemiologic presentation of data for suicide deaths in Denver. With investigator assistance, an in-house database query was performed for all deaths categorized as suicidal in manner, resulting in a total of 1,293 cases collected. The data from cases was compiled into a spreadsheet. Suicide deaths were analyzed given categories available within the database, allowing for comparison of age, race, gender, marital status, method used, possible contribution of ethanol and/or other substances, settings, and time of day of the incident. In this presentation, an in-depth discussion about age distribution is included within each method of suicide, providing a comparison of method of suicide and how the method may have varied over the time period examined. How method varies with race is also discussed. Suicide deaths revealed an upward trend, noting that 2012 had a total of 109 deaths categorized as suicide, the highest number of suicides from 2003-2012. In this presentation, how a person may have one or more risk factors that would put him or her at increased risk for completing a suicide is explored. In 2009, the Denver Office of the Medical Examiner/Coroner became more aggressive in tracking these risk factors. The deaths were recorded in a way that could be better analyzed for identifiable trends to include risk over time. Potential points for further research will be discussed.

This presentation will supply valuable information to the forensic community as it provides an up-to-date and detailed analysis of suicide deaths in a large United States urban population over a significant period of time. This presentation will provide data for further comparison within any notable trends in the upcoming years, and hopefully generate discussion among forensic professionals as to how the city and county of Denver compares to other large urban populations and nationwide statistics.

Suicide, Epidemiology, Urban



Pathology/Biology Section - 2015

H128 Fentanyl-Laced Heroin: A Maryland Public Health Problem

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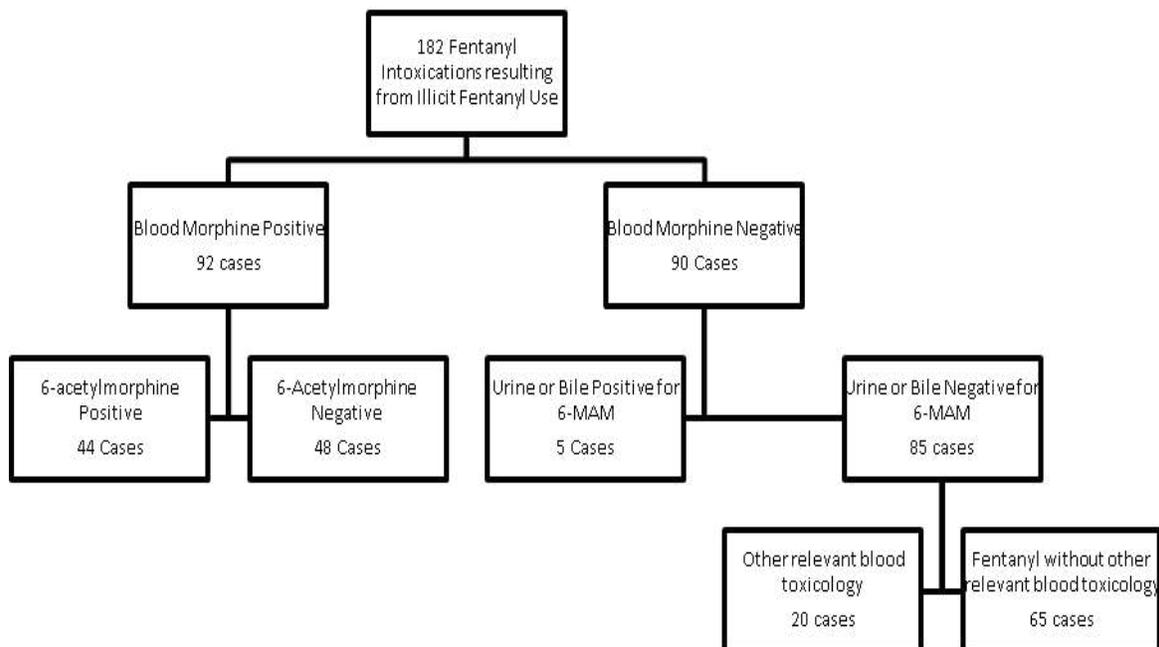
After attending this presentation, attendees will understand the importance and prevalence of synthetic drug combinations and their public health significance.

This presentation will impact the forensic science community by increasing awareness of newly emerging drug combinations and the role of the medical examiner in public health surveillance.

In January of 2014, the Office of the Chief Medical Examiner (OCME) reported that 37 Marylanders had died since September 2013 from fentanyl-laced heroin overdoses. The deaths accounted for 12% of the 318 total overdose deaths in that period. Since then, the cases have markedly increased. Nationally, there has been an increase in the number of deaths from substances variably mixed with fentanyl. In this presentation, deaths related to heroin mixed with fentanyl will be the focus. These deaths raise concerns because fentanyl is 30 to 50 times more potent than heroin and can reach the brain within minutes. Most of the users probably think that they are using just heroin and inject or snort the same amount as usual, but with deadly consequences.

A retrospective analysis of deaths investigated by the OCME from July 2012 through June 2014 (two years) was conducted. A search for cases with “fentanyl” in the Cause of Death field of the death certificate was performed. These cases were then reviewed and any case where fentanyl intoxication was not the cause of death, or those involving the abuse or use of prescription fentanyl, was excluded. This resulted in 182 cases for analysis. The cases were divided into groups based on the presence or absence of morphine and/or other drugs of toxicological significance. Ethanol was not considered for this grouping, as its presence was considered to be contributory rather than a primary intoxicant. This study identified an upward trend. There was an approximate six-fold increase in the number of deaths due to fentanyl.

The distribution of the cases is displayed below. Twenty cases had both fentanyl and quinine present with no other significant substances identified.





Pathology/Biology Section - 2015

Overall, there did not appear to be consistency in the amount of fentanyl in the mixtures with postmortem blood fentanyl concentrations ranging from 3.0ng/mL to 380ng/mL. Most victims were from Baltimore City but other counties throughout the state were also affected. Areas along the Interstate 95 corridor, including Pennsylvania, Massachusetts, Connecticut, and Rhode Island, as well as states as far away as Washington, have also reported similar deaths. The source of the fentanyl is uncertain, however recent Drug Enforcement Agency (DEA) reports indicate that the majority of fentanyl-laced heroin is being illicitly produced in Columbia and shipped to Mexico and then smuggled into the United States. Reasoning behind the use of fentanyl may include: making money for the drug cartels and dealers; persons with addictions using it to ease the pain of withdrawal; or, as a more powerful drug to chase the ultimate high.

This deadly drug trend raises serious public health concerns. Primarily as a result of this trend, the OCME has instituted automatic monthly toxicology data dumps to the Maryland Department of Health and Mental Hygiene and to the DEA. Alerting the public can be a two-edged sword, either encouraging use or saving lives. The Health Department chose to err on the side of saving lives. Alerts have gone out to the press, hospitals, and drug-counseling agencies. If dealers realize that they are killing off their customers, maybe it will not seem as cost-effective for them anymore. The jury is still out on the implications. Emergency departments should also be aware because the urine screen could be negative yet the patients look like they are suffering from heroin overdoses. Hospital staff could then counsel the patients, suggest bystander administered naloxone, analyze the doses of the drugs, or suggest other preventive measures. This presentation will summarize the case findings over the last few years, suggest public health surveillance methods, and include a review of the current literature.

Heroin, Fentanyl, Public Health



H129 Triangulating Time-of-Death With CT Scan, Immunohistochemistry, and Autopsy: An Experimental Study on Murder Case Investigations

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After attending this presentation, attendees will understand how forensic methodology on estimation of time-of-death can be implemented with new technologies and how a correct procedure may solve a case in less than 24 hours.

This presentation will impact the forensic science community by presenting a multidisciplinary approach (forensic pathology, forensic radiology, and forensic histopathology) to the everlasting problem of the chronological assessment of a murder case.

This study presents an unusual murder case where the classic measurements to determine the chronological estimation of death did not agree with the Computed Tomography (CT) scan made of the victim's body. In this case, the use of a CT scan was crucial for the exact estimation of time of death which was a new use of this technology in the forensic examination. The autopsy led to the identification of the murder weapon, thus narrowing the list of suspects and leading to an arrest and a confession by the murderer. Additionally, the immunohistochemistry confirmed a suggested hypothesis that matched the murderer's confession. In this specific case, a discrepancy was detected between the time of the reported attacker's crime and the body temperature of the discovered corpse. In fact, according to these reports, the crime would have to have been accomplished later than suspected and at a time when the suspect was seen in another place. The examination of CT images acquired before the autopsy was performed made it possible to highlight a hemorrhage in the cerebral ventricles that appeared organized, making it possible to chronologically characterize the hemorrhage and determine the time of death. Immunohistochemical staining on skin lesions was also performed to verify the viability and, where possible, the time of production. The comparison between the state of the cerebral hemorrhage and vital reactions, highlighted by immunohistochemical investigations, made it possible to demonstrate that the lesions were produced in a period preceding the time of death determined by traditional forensic thanatology phenomena. The condition of the cerebral hemorrhage and the evolution of the inflammatory phenomena shown by immunohistochemistry allowed tracing the injuries to before the time indicated by the state of rigor mortis and rectal temperature. This data established that the dating of the injuries (and thus the attack) was made at a time when the suspect did not have an alibi. Thanks to the examination of these images and immunohistochemical preparations, it was possible to assume that the victim had not died immediately after the attack but had remained in agony for many hours. This justified the discrepancy between the body temperature at the time of examination and the actual time of the assault. This case shows the possibility of using the forensic radiology, possibly together with the immunohistochemical reactions, to evaluate the state of the injuries and the determination of the time of death.

In conclusion, this study clarifies how the support of a multidisciplinary approach to murder cases, using all the tools available to the forensic examiner, are crucial for a correct legal and medical methodology and, of course, the arrest of criminal suspects.

Time-of-Death Estimation, Immunohistochemistry, CT Scan



Pathology/Biology Section - 2015

H130 Is It Proper to Cite Mitral Valve Prolapse (MVP) as the Cause of Sudden Cardiac Death (SCD)?

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After attending this presentation, attendees will be armed with the required information when dealing with skeptical clinicians who do not accept the opinion that SCD due to MVP is a proper cause of death.

This presentation will impact the forensic science community by raising awareness about the appropriateness of the diagnosis of SCD due to MVP. This presentation will also correct the impression among clinical colleagues in whose opinion patients with MVP only succumb to the disease after developing multiple secondary complications of Congestive Heart Failure (CHF). An index case and the response from the decedent's treating cardiologist are described. This response provoked a ten-year review of SCD cases that were determined to be due to MVP.

The deceased was a 43-year-old man with a past medical history of myocarditis, diagnosed approximately a year prior to the terminal event. He was evaluated by a cardiologist who also diagnosed him with mild mitral valve regurgitation and mitral valve thickening. Terminally, he complained of headache, rapidly developed chest pain, and anoxic-type seizures. He was transported to a hospital in asystole and was pronounced dead after failure of resuscitation.

The autopsy revealed a well-nourished and well-developed man with a body mass index of 24.7kg/m². The external examination revealed no significant physical trauma and was otherwise essentially unremarkable. Internally, there was concentric biventricular myocardial hypertrophy; the heart weight was 549 grams (average heart weight for body weight 371 grams, with a range between 281 and 489 grams). There was generalized mild (less than 25%) atherosclerotic stenosis of the proximal aspect of all major epicardial arteries. There were no changes of acute, subacute, or remote myocardial infarct or significant myocarditis. The mitral valve revealed marked redundancy and, when viewed from the left atrium, looked like what has been classically described as a deployed parachute. The circumferential measurement of the mitral valve was 13.5cm. The anterior leaflet of the mitral valve measured 4cm in length with a thickness of 0.3cm. The mitral valve annulus revealed focal calcification. The chordae tendinae of the mitral valve were thickened. The other cardiac valves were unremarkable. There was pulmonary edema (combined lung weight 1,095 grams) and changes of passive venous congestion. The remainder of the autopsy was essentially unremarkable. The toxicology screen was negative. The evaluation of the conduction system was non-contributory.

The cause of death of this decedent was listed as sudden cardiac arrhythmia secondary to mitral valve prolapse. This opinion was challenged by the decedent's treating consultant cardiologist, in whose opinion, "No one dies suddenly of MVP."

A ten-year look back of cases certified as related to mitral valve prolapse was performed. Fifteen cases, aged between 25 and 62 years (mean 46 years) which included 8 males and 7 females, predominantly Caucasians (ten), few (four) of African descent, and a rare (one) Asian descent were identified. The decedents had varied clinical histories with five cases having no history of medication use or illicit drug abuse. Among the rest, there was one case each with histories of alcohol abuse, warfarin and loratadine use, cocaine abuse, fluconazole and valaciclovir use, escitalopram and metformin use, acetaminophen/oxycodone use/abuse, and the last decedent had a history of the use of tadalafil. Adequate information was unavailable in two cases. None of the decedents had a prior history of cardiac dysrhythmia.

Anatomically, the mitral valve consists of the mitral valve annulus, the anterior and posterior leaflets, chordae tendinae, and the papillary muscles. Histologically, the mitral valve consists of the fibrosa, spongiosa, and atrialis layers. The spongiosa is made up of connective tissue, proteoglycans, and some elastic fibers, all being most prominent at the free edge of the valve. The spongiosa is the major load-bearing layer of the valve and is where one sees the myxomatous degeneration due to accumulation of glycosaminoglycan (dermatan sulfate). This accumulation secondarily attenuates the fibrosa and atrialis layers leading to the "parachuting" that is classically seen in MVP.

Chesler, et al, found that MVP induced endocardial friction lesions with thrombotic lesions at the angle formed by posterior leaflet of the valve and left atrial wall could lead to fatal cardiac dysrhythmias.¹



Pathology/Biology Section - 2015

Reference:

3. The myxomatous mitral valve and sudden death. E. Chesler, R. A. King, J. E. Edwards. *Circulation*. 1983 March; 67(3): 632–639
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Sudden Cardiac Death, Mitral Valve Prolapse, Myxomatous Degeneration



Pathology/Biology Section - 2015

H131 Double Approach to Patients With Brugada Syndrome by a Genetic and Proteomic Point of View

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WITHDRAWN



Pathology/Biology Section - 2015

H132 Sudden Death Due to Undiagnosed Rheumatic Heart Disease in a Child

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After attending this presentation, attendees will better understand an unusual presentation of rheumatic heart disease which may lead to sudden death in young people and, in particular, the histology of heart lesions typically caused by this disease.

This presentation will impact the forensic science community by presenting an uncommon cause of sudden death in childhood and describing how forensic scientists should recognize the signs which can lead to this diagnosis.

Introduction: Sudden death in childhood is rare. The case of a young boy who died from complications of rheumatic heart disease with atypical presentation is presented.

Case report: A five-year-old Caucasian boy was pronounced dead at his home by paramedics. He had a recent medical history that had started one month prior to death with symptoms of gastroenteritis. Twelve days before death he suffered a painless, discrete cutaneous rash on the legs and one hand; scabies was suspected and he was treated accordingly. The following day there was fever and bilateral, diffuse thigh pain. Eight days before death he had trouble walking, handling a pen, and exhibited general psychomotor retardation. He was hospitalized for seven days for evaluation of the neurological symptoms. No explanation was found and all exams were normal, although there was an inflammatory syndrome and the brain Magnetic Resonance Imaging (MRI) showed what seemed to be signal abnormalities in the left centrum semiovale. All symptoms had disappeared during hospitalization and the boy was feeling well, so viral encephalitis was suspected and the boy was authorized to leave the hospital. He was scheduled to have an MRI six months later.

The boy died the day after he was discharged. An autopsy was requested. External examination of the body showed no injury. There were numerous small, crusted skin lesions on the legs. At autopsy, the organs were congested and there was pulmonary edema with enlargement of multiple lymph nodes. Toxicology tests were negative. Histological analysis revealed pancarditis associated with Aschoff bodies, aseptic mitral valve endocarditis, and myocarditis of the septum. The main neuropathological finding was a sub-acute cerebral infarction of the left centrum semiovale. It was concluded that the cause of death was an acute cardiac arrhythmia secondary to heart failure due to rheumatic heart disease. The boy had suffered undiagnosed rheumatic fever. The brain lesion was attributed to ischemic stroke resulting from an embolism secondary to the heart condition.

Discussion: Rheumatic heart disease is the most serious complication of rheumatic fever, a systemic disease that affects children with a previous Group A beta-hemolytic Streptococcus infection. Around 40% of patients with acute rheumatic fever develop some degree of pancarditis with associated heart failure. Acute rheumatic fever and rheumatic heart disease are thought to be due to an autoimmune response, but the exact pathogenesis remains unclear. The prevalence of rheumatic fever has decreased and is less than 0.05 per 1,000 population in industrialized countries, making it an uncommon cause of sudden death in childhood.

In this case, the issue of medical negligence could be raised, because the boy died the day after he had left the hospital with an inappropriately reassuring diagnosis; however, the presentation was atypical, with prominent neurological signs that were not specific to rheumatic fever and that were subsequently attributed to ischemic stroke. Following hospitalization and investigation, viral encephalitis was suspected which was consistent with the medical history. The diagnosis of rheumatic heart disease was made postmortem on the basis of histology. Although rheumatic fever is infrequent, clinicians should consider this diagnosis when confronted with neurological symptoms in a child with a previous history of fever and rash.

Forensic Pathology, Sudden Death, Rheumatic Heart Disease



Pathology/Biology Section - 2015

H133 Cardiovascular Abnormalities Associated With Sudden Unexpected Death in Epilepsy

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After attending this presentation, attendees will better understand the cardiac abnormalities and other pathologic conditions associated with sudden death in young adults with epilepsy without evidence of a seizure episode at the time of death.

This presentation will impact the forensic science community by providing an overview of cardiovascular abnormalities seen in young adults with seizure disorders who died suddenly, which will be useful in identifying potential causes of death in this population.

Many young adults suffer with seizure disorders, such as epilepsy. While people with seizure disorders can die during a seizure, they also have a higher incidence of sudden death (without evidence of a seizure) when compared with the general population. The reasons for Sudden, Unexpected Death in Epilepsy (SUDEP) remain largely unknown. There is evidence that repeated episodes of hypoxia during seizures may make people more prone to cardiac arrhythmias and even that the medications used to treat the seizures may be arrhythmogenic. The goal of this study is to examine the presence of cardiac abnormalities in SUDEP and describe other pathologic findings associated with SUDEP in young adults.

A search of the Cook County Medical Examiner's Office database from 2011-2013 was performed for people between the ages of 18 and 40 years in which "seizure disorder" or "SUDEP" were listed as either the cause of death or as a contributing factor in death. Autopsy reports were reviewed for evidence of cardiovascular, respiratory, and neuropathological abnormalities. For evidence of structural heart disease, heart weight, chamber dilatation, hypertrophy, or presence of coronary atherosclerosis were evaluated.

Between 2011-2013, there were 775 natural deaths in young adults between the ages of 18 and 40 years. Of these 775 natural deaths, there were 40 (5%) in which "seizure disorder" or "SUDEP" was listed as the cause of death or as a significant contributing condition. Of these 40 cases, 15 (37.5%) were female and 25 (62.5%) were male. Only six (15%) were found to have evidence of seizure activity at the time of death (presence of intramuscular hemorrhage in the tongue) versus 34 (85%) with no evidence of seizure activity. All cases showed evidence of pulmonary edema or congestion and 15% had cerebral edema. With regard to seizure medications, 18 (53%) of the 34 with no evidence of seizure activity and three (50%) of the six decedents who had an evidence of seizure at the time of death were on medication for their seizures. The medication status of the remaining cases at the time of death was unknown. Of the 18 decedents without evidence of a seizure who were on medication, ten had therapeutic levels.

Cardiac abnormalities were present in 62% (21 out of 34) of young adults with no evidence of seizure activity at the time of death. The most common abnormality was cardiomegaly, which was found in 85% of cases (18 out of 21 cases). Each of these had a heart weight greater than 400 grams and there were nine cases with heart weights of greater than 500 grams. In the group of 21 cases with abnormal hearts, 52% displayed left ventricular hypertrophy, 33% had dilated ventricles, and 28.5% had coronary atherosclerosis (no significant occlusions). Histologically, cardiac myocyte hypertrophy and interstitial fibrosis were the most common abnormalities seen. Of the 34 decedents with no evidence of a seizure, 27 (79%) were overweight (BMI >25kg/m²), 16 of which were obese (BMI >30kg/m²). Of the 21 young adults with abnormal hearts, 20 (95%) were overweight (BMI >25kg/m²) and 14 were obese (BMI >30kg/m²).

In this study, it was shown that most young adults diagnosed with seizure disorders have no physical signs of seizure activity at the time of death. Most of these young adults dying suddenly with no evidence of a seizure are overweight or obese and have evidence of cardiovascular disease. The most common cardiac abnormalities identified were cardiomegaly, left ventricular hypertrophy, and chamber dilatation. The results of this study provide insight into potential causes of death in young adults with SUDEP and confirm that clinical surveillance of cardiovascular disease in this population is warranted.

Sudden Death, SUDEP, Cardiovascular



Pathology/Biology Section - 2015

H134 Sudden Deaths in Patients With Cardiac Rhythm Devices Via Medical Examiner Surveillance and Systematic Autopsy

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After attending this presentation, attendees will understand that cardiac devices such as pacemakers and defibrillators may contain useful information as to the heart rhythm at the time of death, as well as giving important clues as to why the patient died through interrogation of the device and inspection of the programming details and device settings, as well as uncovering possible malfunctions of the device.

This presentation will impact the forensic science community by reminding members of their role in public safety in finding malfunctions and defects in products placed into patients and their duty to help the living by giving feedback to cardiologists who program and implant these devices into patients.

Introduction: Data on device malfunctions are based on the Food and Drug Administration's Manufacturer and User Facility Device Experience (MAUDE) database, participation in which is mandatory for manufacturers but voluntary for healthcare providers; however, because the vast majority of Sudden Cardiac Deaths (SCDs) occur out of hospital, interrogations and autopsies of SCDs with devices are rarely performed; thus, actual rates of device failure that lead to sudden death are unknown. This study was initiated to determine the Causes Of Death (COD) in SCDs with devices in a prospective autopsy study of all incidents of SCDs in San Francisco.

Methods: In the first 30 months of the San Francisco Postmortem Systematic Investigation of SCD (POST SCD) Study (02/1/2011-11/30/2013), autopsies were performed on 468 of 480 (97.5%) of all incident SCDs and demographically matched trauma death cases captured through active surveillance of all deaths reported to the Medical Examiner (ME). Evaluation for all cases included full autopsy, toxicology, histology, and detailed examination of the heart and cranial vault. Device interrogation was performed on all SCDs and selected trauma controls with a device. A multidisciplinary committee reviewed pre-hospital medical records and autopsy results to adjudicate a final COD by consensus.

Results: Twenty-one of 468 (4.5%) incident SCDs and three trauma controls had devices (SCDs: eight Implantable Cardioverter Defibrillators (ICDs), 13 Permanent Pacemakers (PPMs); Trauma: one ICD, two PPM).

Six of eight ICDs showed a terminal rhythm of Ventricular Fibrillation (VF) with undersensing and delayed shock in SCD cases. Acute COD (e.g., pulmonary embolism, lethal toxicology, acute myocardial infarct) was excluded in all six. Three of six ICDs had delay of shock due to Antitachycardia Pacing (ATP) programming in VF zone. Four of 13 PPM showed a terminal rhythm of VF in SCD cases. Three of 13 (23%) PPMs had evidence of malfunction, acute COD was excluded in all three. Two PPM-dependent patients had evidence of acute premortem lead fracture; one PPM-dependent patient had rapid battery depletion the day prior to death. Search of MAUDE showed no prior deaths reported for the two leads.

All three trauma control cases showed no evidence of device malfunction at or around the time of accidental death.

Conclusions: By prospective ME surveillance, arrhythmic sudden death despite ICD was common, often due to undersensing and/or delay of VF therapy. VF was the most common mechanism of death in PPM SCDs, followed by lead fracture. Without systematic interrogation and autopsy, these events would have been missed, thus current calculated rates of malfunctions are likely substantial underestimates.

Sudden Cardiac Death, Pacemaker, Defibrillator



Pathology/Biology Section - 2015

H135 A Case of Maternal and Fetal Death by Spontaneous Rupture of Splenic Artery Aneurysm (SAA) in a Nulliparous Pregnancy at the Third Trimester

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The goal of this presentation is to examine the clinical and histopathological aspects of a case of SAA in a nulliparous pregnant woman at the third trimester.

This presentation will impact the forensic science community by illustrating the necessity of considering the occurrence of atypical and unrelated symptoms in pregnant women, especially in the third trimester, that could require prompt attention in diagnosing SAA rupture before a catastrophic evolution in hemorrhagic shock, characterized by an increased risk of maternal and fetal death.

SAA is the third most common intra-abdominal aneurysm (after aortic and iliac artery aneurysms), and the most common splanchnic artery aneurysm (60% of all visceral aneurysms). Its incidence is between 0.02% and 10.4% in the general population, with a prominent occurrence in women (4:1 female-to-male ratio), commonly multiparous pregnant women. More than two-thirds of aneurysms of the splenic artery are true aneurysms; infrequently they are pseudoaneurysms. They are usually saccular and occur at a bifurcation in the splenic hilum. The true cause of SAA formation is unclear; however, an increase in splenic blood flow may play a role in the development of SAA in patients with portal hypertension. The increased prevalence in multiparous women may be related to increased splenic blood flow and the effects of estrogen on the elastic tissue of the tunica media. Approximately 80% to 95% of SAAs are asymptomatic until rupture and are incidentally found during evaluation of unrelated symptoms, such as left upper quadrant pain, nausea, and vomiting. The most ominous presentation is hypovolemic shock secondary to aneurysmal rupture; however, the overall risk for rupture is low, about 5%, and it is associated with aneurysm size of at least 2cm, commonly during the last trimester in multiparity pregnancy. Rupture of the SAA is associated with a disproportionately high maternal and fetal mortality rate, 64%-75% and 72.5%-95%, respectively. Fortunately, it is observed to be a rare event. The major contributing factors to mortality are the minimal prodromal symptoms and the misdiagnosis with other common obstetric emergencies, like placental abruption, uterine rupture, or amniotic fluid embolism, as well as pulmonary thromboembolism, cholecystitis, appendicitis, or perforated peptic ulcer disease. In the non-pregnant population, mortality has been reported to be approximately 10%-25%. Fortunately, SAA is more frequently diagnosed today than in the past decades with the advancement and liberal use of imaging modalities.

A 28-year-old nulliparous, 39-week pregnant woman was taken to the emergency room showing 90/60mmHg blood pressure, 58b/min pulse rate, 90% SpO₂, and 15 score Glasgow Coma Scale (GCS). Her past medical history was unremarkable. After 25 minutes, the patient suddenly developed a shock status. She was immediately intubated and, despite the resuscitation efforts and epinephrine, a cardiac arrest occurred and the woman died. The gynecologist decided to deliver the fetus by cesarean section and before incision he described the wall of the uterus as pale but intact. A live fetus was delivered, with zero Apgar Score at birth, and three Apgar Score at five minutes. The exploration of the peritoneal cavity revealed a massive hemoperitoneum and 3,000cc of blood were removed. The prosecutor's officer requested an autopsy to verify if a rescue delay occurred.

The autopsy showed a transverse laparotomy with Pfannenstiel incision. The lesser sac was found to be filled with 30cc of blood and 60 grams of clots; a careful examination showed a 2cm splenic artery saccular aneurysm arising from the distal third of the artery's length, 2cm near the splenic hilum, in which was found a 5mm in length rupture.

The uterus presented a sharp lower uterine segment incision due to transverse cesarean delivery.

A complete histological examination was also performed using Hematoxylin-Eosin (H&E) staining, confirming the typical aspect of a hypovolemic shock, lack of blood in all organs with hypoxic-ischemic changes in particular of cerebellum and heart. The study of placenta after formalin fixation was unremarkable.

In conclusion, the cause of death was attributed to a hypovolemic shock due to the rupture of a splenic artery saccular aneurysm in a 3rd trimester pregnant woman and any responsibility of the rescue team was excluded. The infant died after four months due to hypoxic-ischemic brain injury.

Splenic Artery Aneurysm, Pregnancy, Fetal Hypoxic-Ischemic Injury

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Pathology/Biology Section - 2015

H136 Sudden Death in an 11-Year-Old Child With Epilepsy

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After attending this presentation, attendees will appreciate the potential value of examining the cardiac conduction system in Sudden Unexplained Death in Epilepsy (SUDEP) and will have a better understanding of proposed mechanisms of death in SUDEP.

This presentation will impact the forensic science community by calling attention to a seizure-related death where a cardiac conduction system examination was critical for revealing a pathologic abnormality that explained the likely mechanism of death and by suggesting that routine cardiac conduction system examination may be warranted in unexplained deaths associated with seizures.

SUDEP is the most common cause of death related to epilepsy, yet the precise mechanism of death remains poorly understood. Cardiac arrhythmia induced by seizure, possibly in combination with a genetic predisposition for arrhythmia, is the most commonly proposed mechanism of death in SUDEP. Other proposed mechanisms include asphyxia due to external obstruction of airway and unwitnessed status epilepticus. Notably, each of these mechanisms of death is likely to have minimal abnormal findings at autopsy. As a cause of death, SUDEP essentially remains a diagnosis of exclusion based on a history of epilepsy, findings or witness accounts indicative of a peri-mortem seizure, and lack of another cause of death revealed through complete autopsy, scene investigation, and ancillary studies.

A case of an 11-year-old girl with a history of seizures and attention-deficit-hyperactivity disorder, who first developed seizures approximately 1½ years prior to death is reported. A detailed neurologic evaluation did not reveal the etiology of the seizures, although magnetic resonance imaging of the brain suggested the presence of mesial temporal sclerosis. She was diagnosed with epilepsy and started on levetiracetam, which was effective in controlling her seizures; however, approximately six months later, she suffered a prolonged witnessed seizure followed by cardiac arrest. She was successfully resuscitated without any neurological deficits and was admitted for comprehensive neurologic and cardiology evaluations. No notable abnormalities were found and she was discharged home. Approximately two weeks later, she suffered another witnessed seizure followed again by cardiac arrest. This time resuscitation was not successful and she was pronounced dead.

Postmortem examination, including cardiac conduction system examination, revealed a focal recent, but healing, myocardial infarct of the summit of the ventricular septum abutting the AV bundle. There was granulation tissue reaction within the infarct, compatible with the infarct occurring around the time of the initial cardiac arrest two weeks prior. The remaining histologic sections of the heart were normal. There were no other infarcts of other organs in the body with a similar histologic age. Neuropathologic examination was notable for a remote 0.3cm cerebellar infarct, subpial gliosis consistent with prior seizure activity, and changes consistent with acute hypoxia. Mesial temporal sclerosis was not identified.

The focal myocardial infarct explains the mechanism of the second cardiac arrest as probably a cardiac arrhythmia. The histologic age of the myocardial infarct suggests the infarct occurred around the time of the initial cardiac arrest two weeks prior to death, perhaps due to inadequate circulatory flow and subsequent ischemic injury; however, there were no other organizing infarcts in other regions of the heart or in other organs and the region of the AV node is not known to be particularly prone to ischemic injury; therefore, this explanation is not entirely adequate. Furthermore, the presence of the infarct does not explain the seizures that preceded both episodes of cardiac arrest since witnesses provided compelling testimony that the seizure preceded cardiac arrest rather than vice versa.

A remarkable aspect of this case report is that the only pathologic abnormality in the heart was found in the cardiac conduction system. The cardiac conduction system is not routinely examined at autopsy. Even in cases of otherwise unexplained deaths, medical examiner/coroners will sporadically put through the conduction system for histologic examination as an exercise in completeness with little expectation of finding a significant abnormality; however, this case suggests that routine examination of the cardiac conduction system may be warranted in cases of sudden death associated with seizures.

Myocardial Infarction, Conduction System, SUDEP



Pathology/Biology Section - 2015

H137 Next Generation Sequencing Technology for the Identification of Genetic Markers Associated With Sudden Unexplained Death and Sudden Infant Death Syndrome

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After attending this presentation, attendees will understand how the use of next generation sequencing technology to identify genetic markers associated with Sudden Unexplained Deaths (SUDs) and Sudden Infant Death Syndrome (SIDS) can provide a lower-cost alternative to traditional genetic testing methods.

This presentation will impact the forensic science community by demonstrating the potential advantages of using next generation sequencing to identify genetic markers associated with SUDs and SIDS and the advantages of this approach over existing methods.

In the United States each year, there are thousands of deaths of young adults and infants for which there is no determinable cause of death at autopsy. After postmortem investigation, these cases are often listed as SUDs or SIDS. It is estimated that up to 30% of SUD and 10% of SIDS cases could be attributed to potentially lethal and heritable mutations in genes associated with cardiac function. Tests are currently available to identify these genetic variants; however, due to the labor-intensive nature of the traditional sequencing methods used, the identification of putative SIDS/SUD mutations can cost \$5,000 or more per case to sequence only 11 of these genes. This type of testing is prohibitively expensive for medical examiner's and coroner's offices. The purpose of this project was to develop and validate a cost-effective molecular autopsy tool to aid in the determination of cause of death for autopsy negative SUD and SIDS cases. In collaboration with the Baylor College of Medicine Human Genome Sequencing Center, the exomic regions of 65 genes implicated in cardiac arrhythmia and/or sudden death were sequenced using next generation sequencing on the Illumina® HiSeq platform. This allowed a sequencing of a much greater number of genes with a ten-fold *decrease* in cost. More than 300 decedent samples from a Harris County Institute of Forensic Sciences SIDS and SUD cohort were sequenced for lethal mutations. Within this cohort, over 1,000 potential pathogenic variants were identified. After a comprehensive biochemical and functional analysis, the cases where pathogenic mutations were identified were further analyzed for confirmation by the Baylor College of Medicine Medical Genetics Laboratory, a Clinical Laboratory Improvement Amendments (CLIA) -accredited laboratory. Approximately 3%-5% of the cohort was identified as having a lethal genetic mutation, which is significantly less than previously reported estimations. Studies in collaboration with Baylor College of Medicine Center for Medical Ethics and Health Policy are ongoing to develop criteria for reporting final results to family. This presentation will summarize the methods and the sequencing results as well as the process for confirming the findings and proposed reporting guidelines.

Next Generation Sequencing, SIDS, SUDS



Pathology/Biology Section - 2015

H138 Sudden Cardiac Death Due to Anabolic Androgenic Steroids (AAS): Autoptic, Histopathological, and Toxicological Findings in Four Cases

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The goal of this presentation is to examine the cardiovascular effects of AAS chronic abuse. In all cases of sudden death in apparently healthy bodybuilders, an accurate circumstantial investigation is fundamental in order to confirm the AAS abuse; the autopsy, histological, and toxicological investigation can ascertain the cardiac pathological features correlated to the abuse of this group of substances.

This presentation will impact the forensic science community by providing the pathological relationship between androgenic-anabolic steroid abuse and sudden cardiac death.

Introduction: Bodybuilders use AASs to increase strength, lean body mass, and, in some cases, to improve physical appearance. This class of substances is able to increase the risk of sudden cardiac death. Here four fatal cases are reported.

Case 1: A 32-year-old bodybuilder suddenly lost consciousness and died during a weightlifting workout at the gym. For several months, he had taken testosterone propionate (700mg/week) and nandrolone (200mg/week) parenterally; he had also taken stanozolol (70mg/wk) orally. His medical history was unremarkable.

Case 2: A 29-year-old bodybuilder was found unconscious during the morning lying on the bed in his apartment. Family members and friends reported he had been using anabolic steroids parenterally (250mg/week of nandrolone and 350mg/week of stanozolol) for several months.

Case 3: A 30-year-old competitive bodybuilder, who worked out regularly at the gym, suddenly collapsed at home and died. In an ashtray near the body, a 2ml vial of nandrolone decanoate was found along with a used 2.5ml syringe. All witnesses confirmed that the subject had started using AAS approximately six months before his death.

Case 4: A 28-year-old amateur bodybuilder was found dead by his father lying on the bed in his bedroom. Family members reported that he had a long history of AAS abuse.

For these four cases, a complete postmortem examination as well as histological and toxicological analyses were performed.

Results: *Case 1:* The body was that of a well-built man (height 189cm, weight 90kg) with a prominent muscular build. The 450g heart was normal in shape, with no cavity dilatation. Its dimensions (14x14x4cm) and wall thicknesses all fell within the normal ranges. The coronary arteries were unremarkable. A grayish area in the internal half of the anterior-lateral wall of the left ventricle was found. Histologically, the grayish area corresponded to typical infarct necrosis with a histologic age of approximately 15 days. *Case 2:* The body was of a well-built man (weight 72kg, height 166cm). All organs were normal. In particular, the dimensions of the heart were 11x10x5cm, the weight was 390g. Histology did not reveal any pathological changes, only focal myocardial cells with contraction bands and segmentation of the myocardial cells similar to those seen in case 1, were found. *Case 3:* The body was that of a well-built man (weight 90kg, length 178cm). The autopsy revealed abnormal muscle development, testicular atrophy, and hepatomegaly. The heart weight was 400g. The wall thicknesses were normal. The coronary arteries showed scattered fatty streaks. Histopathological examination of the heart revealed focal myocardial fibrosis. The liver showed cholestasis and vascular gaps compatible with the diagnosis of peliosis hepatis. *Case 4:* The body was that of a well-built man (weight 87kg, length 176cm). The heart had a normal shape and was normal in size (13cmx11.5cmx3.5cm) but weighed 470g. The left and right coronary showed 75%-80% lumen reduction. Histologically, the myocardial samples showed wide fields of myocardial necrosis characterized by hypercontraction of the myocells. In all cases, a complete toxicological analysis confirmed the presence of AAS in the biological specimens, whereas other recreational drugs or ethanol were not detected.

Conclusions: The morphologic findings and the toxicological results are able to explain the deaths of the four bodybuilders, as related to the cardiac effects of AAS abuse. Therefore, the warning against the use of these drugs by athletes is reinforced and heightened surveillance for AAS-related death that may be under-recognized and under-reported in the medical literature is encouraged.

Anabolic Steroids, Sudden Death, Toxicological Findings

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H139 The Challenges Faced in Evaluating a Death Related to Left Ventricular Assist Device (LVAD)

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After attending this presentation, coroners and forensic pathologists will understand the appropriate process involved in the evaluation of a decedent with an implanted LVAD and thus avoid some of the pitfalls experienced by others.

This presentation will impact the forensic science community by raising the awareness about an option that has been recently made available for the treatment of Congestive Heart Failure (CHF): the implantable LVAD, of which there are now a variety of devices in developmental stages in clinical trials and in routine use.¹ The limitations of the process involved in the evaluation performed at the Regional Medical Examiner's Office in Newark, NJ, for a decedent with a LVAD are described. The criteria for the use of LVADs, a brief review of the types and functioning of these devices, and their therapeutic complications will also be outlined.

A 57-year-old man was transported from his residence to the nearest health care center after being found unresponsive by his spouse. His downtime was estimated at 20 minutes. His past medical history was significant for end-stage CHF, status post-LVAD implantation, atrial fibrillation, status post-pacemaker implantation, gout, hypothyroidism, and obesity (Body Mass Index (BMI)=30.7kg/m²). He was later transported to the local area major medical center, where his LVAD had been previously implanted. He was diagnosed with anoxic encephalopathy and pronounced dead after failure of resuscitation.

At autopsy, he had a 942 gram heart with concentric biventricular myocardial hypertrophy. Gross examination of the heart revealed prominent subendocardial fibrosis in the left ventricular outflow tract and partial fusion of the aortic valve cusps, a finding that has been reported in the literature.¹ Evaluation of the heart and the device at autopsy revealed no loss of integrity of the anastomosis and no bleeding or evidence of infection. Additional autopsy findings included pleural and peritoneal effusions, passive venous congestion of the liver, and marked pulmonary congestion with numerous heart failure cells. The toxicology screen was negative. Besides the complete autopsy, the LVAD and pacemaker were evaluated by the respective manufacturers.

CHF affects approximately five million patients in the United States and about a half-million new patients are diagnosed with the condition every year. While there are pharmaceutical options for the treatment of CHF, the survival rates and quality of life with these therapies remains suboptimal. An alternative remedy is cardiac transplantation; however, as is well known to the forensic pathology community, the major limitation for this option is the availability of donors for the procedure. The research into the development of an artificial heart was initiated at the National Institute of Health in 1964. In 1994, the Food and Drug Administration approved the use of LVADs, initially as a bridge to transplantation. The device is currently used as a destination device for those too ill for cardiac transplantation.

Immediate complications of LVAD use include postoperative bleeding, with the necessity of massive transfusions with all its associated risks, and infection involving the operative site resulting in sepsis. A slightly more ominous complication is acute gastrointestinal bleeding due to the formation of arteriovenous malformations in the stomach and intestines. Other reported complications include pulmonary insufficiency with right heart failure and transient cerebrovascular ischemic episodes or strokes. The manufacturers are attempting several innovations to reduce these complications.

Several challenges were experienced while evaluating the cause and manner of death in the decedent described above. While there are several articles on the topic of LVAD in the clinical literature, a review of the forensic literature revealed only one article on this topic.¹

Reference:

1. Padera RF & Mitchell RA: The intervened heart: Cardiac hardware in the Forensic Suite; *Academic Forensic Pathology*; 2011; 1; 166 – 176.

LVAD, Congestive Heart Failure, Sudden Death



Pathology/Biology Section - 2015

H140 Cardiovascular Pathology in Cases of Death Following Autonomic Failure

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After attending this presentation, attendees will be able to identify common, less common, and very rare histopathologic findings of the heart and its conduction system following a sudden death due to a derangement of the autonomic nervous system. Attendees will be taught the appropriate approach for the examination of the heart. Different dissection and sampling techniques, together with different histology, staining procedures, immunohistochemistry, and DNA analyses will also be presented.

This presentation will impact the forensic science community by approaching the concept of “neurocardiology” in a multidisciplinary way. The goal of this study is to provide new tools and guidelines for the medical examiners attending a case in which a failure of the autonomic nervous system is regarded to be the triggering factor in the chain of events leading to death.

Central autonomic failures may result in several cardiovascular complications ranging from reversible Electrocardiogram (EKG) changes to a well-established myocardial infarction. It is well recognized that physical and emotional stress (such as physical pain, anxiety, and anger) may result in sudden death following a major, apparently “inexplicable,” cardiac event. These mechanisms are known to be responsible for more than 300,000 sudden cardiac deaths every year in the United States.

During the presentation, the chain of cellular events resulting either in reversible cell injury or death of the myocytes will be explained briefly. After the explanation of the process involving cardiac physiology and physiopathology, attendees will be shown a series of cases in which the autonomic nervous system was found to be the only trigger factor for the development of a myocardial ischaemia or an infarction. At the time of the autopsy, all of the decedents were found to have patent coronary arteries, no abnormalities to the cardiac conduction system, and negative DNA analyses for the most common channelopathies. Both the macroscopic and the microscopic examination of the organs (with the exception of the heart) were unremarkable. The toxicological analyses were negative and there was no history of previous diseases or alcohol or drug abuse.

The goal of this study is to link the histopathological findings to the concept of neurocardiology. The most common EKG changes (when recorded shortly prior to death) will be shown and compared with the related “neurocardiac lesions” such as myofilament disintegration, coagulation necrosis, colliquative myocytolysis, coagulative myocytolysis (contraction band necrosis) and macroscopically, subendocardial hemorrhages, and/or a well defined myocardial infarction. The attendees will also be taught how to assess the different dating of the above-mentioned injuries using different tools such as the degree of inflammatory response, different histological stains, immunohistochemistry, and DNA analysis. Further “ancillary” analyses, techniques of fixation, and dissection will also be discussed.

After the presentation of several cases, a comprehensive explanation of the biochemical derangements and their underlying intracellular mechanisms, attendees will be able to recognize the different types of “neurogenic heart disease” such as those caused by increased plasma levels of catecholamines, increased or decreased plasma levels of steroids (e.g., fluorocortisol), or a sudden intracellular calcium influx.

Sudden Death, Autonomic Failure, Cardiovascular Pathology



Pathology/Biology Section - 2015

H141 Myocardial Rupture and Cardiopulmonary Resuscitation: Diagnosis and Forensic Issues

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After attending this presentation, attendees will better understand traumatic, particularly myocardial, injuries due to Cardiopulmonary Resuscitation (CPR).

This presentation will impact the forensic science community by helping differentiate traumatic myocardial rupture due to CPR from other causes of rupture such as myocardial infarction and by recognizing risk factors of heart injuries due to CPR.

Chest compression is the main component of CPR and provides minimal blood flow despite cardiac arrest. Since the 1960s, skeletal injuries related to chest compression have been described, in particular, sternum and anterior rib fractures. They are frequent (in 25%-30% of patients) but are usually not fatal. To the contrary, visceral injuries such as lung contusions, heart or hepatic hematomas or ruptures, and splenic ruptures due to CPR are uncommon but may lead to the death of the victim.

Heart rupture, which is a particular autopsy finding, raises several questions: (1) is the heart rupture traumatic due to blunt force trauma or non-traumatic due to a pre-existing cardiovascular condition (e.g. cardiac infarction or myocarditis); (2) if the rupture is traumatic, what kind of mechanism may be involved? In cases of chest trauma, are the lesions due to CPR or to the initial injuries; and, (3) is death due to the primary cause of cardiac arrest or due to the CPR-related heart injury? In order to illustrate and delve more deeply into this matter, this study will present five cases of heart ruptures after CPR. The cases that will be presented include three women aged 35, 34, and 48 years old (Cases 2, 3, and 5), and two men aged 29 and 23 years old (Cases 1 and 5). The three women were underweight (Cases 2, 3, and 5); in two cases, the body weight was within normal range. Cases 1 and 4 had unexplained syncope in the presence of witnesses, whereas Case 5 lost consciousness after facial trauma in front of his assailant. In Cases 2 and 3, the women were found hanged by their partners. CPR was undertaken by medical emergency teams and initially by laypersons in Cases 3 and 5.

Autopsies found right atrium rupture in Case 3, right ventricle rupture in Cases 1 and 2, and left ventricle rupture in Cases 4 and 5. These ruptures were associated with hematoma of the left atrium in Cases 2 and 3, with hematomas of the aortic arch in Cases 4 and 5, of the pulmonary hilum in Cases 2, 4, and 5, of left interlobar fissure in Case 5, and of the descending thoracic aorta in Case 4.

Hemopericardium of variable volume from 50-300ml was found in all cases. Hemothorax less than 100ml was found in Cases 4 and 5. There was no case of lacerations of the pericardium. Two cases (2 and 4) had pericardium hematoma. Four cases presented with anterior or anterolateral rib and sternum fractures. A 70% coronary stenosis was found in Case 4, but without thrombosis or myocardial infarction. Facial bruises with nasal fracture were found in Case 5, but there were no brain injuries.

Microscopic analyses (Cases 1, 2, and 5) did not find cardiovascular pre-existing pathology and concluded that lacerations of the right ventricle (Cases 1 and 2) were postmortem. In Cases 1 to 4, cardiac and mediastinal injuries were found to be related to chest compression during CPR. In Case 5, hemorrhagic infiltrates and myocardial fibers in contraction were found microscopically in the samples of the left ventricle. These observations suggested antemortem injuries due to chest compressions on a still-beating heart.

In conclusion, the probability of CPR-related myocardial rupture is high when associated with sternum and/or rib fractures, when chest compression could be only explained by CPR, and when there is no pre-existing cardiovascular disease. Risk factors are low body weight of the victim, CPR initiated by laypersons, and maneuvers of long duration.

Cardiopulmonary Resuscitation, Heart Rupture, Hemopericardium



Pathology/Biology Section - 2015

H142 Genetic Investigation of Sudden Cardiac Death: The State of the Art in Italy

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After attending this presentation, attendees will understand of the protocols used in forensic practice (genetics and pathology fields).

This presentation will impact the forensic science community by providing a full panoramic view of the state-of-the-art in the forensic investigation of Sudden Cardiac Death (SCD) in order to reach the best practice when being presented with these cases in routine workflow, especially regarding genetic analysis. This presentation will also impact the forensic science community by defining the key role played by the forensic pathologist, especially regarding the available options (i.e., genetics, proteomics) in the research and diagnostic procedures of sudden death cases

Background: Forensic medicine defines unexplained sudden death as a death with a non-conclusive diagnosis after autopsy. Molecular diagnosis is progressively being incorporated in forensics, mainly due to improvements in genetics. New genetic technologies may help to identify the genetic cause of death, even though the clinical interpretation of genetic data remains the current challenge. The identification of an inheritable defect responsible for arrhythmogenic syndromes could help to adopt preventive measures in family members, many of them asymptomatic but at risk of sudden death. This multidisciplinary translational research requires a specialized team. In this presentation, the state-of-the-art of the forensic approach to sudden death will be addressed. Two families with a recurrence of sudden death events investigated by molecular analysis will be presented to discuss current possibilities and limitations.

Material and Methods: Direct sequencing of the major contributing candidate genes in two families, originally diagnosed with Brugada syndrome after the probands experienced cardiac arrest and clinical and genetic analysis in their members, was performed. Pathogenicity of the variants was analyzed using family segregation, allele frequency from public databases, and conservation analysis. Phenotype-genotype correlations were analyzed statistically. Direct sequencing identified two different mutations in the SCN5A gene respectively designated E1784K — missense mutation causing the substitution of Glu by Lys — and an insertion of nucleotides TG in domain II (TGins851).

Conclusions: These results support the use of genetic testing as part of the diagnosis of SCD syndromes and to help in identifying relatives at risk of SCD; however, the identification of genetic variations in the clinical and forensic investigation of single patients using bioinformatic tools can produce erroneous conclusions regarding pathogenicity. In fact, distinguishing pathogenic mutations from rare variants is of critical importance in the interpretation of genetic testing. Therefore, segregation studies are the key to determining causality. Mutation type, mutation location, and ethnic-specificity should be viewed as variants of uncertain significance and prompt further investigation to clarify the likelihood of disease causation; however, mutations in regions such as the transmembrane, linker, and pore areas may be defined confidently as high-probability disease-causing mutations. These findings may have crucial implications for other genetic disorders involving mutational analysis, especially in postmortem setting.

Sudden Cardiac Death, Autopsy, Genetics



Pathology/Biology Section - 2015

H143 Homicide by Stroke: Cholesterol Embolus Induced During a Struggle

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After attending this presentation, attendees will recognize stroke from atheroembolus (cholesterol embolus) as a potential result of blunt trauma during the course of an altercation, as well as the spectrum of natural disease processes that may be precipitated in a homicidal manner.

This presentation will impact the forensic science community by raising awareness of the possibility of stroke by atheroembolus resulting from blunt trauma received during the course of a homicide. As this is the first such report of homicide by stroke due to atheroembolus, it is most likely an under-recognized entity.

Death from natural diseases precipitated by homicidal acts is uncommon, but well documented in the forensic literature. "Homicide by heart attack" was reviewed in recent years by Turner et al.¹ The situation is made difficult by circumstances in which no physical contact occurs between perpetrator and victim but nevertheless involve fatal arrhythmia induced by emotional or physical stressors. Criteria are available for such circumstances. Similar situations may involve physical contact that by itself is non-lethal but still results in sudden cardiac death. Revised criteria are available for these situations as well. In each case, severe underlying heart disease is present in addition to sublethal injuries. This is the first case of homicide by atheroembolus reported in the literature. The decedent was a 62-year-old Caucasian woman who was found incoherent at home. She was transported to the hospital and expired shortly thereafter. The investigation revealed that an altercation took place between the decedent and a man, possibly involving a head-lock maneuver. The autopsy revealed an obese woman with moderate calcific atherosclerosis involving the coronary arteries, aorta, and major branches. Brain examination revealed calcific atherosclerosis involving the Circle of Willis with a clot in the right middle cerebral artery, and an acute ischemic infarct involving all of the right middle cerebral artery territory, with transtentorial herniation and secondary brainstem (Duret) hemorrhages. Sections of the right middle cerebral artery revealed a large cholesterol embolus.

In conclusion, this is the first case report of a cholesterol embolus becoming dislodged during the course of an altercation, which resulted in a large right hemispheric stroke with mass effect and death. Extrapolating from criteria associated with sudden cardiac death and non-lethal injuries, the manner of death in this case is compatible with homicide. Given the frequency of atherosclerosis and the possibility of cholesterol emboli with trauma, careful examination of the Circle of Willis should be performed, especially in cases in which the victim suffers acute neurologic decompensation following an altercation.

Reference:

1. Turner SA, Barnard JJ, Spotswood SD, Prahlow JA. "Homicide by heart attack" revisited. *J Forensic Sci.* 2004 May;49(3):598-600.

Atheroembolus, Cholesterol Embolus, Stroke



Pathology/Biology Section - 2015

H144 An Uncommon Cause of Pulmonary Embolism

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The goal of this presentation is to increase awareness of uncommon sources/locations of thromboemboli in patients with autosomal dominant polycystic kidney disease.

This presentation will impact the forensic science community by highlighting the importance of examining the inferior vena cava and pelvic vessels in patients with polycystic kidney disease.

Case: A 20-year-old African American female was found unconscious and pulseless in her bedroom. She was last known alive ½ hour prior to discovery. When an ambulance arrived, paramedics intubated her without difficulty and initiated advanced cardiac life support. On arrival at the emergency department, an intraosseous line was placed and the correct position of the endotracheal tube verified. Despite these efforts she remained pulseless, without respirations and blood pressure, and was pronounced dead after 15 minutes.

Prior to the autopsy, the only available medical history indicated that three days before her death she complained of cold-like symptoms and began taking an antibiotic.

The external examination revealed an African American female measuring 5 feet 3 inches in height and weighing 233 pounds. The lower extremities were symmetrical and did not have edema.

The internal examination found large polycystic kidneys occupying the abdominal cavity and extending into the pelvis. The right kidney weighed 1,421 grams and measured 22cm in length and 12cm in width. The left kidney weighed 1,456 grams and measured 19cm in length and 13cm in width (normal range for women — weight: 115-155 grams, length: 11-12cm, width: 5-7cm). The right lung weighed 313 grams and the left lung weighed 299 grams. The pleural surface of the left upper and lower lobes revealed darker red/tan areas respectively measuring 3cm and 3.5cm. The cut surfaces through these areas were consistent with the appearance of pulmonary infarcts.

Opening the pulmonary trunk and the right and left pulmonary arteries revealed thromboemboli which ranged from 1cm to 6cm in length and up to 1cm in width. The proximal right and left pulmonary arteries had adherent thromboemboli. The right and left iliac veins and the inferior vena cava had adherent thrombi. Examination of the veins of the lower extremities was negative for thrombi.

There was mild cardiomegaly (heart weight: 396 grams; LV: 1.6cm) with normal valves and bilateral polycystic ovaries. There were no cysts involving the liver, pancreas, or spleen. There were no aneurysms of the aorta or Circle of Willis. Toxicology was negative for ethanol, cocaine, and heroin. Vitreous electrolytes had a normal postmortem pattern. A nasopharyngeal viral culture was negative.

The patient's mother confirmed that her daughter had polycystic kidney disease and was followed by a physician for her disease but had been putting off going to see her doctor for over a year because she had no complaints, such as fullness in her abdomen, leg pain, and/or swelling.

Autosomal Dominant Polycystic Kidney Disease (ADPKD) is one of the most common inherited disorders and is the most frequent genetic mutation leading to renal failure in adults, accounting for % to 8% of patients on dialysis in the United States. The disease is progressive, can affect multiple organs systems, and is characterized by the formation and enlargement of cysts in the kidneys. Extra-renal cysts are most commonly found in the liver. The clinical features usually begin in the third to fourth decade of life, although cysts can be detected in childhood and *in utero*. Although the presentation of the disease is highly variable, the most common initial complaint is pain and/or discomfort in the abdomen, flank, or back, typically as the result of liver and kidney cysts exerting pressure in these areas.

ADPKD is caused by mutations in one of two genes — PKD1 which codes for polycystin 1 and PKD2 which codes for polycystin 2. Approximately 85%-90% of patients with ADPKD have the PKD1 mutation (ADPKD 1) located on the short arm of chromosome 16 (16p13.3). ADPKD 2 accounts for 10%-15% of cases. The mutation is found on the long arm of chromosome 4 (4q21-22). Individuals with ADPKD 1 have an earlier mean age of onset of end-stage renal disease (53 years), compared to those with ADPKD 2 (74 years).



Pathology/Biology Section - 2015

Two studies have described the causes of death in 217 patients with ADPKD. Prior to 1975 and the use of renal replacement therapy, the average age of death was 51 years, increasing to 59 years by 1995 and 60.5 years by 2009. Cardiac disease is responsible for most deaths, followed by infection and central nervous system causes. A total of eight individuals died as the result of pulmonary embolism. Although detailed information was not available for these patients, there was no thrombosis of the vena cava or renal vein noted in seven of the eight patients.

Only a handful of cases of pulmonary embolism due to thrombosis of the inferior vena cava and/or ileofemoral vessels caused by compression of the infra-hepatic inferior vena cava by renal cysts have been reported. None of these were fatal and the development of thrombosis of the inferior vena cava was independent of renal function.

The risk factors for the development of a pulmonary embolism are well known. There was no history of coagulation abnormalities, immobility, or cancer in this patient. Although this patient had a BMI of 41.3, placing her in the obese category, it is proposed that thrombosis of the inferior vena cava and iliac veins was a result of extrinsic compression by the kidneys and death was due to pulmonary embolism caused by compression of the inferior vena cava and pelvic vessels due to polycystic kidney disease.

Polycystic Kidney Disease, Pulmonary Embolism, Inferior Vena Cava



Pathology/Biology Section - 2015

H145 A Novel Approach to a Quality Program in a Medical Examiner's Office

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After attending this presentation, attendees will understand a robust quality program implemented in a statewide medical examiner's office.

This presentation will impact the forensic science community by setting a high standard of quality in a medical examiner's office using concepts of reasonableness that are independently reviewable.

Forensic pathology is a sub-specialty of pathology responsible for the determination of cause of death in individuals dying suddenly, unexpectedly, or violently. Just like other branches of pathology and laboratory medicine, a robust quality control and quality assurance program is essential to ensure the product generated is of the highest quality to best serve the families of the deceased and the medicolegal communities. Although there are no minimum standards to which medical examiner/coroner offices must adhere, the National Association of Medical Examiners (NAME) offers a voluntary accreditation that attempts to ensure that offices meet a minimum set of criteria for death investigation. Having a functional quality assurance program is a component of this accreditation; however, NAME only dictates that a quality program is in place and documented; it neither specifies how the program should be structured nor does it specify any details such as a percentage of cases to be reviewed or whether reviews are to be prospective or retrospective. The New Mexico Office of the Medical Investigator have developed one of the most comprehensive and robust Quality Programs (QP) in the field of forensic pathology.

The QP has two arms: a Quality Assurance (QA) arm and a Quality Control (QC) arm. QA is defined as a prospective activity to ensure that the death investigation report is of the highest quality with reasonable conclusions as to the cause and manner of death. This program defines QC as a retrospective activity to ensure completed death investigation reports have met a high standard of quality with reasonable conclusions as to the cause and manner of death.

Reviews focus on the concept of what is "reasonable" because it is recognized that forensic pathologists can have a legitimate difference of opinion regarding cause and manner of death and it is also possible that both opinions are reasonable. Even though a peer reviewer may disagree with the opinions of the original pathologist, the conclusions are deemed reasonable if the reviewer can follow the logic used and understand how decisions and diagnoses were made. Another key component of the program is to ensure that all reports are "independently reviewable," which means that another forensic pathologist would have a sufficiently objective dataset (e.g., photographs, microscopic slides) to come to his/her own conclusions about the case and be able to agree or disagree with the conclusions reached by the autopsy pathologist. This is absolutely necessary to enable a quality review and also ensures that the report would be able to be reviewed by an external reviewer or another expert witness in the future.

The QA component reviews all homicides, all cases with an undetermined manner of death, and all deaths of children younger than five years (60 months) of age. This review is performed prior to completion of the case. The QA review of the pediatric cohort is slightly different: a committee chaired by the Director of Pediatric Forensic Pathology and consisting of the QP pathologist for the month and the forensic pathology fellows reviews the cases and forms a consensus opinion.

The QC arm is an administration-directed retrospective review of completed death investigations including Office of Medical Investigator (OMI) jurisdictional and consultation autopsies, pathologist external examinations, and investigator external examinations in cases with natural, accidental, and suicidal manners of death. In practice, with seven full-time forensic pathologists at the OMI, the QC arm will review 252 cases in this cohort annually.

The QP has not yet been implemented for a full year, but in 2012 (the most recent year with available data), there were 160 homicides, 1,578 natural deaths, 419 suicidal deaths, 1,458 accidental deaths, and 98 deaths of undetermined manner certified by forensic pathologists at the OMI. Included in these cases were 181 deaths of children under five years of age regardless of manner of death. In 2012, therefore, the two arms of the QP collectively would have reviewed 658 death investigations, or approximately 17% of the death investigations performed by the OMI.

A robust QP that demonstrates and ensures a commitment to producing quality death certifications and death investigation reports has been implemented at the OMI.

Quality Control, Quality Assurance, Medical Examiner/Coroner

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Pathology/Biology Section - 2015

H146 **Worked to Death: A Detailed Discussion of At-Work Deaths in Harris County, Texas**

Kathryn H. Haden-Pinneri, MD, Harris Co Inst Forensic Sciences, 1885 Old Spanish Trail, Houston, TX 77054*

After attending this presentation, attendees will better understand the importance of investigations of deaths at work and the proper procedures for this type of investigation.

This presentation will impact the forensic science community by providing instruction on the proper steps to take in the investigation of at-work deaths and by increasing awareness of the importance of a thorough investigation, including collaboration with regulatory agencies, such as local Occupational Safety and Health Administration (OSHA) representatives.

Deaths occurring at work are very disconcerting for all parties involved. Finding a co-worker dead or witnessing an accident or event that results in death can be emotionally traumatic. The employer is responsible for maintaining the safety of their employees, so when a fatal accident occurs, they are potentially liable for the death. Regulatory agencies are charged with monitoring and enforcing safety and health regulations in the workplace and the type of workplace determines which agency will be involved. A multi-agency death investigation has many challenges, including proper communication and information sharing. Lack of either may result in further harm to other employees of the business or harm to those doing the investigation and subsequent autopsy.

Harris County, TX, which includes the city of Houston, has 31 different law enforcement agencies and a population of almost 4.1 million people (as of the 2010 United States census). The Harris County Institute of Forensic Sciences (HCIFS) investigates approximately 4,000 deaths each year, of which approximately 3.5% are considered to be deaths at work. Of the at-work deaths, approximately half are due to accidental injury and the other half due to underlying natural disease processes. Accidental injuries can include falls from heights, malfunctioning or improperly utilized machinery, electrocutions, motor vehicle accidents while driving, or exposure to toxic chemicals. Many of these incidents result in the individual being transported to the hospital; therefore, it is critical to have someone respond to the scene of the incident for proper investigation and documentation. Determining the level of training/experience the worker has with the particular equipment being utilized is important and could reveal improper techniques being utilized or uncover the need for better training in order to prevent further injuries. This presentation will review some typical as well as some atypical at-work deaths that were investigated at the HCIFS. Injuries documented during the autopsy should be compared with scene photographs and investigative statements to ensure correct interpretation of the trauma.

Toxicology testing is performed on all at-work deaths reported to the HCIFS. This testing includes, but is not limited to, an alcohol screen and full Enzyme-Linked Immuno-Sorbent Assay (ELISA) screen (cocaine metabolite, amphetamines, Phencyclidine (PCP), opiates, benzodiazepines, methadone, and barbiturates). Due to the prevalence in this region, screening is also performed for carisoprodol/meprobamate and marijuana metabolites. Other substances are tested for as indicated by the scene investigation. Harris County is home to many chemical manufacturing plants; therefore, specific chemical analyses are performed to identify potential exposures. The HCIFS has a collaborative working agreement with local emergency responders to ensure the safety of their employees when responding to scenes involving potentially hazardous materials.

Proper handling of at-work deaths is critical for many reasons, not the least of which is monetary. The potential financial concerns of the employer, the employer's insurance carrier, the family, and the possibility of workman's compensation reimbursement may affect the ability to conduct the investigation. It is important to maintain a fact-based presence with all parties involved in order to obtain the necessary information. Inappropriately maintained equipment found to be implicated in the death of an individual may result in significant fines and reprimands for the employer. On the other hand, the family of an individual who has an accident while impaired by drugs or alcohol may not be entitled to any financial reimbursement. Litigation in these instances can be costly, time consuming, and may hinge on details or information that may not initially seem important. Good communication with all parties involved in a timely fashion could help prevent additional accidents and injuries as well as potentially reducing the number of unnecessary lawsuits.

At-Work Deaths, Accidental Injuries, Scene Investigation



Pathology/Biology Section - 2015

H147 Quality Assurance Projects at the Los Angeles Medical Examiner/Coroner's Office

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After attending this presentation, attendees will understand the importance of having a Quality Assurance (QA) process in place so the office can: (1) provide a complete, accurate, timely, usable medicolegal death investigation and report to the citizens; and, (2) ensure all reportable contagious diseases are reported to public health agencies and the needs of the public are met, preserving the confidentiality of results as required by law.

This presentation will impact the forensic science community by helping Medical Examiner/Coroner (ME/C) offices provide quality autopsy reports to the public and improve communication between ME/Cs and public health officials, which will be valuable in the response to a potential bioterrorism event.

The CME-Coroner (CME-C) (1992-2013) coordinated the then Department of Coroner (DOC) staff to obtain accreditations from the National Association of Medical Examiners, American Society of Crime Laboratory Directors-Laboratory Accreditation Board-ISO, Accreditation Council for Graduate Medical Education, and the California Medical Association-Institute for Medical Quality and Peace Officer Standards and Training certification.

A standard operating procedure for quality assurance was developed as the goal was to provide a complete, accurate, timely, usable death investigation report to the citizens served. Through DOC/Medical Division QA committees, reporting and investigation mechanisms for sentinel events and procedures to ensure that all department work products meet minimum specifications was established. When a significant quality problem was identified, measurable corrective action was taken and identified errors were brought to the attention of the persons responsible.

As required by county ordinance, all physicians will receive Performance Evaluations (PE) annually. PEs are prepared by CME-C and physician managers. In order to make the physician PE's less subjective and more objective, raters were directed by the CME-C to use the "QA Case Review" forms as a minimum objective guideline when evaluating cases. These forms assure a basic uniform quality in the autopsy reports (i.e., presence or absence of needle track scars in drug overdose cases, bullet trajectory, skull beveling in deaths from firearms, as well as origin of coronary arteries, etc.). The QA forms were developed with templates from the ME offices in Chicago and San Diego. The draft templates with additional quality indicators were discussed at the Medical QA and in the Physician Business meetings and were approved. The forms were placed in the shared drive for use by physicians on a daily basis during the conduction of autopsies for reference to ensure all minimum quality indicators are addressed. Also the Deputy Medical Examiner (DME) manual was revised to provide guidelines on what needs to be done for a specific type of case.

The measures to be discussed in this presentation include assurance of a basic uniform quality in evaluating cardiovascular status in natural deaths, gunshot wound-related deaths, and drugs of abuse-intoxication/overdose-related deaths. The other measure will outline details on how the ME-C carried out a quality assurance project to improve reporting of contagious diseases to public health agencies. The data before and after intervention (process used) and project completion and outcome will be discussed.

Quality, Autopsy, Communication



Pathology/Biology Section - 2015

H148 Forensic Pathologist Concurrence in the Interpretation of Images of Patterned Injuries

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After attending this presentation, attendees will have a better, and quantitative, idea of the degree in which forensic pathologists agree on the interpretation of images of patterned injuries of the skin.

This presentation will impact the forensic science community by providing an estimate of precision and verification of forensic pathology diagnoses from images.

A common problem in forensic pathology practice is the evaluation of patterned injuries of the skin. These injuries can provide important information about the nature of an object that was used to inflict trauma on a victim. In some cases, such as gunshot wounds, there is a rich literature on the proper characterization of the injuries and the inferences that can be made; however, in most cases, the interpretation of these patterns is primarily a matter of personal experience and training.

Traditionally, the ability of a forensic pathologist to interpret patterned injury of the skin has been relatively unquestioned at trial; however, over the past few years, there has been an increasing emphasis on more stringent application of *Daubert* criteria. This presentation represents the results of the first of a series of studies to address the basic issue of the ability of pathologists to interpret patterned injuries. This is a preliminary study in which participants were asked to identify “classic” injury patterns. Images were selected from known cases and teaching sets and were vetted by an expert panel. Since the provenance of each image was not investigated independently, this should be considered a verification study rather than a validation study.

A survey was constructed using 68 “classic” patterned injuries. The participants were presented with the images and asked for a diagnosis. The questions were asked in three tiers, with the first question asking for a diagnosis of the general class of injury (sharp versus blunt versus thermal, etc.), the second asking for a class diagnosis within the general class (e.g., laceration versus abrasion versus contusion for blunt trauma), and the third asking for a specific diagnosis (e.g., hammer blow versus baton mark versus tire mark, etc.). In addition, each participant was asked to rate his or her level of certainty.

Participants were recruited through the National Association of Medical Examiners. Emails were sent to all members of the organization. Approximately one-third of those emailed started the survey (363 people) and 210 people completed the entire survey.

This survey was constructed as a starting point for cases in which there would be a uniformly high consensus. Following surveys were expected to present modified images to see how that affected the degree of agreement; however, there was a surprising lack of uniformity of consensus, ranging from 100% as a high to 27% as a low. In addition, for at least one question, the most common tier 3 answer was that the respondent did not know what object caused the injury, but could match it to an exemplar.

A number of statistically significant findings are noted in the preliminary statistical evaluation. For example, physicians, those who had anatomic pathology training, those who performed autopsies as their primary work product (as opposed to administration, investigation, etc.), and board-certified forensic pathologists were more likely to choose consensus answers. When comparing the percent of consensus answers with mean confidence level for each question, there was a high correlation (r between 0.8 and 0.9) for each tier. There was a moderate correlation between providing a consensus answer and confidence at the individual respondent level.

The degree of consensus was significantly higher for tier 1 (most general) than tiers 2 or 3; however, there was a higher level of consensus for tier 3 “most specific” than for tier 3 “class within a particular injury type.” Comments by participants suggest that a possible reason for the higher consensus at the more specific diagnosis was due to the presence of multiple injuries. For instance, in one case, the same blunt object caused both lacerations and abrasions. The tier 2 question asked what injury was more prominent; about half noted lacerations and half noted abrasions, resulting in a low level of consensus. In contrast, the consensus on the specific object was high.

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Patterned Injuries, Forensic Image Analysis, Forensic Pathology

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Pathology/Biology Section - 2015

H149 Gone and Forgotten: A Medical Examiner's Nightmare

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After attending this presentation, attendees will better understand the importance of integrating forensic anthropology into a state medical examiner's office. The focus of this presentation will be on the vast progress made with the accumulation of unmanaged skeletal remains at the Mississippi State Medical Examiner's office due to collaboration with the Mississippi State University's Department of Anthropology and Middle Eastern Cultures (AMEC).

This presentation will impact the forensic science community by influencing protocol for skeletal remains in state medical examiners' offices so individuals do not remain unidentified and unanalyzed for numerous years.

In 2010, the state of Mississippi abandoned its policy of nearly 30 years of contract medical examiner services, consisting primarily of non-board certified pathologists and minimal anthropological consultation. Human remains were recovered by coroners and law enforcement agencies with limited to no training. There was no consistent relationship established with a forensic anthropology program, resulting in an accumulation of unidentified and unmanaged skeletal remains.

Many of the remains had limited to no documentation, were lost or never returned by the private consultant, and lacked chain of custody and documentation of follow-up communication between the attending pathologist and the anthropologist to establish a cause and manner of death. In 2011, Mississippi established a State Medical Examiner's office consisting of five board-certified forensic pathologists performing approximately 1,400 autopsies per year. A relationship was established with AMEC to provide professional consultation and a graduate anthropology internship within the State Medical Examiner's Office.

The primary goals of the internship were to address 28 skeletal cases that had accumulated at the Mississippi State Medical Examiner's office, obtain data for the Forensic Osteology (FOROST) trauma metabase, update entries in National Missing and Unidentified Persons System (NamUs), and assist with autopsy examinations. After the initial inventory, the lack of documentation was clear and investigative agencies were contacted to obtain available case histories. Within two months, all remains were inventoried, examined, photographed, and radiographically imaged when necessary. Eight skeletal cases have been identified and released or will be held at the medical examiner's office until directed otherwise, two autopsy reports were generated with cause and manner of death, three cases were entered and one was closed in NamUs, two cases were sent for DNA analysis, and six cases have been documented for entry into FOROST. Additionally, a partially mummified individual, recovered in 2002, was processed for analysis. Examples of these cases, displaying the importance of protocol and anthropological assistance when dealing with human skeletal remains in a medical examiner's office, will be presented.

The Mississippi State Medical Examiner's office seeks to obtain funding for a State Forensic Anthropologist within the State Medical Examiner's office, maintain the Forensic Anthropology internship program, develop a human remains recovery team, train coroners and law enforcement agencies for field recovery, and establish standards within the state to properly search, document, and recover human remains. In conclusion, it is necessary for a medical examiner's office to establish and maintain a structured relationship with a reliable and experienced forensic anthropology program.

Medical Examiner, Skeletal Remains, Anthropological Consultation



Pathology/Biology Section - 2015

H150 Quality Assurance of Autopsy Cultures

*Christopher B. Rogers, MD**, Los Angeles County MEO, 1104 N Mission Road, Los Angeles, CA 90033; and *Nicole Ellis, DO*, Orange County Coroner, 1071 W Santa Ana Boulevard, Santa Ana, CA 92703

After attending this presentation, attendees will be able to implement a quality-assurance system for autopsy culture results and list factors that influence interpretability of cultures.

This presentation will impact the forensic science community by providing information on a quality-assurance program for microbiology specimens taken at autopsy.

Results of bacterial cultures from autopsies are frequently difficult to interpret because of polymicrobial contamination. The forensic literature emphasizes the importance of proper collection technique, as well as interpreting culture results in the clinical context of the case. The most productive areas for culture are blood, lung, and cerebrospinal fluid.

Cultures from autopsies at the Los Angeles County Coroner's Office were retrospectively reviewed for a five-month period to look for factors that are associated with polymicrobial culture results. During the study period, there were 65 bacterial cultures from 47 cases. Fifteen pathologists obtained cultures, with a range of 1-12 cultures per doctor.

The bodies were refrigerated after arriving at the coroner's office, but the time before refrigeration and the time between death and autopsy varied. The preferred technique for obtaining cultures was to heat a scalpel blade and apply it to the surface to be cultured in order to sterilize it. For cultures of skin lesions, the area was sterilized by swabbing with isopropyl alcohol. Blood cultures were taken from the inferior vena cava and other cultures were taken from areas suspicious for infectious lesions. Cultures were taken using either swabs put into transport media or by using a sterile needle and syringe to inoculate blood culture bottles. Cultures were transported within a few hours to a microbiology laboratory.

Highly specific cultures, including cultures for enteric pathogens, acid-fast bacilli, fungi, and results obtained by molecular probes were excluded because of insufficient numbers of specimens and the rarity of false positive results. Also excluded from the study were organisms that are usually contaminants, including coagulase-negative Staphylococci, Diphtheroids, *Streptococcus viridans*, and *Enterococcus*. Cultures were considered interpretable if they either showed no growth or showed a single pathogenic organism.

The most important factor in predicting whether a culture result would be interpretable was the pathologist taking the culture. Results sorted by pathologist showed a range from 0/8 to 8/8 interpretable cultures. The most common postmortem cultures were of blood (6/8 interpretable), bronchus or lung (11/25 interpretable), and meninges or brain (5/7 interpretable). Conditions that were most associated with interpretable cultures included intravenous drug use (5/6 interpretable), meningitis (3/3 interpretable), and abscess (3/5 interpretable). The data did not show an effect of time between death and autopsy, perhaps because of the small number of cases.

The conclusion is that careful culture technique is very important in obtaining interpretable results. Highly selective culture media or molecular probes are helpful in avoiding confusing culture results.

Microbiology, Autopsy, Quality Assurance



Pathology/Biology Section - 2015

H151 A Comparison of Deaths From Diabetic and Alcoholic Ketoacidosis

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After attending this presentation, attendees will understand the patterns of death associated with diabetic and alcoholic ketoacidosis.

This presentation will impact the forensic science community by increasing the understanding of the similarities and differences between deaths due to diabetic and alcoholic ketoacidosis.

Ketoacidosis is an important cause of death encountered at autopsy. Ketoacidosis is most commonly recognized in diabetes mellitus, that is, Diabetic Ketoacidosis (DKA), which is a medical emergency. It may also be seen as a consequence of alcoholism — Alcoholic Ketoacidosis (AKA). Other causes of ketoacidosis include starvation and certain diets. The diagnosis of ketoacidosis is made at autopsy by the presence of ketone bodies (acetone, b-hydroxybutyrate, and acetoacetate). Diabetes mellitus can be identified by history, pathological features (diabetic nephropathy), and by measurement of vitreous glucose or hemoglobin A1C. Alcoholism is typically diagnosed by history and scene findings, which can be supplemented with pathological findings.

This study analyses a series of 151 deaths where ketoacidosis was given as the cause of death in a period from November 2009 to January 2014 in Toronto and Ottawa, Canada. There were a total of 9,332 autopsies, so ketoacidosis represented 1.6% of all deaths. Of these 151 deaths, 82 were reported as deaths due to DKA (0.9% of all deaths), 48 due to AKA (0.5% of all deaths), and 21 as ketoacidosis not otherwise defined or were both diabetics and alcoholics.

Of the 130 DKA and AKA deaths, four DKA deaths and nine AKA deaths were excluded because of insufficient data for analysis or because the principal cause of death was not DKA or AKA, but ketoacidosis was a contributing cause.

Of the 117 deaths, 71 were male and 46 female. The age range was 19-79 years with a mean age of 50.6 years and a median age of 51 years. In relation to the time of year, the deaths were recorded in three-month blocks: March-May=18.8%, June-August=23.9%, September-November=22.2%, and December-February=35%.

The Body Mass Index (BMI) was analyzed with a range of 11.3-52, mean 22.7, and median 21.2.

The main ketone body measured throughout the period of the study was blood acetone. The range varied from not detected to 138mg/100mL with a mean concentration of 28.04mg/100mL and a median of 22mg/100mL.

Of the 78 DKA deaths, 51 were male, 27 female. In 20 deaths, there was no prior history of diabetes mellitus (25.6%). Of the 39 AKA deaths, 20 were male, 19 were female. Comparing DKA and AKA and analyzing the data statistically using the Two-Sample T-Test, the mean age of DKA victims was 49 years, AKA 53.7 years ($p=0.04$), mean BMI in DKA victims was 23.0 and in AKA 22.2 ($p=0.55$). With respect to acetone concentrations, the mean concentration in DKA victims was 33.3mg/100mL and in AKA victims 17.8mg/100mL ($p=0.001$).

In conclusion, DKA is the most common cause of ketoacidosis at autopsy, representing nearly 1% of all deaths and was twice as common as AKA. In 25% of deaths from DKA, there was no prior history of diabetes mellitus. Deaths associated with ketoacidosis were most common between December and February. Victims of DKA and AKA had similar BMIs of 23.0 and 22.2. Mean acetone levels were significantly different between the two groups, being 33.3mg/100mL in DKA and 17.8mg/100mL in AKA victims.

Diabetic, Alcoholic, Ketoacidosis



Pathology/Biology Section - 2015

H152 The Speckled Polarized Lung: Microcrystalline Cellulose Pulmonary Granulomatosis as a Complication of Intravenous Drug Abuse

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The goal of this presentation is to examine the histopathologic findings of microcrystalline cellulose pulmonary granulomatosis from intravenous drug abuse.

This presentation will impact the forensic science community by discussing the case of a lung transplant patient with histopathologic findings of intravenous drug abuse. This case also emphasizes the necessity of suspecting intravenous drug abuse in patients without a reported history in the setting of lung transplantation. Additionally, the pulmonary complications and histopathologic findings of intravenous drug abuse in the setting of lung transplantation will be discussed.

The medical complications of drug abuse are diverse, involving almost any organ and varies with the type of substance and route of administration. Pulmonary foreign body granulomatosis is caused by intravenous injection of pulverized pharmaceutical tablets or more uncommonly by nasal inhalation of drugs. Oral medications commonly contain insoluble binding filler agents that may include microcrystalline cellulose, talc (magnesium silicate), or potato or corn starch. Respiratory complications may involve lung parenchyma, upper airways, pleural space, and pulmonary vasculature.

This study presents a case of a 42-year-old female with a history of chronic obstructive lung disease status post-single lung transplant. Post-operative complications were related to anastomosis, granulation tissue, and narrowing of the right bronchus intermedius requiring frequent dilatation. She was admitted for worsening dyspnea on exertion six months following transplantation. Her hospitalization was complicated by appendicitis, Cytomegalovirus (CMV) viremia, and *Klebsiella* bacteremia. Additionally, she experienced symptoms of non-specific chest pain and tightness. An echocardiogram showed normal Left Ventricular Ejection Fraction (LVEF) with no wall motion abnormalities. Chest radiographs demonstrated non-specific changes. The decedent fell in her room striking her head and was found pulseless. Despite extensive cardiopulmonary resuscitation, the patient expired and an autopsy was requested by her family.

Histopathologic examination revealed diffuse polarizable crystalline deposits with numerous foreign body granulomatous changes involving both the native and transplanted lung due to diffuse microvascular pulmonary microemboli. The rodlike particle shape and size and birefringence with polarized light were consistent with microcrystalline cellulose. No evidence of acute inflammation or rejection was identified in the transplant lung. A review of numerous sequential transbronchial biopsies demonstrated foreign body type granulomatosis beginning three months following transplantation. The presence of microcrystalline cellulose in the lungs was highly suspicious of intravenous drug abuse despite a negative clinical history of abuse.

Intravenous drug abuse can cause numerous pulmonary complications including pulmonary granulomatosis and microemboli with severe respiratory compromise. Histopathologic examination is of paramount importance in determining cause of death in patients who reportedly have no history of drug abuse. This presentation discusses a case of serendipitous intravenous drug abuse in a lung transplant patient.

Lung Transplantation, Pulmonary Granulomatosis, Intravenous Drug Abuse



Pathology/Biology Section - 2015

H153 Death Scene Findings Associated With Accidental Toxicity Deaths in New Mexico, United States, From 2010 to 2011

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After attending this presentation, attendees will be able to identify specific scene findings that are directly associated with accidental toxicity deaths in New Mexico. In addition, attendees will have a better understanding of changes and current trends in toxicity deaths using an evidence-based approach of more than 576 actual cases.

This presentation will impact the forensic science community by presenting an evidence-based methodology in determining likely overdose cases based on scene findings.

Understanding the specific markers of toxicity will allow medicolegal death investigators and other death scene responders to more easily triage potential overdose cases in sudden, non-violent deaths in adulthood.

In accidental overdose deaths, toxicology will always be considered the final arbiter; however, before toxicology testing can ultimately confirm or expose the toxic nature of a death, these cases need to be appropriately flagged and triaged into the medicolegal system. Medical examiners/coroners depend on initial information and scene findings to determine the level of medicolegal scrutiny that cases will receive. The quality of this information and appropriate understanding of the findings themselves are integral to the process that ultimately leads to these cases being autopsied and toxicology testing being performed. The overwhelming majority of this information will come from observations and interviews conducted on scene.

The objective of this study was to identify common death scene indicators and catalog potential markers for toxicity found on accidental overdose scenes. Death scene reports and scene investigations in 578 unintentional toxicity deaths over a two-year period (2010-2011) in New Mexico were systematically reviewed to identify scene indicators and document patterns suggestive of toxicity. More than half (54%) of the deaths were due to prescription drugs and 10% were attributed to a mixture of prescription and illicit drugs. Women represented more than half (53%) of the prescription overdose deaths, but only 17% of illicit overdose deaths. Decedents dying of a prescription drug overdose were significantly more likely to be White, non-Hispanics ($p < 0.0001$) and were also significantly older than people dying from illicit substance overdoses ($p = 0.01$). Almost one-third (31%) of prescription overdoses were in people more than 49 years of age. More than half (56%) of these decedents had three or more prescriptions for pain medication, and 55% had medications with counts not within expected limits, making access to medication slightly more prevalent than tangible evidence of misuse. Decedents were found in a position of sleep in fewer than half (46%) of prescription overdose deaths more than 49 years of age, and evidence of tampering with the scene was found in 9% of overdoses more than 49 years of age, which was greater than in cases 49 and younger (6.9%).

It was also determined that there was a significant incidence of history of potentially lethal natural disease in prescription toxicity deaths in older persons (67% for ages 50 and over). Thus, the presence of natural disease should not be solely used as a disqualifier for autopsy or toxicology testing. Even in cases with natural disease and little evidence of substance abuse, particularly in decedents more than 49 years of age, investigators must maintain a high index of suspicion for prescription overdose deaths if access to potentially abused medications is present.

The landscape of toxicity is rapidly evolving and understanding how toxicity deaths present themselves on scene provides an opportunity to refine the triage process using an evidence-based approach for future cases, both for death-scene investigators and pathologists. This presentation will assist death-scene investigators and pathologists alike in recognizing and understanding the association between specific scene findings and accidental toxicity.

Overdose, Accidental, Scene



Pathology/Biology Section - 2015

H154 **An Analysis of the Causal Relationship Between Maternal/Prenatal Cocaine Use and Stillbirth: Results of a National Hospital Database Study**

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The goal of this presentation is to describe the contribution of maternal/prenatal cocaine exposure to stillbirth risk using national hospital data. After attending this presentation, attendees will better understand an issue increasingly encountered by medical examiner offices: whether maternal/prenatal cocaine exposure is a cause of stillbirth and, when such exposure is found in the instance of a stillbirth, whether the exposure can be ruled the sole cause of the stillbirth.

This presentation will impact the forensic science community, as well as those who represent disadvantaged populations, by elucidating the causal association between maternal/prenatal cocaine exposure and stillbirths.

Prenatal illicit substance use, including the use of crack cocaine, has become the focus of recent legislative attention in a number of states in recent years. In some states, the occurrence of a stillbirth coinciding with maternal and thus fetal exposure to cocaine, is considered evidence of homicide. Because stillbirth occurs both with and without maternal/prenatal cocaine exposure, it is fallacious to consider such exposure the entirety of the cause of a subsequent stillbirth without first considering the magnitude of competing causal factors. Without accurate information on cause, there is no way to determine the manner of death in a case of stillbirth following maternal/prenatal cocaine exposure (absent toxicology findings indicating a fatal dose).

Stillbirth, defined as a fetal death occurring at 20 or more weeks of gestation, is a relatively common adverse pregnancy outcome in the United States.¹ In 2005, a stillbirth rate 6.22 fetal deaths per 1,000 live births was reported in the United States, totaling 25,894 deaths.² Stillbirth occurs disproportionately among the disadvantaged and women of color.¹ Although the etiology of stillbirth in individual cases is often unclear, a number of associated factors, including poverty, single motherhood, inadequate prenatal care, maternal age, infection, obesity, diabetes, thrombophilia, fetal genetic or structural abnormalities, and umbilical cord abnormalities have been identified. Maternal syphilis infection is strongly associated with stillbirth, but it is a rare case; in 2008, there were 431 cases of congenital syphilis reported in the United States, of which only 25 (6%) were stillborn.³

The relationship between maternal cocaine exposure and stillbirth risk is incompletely explored at the present time. Using a case-control design Miller and colleagues examined stillbirths occurring at an urban hospital in Louisiana in 1994.⁴ That study reported the odds of stillbirth among the cocaine-exposed group as a non-significant 1.18 (95% CI 0.45, 5.07). In a later study linking birth and death certificates, Wolfe et al. reported a non-significant odds ratio of 1.2 (95% CI 0.99, 1.42) for fetal death and fetal cocaine exposure in the univariate analysis.⁵ A multivariate analysis that accounted for confounding variables demonstrated a counterintuitive protective effect (OR 0.2, 95% CI 0.19, 0.30). Flenady and colleagues reported a significant association between illicit drug use and stillbirth, but did not provide an analysis of cocaine use individually.⁶

Despite the fact that cocaine use during pregnancy is associated with adverse outcomes such as low birth weight, preterm birth, and small-for-gestational-age status, there are several issues that obscure the relationship between cocaine use and stillbirth.⁷ Polydrug use and socioeconomic factors make it challenging to distinguish the effects between cocaine and other socioeconomic, ethnic, and health factors commonly associated with cocaine use. Additionally, the presence and degree of maternal cocaine use is difficult to measure.

To further explore this relationship, an analysis of hospital inpatient birth data was performed. Data from the Nationwide Inpatient Sample Database (NIS) of the Healthcare Utilization Project of the Agency for Healthcare Research and Quality of the United States Department of Health was accessed. The NIS is a publicly held database containing data from approximately eight million United States hospital stays each year in 45 states, or approximately a 20% sample of all hospital discharges. The NIS data allow for a national estimate of the incidence, risk factors, outcomes, and other variables pertaining to all conditions seen in US hospitals. A univariate analysis of the contribution of maternal cocaine presence to stillbirth risk, along with other known risk factors, was conducted. These findings were used to construct an adjusted model of cocaine exposure as a cause of stillbirth, using binomial logistic regression.

The preliminary results of this analysis resulted in an odds ratio of 1.58 (95% CI 1.02, 2.45), equivalent to an attributable risk or probability of causation of 37%. Thus, the result of this preliminary analysis indicates that the presence of maternal/prenatal cocaine use in a case of stillbirth does not even account for more than 50% of the cause of the stillbirth. Further analysis is needed to examine the interaction of a wider variety of predictive factors.



Pathology/Biology Section - 2015

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Cocaine, Stillbirth, Forensic Epidemiology



Pathology/Biology Section - 2015

H155 Heroin Trends in Cuyahoga County, 2013

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After attending this session, attendees will understand the emerging problem of heroin abuse and its relationship to prescription narcotic abuse. Attendees will gain an appreciation of significant risk factors for heroin overdose.

This presentation will impact the forensic science community by highlighting an emerging national epidemic of heroin abuse. By defining risk factors, public health interventions may be formulated. A model for data collection, analysis, and intervention planning is presented which may serve as a template for other communities experiencing this problem.

Heroin has been a cause of overdose fatalities for several years with intermittent cycles of increased popularity. Recently, abuse of Opioid Pain Relievers (OPR) has been linked to an alarming rise in mortality on a national level and several efforts have been made to address this problem. In Cuyahoga County, OH (metropolitan Cleveland), heroin mortality has re-emerged and overshadowed all illicit and legal drug-related fatalities, including OPR. In 2013, there were 194 overdose fatalities involving heroin, either alone or in combination with other drugs, representing approximately 60% of all overdose deaths.

Analysis of demographic data revealed that the majority of the deaths involved the White race (85%) and males (73%). The most common age group was 45-60 years of age (38%) and most victims were single or divorced (86%). Recent trends in the county have shown an increase in the percentage of women and younger addicts, each of which now represent approximately 25% of all deaths. There was a slight majority of suburban deaths (outside of Cleveland city proper), both in injury incident location as well as decedent residence. Approximately 12% of deaths were veterans of the armed services.

In early 2013, The Cuyahoga County Medical Examiner's Office convened a Poison Death Review Committee to prospectively review heroin fatalities in greater depth. This multidisciplinary committee includes members from medical and law enforcement/judicial communities sharing primary source information on a number of variables to provide data to inform policy decisions. These variables included whether the decedent was using drugs with others (12%), whether others were present but not using drugs when the decedent overdosed or proceeded through the terminal stages of intoxication (58%), whether first responders administered the opiate overdose antidote naloxone (28%), whether drug paraphernalia was present at the scene of death (53%), and whether the decedent had a previous history of illicit drug use (95%), especially intravenous drug abuse (62%). Review of recent decedent interactions with various systems revealed that within two years prior to death, 64% had received some type of medical treatment, 45% had some mental health history, 48% had received detoxification/rehabilitation services, and 40% had been incarcerated.

To explore the relationship between heroin mortality and prescription medications (including OPR), data from the Ohio Automated Rx Reporting System (OARRS), the Ohio prescription drug monitoring program was reviewed. OARRS data is limited to two years prior to the date of the search. The majority of Cuyahoga County heroin victims had a legal prescription for a controlled substance within two years of their fatal overdose (73%), with 65% having a prescribed opioid and 32% with a prescribed benzodiazepine. Of note, 36% of decedents met criteria for "doctor shopping," defined as five or more prescribers of controlled substances within a one-year period.

These data have been employed to support public health interventions in the county and indicate there are significant opportunities for educational efforts on several fronts to address this epidemic.

Heroin, Mortality, Epidemiology



Pathology/Biology Section - 2015

H156 **Obstruction of Aqueduct of Sylvius Following Spontaneous Intra-Ventricular Hemorrhage and Meningitis in a Premature Infant Leading to Hydrocephalus and Cystic Cerebellar Degeneration With Polymicrogyria: A Case Study With a Review of Literature**

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After attending this presentation, attendees will better understand the etiology, pathological findings, genetic associations, clinical features, and forensic presentations of blockage of the aqueduct of Sylvius.

This presentation will impact the forensic science community by raising awareness regarding distant complications and the long-term outcomes of spontaneous intra-ventricular hemorrhage and blockage of the aqueduct of Sylvius.

The aqueduct of Sylvius is the narrowest part of the ventricular system and its obstruction leads to hydrocephalus (dilatation of the lateral ventricles of brain). Obstruction of the aqueduct is usually associated with sporadic stenosis, atresia associated with Arnold-Chiari malformation, gliosis following infection or hemorrhage, vascular malformation, or X-linked hydrocephalus spectrum. A review of the literature led to rare case reports of gliosis related to various infectious etiologies including mumps, toxoplasmosis, tuberculosis, parasitic granuloma, and aspergillus.

A case is reported of a premature eight-month-old girl who developed hydrocephalus and cystic cerebellar degeneration due to obstruction of the aqueduct of Sylvius following the development of spontaneous intra-ventricular hemorrhage and meningitis. The infant was born at 24-weeks gestation by spontaneous vaginal delivery and was 860 grams at the time of delivery. The pregnancy was complicated by chorioamnionitis. The early clinical course of the infant was significant for *Streptococcus agalactiae* and *Escherichia coli* sepsis, meningitis, and Grade III intra-ventricular hemorrhage with placement of an intra-ventricular shunt. Her family at home cared for her and she required tube feeds for nutrition. Early one morning, her mother checked on her and found her apneic. She was pronounced dead on arrival at the hospital. An autopsy revealed no external or internal evidence of injury. There was bilateral acute pneumonia. The brain weight was 387 grams (normal adjusted brain weight=516 grams). The cerebral cortex appeared atrophic and the cerebral hemispheres showed polymicrogyria. On cut surface, there was yellow discoloration of the periventricular white matter. There was obstruction of the aqueduct of Sylvius with dilation of the lateral and third ventricles and cystic degeneration of the cerebellum. Microscopic examination revealed gliosis and hemosiderin-laden macrophages within the periventricular white matter and adjacent to the aqueduct of Sylvius. The cause of death was certified as pneumonia and hydrocephalus as complications of prematurity.

Aqueduct of Sylvius, Blockage, Hydrocephalus



Psychiatry & Behavioral Science Section - 2015

II Medicolegal and Criminological Suicide Diagnosis in Historical Cases: A New Methodology

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After attending this presentation, attendees will be informed about a new method for the medicolegal and criminological diagnosis of suicide based on five empirical and statistical criteria.

This presentation will impact the forensic science community by proposing how the application of five different criteria for suicide diagnosis can also be useful and reliable for the investigation of both historic and cold cases, taking into account the incompleteness of the evidence.

The forensic practice clearly demonstrates that a predominant number of suicides have typical or frequent distinctive characteristics (i.e., common wound topography in the case of firearm injuries by short- or long-barrelled weapons); however, some suicides show atypical or unusual features (i.e., multiple gunshot wounds or incomplete hanging). According to the International Association for Suicide Prevention (IASP), in 2013, the number of suicides worldwide was estimated to be one million; this number raises the urgency to provide crime scene operators (prosecutors, police officers, investigators, pathologists, and forensic anthropologists) with an investigative technique that allows them to recognize and distinguish suicide from other manners of death with a high degree of scientific probability.

This presentation proposes the application of a new combination of criteria to emblematic historical cases.¹ This new method, based on analyses of Italian case reports, can be summarized by the answers to five different questions, as evaluated by a specific scoring system: (1) did the victim use a suicide method frequently observed during that specific era and in the nation/culture of origin?; (2) are the method and injuries compatible with suicide?; (3) was a singular or multiple method used?; (4) is evidence from the environment and crime scene consistent with suicide?; and, (5) are there any indications of psychiatric disorder in the victim's medical history?

Since ancient times and across almost all cultures, suicide has been a widespread practice, even though some communities do not seem to acknowledge self-inflicted death, at least from a statistical standpoint. Nevertheless, even a minimal investigation of suspicious/equivocal/undetermined death is virtually unknown throughout antiquity. For this reason and to test the applicability and limitations of the five criteria, three cases were selected: Judas Iscariot, Antonius and Cleopatra, and Vincent Van Gogh. The selection has been oriented toward well-known characters from different socio-cultural contexts and epochs to tackle the issue of suicide without prejudice.

As result, the test confirms the potential in applying the five diagnostic criteria in historical cases as well to better understand distant epochs and episodes. Consequently, this study demonstrates how this approach also became relevant and useful in the investigation of cold cases with active forensic interest; however, in this scenario, it is essential to have as much data as possible to permit a scientifically valid examination of all aspects describing the case. To confirm this last assumption, the example of Van Gogh is meaningful. In Van Gogh's case, the availability of circumstantial information related to suicide and anamnestic information derived from the painter's letters to his brother make the manner of death effectively clear and understandable.

This study will present a new approach for the investigation of doubtful manners of death with the emphasis on addressing the relevance of each singular aspect of the methodology in different scenarios.

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Suicide, Crime Scene Investigation, Suspicious Death



Psychiatry & Behavioral Science Section - 2015

I2 Malingering Detection With the Autobiographical Implicit Association Test

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After attending this presentation, attendees will be familiar with one of the most popular tests in Italy used to establish whether an autobiographical memory trace is encoded in the respondent's mind.

This presentation will impact the forensic science community by increasing awareness of a new test that can be used in a forensic setting to establish if the respondent is lying and if the fact that the respondent says he/she does not remember is in his/her implicit memory.

A new method that can be used to identify a true autobiographical memory (intentions and reasons that motivate an act) is the autobiographical Implicit Association Test (aIAT).¹ It is a variant of the Implicit Association Test that is used to establish whether an autobiographical memory trace is encoded in the respondent's mind/brain.²

With the aIAT, it is possible to evaluate which of two autobiographical events is true. The method consists of a computerized categorization task that is structured in five blocks; three simple blocks (1, 2, 4) and two combined categorization blocks (3 and 5). In simple blocks, each response button is used to classify sentences related to only one category. In double blocks, each response button is used to classify sentences related to two different categories.

In Block 1, participants have to classify true and false sentences (e.g., I am in front of a computer vs. I am in front of a television) using two response keys, one on the left and one on the right of the keyboard. In Block 2, participants have to classify autobiographical sentences (e.g., I went to Paris for Christmas vs. I went to New York for Christmas) with the same two response keys. In Block 3 (double categorization block), true sentences and sentences related to the first autobiographical event (e.g., Christmas in Paris) are paired on the same response key and false sentences and sentences related to the second autobiographical event (e.g., Christmas in New York) are classified with the other response key. In Block 4, only autobiographical events are reversely classified with the two response keys. Finally, in Block 5, participants have to classify both true sentences and sentences related to the second autobiographical event (Christmas in New York) with the same response key, and false sentences and the first autobiographical event (Christmas in Paris) with the other key.³

The true autobiographical event is identified because, in a combined block, it gives rise to faster Reaction Times (RT's) when it shares the same motor response with true sentences. Validation experiments have documented very high classification accuracy over a wide range of tests, with average accuracy rates exceeding 90%. The aIAT has been validated both in forensic and in clinical settings. In this study, the aIAT main applications (it distinguishes between witnessed and non-witnessed detail, detection of intentions, detection of Reasons Underlying Lies, malingered whiplash syndrome, malingered depression, etc.) are reviewed and one of the most popular cases in which it has been used in Italy is presented: The Case of Como.³

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Autobiographical Memory Trace, Implicit Association Test, Malingering Detection



Psychiatry & Behavioral Science Section - 2015

I3 Criminal Behavior and Single Nucleotide Polymorphisms in Genes Related to Dopamine and Serotonin Modulation

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After attending this presentation, attendees will understand how principles of behavioral genetics can be applied to investigate antisocial or criminal behavior.

This presentation will impact the forensic science community by providing a better understanding of the manner in which certain genes related to modulation of neurotransmitters influence criminal behavior.

Little is known about the biological mechanism that regulates antisocial behavior; however, a number of dopamine-related genes have been implicated in the etiology of violent behavior and conduct problems. These genes include Monoamine Oxidase A (MAOA), Monoamine Oxidase B (MAOB), Catechol-*O*-Methyl-Transferase (COMT), Dopamine Beta-Hydroxylase (D β H), and Tryptophan Hydroxylase 1 (TPH1). A landmark study in molecular genetics reported that individuals with low-functioning alleles within the MAOA gene were more likely to develop antisocial behavior following maltreatment. Although the literature concerning behavioral genetics is accumulating, there is limited information concerning the neurobiological mechanisms influencing criminal behavior in humans

The focus of this research is to investigate polymorphisms in genes associated with dopamine and serotonin modulation in an incarcerated population and a control group. This study investigated 13 single nucleotide polymorphisms (SNPs) in five genes involved with dopamine and serotonin regulation. The following SNPs were selected: rs909525, rs3788862, rs979605 (MAOA), rs1799836, rs2283729 (MAOB), rs740603, rs737865, rs165599, rs4680 (COMT), rs739398, rs1611115, rs129882 (D β H), and rs1800532 (TPH1).

DNA was extracted from buccal swabs collected from male inmates incarcerated in a southern Texas jail (N=100) and from control male students (N=93). All the protocols used in this study were approved by the Institutional Review Board at Sam Houston State University. DNA was quantified by real-time Polymerase Chain Reaction (PCR) and samples were amplified and subjected to single base extension. Extended products were detected by capillary electrophoresis with fluorescent detection.

After correction for multiple comparisons, departures from Hardy-Weinberg equilibrium were not observed in any group. Linkage disequilibrium was strong in MAOA and COMT genes ($D' > 0.8$), moderate in MAOB ($D' = 0.65$) and weak in D β H ($D' = 0.30$). Although no single genetic variant in any of the five genes differentiated individuals in the investigated groups, significant haplotype differentiation ($p < 0.05$) was observed for MAOA, MAOB, and COMT markers.

Moreover, gene-gene interaction was identified between MAOA rs3788862-rs979605, COMT rs165599-rs740603, and COMT rs4680-D β H rs739398.

This evidence suggests that defined haplotypes and Single Nucleotide Polymorphism-Single Nucleotide Polymorphism (SNP-SNP) interactions in MAOA, MAOB, and COMT SNPs are associated with criminal behavior. Overall, the study attempts to understand the biological basis of complex behaviors, such as antisocial and criminal behaviors, by identifying relevant genes and promoting future research on the genetic influence on criminal behavior.

Behavioral Genetics, Criminal Behavior, Single Nucleotide Polymorphism



Psychiatry & Behavioral Science Section - 2015

I4 Forensic Analysis of a Chiropractor Accused of Sexual Assaults on His Patients: A Case Report

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After attending this presentation, attendees will be able to discuss how to approach a psychiatric evaluation of a subject with paraphilic sexual behavior and unspecified personality disorder.

This presentation will impact the forensic science community by providing a discussion of the reasons why the subject was not considered mentally insane at the time he committed the crime.

This presentation concerns a psychiatric-forensic assessment of a health care expert, a chiropractor, accused of having drugged and raped a large number of male patients. These criminal actions went unnoticed for many years until he made an error in medicating a patient, who awoke while being manipulated by the chiropractor. Newspapers and television programs described the subject as a very aggressive, violent serial sex offender. The local population characterized him as a “monster” and some people also took violent action against him and his properties by, for example, setting his house on fire. When the man’s criminal behavior was discovered, he was accused of repeated sexual assaults and the judge requested an evaluation of the subject’s state of mind at the time the crime was committed.

The goal of this presentation is to describe how examiners approached such a case and the results of the forensic psychiatric assessment. It was discovered that criminal sexual intercourse as described above was the only type of sexual relationships the subject had during his life. The evaluation also revealed that the subject did not develop a mature sexual identity and wasn’t able to properly indicate his sexual preferences. Despite his being accused of rape, he described his intercourse with his victims in terms of genital manipulation, without penetration. He also took numerous pictures of his patients while they were naked and drugged. The subject’s criminal behavior was very organized and appeared not to be simply instinctual. In fact, his crimes were discovered because he finally committed an error and one of his patients woke up while being raped.

Trying to analyze the subject’s psychic functioning model, the examiners discovered a disorder of sexual preference, with enduring unusual sexual needs and activities; however, none of the criteria for a specific paraphilia were identified, so the subject was considered to have an unspecified sexual preference disorder with problems in sexual development and orientation. The forensic psychiatric assessment also revealed the coexistence of an unspecified personality disorder. Answering the judge’s requests, the examiners considered that the accused’s criminal actions, which quite clearly represented symptoms of the subject’s disorder, could not be regarded as a sign of an unconsciously manageable pathological impulsiveness.

Sexual Offender, Paraphilia, Personality Disorder



Psychiatry & Behavioral Science Section - 2015

I5 Aggression and Interpersonal Violence in Hospital Emergency Departments

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After attending this presentation, attendees will be aware that hospital-based Emergency Departments (EDs) rank among the most dangerous working environments in the country. Attendees will gain an understanding of the association between chief clinical complaints (reasons for hospital visit) and specific types of aggression (i.e., verbal or physical assault).

This presentation will impact the forensic science community by offering insight into underlying causes of aggression via differentiating between physical violence and verbal assaults/threats, thereby increasing the competence of those working in hospital settings.

Given the high prevalence of violence in ED settings, it may be useful to examine the association between the types of chief clinical complaints that bring a patient into the hospital and the nature of ensuing aggression with the purpose of identifying potential risk factors for violence. The current study examines the association between specific types of aggressive incidents (i.e., verbal or physical) and chief complaints voiced in an emergency department setting.

Physical violence and verbal incidents from six EDs were recorded and compiled into a large dataset ($N=813$). Each incident was classified for the presence of verbal threat/assault and various types of physical aggression (e.g., hitting, spitting, kicking, pushing). A count variable was calculated based on the number of aggressive incidents per patient. Medical staff (e.g., physicians and nurses) provided a qualitative appraisal of the cause/underlying nature of the aggressive incident. These data were then classified into various broad categories that included, among others, intellectual disability, mental illness, neurological issues (e.g., head injury), medication issues, drug/alcohol intoxication, mental status changes (e.g., dementia, psychosis), suicidal ideation, homicidal ideation, frustration with wait time at the ED, refusal to leave the ED, and pain symptoms.

Point-biserial correlations were calculated to examine the association between the number of aggressive incidents per patient and each of the broad categories describing the medical staff's explanation of the incident. Small, but significant, positive correlations were found between the presence of intellectual disability and incidents of aggression ($r=.103, p<.005$), as well as presence of homicidal ideation ($r=.104, p<.005$). Pain symptoms were negatively correlated with overall level of aggression ($r=-.090, p<.05$).

Chi-square analyses were calculated to examine the association between verbal vs. physical aggression and each of the broad categories used to explain the incident. Patients rated as having intellectual disability ($\chi^2=4.77, p<.05$), neurological issues ($\chi^2=4.40, p<.05$), changes in mental status ($\chi^2=4.14, p<.05$), and homicidal ideation ($\chi^2=6.02, p<.05$) were significantly more likely to exhibit physical vs. verbal aggression. Patients with pain symptoms ($\chi^2=5.16, p<.05$) and those who experienced frustration due to wait time ($\chi^2=10.32, p=.001$) were significantly more likely to exhibit verbal vs. physical aggression.

In summary, the presence of intellectual disability and homicidal ideation were associated with a greater number of aggressive incidents per patient, whereas pain symptoms were associated with fewer aggressive incidents. Several factors were also able to significantly discriminate between verbal and physical features of aggression. For instance, intellectual disability, neurological issues, changes in mental status, and homicidal ideation were associated with physical aggression, whereas pain symptoms and frustration due to wait times were associated with verbal aggression. Although pain symptoms were related to lower levels of overall aggression, they were nevertheless related to higher levels of verbal vs. physical aggression. These results have implications for risk assessment in medical settings.

Violence, Threats, Risk Assessment



Psychiatry & Behavioral Science Section - 2015

I6 Could Cases of Abusive Head Trauma (Shaken Baby Syndrome) Historically Have Been Misdiagnosed as Pervasive Developmental Disorders?

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The goals of this presentation are to: (1) review original cases used to establish the diagnosis of Childhood Disintegrative Disorder (CDD) (Heller syndrome); (2) compare overlap of symptoms of CDD with those of abusive head trauma (shaken baby syndrome); (3) consider whether CDD and other Pervasive Developmental Disorders (PDDs) may have historically included missed cases of abusive head trauma; (4) evaluate whether or not it is possible to distinguish symptoms of abusive head trauma in children with the use of the new criteria of Autistic Spectrum Disorder in the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5); and, (5) examine whether child evaluators using DSM-5 receive enough training to distinguish abusive head trauma symptoms from Autistic Spectrum Disorders (subsuming CDD and other pervasive development disorders).

This presentation will impact the forensic science community by informing attendees of the possible overlap between pervasive developmental disorders and abusive head trauma since the origin of the diagnosis in historical literature was used to formulate the criteria before the recognition of child abuse.

PDD, a diagnosis established in the first part of the 20th century, may subsume unrecognized cases of abusive head trauma (shaken baby syndrome) if providers utilizing the DSM-5 are not trained in the recognition of child abuse.¹

PDDs are characterized by serious and persistent impairment in several areas of development, which are described as occurring after initially normal development. The same can be said for some cases of abusive head trauma (also known as shaken baby syndrome, shaken-slam syndrome, or inflicted traumatic brain injury). Despite overlap in their symptomology and presentation, disorders that have most recently been classified in the DSM-5 as Autistic Spectrum Disorders were first described and labeled decades before abusive head trauma was a regularly considered differential for medical diagnosis.

Review of the historical cases used to establish and describe PDD, in particular CDD, was undertaken to determine whether methods used were sufficient to rule out loss of developmental milestones from inflicted head trauma and, in particular, whether providers without medical training in child abuse will be able to differentiate the two when utilizing the DSM-5 to make diagnoses.^{2,3}

Writers and researchers who developed the DSM-5 now believe a single umbrella of Autistic Spectrum Disorders will improve the diagnosis of PDDs without limiting the sensitivity of the criteria or substantially changing the number of children diagnosed. If the criteria are based on historical cases that may have included missed cases of child abuse, psychologists and other evaluators undergoing training in the diagnosis of Autistic Spectrum Disorder may not be equipped to separate symptoms of abusive head trauma in infants and young children from late-developing autistic spectrum disorders. Recognition of this issue may aid in preventing misdiagnosis of future cases of inflicted head trauma as Autistic Spectrum Disorders.

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Child Abuse, Developmental Disorders, Autistic Spectrum



Psychiatry & Behavioral Science Section - 2015

I7 Assessment of Children Who Are in Conflict With the Law: Çukurova University Department of Child and Adolescent Psychiatry Between 2006 and 2011

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After attending this presentation, attendees will better understand the data relating to the sociodemographic characteristics of children driven to crime, their mental health, and the crimes allegedly committed.

This presentation will impact the forensic science community by providing information about the attention given to upcoming policies developed to protect children.

According to international law, 18 years of age is considered to be the age of maturity and consent. Children grow up both physically and mentally and need care, education, and protection from outside influences during this phase.

Juvenile delinquency can be described as illegal behavior by children. The child who is having a hard time adapting to rapidly changing phases during puberty can commit illegal acts because of social inexperience. A child may not realize the act is a crime or that the crime will affect his/her future life. Legally the term “delinquent child” was favored over “criminal child/child who has committed a crime.”

Doctors are asked to determine if the delinquent child has a criminal responsibility. The primary questions to analyze are whether the child was mentally competent to understand the meaning of the action, had reached a mentally developed stage to understand the results of his/her actions, and understood what shaped his/her behaviors.

In this study, 141 case reports were retrospectively evaluated. This study included 134 boys (95%) and seven girls (5%) with an average age of 13.6+1 for girls and 13.1+1.1 for boys. In terms of education, 76 of the 141 cases were continuing their education, 65 cases were not continuing their education, five cases had no education, and eight cases continued their studies in a private educational institution. Fifty-six cases (39.7%) indicated their mothers had no education, 56 cases (39.7%) indicated their mothers graduated from primary school, 20 cases (14.2%) indicated their fathers had no education, and 71 cases (50.4%) indicated their fathers graduated from primary school. The average number of siblings was 4.2+2.6, and there were 13 siblings at most in one case. Both parents were alive in 131 of 141 cases (92.9%); there wasn't sufficient information for this determination in four cases (2.8%). Forty cases (28.4%) had a history of neglect in some area and 27 of these cases (19.1%) had a history of neglect in more than one area. When analyzed according to areas of neglect, 25 (17.7%) education cases, 27 (19.1%) discipline and social needs cases, 19 (13.5%) sanitation-care/nutrition cases, and 20 (14.2%) health cases were reported. There were 49 cases reporting violence in the family (34.8%) with nine of them (6.4%) reporting violence between parents. When the alleged crimes were analyzed, there were 50 cases (35.5%) of robbery, followed by 25 cases (17.7%) of sexual abuse. Crimes against life were more common with girls, while crimes against property, terror crimes, and sex crimes were more common with boys.

In childhood, the features of criminal acts are strongly affected by gender differences. Boys have an increased likelihood of acting more severely and engaging in personal forms of criminal behavior.¹ From 12 to 15 years of age, the lack of self-control is more important than perception; in most situations, it is known that children are unable to shape their behaviors as the law necessitates.²

It should be taken under consideration that if a child continues his/her education in an educational institution, that child will be less likely to engage in criminal behavior as he/she will not be in a criminal environment.^{3,4} Looking for happiness in another place is natural for a child who has a bad family environment; inevitably, the child is also looking for attention in a criminal environment.⁵ If a child took part in a crime, punishment is not a recommended method of prevention. Criminal law must be the last process to use with these children.

The people who will either enhance or diminish the future are the children of today. For a better future, it is our social and public duty to ensure physical, mental, social, and cultural development for our children.



Psychiatry & Behavioral Science Section - 2015

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Child, Crime, Turkish Criminal Law



Psychiatry & Behavioral Science Section - 2015

I8 Violence in the Inpatient Psychiatric Unit: A Case Study and Review of the Literature

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After attending this presentation, attendees will understand the multitude of factors predisposing inpatients for violent and aggressive behavior, including the role played by the surroundings and staff in mitigating or inciting such behavior. Emphasis will be given to the incident that is being investigated for this case study while signifying the role of the inpatient psychiatrist and support staff, once such an incident happens.

This presentation will impact the forensic science community and inpatient psychiatrists by clarifying the roles of individuals as part of a multidisciplinary team when faced with a violent incident involving grievous injury in an inpatient setting. The review of literature on this subject could prove instrumental in being able to anticipate such incidents and take necessary precautions to foster a safe environment for patients and staff.

Violent incidents are common in inpatient psychiatric units compared to lower levels of care. They constitute a major hazard for both the staff and the patients and tend to be unpredictable. The factors influencing these incidents are often deduced by their post-hoc analysis.¹ Although male and female patients carry a close relative risk of perpetrating such events, the majority of incidents with severe bodily injuries or fatalities involve a male patient being the assaulter.² This presentation will discuss a female patient who assaulted a male patient, resulting in significant bodily injury, and the sequence of events that ensued. The predetermined role of hospital personnel in handling such incidents will be discussed. A summary of the review of literature on this subject will also be presented.³

The specific goals of this case report and review of the literature are to: (1) deduce the patient-, surrounding-, and staff-related factors identified from previous studies of aggression and violence in inpatient psychiatric units, which are specifically pertinent to the case under discussion; (2) propose an approach for documenting such incidents as part of a registry to be available for analysis and determination of specific factors responsible in a hospital or an institution. This could prove instrumental in devising specific interventions necessary to create a predictable environment for psychiatrists and a safe environment for patients; and, (3) propose that this data be used in the post hoc analysis of such events in order to understand nuances of environmental and staff factors specific to the community hospital at which these incidents took place. The factors that will be focused on are the timing of the shift changes, experience of the staff, diagnosis of the patient's involved, voluntary/involuntary admission, day of the week, time of the day, and a history of similar incidents in the past based on the available records. Identifying these factors before data collection will prevent any biases and will ensure a uniform sample for analysis.²

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Violence, Inpatient, Safety



Psychiatry & Behavioral Science Section - 2015

19 Violent Behavior and Protective Factors: A Retrospective Study of a Psychiatric Patient's Cohort in Southern Italy

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After attending this presentation, attendees will focus on possible psychosocial factors that may be specifically associated with the various modalities of violent behaviors enacted by the sample cohort examined (directed against others, directed against objects, and self-directed) in order to identify targeted strategies of prevention and treatment.

This presentation will impact the forensic science community by assessing the role of psychosocial factors in the etiology of violent behavior.

In recent years, numerous investigations have confirmed that patients suffering from severe mental illness are at greater risk of engaging in violent behavior. Several risk factors that increase the probability of psychiatric patients committing violent acts have been identified. Some of the factors associated with violent behavior are also psychosocial, including: the occurrence of stressful events, belonging to a “pathogenic” or “disinterested” family, a family history of substance abuse and violent behavior, and poverty and deprivation of the family of origin. There is evidence in the literature to suggest that some psychosocial factors exert a protective effect.

Method: The four different public psychiatric outpatient facilities taken into consideration met all the same criteria of homogeneity. In addition, the local social service agencies in each municipality were involved in the investigation. The sample consisted of 1,582 subjects. The resulting data were stored in a dedicated database. Psychosocial factors and various modalities of violent behavior were the main focus in the sample.

Results: Most patients had long clinical histories (70% of the cases had more than 10 years of history) and had often been hospitalized (sometimes forcibly) over the years. The most common diagnosis was mood disorder (41.2%). After the first contact with the facility, virtually all patients were placed on psychopharmacological treatment (84%).

Violent behavior emerged in the clinical histories of more than one-third (36.3%) of the patients in this sample. Males were clearly predominant among patients exhibiting violent behavior (65.74%). In the majority of cases, this behavior was directed exclusively toward others (76.7%). There was a significant correlation between violent behavior and a family history of either substance abuse or violent behavior. In approximately half of these cases, the victim of the violence was one of the parents.

One episode of violent behavior toward other persons was recorded in 27.9% of the total sample. For the most part, this behavior was enacted by men (69%). Indeed, male sex proved to be a risk factor for violent behavior toward others. Having a supportive family reduced the likelihood of enacting violence against others. By contrast, this probability was increased by the presence of a disinterested family or a “pathogenic” family. Good intra-familial relationships were seen to reduce the probability of committing acts of violence against others, while poor intra-familial relationships increased this probability. Likewise, the probability of engaging in violence against others was reduced when extra-familial relationships were good or satisfactory. By contrast, poor extra-familial relationships increased the likelihood of violence against others. Having a good psychosocial level was seen to reduce the probability of engaging in violence against others as was a satisfactory psychosocial level.

Discussion: Generally speaking, a significant prevalence of violent behavior emerged in this sample, especially among more seriously ill psychiatric patients (psychotic disorders). Moreover, as this study unequivocally demonstrates, the phenomenon investigated is, in terms of magnitude and gravity, considerable. This first finding, which has a direct and immediate impact, requires consideration in order to draft adequate treatment programs.

The importance of psychosocial protective factors is therefore confirmed. In agreement with the literature data, this study confirmed the general finding that protective factors play a significant role in preventing the enactment of violent behavior.



Psychiatry & Behavioral Science Section - 2015

Apart from the results yielded by the present research, many of which are in agreement with the literature, some of the limits typical of a retrospective study must be taken into account, in particular the partial nature of the medical documentation available. Moreover, episodes of violent behavior on the part of patients in public health service care are not always reported in clinical records. It therefore seems likely that the phenomenon was underestimated in the population sample studied.

Mental Illness, Violent Behavior, Protective Factors



Psychiatry & Behavioral Science Section - 2015

I10 The Acute Stress Response to Threats and Physical and/or Verbal Aggressions: The Influence of Epigenetics on Defensive Strategies of Human Behavior

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After attending this presentation, attendees will be informed about current perceptions of epigenetics on the issue of human capacity/ability to react to a threat.

This presentation will impact the forensic science community by reviewing the results of many important works concerning the acute stress response to threats in the hope that studies on the pairing of fear and epigenetics may shed more light on this subject.

It is a known fact that the behavioral responses — primordial and animal responses — to a dangerous stimulus are diversified among individuals as well as among subjects of varied age and gender.

In particular, the defensive (animal) forms of behavior are shown to be integrated within a complex and flexible mechanism which is distance-dependent (Eilam) — it is known that human behavior is organized in modules that are relatively independent from one another (Tooby and Cosmides).^{1,2}

Over the years, Cannon's intuition (fight or flight) about the mechanism of human behavioral response to threat, Marks' behavioral sequence (flight, freeze, fight, and appeasement), Gray's sequence (freeze, flight, fight) as studied in animals and non-human primates, the theory of Gray and McNaughton (clear distinction between fear and anxiety), and the variant thereof put forward by Bracha (freeze, flight, fight, fright) have been reviewed and studied.

However, why does a subject take to flight in the face of an aggression while another individual will fight? Why is it that one woman runs away while another reacts by putting up a fight? Why does one child freeze when confronted with a dangerous situation while another flees? Why does a particular individual, when placed in front of the same threats, fight at one moment (or stage) of his life while at a different moment he escapes or, given the same scenario, he first escapes and then fights? The differences depend on a multiplicity of factors. (i.e., age, bio-social-genetic characteristics of the victims, etc.).

But how, for the sake of the survival strategy, does the environmental variability (Levins and theory of evolution in a fluctuating environment) interact with the genes?

The study of epigenetics may show how gene changes occur in accordance with the type of environment in which the individual finds himself interacting; for instance, "ill-treating environment" or "beneficial environment." A genetic variability is produced, one on which the evolutionary factors are capable of acting by molding the individuals' adaptation capacity; thereby, empowering the suited phenotypes to self-reproduce and be transmitted across generations. Simultaneously, the same mechanism prevents the unsuited phenotypes from gaining ascendancy, with a resultant decrease in the genotypes lying at the base. Notwithstanding that, studies carried out in the last decades have proven that the genomic variations are not merely "casual" but also "causal" (i.e., they are induced by the environmental impact, or better still, guided by whatever surrounds the organism (natural and social habitat)).

The suggested study hypothesis is as follows: in order to attain deep knowledge of all the aspects relating to acute response to a noxious (dangerous/lethal) stimulus as well as if and how such reaction is going to change in the future, one must be profoundly acquainted with the mechanism of fear, the way it is memorized, and the mechanism of decision making through epigenetics. Thus, one could understand the real assessment a human subject makes in response to external stimuli; value may be assigned to such a response with a view to exempting the subject from legal accountability in the event of a criminal trial against him/her.

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Epigenetics, Fear, Threats



Psychiatry & Behavioral Science Section - 2015

II1 Mental Health Services Within California's Prison Realignment Act (Assembly Bill 109): Challenges and Recommendations

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After attending this presentation, attendees will be familiar with: (1) the legal background that led to the development of California's Assembly Bill (AB) 109, implemented October 2011; (2) the mental health services provided under AB109 in Los Angeles County; and, (3) the potential challenges faced by AB109 probationers in terms of accessing mental health treatment in Los Angeles County.

This presentation will impact the forensic science community by raising awareness of the barriers to accessing mental health services under AB109 and by identifying potential solutions to improving access and thereby decreasing recidivism.

AB109, commonly referred to as "realignment," came as a result of two major class action lawsuits in California: *Coleman v. Brown* in 1990 and *Plata v. Brown* in 2001, in which the United States Supreme Court ruled that inadequate medical and mental healthcare in California state prisons was a violation of inmates' eighth amendment rights protecting them from cruel and unusual punishment. These deficiencies were attributed to state prison overcrowding and the United States Supreme Court ordered California to reduce the number of its prison inmates by 33,000 within two years. The state of California responded by enacting new legislation, AB109, which overall resulted in the transfer of responsibility of low-level offenders from the state to local counties. AB109 resulted in three major changes in the California criminal justice system: (1) individuals convicted of non-serious, non-violent, non-sexual crimes now serve time in county jail rather than state prison, even if the sentence length is longer than one year, as long as they do not have any prior serious or violent convictions; (2) parole violators are sent to county jail rather than to state prison; and, (3) if the most recent offense was non-serious, non-violent, and non-sexual, then the offender released from state prison is placed on county probation (post-release community supervision) rather than state parole, regardless of prior convictions, which may be serious, violent, and/or sexual.

AB109 legislation has major implications for mentally ill probationers. It is estimated that nearly 30% of California prison inmates suffer from mental illness, and the recidivism rate is higher among offenders with mental illness, as compared to those without mental illness. Since the passage of AB109, more than 18,000 former state prisoners are now under Los Angeles County probation supervision, and approximately 8,000 are in Los Angeles County's Department of Mental Health database because of a history of mental illness. It should be noted that AB109 does not mandate that counties provide re-entry programs that offer rehabilitation; also, the allocation of funding to rehabilitation is left to the discretion of each individual county. Given the high prevalence of mental illness in California prisons, the question becomes whether local counties will be able to adequately fund rehabilitation programs to meet the needs of this at-risk, mentally ill offender population. AB109 could potentially be part of the solution to decreasing the "revolving door" of recidivism; however, this will depend on post-release mental health services and accessibility.

This presentation will include case examples that illustrate some of the challenges faced by AB109 probationers in terms of accessing mental health treatment. The presentation will include information obtained from individual semi-structured interviews with mentally ill patients on AB109 probation, regarding their mental health history, legal history, post-release mental health treatment course, and outcomes. Information from interviews with staff at a Los Angeles mental health treatment program for AB109 probationers will also be presented to demonstrate the perspective of treatment providers on the challenges and facilitators to providing mental health services to this population. In order to conduct the study, approval was obtained by the University of Southern California Institutional Review Board. Finally, recommendations will be made for ways to potentially improve the delivery of mental health services to this unique population.

Realignment, Assembly Bill 109, Mental Health



Psychiatry & Behavioral Science Section - 2015

I12 Stalking of Health and Social Services Workers: Preliminary Results of a Study Conducted by the Region of Apulia, Italy

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After attending this presentation, attendees will be more aware of the impact that stalking of healthcare professionals has on workers in the helping professions.

This presentation will impact the forensic science community by demonstrating that further, well-designed studies are needed in order to confront this underestimated but very serious problem facing healthcare professionals.

The helping professions, which include those disciplines whose workers' goals are to help others (e.g., general physicians, psychiatrists, psychologists, educators, and social workers) all have one criminologically relevant feature in common: a high risk for being the target of stalking.

A study conducted by Galeazzi, Elkins, and Curci has shown that out of 108 psychiatrists, psychologists, and graduate students, 20% were the target of some sort of stalking for a period of one month or longer, with more than ten single episodes of intrusion.¹ According to this study, the incidence of stalking in the helping professions may be attributed to a series of reasons. For example, the professional may become a "good" or "bad" person in the eyes of the client, and based on such fantasies, this can give rise to "troubling and disturbing" behaviors on the part of the patient who seeks to attract the attention of the professional with the hope of never being separated from him or her. Professionals in the field of social work, psychology, and psychiatry come to know the most intimate needs of their clients; consequently, they can easily become the object of the patient's projections, affections, and fantasies of all types to such an extent that gratitude may be progressively transformed into a desire for an affective or friendly bond that they cannot do without.

Patients who harass their therapists are inclined to mistake a purely professional relationship for a personal one. Feeling the frustration that arises from this expectation, they may begin to act out intrusive behaviors in order to establish contact. In such situations, the relationship boundaries imposed by the professional are breached. A patient may begin to telephone repeatedly, send letters, and show up at the professional's office without an appointment claiming that they are not understood and that they are being treated badly. Other contributing factors to such situations may include errors, deficiencies, and limitations on the part of the helping professional in establishing and creating a proper setting for their patients. When there is a lack of clear-cut professional rules (e.g., excessive intimacy, closeness with the patient and his family, an absence of well-defined boundaries within the professional relationship, and requests by the worker to become more personally involved), the creation of communicative distortions and very real misunderstandings, which can be the trigger for stalking behavior, may emerge.

This study was carried out in both the public and private sectors of health and social services facilities in the region of Apulia, Italy, both in the public and private sectors. All workers at the Psychiatry and Child Neuropsychiatry Units at the Policlinic of Bari, as well as all other workers in private social services facilities that are affiliated with the national healthcare system, were interviewed. Adult consumers of these facilities include those with psychosocial problems, physical disabilities, victims of all types of violence, and those who suffer from psychological dependence. All of the subjects in this study were given a 20-question, multiple-choice questionnaire to be completed individually and anonymously. Sociodemographic data on both victims and presumed perpetrators of harassment, along with the circumstances surrounding the events, and the victims' emotional reactions were collected via this questionnaire on a sample of 101 subjects, all of whom were professionals in the field of social and healthcare services (i.e., medical doctors, psychologists, nurses, and social healthcare workers), in both the public and private sectors.

The subjects in this study who reported harassment attributable to the crime of stalking are represented as n=30 subjects out of n=101 of the total sample (29.7%). Females are more highly represented with F=22/30 (73.3%) with respect to males (M=8/30 (27.6%). With regard to women workers, 30.14% of them (22/73) reported having endured some type of harassment and 28.7% of male workers (8/28) reported the same.

The data derived from this study reveal that little attention is paid to this phenomenon and that the victims of harassment in this study tend to have little faith in the institutions where they work addressing these concerns. Other factors that play a role in these events include a health and social services system that, when it comes to stalking, its prevention, and how to best deal with it, is rather outdated. Only a small part of these crimes (16.1%) is reported to the authorities or anti-violence centers.



Psychiatry & Behavioral Science Section - 2015

This study highlights that there is a significant lack of information available regarding the phenomenon of stalking and its psychological repercussions on workers who encounter it. After having conducted this study, the necessity for training that informs health and social services workers, both in the private and public sectors, about the most suitable ways of avoiding and/or confronting stalking, has become even more evident.

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Stalking, Helping Professions, Harassment



Psychiatry & Behavioral Science Section - 2015

I13 Parricide, Abuse, and Emotional Processing

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After attending this presentation, attendees will better understand the extremely rare phenomenon of parricide and many of its implications.

This presentation will impact the forensic science community by demonstrating that further, well-designed studies are needed in order to confront this underestimated but very serious problem facing families.

Parricide is a very rare phenomenon and represents only a small percentage of all homicides committed. In Europe and the United States, it is estimated that the occurrence of this crime makes up between 2% and 4% of all murders in those regions, with patricides outnumbering matricides.¹⁻⁷ In Italy, the 2008 European Employment Services-Agenzia Nazionale Stampa Associata (EURES-ANSA) Report observed that the rate of parricide in Italy makes up 3% of all homicides committed, with the rate of matricide being significantly higher (59%) than patricide.

Homicide occurs more frequently in homes where the victim and perpetrator cohabit.^{8,9} When a minor commits parricide, he or she does so in a cold and calculated manner and does so in such a way as to avoid confrontation with the victim; for example, when the parent is sleeping, watching television, or engaged in some other activity.¹⁰ Parricide is rarely committed during a violent altercation between parent and child.

Males are much more likely to commit parricide.¹¹⁻¹⁶ For example, when Marleau et al. investigated parricide cases committed in Canada between 1961 and 1989, they observed that males committed 90% of these killings.¹⁷ A vast majority of the literature regarding young perpetrators of parricide shows that minors kill in order to bring years of abuse to an end. Other factors that may influence this phenomenon have also been identified, in particular, the presence of psychiatric disorders and the antisocial tendencies of minors.^{18,19} These seem to be important aspects in the implementation of this crime. Based upon this, it is possible to identify three categories of juvenile perpetrators of parricide: (1) adolescents who suffer from serious mental pathologies; (2) antisocial adolescents; and, (3) adolescents who have endured severe abuse and who kill as a reaction to their circumstances.²⁰ Minors who are systematically punished, criticized, treated with hostile rejection, or ignored by their primary caregivers tend to believe that they are at fault, unwanted, bad, or unlovable.²¹ Such global and negative beliefs about oneself elicit shame because, when shame is experienced, the sense of self is threatened and attention is focused internally.²² In this way, minors who have been or who are currently being treated in a punitive manner seem to develop a different disposition regarding the emotion of shame and their reactions to other life events when compared to those minors who have not been maltreated. For example, Alessandri and Lewis pointed out that maltreated minors (i.e., neglected; physically, sexually, and emotionally abused), from 4-5 years of age, exhibit much more shame when they make a mistake and show less pride when they do well with respect to their non-maltreated peers.²³

Lack of affection, brutality, various forms of violence, and coercion coming from one or both parents may be elements that facilitate the formation of highly discordant and unrestrained personality structures. Furthermore, the presence of a generation gap may be unbridgeable in more serious situations. This is characterized by reciprocal incomprehension and an absence of clear and direct forms of communication regarding central aspects of relational life. In addition, one fact that this study would particularly like to underscore relates to the presence of emotional imbalances and alterations that may act to compromise the formation of a mature self that is endowed with healthy sensitivity and the capacity to foresee the consequences of one's own actions as well as those of others. For this reason, it is necessary to view parricide as a process that is comprised of precipitating risk factors that need to be identified early on, both at the clinical level and at the criminological and forensic psychiatric level as well, in an attempt to avoid tragic and irreversible "acting out."



Psychiatry & Behavioral Science Section - 2015

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Parricide, Patricide, Matricide



Psychiatry & Behavioral Science Section - 2015

I14 Elder Abuse: Risk Factors and the Role of the Nurse

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After attending this presentation, attendees will better understand the very serious and under-reported crime of elder abuse.

This presentation will impact the forensic science community by demonstrating that further, well-designed studies are needed in order to confront and more fully comprehend this problem facing our aging population.

Currently, elderly people are more active, independent, and likely to be in good health in addition to having a longer life expectancy than in the past. In spite of this, abuse, exploitation, and abandonment of the elderly still remains a hidden and underestimated problem throughout the world. Often the people responsible for these abuses are the victims' relatives or, in cases where the victim resides in some sort of institution or facility, the abusers are often the healthcare workers themselves. Abuse affects the physical and psychological well-being of the aged and may lead to a serious public health problem. From the available data regarding the elderly in Europe, the World Health Organization (WHO) has disclosed that in 2004, about four million people over 60 years of age experienced physical abuse; one million experienced some form of sexual abuse; six million were the victims of financial abuse, and a solid 29 million endured psychological abuse; however, such statistics do not accurately reflect the problem. Often, attention is paid to this phenomenon only when news of medical malpractice is reported by the mass media that tend to report only a part of the overall problem, thus leading to incorrect and inadequate conclusions regarding healthcare workers and the general nature of the phenomenon.

Goal of the Study: Beginning with these assumptions, the goal of this study is to examine the available literature in order to define the role of the nurse regarding elder abuse, focusing on the possible types of abuse perpetrated, the possible reasons for such, and possible preventive interventions.

Materials and Methods: Articles regarding elder abuse taken from international literature from the last five years were selected. This study was carried out using well-known scientific databases such as PubMed®, The Cochrane Library, MEDLINE®, EMBASE®, Medscape, and www.clinicaltrials.gov. The WHO and The National Center for Elder Abuse (NCEA) websites were also used. The following key words were entered into each database: "elder abuse," "elder mistreatment," "abuse and neglect of the elderly," and "elder abuse in nursing homes."

Results: Most scientific studies that deal with the abuse and exploitation of the elderly have been published in the PubMed® databases, whereas in other scientifically recognized sources such studies are relatively scarce. Nevertheless, on the WHO and NCEA websites, this topic is recognized as a serious problem and demonstrates the need for more well-designed and analytical studies in order to improve the approach to this phenomenon. Research into this issue is still lacking even though there has been an increase in published studies on the topic in recent years. Summarizing the data that has emerged from PubMed® regarding elder abuse and utilizing specific key words, it has been deduced that in the last five years approximately 750 articles have been published. These findings have been divided into subcategories and shown in Table 1.

Table1: Distribution of scientific articles on elder abuse in various databases

	PubMed®	Cochrane Library	Medscape
Elder abuse	460	3	4
Elder abuse in nursing homes	60	0	0
Elder mistreatment	80	0	1
Abuse and neglect in elderly	150	0	2



Psychiatry & Behavioral Science Section - 2015

Conclusions: Analysis of the selected scientific literature shows that the problem of elder abuse is progressively increasing as the population ages. Scientific articles on the subject are relatively scarce and the vast majority of these studies are quite recent, having been published in the past ten years. Though there may be a general misperception of this phenomenon, one that points the finger of elder abuse at healthcare workers, the truth is that most instances of elder abuse happen within the family environment, and even to this day speaking about it is considered to be a real taboo. The studies examined provide some indications that may be utilized in confronting the problem of elder abuse by acting on risk factors and swaying public opinion toward the recognition of the existence of this problem, which in turn could help in developing more effective programs through well-designed research studies.

Elder Abuse, Role of Nurses, Aging Population



Psychiatry & Behavioral Science Section - 2015

I15 Qualitative Analysis on the Ability to Provide Consent of Treatment to Patients With Chronic Neurodegenerative Diseases — Alzheimer's Disease

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After attending this presentation, attendees will recognize characteristics in the use of “off-label” medical treatments in neurocognitive disorders.

This presentation will impact the forensic science community by increasing awareness of the competence of patients with neurocognitive disorders to consent to off-label use of atypical antipsychotic drugs.

In neurocognitive disorders, psychotic symptoms and behavioral dysfunctions are common and atypical antipsychotic drugs are considered, at the moment, preferred treatments; however, in Italy their use in patients (including older adults) with behavioral abnormalities is considered off-label. In Italy, off-label medical treatments are regulated by Law No. 94/98 which authorizes their prescription only after the obtainment of the patient's written informed consent. As is commonly known, informed consent is an essential prerequisite for any treatment. A valid consent may be given, in accordance with the ethical and legal standards of good clinical practice, only after a patient has received adequate information and included their health condition, the risks and benefits of the therapies proposed, possible alternatives have been evaluated, and their intact decision-making capacity (“competence”) has been verified. Informed consent is usually in oral or written form, as provided in the recent Italian Code of Medical Ethics (Article 35), in the case of off-label treatments.¹

The ISTAT (National Institute of Statistics) 2009 data show there is a progressive increase in the number of patients affected by dementia. The most common dementias are among neurocognitive disorders; in this context in recent years, new criteria has been developed for the clinical diagnosis that took into account the findings of research and a better understanding of the neuro-pathological disease.² The typical pathological lesions of Alzheimer's disease can be found in cognitively normal subjects, in those who present with Mild Cognitive Impairment (MCI), or in people with full-blown dementia. In these patients, where the risks associated with the recommended treatment are especially high, it is necessary to document the actual will of the person to accept the proposal of the doctor, as in the case of off-label treatments in behavioral disorders. Evidence in the literature suggests that even patients affected by severe chronic degenerative diseases still possess valid levels of competence to make some or all of the decisions about their treatment and thus no state of incompetence should be simply presumed.^{3,4} Patients regarded as legally competent to make their own decisions may not be able to completely understand the medical proposals and choices in order to make a truly valid decision and give aware consent. Even the recent Code of Medical Ethics (Article 33) expressly provides information concerning “understandable and comprehensive” therapy. Accordingly, the view is that the failure to give consent must be considered a priori not related to a mental illness, a particular diagnosis, or nosological category; it must be assessed on a case-by-case basis and go beyond the stage of the progression of the deficit and regard the possible impact of the disease on neurocognitive skills that are the basis of decision making. This is to ensure compliance with the rules of law and ethics, but also to provide ethical access to care by all patients in accordance with “freedom of care.”

For this reason, formats for adult persons and Parents/Guardian Ad Litem/Custodian/Administrative Support for interdicted or incapacitated beneficiary were designed. In the forms, scientific evidence of efficacy and the safety of proposed off-label treatment reported in the literature are explained. This important work fills a gap in the implementation of bioethical principles in the clinical setting.



Psychiatry & Behavioral Science Section - 2015

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Neurodegenerative Diseases, Off-Label Treatment, Informed Consent



Psychiatry & Behavioral Science Section - 2015

I16 An Unusual Patricide: The Woman Who Dismembered Her Father

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After attending this presentation, attendees will have a better understanding of patricide.

This presentation will impact the forensic science community by discussing patricides. In this case study of patricide, the victim, a father, was killed by his child. In general, the murderer often suffers from mental illness and the homicide usually takes place in the home. In Italy, patricide is a rarely reported murder (about 3%) with the typical offender being a young, unemployed male who lives with the victim.

As an elderly man had been missing for about a month, his apartment was searched by the police. They found seven boxes containing body segments, covered by feminine clothing, surrounded by lime, and covered with cellophane. There was no blood or larvae present. The man's daughter said it was the corpse of a stranger and claimed not to have reported the discovery for fear of being accused of murder. She added that her father had been away from home and she did not know where he was. A postmortem computed tomography examination of the decedent showed multiple fractures of the head, inflicted by an object with a cutting edge. During the autopsy, the body was identified and the presence of nine dissected body segments was revealed. The right side of the skull was damaged and there was leakage of a completely liquefied brain. The soft tissues were removed through maceration techniques and chemical treatment to highlight the bones' margins in order to determine the weapon used, which was found at the crime scene, and the manner of death. The macroscopic analysis of bone margins with radiological and histological studies helped to assess the force of the injuries. The examination of the injuries inflicted on the head indicated the trauma occurred while the subject was still alive. An autopsy confirmed that the victim was the daughter's 72-year-old father. His 38-year-old daughter had been a medical school student and for years lived in another city where she attended the university. She suffered from psychiatric disorders and was not treated with drugs. After the death of her mother, she returned to live with her father. The daughter was arrested, but for months continued to deny that she killed her father. Finally, she wrote a letter to the judge in which she confessed to the murder.

The court ordered a forensic psychiatric assessment, against the advice of the attorney who was suspicious of the fact that the woman would possibly inherit her father's large estate. The prosecutor pointed out the woman's shortcomings in an attempt to prevent her from inheriting family property; however, experts concluded that at the time of the crime, the woman was suffering from schizophrenic disorders and was not guilty by reason of insanity. The woman had been told by her mother, who died about a year prior, that she and her husband were members of a satanic cult and that all members of this sect abused her from the time she was two to three years of age. She added that she had no recollection of any abuse because the sect members administered drugs that rendered her unconscious. The abuse took place only at night; by day, her parents led an exemplary life. After the death of the mother, the father had demanded they have sex, but she resisted, at which point, the father threatened to kill her. In the course of yet another clash with her father, where he attempted to rape her, the woman defended herself and finally struck him on the head with a heavy glass object. She then decided to cut the body into pieces in order to transport the remains and bury them more easily. In fact, the judge said she did not intend to make her pay for a crime committed as a defense against her father's abuse. The woman, who had claimed to have never had a romantic relationship, was not a virgin.

Patricide, Forensic Pathology, Forensic Psychiatry



Psychiatry & Behavioral Science Section - 2015

I17 Profiling in Violent Crimes: The Perpetrator and the Victim in Portuguese Cases of Filicide

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After attending this presentation, attendees will understand how filicide, the murder of a child by a parent, is a multifaceted phenomenon with various causes and characteristics.

This presentation will impact the forensic science community by highlighting the present state of knowledge regarding filicide perpetrated by either the mother or father.

Goal: The goal of this study is to describe the homicide of children in Portugal and to examine the gender differences in filicidal offense characteristics and associated variables in order to establish a common profile of filicidal offenders and victims. Limitations of the current study and future directions for research will be presented.

Methods: This retrospective study was approved by the Ethics Committee from the Faculty of Medicine of the University of Coimbra and the National Institute of Legal Medicine and Forensic Sciences (NILMFS). The material of the present study was register-based, comprehensive, and nationwide. The information concerning all deaths certified as homicide or with an undetermined/unknown cause in 29 Portuguese medicolegal and forensic offices, for children under 18 years of age, for the period 2004-2013 was examined. Forty-two cases of filicide were analyzed with the Statistical Package for the Social Sciences (SPSS) 19.0 statistical software package. The assessed variables related to the perpetrator included demographic characteristics (e.g., age, sex, ancestry, marital status, and residence area). The assessed variables related to the victim included age, sex, ancestry, circumstances of birth, and number of victims. The variables related to the crime were correlated with the sex of the perpetrator (e.g., crime type, method, motive, post-offense behavior, suicide, and moment of the crime).

Results: A total of 39 perpetrators (six fathers and 33 mothers) killed 42 child victims (21 were male and 21 were female). Statistically significant differences were found between the sex of the perpetrator and the post-offense behavior ($r=.383$), the method ($r=-.323$), and the circumstances of the birth of the child ($r=.394$) to a level of significance of 0.05.

Conclusions: Most of the crimes were committed by young, unmarried Caucasian females between 21 and 30 years of age. The victims were mostly newborns ($n=17$) in non-hospital settings ($n=16$) and resulted from unintended pregnancies. Neonaticide was the most predominant crime with the use of manual and impersonal methods (i.e., fall, suffocation, drowning, strangulation, and intoxication). Males were older, married or divorced, who killed using more violent methods (i.e., firearms and knives) for revenge or retaliation against their companions. Their victims were also older. A better understanding of potentially fatal parental/familial dynamics leading to filicide could facilitate the identification of risk and enable effective intervention strategies.

Acknowledgements: This study wishes to thank all medicolegal and forensic offices of the Portuguese NILMFS for their cooperation and for having made this research possible.

Profiling, Filicide, Infanticide



Psychiatry & Behavioral Science Section - 2015

I18 Death Due to Neglect in the Elderly: A Sad Reality

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After attending this presentation, attendees will be able to describe the impact of forensic science in cases of abandoned elderly people.

This presentation will impact the forensic science community by demonstrating the crucial role of social and familial relationships in cases of neglect in the elderly.

In Italy, there are about 12 million people older than 65 years of age. With the continuous rise of life expectancy, the number of elderly citizens is steadily increasing.

A great number of older people live at home alone and many of them are completely abandoned despite poor medical conditions. Thus, it is very common for elderly people to die alone in their homes not having received medical assistance. Frequently, the discovery of the bodies may be several days after death. No one notices their disappearance and the alert is given late, often by neighbors. Consequently, many of the bodies are in an advanced state of decomposition.

At autopsy, the most frequent causes of death are represented by domestic accidents or cardiovascular disease such as myocardial infarction, ictus, or pulmonary embolism. Many of the deaths would be avoidable with an early warning and a timely rescue.

The reports of the autopsies performed at the Institute of Forensic Medicine of Catanzaro were analyzed and the cases of 20 patients who died between 2013 and 2014 were selected. All cases were of elderly people (nursing home patients with nervous system diseases and mental health issues) who were found dead in their own homes or in rural areas after they disappeared from the care facilities. For each case, the following was carried out using the psychological autopsy method: an inspection of records, an autopsy, and an investigation into a family member. Data analysis has highlighted that in every death, the body showed obvious phenomena of putrefaction. In addition, the autopsies revealed that the causes of death were: from cardiac origin (50%), from asphyxia (esophageal food bolus obstruction) (30%), from traumatic causes (15%), and from malnutrition (5%). The background data indicated a complicated family situation in which the elderly person usually lived alone and was not controlled by the family or by the staff of the nursing home in which he was bedridden. For this reason, it was concluded that the data collected during the judicial inspection and those shown by the medical, legal, and psychological autopsy revealed a deep state of abandonment of the elder before death as well as social and family loneliness. Such cases allow the conclusion that there is a real death-by-neglect in the elderly. Therefore, it emphasizes the importance of family and social integration of the population between 60 and 80 years of age in order to: (1) prevent fatal events related to the lack of prevention of curable diseases which, in a state of neglect, are not treated; (2) avoid accidental events related to lack of control of older people with mental disorders and central nervous system patients at nursing homes or hospitals; (3) increase control strategies in nursing homes or hospitals for the mentally ill or for elderly patients with neurological diseases; and, (4) increase levels of social and family integration distributed throughout cities and towns. These incentives will reduce the exponential increase in deaths from neglect in the elderly.

Forensic Science, Elderly, Neglect



Psychiatry & Behavioral Science Section - 2015

I19 Critique of New Sex Offender Management in California: Assessment, Containment, and Treatment

Susie Morris, MD, USC Institute of Psychiatry and Law, PO Box 86125, Los Angeles, CA 90086-0125*

After attending this presentation, attendees will have an understanding of the sociopolitical history predicated current sex offender evaluation, treatment, and supervision rationale in the state of California. Attendees will also gain a basic understanding of the “containment model,” the codified model for sex offender management in California since 2012, and the roles of therapists, law enforcement, and polygraph examiners within that model. Attendees will be able to: (1) assess the evidence for and against California’s model for sex offender management; (2) draw conclusions about the efficacy of the model; and, (3) consider what further information is needed to bolster the system’s ability to reduce sex offender recidivism, provide adequate supervision, facilitate safe community re-entry, and furnish ethical mental health care to the sex offender.

This presentation will impact the forensic science community by: (1) generating ideas regarding areas where more research is needed with respect to sex offender risk assessment; and, (2) developing novel approaches to improve the current model in sex offender treatment, management, and community re-integration.

In 2008, a state-appointed task force evaluated California’s methods for management, treatment, and registration of sex offenders. This report, prepared jointly by the California Coalition Against Sexual Assault and the California Sex Offender Management Board, indicated areas for improvement in state management of sex offenders. Deficient areas cited were: (1) lack of specific accountability of government agencies with respect to assessing recidivism risk; (2) absence of data on recidivism of sex offenders within the state; (3) inadequate numbers of treatment providers for both incarcerated and non-incarcerated sex offenders; and, (4) no codified guidelines for either risk assessment or treatment of sex offenders. Following this report, Penal Code (Section 290-294) was amended, establishing the State-Authorized Risk Assessment Tool for Sex Offenders (SARATSO) Review and Training Committee.

The SARATSO committee’s function is to select risk assessment instruments to be employed by trained professionals to assess the individual risk of sex offenders with respect to re-offense. This information is meant to be provided to law enforcement and the judiciary in formulating appropriate sentencing, deciding upon post-incarceration supervision (parole or probation), recommending treatment programs, and evaluating the progress of the offender (via observation of assessment score patterns). The ideal assessment tool should reliably predict re-offense risk, accurately assess static and dynamic risk factors, and demonstrate good inter-rater reliability. Currently, the Static-99 and the Juvenile Sexual Offence Recidivism Risk Assessment Tool-II (JSORRAT-II) are the recommended assessment tools for adult male and juvenile male offenders, respectively.

A discussion of how the adult assessment tool scores impact treatment, placement, and community integration will be presented. A critical analysis of California’s sex offender management will be provided from the vantage point of the mental health care provider, and conclusions will be drawn regarding both the merits and potential deficiencies of the containment model within California. The goal will be to generate ideas regarding areas where more research is needed. In addition, with the goals of balancing community safety and providing the sex offender with effective mental health treatment, novel approaches to improve the current model will be posited.

Sex Offense, Recidivism, Risk Assessment



Psychiatry & Behavioral Science Section - 2015

I20 Evaluation of Sexual Abuse Crimes Committed By Teachers Against Students in Terms of Socio-Demographic Characteristics and Related Parameters

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The goal of this presentation is to protect school-age children. Guided by the results of this study, it will be possible to plan treatment for child victims and implement social support mechanisms.

This presentation will impact the forensic science community by increasing awareness concerning unanticipated suspects in sexual abuse crimes.

Introduction: Sexual crime against children, committed by teachers responsible for their education and care, is the main topic in this study. Such crimes are severe social problems and suspects are teachers who are trusted by children and their families. Evaluation of the suspects and the victims separately makes this study unique.

Material and Method: In 2013, the forensic investigation files of the sexual abuse victims came from different regions of Turkey to the Specialization Council of the Istanbul Forensic Medicine Institute, which specializes in forensic psychiatry and sexual crimes, and were evaluated retrospectively. Cases involving educators as suspects were included in the study. Socio-demographic variables such as local regions, ages of the victims and suspects, type of school (public or private), sex differences, and multiple victim situations were evaluated. Mental levels and detailed psychiatric examinations of victims and the types and experiences of suspect teachers were also evaluated. Statistical Package for the Social Sciences (SPSS) 15.0 was used for statistical analysis.

Results: In 2013, 6,310 cases were evaluated in the Specialization Council of the Istanbul Forensic Medicine Institute. These cases are composed of child and adult sexual assault victims, juveniles driven to crime, and other sexual crime cases. One hundred thirty-four of these cases involved children abused by a teacher or educator. Two hundred two children were abused in 134 cases. One hundred eighty-one (89.6 %) were girls and 21 (10.4%) were boys. In 166 (82.17 %) of these cases, the crime was simple sexual abuse (such as touching, kissing, verbal abuse, etc.) and in 36 (17.83%) of the cases, the crime was qualified sexual abuse (such as sexual assault with penetration or ejaculation). The average age of the victims was 11.36 years of age and the majority (113) of victims (55.94%) were in primary and secondary school (first- through eighth-grade students). Ten victims' parents were divorced, 182 had normal mental capacity, 11 demonstrated low average Intelligence Quotient (IQ) scores, five had mild mental retardation, and four had moderate mental retardation. It was detected that seven victims attempted suicide after abuse. Due to the Turkish Criminal Code, psychiatric evaluations reported if there was impairment in mental health as a result of sexual abuse. According to the psychiatric evaluations, 17 (8.41%) of the victims were reported to have impairment in mental health, and 31 (15.34%) were reported to have no impairment detected in their mental health; however, the rest of the victims were reported to have some deterioration in mental health because of sexual abuse.

In terms of the perpetrators: 66 (98.50%) of the 67 suspected teachers were male, with an average age of 41.37 years; 47 of the teachers had been teaching more than ten years; 42 of the teachers worked in public school, while 24 worked in private school; one was an unknown employee; 20 (29.85%) were class teachers, 12 (17.91%) were math teachers, seven (10.44%) were physical education teachers, and eight (11.94%) were managerial. It was found that 30 (44.77%) of the teachers had abused more than one child.

Discussion and Conclusion: There are few worldwide studies in literature examining both sexual crime victims and perpetrators. The goal of this study is to protect school-age children. As a result of this study, planning victim children's treatment and social support mechanisms will be facilitated.

Istanbul Forensic Medicine, Sexual Abuse, Teachers



Psychiatry & Behavioral Science Section - 2015

I21 Assessment and Treatment of Problematic Sexual Behaviors: An Update

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The goal of this presentation is to provide an update on the latest evidence-based approaches to assessment and treatment of adults with problematic sexual behaviors.

This presentation will impact the forensic science community by updating attendees on the evidence-based approaches to assessment and treatment of problematic sexual behaviors for practical application. In addition, it will ideally serve as an interactive method for the collection and exchange of methods and ideas among forensic professionals in the furtherance of developing a standardized guide with a wide consensus.

Although there are common recommendations for the content and methods of assessing and treating persons who have engaged in sexually offensive behavior, there is no universal standard or practice parameter. National and international associations have promulgated guidelines in the past, but not all who are engaged in assessment and treatment are members of such organizations; even among those who are, not all follow the recommendations. Consequently, practitioners and members of the legal community recommending, receiving recommendations for, and overseeing risk management activities are left with minimal, at times inadequate, and even conflicting guidance.

This presentation is presented by forensic practitioners who have experience with clinical, research, and educational facets of both assessment and treatment. This study will provide content that is evidence-based and in keeping with health care and forensic evaluation ethical standards. At the completion of this presentation, attendees will be provided an overview as well as the foundation to be able to: (1) identify the goals of assessing adults with sexual problems/offending behaviors; (2) identify components of comprehensive adult sexual problems/offending behavior assessments; (3) discuss strengths and weaknesses of assessment methods; (4) identify treatment targets; (5) develop a basic treatment plan addressing the application of both psychological and biological interventions; and, (6) discuss efficacy of various interventions and their respective strengths and shortcomings. Clinical, psychometric, and physiological assessment methods will be addressed to include penile plethysmography and polygraphy. For the treatment component, medication management will be included with attention given to use of antiandrogens and antigonadotropics for management of paraphilic symptoms. This presentation is intended to give behavioral science practitioners an overview of the tools available and procedures for their use in assessing and treating problematic sexual behaviors. Additionally, conducting this presentation interactively will serve as a means to elicit input from other forensic professionals in attendance as to their methods of conducting assessments and providing treatment to a unique population as there is active engagement in collecting such input with the goal of contributing to an eventual consensus practice guide.

Sex Offense, Assessment, Treatment



Psychiatry & Behavioral Science Section - 2015

122 Transgender Inmates: Considerations in Placement, Management, and Treatment in Correctional Facilities

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After attending this presentation, attendees will understand the unique legal, medical, and psychiatric considerations inherent in the care of transgender inmates in the correctional setting. Attendees will also become familiar with the evolution of United States case law with respect to this population and how changes in the legal framework have impacted correctional regulations and treatment requirements regarding transgender inmates' placement, management, and treatment.

This presentation will impact the forensic science community by highlighting key legal, medical, and psychiatric considerations involved in the care of transgender inmates with the goal of identifying the necessary elements to providing comprehensive care that balances the individual's needs and safety against those of the correctional institution and of other inmates.

Transgender inmates pose unique challenges to correctional systems with respect to legal, medical, and psychiatric considerations involved in their care. A transgendered person is an individual whose "inward gender identity and outward gender expression differ from the physical characteristics of the body at birth." Thus, a male-to-female transgender person is one who is born with a male body but identifies with a female gender identity, and a female-to-male transgender person is one who is born with a female body but identifies with a male gender identity. For the vast majority of people, sex and gender are congruent. Transgendered individuals may suffer from *gender dysphoria* which the *Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-5)* defines as a significant level of distress that may accompany the incongruence between one's experienced or expressed gender and one's assigned gender. Several studies have found high rates of psychiatric comorbidity in the transgender population as well as a nine-fold higher risk of suicide than that of the general United States population.

Transgendered individuals constitute a minority in the community. International studies estimate a prevalence ranging from approximately 1:10,000 to 1:45,000 male-to-female and 1:30,000 to 1:200,000 female-to-male transgender individuals; however, they are disproportionately represented in the incarcerated population. A 2003 study of the transgender community in San Francisco, CA, for example, found that nearly 14% of transgender individuals had been incarcerated at least once, a rate double the national incarceration average. Moreover, transgender inmates are at increased risk of victimization — in particular, sexual assault — within the correctional setting. A 2007 California prison study found that the prevalence of sexual assault in transgender inmates was more than ten times that reported by a random sample of inmates. This, in combination with evolving case law, has forced correctional systems to create institutional changes to meet the needs of this growing population.

Recent liberal case law has expanded transgendered inmates' rights to healthcare access and safe housing. For example, courts have renounced the use of the "freeze-frame" approach to hormone therapy which essentially freezes the dose of an inmate's hormone therapy treatment during incarceration and prohibits the initiation of hormone therapy. Despite case law, several states continue to utilize this method. Additionally, few correctional facilities in the United States have enacted policy changes to better address issues of placement, management, and treatment of transgendered inmates. Unlike more progressive correctional institutions in Australia, which take into account several risk factors to ensure safe and appropriate housing for their transgendered inmates, the vast majority of United States correctional facilities continue to assign housing solely based on an inmate's external genitalia, not gender identity. There are notable exceptions in California that are managed on the county, state, and federal levels, respectively: the K6G Unit at Men's Central Jail in Los Angeles, the California Medical Facility in Vacaville, and the Metropolitan Detention Center in Los Angeles. Regarding management, there are no uniform guidelines as to how to address a transgendered inmate. Most facilities allow bodily searches of an inmate to be performed by an officer of the same sex and prohibit gender-specific clothing and makeup. Finally, medical and psychiatric treatment focus on addressing access to hormone therapy and/or gender reassignment surgery as well as a transgender inmate's increased risk for psychiatric comorbidities and Human Immunodeficiency Virus (HIV) infection.

This presentation will review the literature on the housing, management, and placement of transgender inmates with respect to key legal, medical, and psychiatric considerations that inform their treatment. Finally, this presentation will present the necessary elements that constitute comprehensive care of these individuals.

Transgender, Corrections, Forensic Psychiatry



Psychiatry & Behavioral Science Section - 2015

I23 Zoophilia and the Law in the United States: Legal Responses to a Rare Paraphilia

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After attending this presentation, attendees will understand the history and phenomenology of zoophilia, recent research findings on individuals who engage in human-animal sexual behaviors, pertinent case and statutory law about human-animal sexual contact in the United States, and the forensic implications of this body of law.

This presentation will impact the forensic science community by teaching useful epidemiological and phenomenological information about this rare paraphilia, and courts' and legislative bodies' historical response to human-animal sexual contact. It will also point out significant gaps in the current understanding of zoophilia as a risk factor for sexual violence recidivism. Attendees' competence at assessing individuals who have engaged in human-animal sexual contact will improve.

Sexual contact between humans and animals has occurred since the earliest recorded history and continues to this day. Alfred Kinsey's original research suggested that human-animal sexual contact was a relatively common phenomenon, but it is unclear to what degree his data are generalizable to the current population. More recent research has focused on self-identified "zoophiles" and their reported reasons for engaging in sexual intercourse with animals. Commonly identified reasons include being sexually attracted to animals and desiring to express love and affection for animals. In the Diagnostic and Statistical Manual, Fifth Edition, zoophilia is classified under the diagnosis of Other Specified Paraphilic Disorder and the diagnostic criteria require that an individual's paraphilia must cause distress or impairment, or result in harm or risk of harm to self or others.

Though societies' responses to bestiality have varied internationally, the response in the United States has typically involved condemnation and prosecution. Currently, there are 31 states with statutes prohibiting human-animal sexual contact. These statutes vary widely in terms of charge severity, potential punishments, and terminology used to describe zoophilic behavior. Despite the prevalence of anti-bestiality legislation, there is limited case law in the United States. Most commonly, bestiality arises in legal cases involving Sexually Violent Predator (SVP) civil commitments. In none of the identified cases has an individual diagnosed with zoophilia had his SVP status removed. Identifying offenders who commit acts of bestiality is important since these individuals may be at an increased risk of committing a variety of other sexual and non-sexual violent acts against humans; however, due to different laws between states, commonly used forensic risk assessment tools for sexual recidivism can yield different scores for individuals charged with or convicted of bestiality offenses. Forensic evaluators should consider this factor when conducting risk assessments. State legislatures should also consider modernizing their bestiality statutes to accord with current terminology and objectives for such laws.

Zoophilia, Sexual Offending, Recidivism



Psychiatry & Behavioral Science Section - 2015

I24 A Pilot Study Comparing Hands-On and Hands-Off Child Pornography Consumers

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After attending this presentation, attendees will understand the difference between hands-on and hands-off child pornography consumers. Attendees will recognize the key characteristics to highlight in order to prevent an escalation and will also be aware of the criminal history of hands-on child pornography consumers.

This presentation will impact the forensic science community by assisting forensic psychiatrists and forensic psychologists to assess the risk these types of offenders pose to public safety.

Introduction: Child pornography is mostly considered as a hands-off offense. For more than a decade, it has been extensively described in an effort to try to understand the reasoning for downloading pornographic images or videos involving minors. For those who engage in these offenses, the motivation is not only driven by their deviant fantasies, the images are sometimes used to enhance contact with a minor. Interestingly, it was found that there are pedophiles who seek child pornography online and subsequently make contact with minors, but there are also child pornography consumers with clear pedophilic fantasies, but without any interest in acting out their urges. In that case, the main questions to ask are if they can decide at some point to assault a child and what could be the reason for the behavioral change. Therefore, it is interesting to compare both populations to know if differences can be highlighted in terms of childhood history, environment, and psychopathology.

Methods: A study was conducted based on a cohort of child pornography consumers with pedophilia diagnoses ($n=34$). The cohort was divided into two groups: those who committed sexual offenses toward children ($n=14$) and those who had never been convicted of anything other than downloading child pornography ($n=20$). Emotional and relational aspects, interpretation of the index offense, and quality of the interview were compared.

Results: No statistically significant difference between both groups in terms of their relationship or sexuality were found; however, statistical differences regarding their attitude toward the offense ($p=0.023$) and the sense of unreality they experienced during the consumption of the images online ($p=0.019$) were found. With respect to the assessment interviews, statistical differences, notably in cooperation ($p=0.001$) and capacity of introspection ($p=0.019$), were found.

Discussion: This study highlights significant differences between the hands-on and the hands-off pedophile. The hands-on offenders are less integrated and seem to have more cognitive distortion than the other group. The hands-off offenders have more insight toward the victims and the consequences of the offense. If they realize their behavior is inappropriate, it would be interesting to know why they do not seek help and cannot restrain their urges to download child pornography images. This study questions the existence of a subgroup of child pornography consumers, the risk they pose to the safety of the public, and how this risk can accurately be assessed. Further studies need to be conducted to understand the triggers that lead to offenses escalating from hands-off to hands-on.

Child Pornography, Risk, Hands-On Sexual Offense



Psychiatry & Behavioral Science Section - 2015

125 **Pedophilia: A Crime or a Disease? How Should the Courts Address This Problem? A Case Study**

Thomas V. Brady, DMD, 1823 Boston Post Road, PO Box 622, Westbrook, CT 06498*

After attending this presentation, attendees will have a better understanding of paraphilias, specifically pedophilia, and will also be educated on what is integral for a proper investigation of a child abuse crime.

This presentation will impact the forensic science community by presenting information by which to view pedophilia in a different light. Hopefully, proper investigation, observation, and evidence collection will be used and the accused will be evaluated via new criteria.

Pedophilia or “The Sandusky Dilemma” — This discussion in no way condones or justifies Mr. Sandusky’s actions. A very intelligent individual made the observation that an inordinate percentage of senior male (mostly) adults that were being incarcerated were accused pedophiles. These were men with no prior criminal history, who started committing this heinous crime. Why? Studies vary, but a common statistic is 42%-50% of first-time senior offenders were classified as pedophiles.

Previously, pedophilia was considered a condition that a person accepts as their sexual preference. More recently, pedophilia has been increasingly viewed as a brain disorder. Pedophilia is defined as a sexual attraction to prepubescent children generally below 13 years of age. A child molester is not necessarily a pedophile. In general, the diagnosis of a pedophilia includes intense sexual fantasies and/or involvement with a prepubescent child for six months or more, must be at least 16 years old, and five years older than the victim(s). Hebephilia, ephebophilia, and teleiophilia are the other age-related sexual preferences.

Neuroscience is the multidisciplinary study of the brain, spinal cord, and associated neurons. Neuroscientists have performed **functional Magnetic Resonance Imaging (fMRI)** sexual preference studies on the brain stem, hypothalamus, hippocampus, and the prefrontal cortex. The fMRI is a magnetic resonance imaging instrument that measures blood oxygen levels at specific sites in the body. The measurement of the blood flow and volume is known as Blood-Oxygen-Level-Dependent (BOLD) imaging.

Studies on convicts showed that when suspected senior pedophiles were shown normative sexually arousing pictures, there was a lack of activation to the hypothalamus due to low levels of blood oxygen; however, when shown similar pictures of children, the blood levels increased. “Healthy” adult males in the study had the reverse findings. In other words, deficits in the activation of the prefrontal cortex were associated with pedophilic behavior. Pedophiles had reduced reactions in the pleasure centers of the brain indicating an altered sexual interest.

Proposed possible contributors to pedophilia include: genetic predisposition, head injury, brain oxygenation issues, tumors, arteriosclerosis, and other illnesses. So, is pedophilia a crime or a disease or both? How can a disease be a crime?

In a 2008 case of child abuse, a three-and-a-half-year-old boy was taken to the hospital with injuries to his penis. The mother’s story changed. At first, she claimed that the cat scratched the child then later claimed that the injury to the penis was due a fall from the toilet in the bathroom. The doctor sutured the penis and was ready to let the child leave when a nurse pointed out other injuries on the boy’s body. The police were called and during their investigation the hospital personnel and the police took multiple pictures of the injuries on the child; however, no ruler was used to take measurements nor swabs taken to look for DNA or amylase found in saliva.

The police continued their investigation at the child’s home. When the bathroom was searched, blood splatter was found on the wall opposite the toilet and photographed, using rulers. The wall was three feet from the toilet. The boy was less than 39 inches tall. Bloody tissue was noted in the wastebasket, but not taken into evidence.

Among the injuries were two apparent bitemarks on the boy’s right leg. The mother said the boy’s five-year-old brother was the biter. With image enhancement software, the apparent bitemarks were examined and measured. The hospital had failed to notice an apparent bitemark at the base of the penis. The lesion was also examined using image enhancement software. Models were taken from the mother and boyfriend. The brother had been sent to Puerto Rico but bitemark models were obtained and included in the examination. An overlay of the models eliminated the brother and the mother, but seemed to coincide with the boyfriend.

When confronted with the evidence, the boyfriend admitted to abusing the child. He was sentenced to ten years in jail, which was suspended after three years for risk of injury to a minor. He was not charged with sexual assault because no rulers were present in the pictures. He is already out of jail and does not have to register as sex offender.



Psychiatry & Behavioral Science Section - 2015

Is he a pedophile?

Pedophilia, Blood Oxygen Level, Incarceration Modalities



Psychiatry & Behavioral Science Section - 2015

I26 **Contemporaneous Assessments of Testamentary Capacity and Undue Influence: Strike While the Iron Is Hot**

Daniel A. Martell, PhD, Park Dietz & Associates, 2906 Lafayette, Newport Beach, CA 92663*

After attending this presentation, attendees will gain a better understanding of the advantages offered by contemporaneous evaluations of testamentary capacity and undue influence at the time a will or estate document is executed.

This presentation will impact the forensic science community by offering a new standard of care for conducting testamentary capacity and undue influence examinations that can reduce the risk of future litigation and related unwanted outcomes.

Contested wills, trusts, and estate plans are extremely expensive to litigate and can significantly erode the corpus of the estate while simultaneously undermining the wishes of the testator and creating turmoil among surviving family members and remaindermen. This is particularly true when late changes are made to an existing will or estate plan. Planning for the possibility of a future will contest is both a prudent and cost-effective strategy that holds the potential to avoid these typical adverse outcomes.

A careful and well-documented assessment of the testator's capacity prepared at the time that a will is executed or any significant changes are made can document and preserve evidence of the testator's competency and freedom from undue influence. This simple step can prevent years of litigation and unnecessary delays in executing the client's desires.

International standards and practices in this area of mental health law will be presented with a focus on the laws in Great Britain, where contemporaneous examinations of testamentary capacity are the norm, and the practice is referred to as "The Golden Rule" following the landmark case of *In Re: Simpson Deceased* (1977). The legal criteria for such assessments will be presented, flowing from *Banks v. Goodfellow* (1870), with a focus on the application of contemporary forensic behavioral science methods to these well-established legal standards.

A proposed standard of care for such evaluations will be presented which includes both psychiatric examination and neurocognitive testing. Evaluations are generally conducted as close in time as possible to the date that a will or trust will be signed and can usually be completed in one day. The process includes: (1) a careful review of the medicolegal record; (2) objective psychodiagnostic and neuropsychological testing; (3) a meticulous forensic psychiatric examination of the testator(s); and, (4) interviews of significant others as needed.

A customized neuropsychological test battery is administered, tailored specifically to those cognitive functions most relevant to testamentary capacity, including attention, concentration, memory, and executive functioning. Psychodiagnostic testing is used to evaluate psychiatric symptoms. The forensic psychiatric examination includes taking a complete history, mental status examination, careful documentation of the testator's competency, and assessment of the factors that increase susceptibility to, or protect against, undue influence. The evaluation can be digitally recorded if desired. A comprehensive report is then prepared to be filed with the estate plan.

In an effort to illuminate the advantages of the proposed model of contemporaneous examinations, case examples will be used to illustrate the complexities and complications of waiting until after the death of the testator to evaluate capacity. Problems and pitfalls likely to be encountered by adopting this approach will be discussed and their potential remedies and advantages will be explored. By changing the thinking about how and when to evaluate testamentary capacity and the possibility of undue influence, there is great potential to safeguard both the estate's financial resources and the family's peace of mind.

Testamentary Capacity, Undue Influence, Neuropsychological Testing



Psychiatry & Behavioral Science Section - 2015

127 Extreme Emotional Disturbance Defense: From the Heat of Passion to a Reasonable Explanation

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After attending this presentation, attendees will: (1) understand the history of the Extreme Emotional Disturbance (EED) defense in the mitigation of prosecutions for murder; (2) compare different states' statutes; (3) examine jury instructions for this defense; and, (4) engage in a discussion of the New York State case, *People v. Sepe*, as a case example of the EED defense. Mr. Sepe was charged with murdering his wife and used the defense that the stress regarding the preparation of a family Easter dinner led him to commit the murder.

This presentation will impact the forensic science community by increasing the understanding of the EED defense and improving competence in psychiatric assessment of defendants.

In 1967, the New York Penal Law dealing with mitigation of prosecutions of murder was revised. The criterion that the offense occurred in the "heat of passion" was replaced with "extreme emotional disturbance for which there is reasonable explanation or excuse."¹ As an affirmative defense, the defendant must prove he or she was provoked by the victim's actions and that a reasonable person would also be provoked.¹ This presentation will discuss both the history of this defense and the current New York State law. This law will be compared with the laws in other states.

A discussion of the jury instructions provided for this defense will be explored. These include: (1) the defendant must have had an extreme emotional disturbance; (2) in committing the homicide, the defendant must have acted under the influence of that extreme emotional disturbance; and, (3) there must have been an explanation or excuse for such extreme emotional disturbance that was reasonable.

There have been many studies describing the use of the "Not Criminally Responsible" or "Insanity Defense"; however, there are very few studies describing other psychiatric defenses. One such study looked at the New York County criminal justice system. The results of the study showed that similar to the insanity defense, EED defense is rarely used by defendants in the criminal justice system. The plea rate for this particular defense by criminal defendants was 0.84%.³ Furthermore, the defense won the case in only one instance when in a jury trial.² In this sample, the EED defense success rate was 39% of the times that it was entered; however, it was noted this was usually when the prosecutor accepted the particular argument offered by the defense.³

The discussion will conclude with a review of the New York State case *People v. Sepe*, a prominent case utilizing the EED defense from 2009.³ The judicial reasoning behind the verdict, which exemplifies the challenges of using this defense, will be highlighted.

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Extreme Emotional Disturbance, Murder, Defense



Psychiatry & Behavioral Science Section - 2015

I28 The Power of Intuition in Deception Detection

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After attending this presentation, attendees will be competent in identifying the strengths and limitations of using intuition for detecting deception and learn how intuition can be applied to different fields in forensic science. Drawing from recent research in several different disciplines, this presentation proposes that the ability to detect lies can be improved by harnessing the power of intuition.

This presentation will impact the forensic science community by integrating knowledge and research from several different disciplines to provide a clear and coherent picture of the latest understanding of the use of intuition for detecting deception.

Humans tell lies all the time. The lies told range from minor, inconsequential white lies to major, high-stakes lies. Humans are also extremely poor lie detectors and fare no better than chance at telling whether or not someone is lying or telling the truth. Although humans are poor at ascertaining when someone is lying or telling the truth, there is some evidence that a small number of individuals are particularly skilled at detecting deception. Some researchers have postulated that these individuals are able to deliberately look for and find ultra-fast, subtle changes in facial expressions (microexpressions) and other behaviors that serve as cues that someone is not being genuine; however, other researchers argue that there is no difference in individuals' abilities to detect deception and question whether people are really able to detect different cues.

This presentation proposes that accurate deception-detection ability does not rely on any conscious and deliberate discovery of cues that someone is lying, but instead relies on intuitive or unconscious processes. Dual-Process Theory postulates that humans operate using two types of thinking or "systems" when making decisions — one intuitive and the other deliberate. Intuitive thinking is considered a fast, automatic, unconscious process, whereas deliberate thinking is considered a slow, conscious, analytic process. This presentation explores the potential application of intuition to detecting deception. Recent research demonstrates that by forcing people to use their intuition, accuracy of deception judgments increases substantially. Implications of these findings and future potential for intuition in different fields will be discussed.

In the spirit of the 2015 American Academy of Forensic Sciences (AAFS) Annual Scientific Meeting, *Celebrating the Forensic Science Family*, and the three major planks of the meeting, this presentation is a collaboration between a mentee and mentor, involves learning from a variety of disciplines including psychiatry, psychology, law-enforcement, and evolutionary biology, and is designed to stimulate discussion and future research into the field of deception detection.

Intuition, Deception, Detection



Psychiatry & Behavioral Science Section - 2015

I29 Incapacity of the Mind Secondary to Medication Misuse as a Not Criminally Responsible (NCR) Defense

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After attending this presentation, attendees will be aware of the types of Not Criminally Responsible (NCR) defenses according to the Criminal Code of Canada. Attendees will learn about the concept of non-insane automatism and will be informed about an atypical case of non-insane automatism.

This presentation will impact the forensic science community by providing specific knowledge about the possibility of raising an NCR defense in a case of Parkinson's disease, which has not been previously described.

Introduction: Non-insane automatism can be an important factor for consideration in an NCR assessment. The literature provides numerous examples of this including hypoglycemia, postictal state, or sleep walking. To be able to raise this possibility and make a sound case, there needs to be a clear-cut relationship between the medical condition and the behavior during the offense.

Case Report: This study describes the case of a 77-year-old man, with no previous criminal history, suffering from Parkinson's disease, who was charged with an unprovoked attack upon his wife. During the NCR assessment, his presentation was unremarkable for any aggressive behavior or psychotic symptoms. During the evaluation, it became evident that the patient had increased the dosage of his medication to better control the motor symptoms associated with his Parkinson's disease. The impact of this was captured on two videos: the first on his admission to a general medical unit and the second two days following medication adjustments, with remarkable differences in his Parkinson symptoms and behavior. The first video showed a patient with better control of his movements and an ability to walk easily, but who seemed disinhibited. In the second video, when he took the regular dose of his medication, he had more tremor and a poor facial expression. While reviewing the events preceding, during, and following the index offense, the conclusion was that the unfortunate and non-deliberate misuse of the medication distorted his reality and judgment sufficiently to alter his ability to appreciate the nature and quality of his actions.

Discussion: The misuse of a medication can hardly lead to an NCR defense, unless proven that the medication provoked a confusion state or non-restrainable behavioral symptoms and that the offender was unaware of the negative consequences of misusing. This argument is often used, in relation with benzodiazepine or antidepressant. The analysis of the behavioral pattern of the offender can allow a better understanding of the role of the medication before or during the incident. As far as is known, misuse of a medication used to treat Parkinson's disease has never been previously described as an NCR defense. With this explanation, in consultation with the victim, the individual was acquitted.

Non-Insane Automatism, Not Criminally Responsible, Parkinson's Disease



Psychiatry & Behavioral Science Section - 2015

I30 Unintentional Child Neglect: Literature Review and Observational Study

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The goals of this presentation are to help attendees better understand the issue of unintentional child neglect, evaluate risk factors leading to neglect, and evaluate the consequences of child neglect.

This presentation will impact the forensic science community by instilling a need for further research into the issue of child neglect, specifically unintentional neglect. With a more consistent and cohesive definition of neglect and a better understanding of its associations and risk factors, it will be possible to better address the problem.

Child abuse is a problem that affects over six million children in the United States each year. Child neglect accounts for 78% of those cases. Despite this, the issue of child neglect is still not well understood, partially because child neglect does not have a consistent, universally accepted definition. Some researchers consider child neglect and child abuse to be one and the same, while other researchers consider them to be conceptually different. Factors that make child neglect difficult to define include: (1) cultural differences; motives must be taken into account because parents may believe they are acting in the child's best interests based on cultural beliefs; (2) the fact that the effect of child abuse is not always immediately visible; the effects of emotional neglect specifically may not be apparent until later in the child's development; and, (3) the large spectrum of actions that fall under the category of child abuse.

Some of the risk factors for increased child neglect and maltreatment have been identified. These risk factors include socioeconomic status, education level, family composition, and the presence of dysfunctional family characteristics. Studies have found that children from poorer families and children of less-educated parents are more likely to sustain fatal unintentional injuries than children of wealthier, better-educated parents. Studies have also found that children living with adults unrelated to them are at increased risk for unintentional injuries and maltreatment. Dysfunctional family characteristics may be even more indicative of child neglect. Parental alcohol or drug abuse, parental personal history of neglect, and parental stress greatly increase the odds of neglect. Parental depression doubles the odds of child neglect; however, more research needs to be done to better understand these risk factors and to identify others. Having a clearer understanding of the risk factors could lead to prevention and treatment as it would allow for health care personnel to screen for high-risk children and intervene before it is too late. Screening could also be done in the schools and organized after-school activities. Parenting classes have been shown to be an effective intervention strategy by decreasing parental stress and the potential for abuse; but there has been limited research on this approach. Parenting classes can be part of the corrective actions for parents found to be neglectful or abusive, but parenting classes may also be useful as a preventative measure, being taught in schools or readily available in higher-risk communities. More research is needed to better define child abuse and neglect so that it can be effectively addressed and treated.

Child Neglect, Unintentional Injuries, Maltreatment



Psychiatry & Behavioral Science Section - 2015

I31 Assessing and Addressing Preteen Violence

John L. Young, MD, 203 Maple Street, New Haven, CT 06511-4048*

After attending this presentation, attendees will understand the unique, relevant features characterizing the very youngest perpetrators of violence, including possible causes and means for prevention.

This presentation will impact the forensic science community by facilitating the direction of critical professional attention to the social impact of violence by pre-adolescent youths.

Traditionally, seven years of age is regarded as the “age of reason,” the time by which a substantial level of moral discernment can be expected. There is a long legal history that at least loosely follows this notion. Yet it is known from many investigators, including James, Kohlberg, and Piaget, that for many purposes, it is an oversimplification. Moreover, current work by Paul Bloom has been uncovering the ability to express morally based preferences in infants during their first year.

While still quite young, children may in extreme cases give violent expression to their choices. Examples include a first-grader in Michigan shooting a classmate, two English schoolboys killing toddler James Bulger, and an 11-year-old Chicago boy accused in the slaying of a 14-year-old girl. These perpetrators are all preteens and appear to form a class distinct from the likes of adolescent school shooters, bullies, hazers, or so-called gang bangers. Unlike their older counterparts whose violence surprised most of those who knew them, these younger children are more likely to show serious disturbances as early as their fourth year. Fortunately, they are apparently rare, but hard to count accurately or to even discuss.

Suggested causes for pre-teen violence range widely. Brain imaging and genetic techniques so far have not yielded any distinguishing findings. Early attachment disturbances are suspect. A wide range of substances misuse may be a factor, including herbal remedies that appeal to the very young. The easy availability of guns may play at least a secondary role. Significant controversy continues regarding the causal role of media violence, whether through the internet, films, television, radio, video games, printed periodicals, or even books.

Potential remedies are many and many can be applied simultaneously. Preventive measures focus on decreasing violence in the environment with alternative positive opportunities to socialize. Parental training efforts have proven welcome and helpful. Introducing enforcement of existing but ignored laws at all levels can be effective. Close attention to day-care operations is probably warranted, in view of a study showing that length of exposure to day care may correlate at least slightly with disruptive behavior in the early years of school. School-based programs can systematically encourage appropriate responses to routine experiences that would otherwise elicit violence. When prevention fails, there is a minority advocating school-based corporal punishment.

Finally, pre-teen violence does not respect international boundaries. Thus, it is important to make the effort needed to compare relevant concerns and experiences across diverse cultural and political contexts. History judges societies according to their treatment of their most vulnerable members.

Violent Children, Violence Prevention, Assessment



Psychiatry & Behavioral Science Section - 2015

I32 Perception of Police Among Adolescents in Istanbul

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The goal of this presentation is to help attendees identify Turkish adolescents' perception of the police. Attendees will critically examine the factors shaping adolescents' perception of police in Istanbul.

This presentation will impact the forensic science community by highlighting the importance of police-citizen relationships in order to improve police response to crime.

With the rise of community policing around the globe, many studies have started to focus on the police-community relations to improve police departments' response to community needs. Thus, citizens' perception of the police has become a primary focus for scholars. It is believed that citizens who are satisfied with the police are more likely to cooperate with the police, which may increase the police effectiveness in crime control. Considerable research has shown that personal experiences with the police, demographic characteristics including gender, age, socio-economic status, etc., and neighborhood characteristics are correlated with the perception of police, indicating that those living in poor neighborhoods, those who are young, and having negative contact with the police resulted in low levels of satisfaction with the police. Because many of the studies were conducted outside of Turkey, there is a lack of empirical evidence that measures adolescents' perception of police in Istanbul. The main goal of this study is to empirically assess Turkish adolescents' police perceptions in Istanbul.

The goal of this study was to examine high school students' perceptions of police in Istanbul, Turkey. Data were analyzed based on 1,785 surveys conducted with Turkish adolescents attending middle and high school in Istanbul between October and December 2013. The survey instrument used to collect the data for this study was a two-page questionnaire that consisted of 49 questions and statements. Twenty items were related to perception of police, including the following statements: "the police are dishonest" and, "the police are biased." The remaining questions asked about the students' demographic characteristics, socio-economic status, and perceptions of safety in their community.

In the sample, 56.7% of the respondents were female. The average age of the adolescents was approximately 12.5 years old. Almost all lived with their parents. Approximately 13% had previous contact with police. Of these, 53% perceived their neighborhood as safe in terms of crime. The average value on the 20 items relating to the police was 4.2, indicating that adolescents reported slightly positive perceptions of police. Female students' ratings of the police were significantly higher than males, which was consistent with the studies.

In general, the results showed that students who had previous contact with police rated police more favorably than those who had no contact with the police in the past. Overall, adolescents reported positive perception of police regardless of gender, neighborhood characteristics, and background. Consistent with the previous studies, adolescents living in low-income areas perceived crime as a problem in their neighborhoods and recognized the importance of the police as a solution to crime. This research is the first to examine Turkish adolescents' perception of police in Istanbul. These findings can help the police to improve their services and to promote better police-citizen relationships.

Perception of Police, Adolescents, Turkey



Psychiatry & Behavioral Science Section - 2015

I33 Behavioral Characteristics and Personality Traits of the White-Collar Organized Criminal and the White-Collar Organized Community

Janet M. Schwartz, PhD, PO Box 36058, Canton, OH 44735-6058*

After attending this presentation, attendees will be able to: (1) describe a milieu of perspectives on white-collar criminals including that of Main Street media, history, and criminal justice; (2) list five categories of 21st-century white-collar crime; (3) identify behavioral characteristics and personality traits of the white-collar organized criminal and the white-collar organized community; (4) demonstrate how to assess the presence of psychopathy and the risk of dangerousness in individuals when preparing for litigation or throughout an investigation; (5) understand the significance of the psychopathic continuum; (6) learn tactics such offenders use to avoid retribution; and, (7) list ways to protect oneself, one's family, and one's community from falling prey to these social predators.

This presentation will impact the forensic science community by educating attendees with a comprehensive overview of white-collar crime and ways to detect patterns of corruption. Developing an awareness of the behavioral characteristics and personality traits of white-collar organized criminals and the white-collar organized community is a preventative measure against future victimization.

Forensic Fraud Research, Inc. is a non-profit, not-for-fee investigative firm that performs intelligence-gathering efforts and works collaboratively to help strengthen our government. Through work at Forensic Fraud Research, Inc., a gateway of opportunities to perform investigations and to gather semi-structured interviews with 300+ victims, whistleblowers, alleged offenders, offenders, alleged offenders' spouses and family members, witnesses, and bystanders was possible. The results of this study have yielded the "Behavioral Characteristics and Personality Traits of the White-Collar Organized Criminal and the White-Collar Organized Community" (Note: When the study had conducted 100 interviews, the United States Department of Justice requested permission to videotape the presentation for educational purposes and for use on their Justice Television Network (2005)). Of particular interest is the role of the manipulative cycle in the decision to deceive and to carry out the deceptive act.

Learning when to intervene is essential in order to prevent the contemptuous delight of the white-collar organized criminal. Rich anecdotal data gleaned from working on the frontline substantiates the findings. As security incidents are frequently an aspect of this work, determining the presence of psychopathy and assessing the risk of danger is critical. The criteria utilized will be shared. While there is no universal definition of white-collar crime, the accepted definition used by the United States Department of Justice and the "consensus definition" offered by the National White-Collar Crime Center (NW3C) will be discussed. The definition of organized crime as provided by Investigator John Clark, High Intensity Drug Trafficking Areas (HIDTA) -Money Laundering Unit of the Federal Bureau of Investigation (FBI), clarifies the link of collusion in corruption. According to the Association of Certified Fraud Examiners' 2014 Report to the Nations, "When collusion is involved, median losses due to fraud increase substantially." Attendees will be empowered with helpful information that may prove to be useful in both their professional and personal lives.

White-Collar Crime, Psychopath, Characteristics



Psychiatry & Behavioral Science Section - 2015

I34 Honor-Based Violence: A Cultural Problem!

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After attending this presentation, attendees will gain an understanding of the relationship that exists between the individual environmental factors of honor-based killings committed by family members and the reasons behind such acts in various cultural contexts, thus assisting in the development of preventive interventions.

This presentation will assist the forensic science community by identifying the causes of culturally and traditionally accepted crimes committed against female members of the family by their male relatives and will assist in the development of preventive programs by preparing the grounds for the establishment of policies and strategies that will eventually pave the way for preventive and ameliorative measures.

Honor can be defined as the commitment to moral principles and social values such as chastity.¹ In particular, its meaning in Turkish refers to the rules regarding sex in the Islamic religion. In Turkey, honor is considered generally to be synonymous with sexual purity.^{2,3}

Despite the different perceptions among different people, it has been observed that the most common trend is to consider women, their bodies and their sexuality, and their control as elements of honor. In this context, to the male members of the family, honor relates to a man's wife, sister, mother, other family members, and even to the other women in his circles. As such, he has a duty to keep an eye on all of these women. This increases the pressure on women, especially in areas where tribal and kinship bonds are strong or where small-scale and face-to-face relationships are established. By controlling her sexual behavior, a woman provides information to society not only about her own honor but also about the honor and pride of her family.⁴

Behavioral repertoires that can be considered as components of a woman's sexuality comprise not only her sexual relationships but also many other forms of behavior, such as her way of dressing, holding hands with the opposite sex, requesting a song for a man on television or radio, kissing, and flirting.⁵

Honor killings are committed as a social reflection of the cultures in which women are considered a commodity.^{6,7} If a woman in Turkey defames her own honor or the honor of her family through her behavior, she may be punished in various ways (verbally and/or physically), as in many other countries. In the case of honor killings, a single perception is enough to trigger a crisis.⁸

According to research conducted by the United Nations Population Fund, more than 5,000 women are killed every year in honor-based killings around the world.⁹

According to The Grand National Assembly of Turkey (TBMM), 1,091 honor-based killings took place between 2000 and 2005, and 322 of those were reported as murders committed in the name of honor. Unfortunately, as the statistical data related to crimes are classified according to the terms of crime, as stated in the Turkish Penal Code, honor killings are recorded only as homicides in the statistics, making it difficult to compile concise data related specifically to honor killings.¹⁰

This study collected only the ten files that were sent to The 2nd, 3rd, and 4th High Criminal Courts of the Adana Courthouse between June 2008 and June 2014 and that were registered in the Adana Courthouse, the National Justice Network Information System.

Providing a clear overview to attendees, focus will be on the individual cases, the cultural infrastructure of the geographical regions in which the killings took place, and the local social attitudes. The cases presented here have all been finalized with court decisions. Focusing only on socio-economic conditions and social pressure as the causes of honor killings may, in a way, cause the violence committed by a person or people to be overlooked. Prevention of honor killings should start by setting aside the current perspective toward the concept. In order to change the traditional perception and judgments of gender, there are a number of necessary steps that must be taken, primarily by public institutions and establishments, who must take effective measures to ensure that the security of life for women is a priority.

Violence against women has been the subject of research for the last 50 years, while forensic science has been taking an interest in the issue for the past 30 years; despite this, significant developments have only been realized within the last ten years. For this reason, it is vital that this matter be a subject of perpetual discussion in the field of forensic sciences so as to improve the level of awareness/consciousness not only in the field of forensic sciences, but also in social development. Accordingly, this study recommends that these cases should be considered on a much broader base and discussed more at an international level.



Psychiatry & Behavioral Science Section - 2015

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Honor, Violence, Cultural Problems



Psychiatry & Behavioral Science Section - 2015

I35 Paternal Filicide for Spousal Revenge: The Male Side of Medea's Syndrome in the Italian Population Over the Last Ten Years

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After attending this presentation, attendees will understand Medea's syndrome as it applies to fathers, by observing the prevalence of this syndrome in Italy during the last ten years (from 2005 to 2014).

This presentation will impact the forensic science community by demonstrating that a substantial proportion of cases due to Medea's complex are attributable not only to mothers but also to fathers. Although it is traditionally thought that mothers kill their children for revenge against their fathers, the opposite can also happen.

By consulting the archives of an important Italian national newspaper, "Corriere della Sera", this study chronicled all the cases of children or teenagers killed by their fathers due to spousal revenge. Twenty-eight cases were analyzed, focusing on spatial and temporal distribution, age, nationality of the fathers and children, gender of the children, the fathers' occupations, the methods of killing, and any association with suicide.

Results showed that 64.5% of cases occurred between 2005 and 2009, mostly in February, July, and December; in fact, these are holiday months (Valentine's Day, summertime, or Christmas, respectively), which may explain the high concentration of cases during this period. Regionally, 57.1% of cases occurred in Northern Italy, but major population density in other regions must be taken into account; 82.1% of perpetrators were Italian with others being from Egypt, Slovenia, Romania, and Nigeria, demonstrating that it is not a phenomenon linked to immigration. Additionally, 42.8% of these fathers were employed, 25% were unemployed, and 21.4% were self employed, with most experiencing financial difficulties, showing this may be a risk factor. Fifty-four percent of the children killed were between one and ten years of age. This differs from female perpetrators, who generally kill their children when they are less than one year of age. Moreover, 67.6% of the children killed were male, as if there were a sort of gender identification with the victim. The methods of killing were mostly violent: 39.3% by blunt force trauma; 21.4% by gunshot trauma; 21.4% by sharp force trauma; only 14.3% were due to asphyxia, which is a less violent method generally used by maternal killers. In 51.3% of the cases, a relationship separation existed, evidencing a possible strong correlation between these crimes and jealousy or revenge. In 24.3% of the cases, perpetrator depression was associated with the act and in only 8.1% of the cases, other contributors such as substance abuse or cultural or religious divergences were noted. In 64.3% of the cases, a perpetrator suicide or attempted suicide was involved, possibly indicating guilt or a great sense of inadequacy and unease rooted in the father who committed the act.

This study sheds light on an almost-forgotten segment of fathers who murder their own children simply for revenge against their partners, representing the male side of the so-called Medea's syndrome.

Medea Complex, Filicide, Revenge



Psychiatry & Behavioral Science Section - 2015

I36 Suicide Among Turkish Police Officers From 2003 to 2013

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The goal of this presentation is to help attendees understand police suicide trends in Turkey between the years 2003 and 2013. Attendees will critically examine the relationship between police suicides and demographic variables in Turkey.

This presentation will impact the forensic science community by describing police suicide trends in Turkey over a ten-year period, covering the years from 2003 to 2013.

Police work is one of the most stressful occupations; research found a significant correlation between stress and suicide among police officers. Thus, suicide among police officers has been extensively studied. It is alleged that police are at higher risk of suicide than the general population; however, studies have shown that police suicide rates are lower than those of the general population. It is widely argued that police suicides are vastly under-reported due to social stigma, insurance and religious reasons, and loyalty to the department. Regardless of police suicide statistics, the death of a police officer has a significant impact on the family of the victim and coworkers within the department.

Most of the studies on police suicide are focused on United States police forces and there are few articles based on other countries, including England and Canada; therefore, the goal of this study was to examine police suicides in Turkey between 2003 and 2013. This study also considered police suicides in the context of general population suicide rates to determine whether police suicide rates are higher or lower than the general population. A secondary purpose was to explore the relationship between police suicide rates and gender, age, marital status, reasons, method of suicide, and years-in-service. Data for this study was gathered from the Turkish National Police Department. The annual number of police suicides were stratified by gender, age, degree, marital status, reported reasons for suicide, years-in-service, and method of suicide during the study period, 2003-2013. The annual suicide rate was calculated as the number of suicides per year by dividing the number of officers on the force.

Over ten years, the total number of police suicides was 277. The overall suicide rate among police officers during the period was 17.6 per 100,000 person-years. Rates in married police officers were an average of 40% higher than other groups including divorced and single officers. Recognizing the many stresses associated with police work, it is not surprising that suicide among married police officers is higher. Almost all police suicides resulted from firearms due to the easy access and socialization of police officers to handguns. During the entire period from 2003 to 2013, there were fluctuations in the rates of police suicides; there were no trends. Compared to the demographically adjusted suicide rate for the Turkish population (3.98 per 100,000 person-years), the police officer suicide rate was higher than the general population.

The study findings showed that police suicide rates are much higher among male and married officers. The higher rates of suicide among married police officers can be interpreted by considering that police work affects the family of police officers in substantial ways, particularly shift work and temporary assignments which disrupt family life. It should be noted that the fluctuations in police suicide over this ten-year period in Turkey reflects cultural changes, especially changing attitudes toward the police.

Police Officers, Turkey, Suicide



Psychiatry & Behavioral Science Section - 2015

I37 Parricides and Crime Scene Behaviors — Do They Tell the Story?

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After attending this presentation, attendees will be more knowledgeable about psychological motivations for parricide cases and how crime scene analysis can aid in understanding the crime and the possibilities of preventing it.

This presentation will impact the forensic science community by expanding knowledge regarding the victims and offenders of parricide cases by looking at the crime scene behaviors for insight into family dynamics and motives for the crime.

Parricides, while being a rare event, are one of the most sensational crimes that attract the attention of the media, clinicians, and researchers worldwide. Much research has been done on the topic of parricide despite the incidence being only about 1.5% to 2.4% of all homicides yearly. Often research focuses on the offender's and the victim's characteristics in order to evaluate the crime and motive; however, there is very limited research into the crime scene behaviors. Basic knowledge of parricide in multiple studies shows that offenders are typically adult White males with mental health issues who often target their mothers. Differences between adults and adolescents who commit parricide show that adults have a higher incidence of schizophrenia or a psychotic process being present at the time of the crime.^{1,2} Some studies have pointed to abuse as the motivation in adolescents who commit parricide. Dr. Kathleen Heide has shown in multiple studies the differences between adult and adolescent offenders, such as most single victim-single offender cases of parricide involve adult males of White, non-Hispanic ethnicity. Studies have shown that the age of the offender is significant as to which parent is targeted as the victim (i.e., offenders less than 30 years old target the father, stepfather, and stepmother, while offenders who are older target their mothers as the victim).³⁻⁵

While research has focused on demographics, motive, social, legal, and psychological factors such as mental health and abuse history, minimal research of crime scene behaviors in parricide cases have been explored. Other types of homicides are often profiled based on crime scene analysis, so why not open this door when looking at parricides as well? Previous analysis of crime scene research in parricides is limited to looking at the weapons the offender used and signs of overkill. Crime scene analysis and profiles have been invaluable tools for investigators and police to help understand the offender of violent crimes, but this concept has not been used to look at parricide cases in order to better understand the events and circumstances that lead to an individual taking their parent's life. To examine the components of crime scene behaviors and its implications within parricide, data from adolescent parricides will be presented. Due to the high incidence of psychosis in adult cases of parricide, this data was excluded from this study. This study will present preliminary data about adolescent parricide offenders and crime scene behaviors such as condition of body when found (covered, face covered, and moved or hidden); type of attack (blitz, surprise, or con/deceived); presence of defensive wounds; signs of overkill; weapon used; confession type/plans to avoid detection; and location of the attack. The hope is that by examining these unique crime scene behaviors, better insight into parricide can be acquired.

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Parricide, Crime Scene Behaviors, Body Covering



Psychiatry & Behavioral Science Section - 2015

I38 Suicide by Suffocation: A Case Report of an Elderly Woman Who Completed Suicide by Suffocation

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After attending this presentation, attendees will be informed about rare methods of committing suicide and, in particular, learn about suicide by suffocation/asphyxiation. Attendees will also be alerted to forensic issues related to patient suicide; additionally, risk factors for completing suicide will be reviewed. Recommendations regarding psychoeducation to health care providers and patients will be explored.

This presentation will impact the forensic science community by educating attendees about rare methods of suicide and populations vulnerable to suicide by suffocation.

Each year, approximately 38,364 people die by suicide, making it a leading cause of preventable death in the United States, surpassing automobile accidents (33,687). Studies also report a 16% increase in death by suicide during the period 2000-2010. This has resulted in 12.6 people dying per day by suicide in the United States. Having a mental illness or engaging in substance use puts one at a significantly higher risk for suicide completion. While firearms continue to be the most common method for suicide completion, studies suggest that suicide by hanging or asphyxiation showed the most dramatic increase. In 2000, 19% of all suicides were completed by hanging or asphyxiation and in 2010, it rose significantly to 26%. While suicide by hanging or asphyxiation is on the rise, specifically, suffocation using a plastic bag remains an extremely rare method of suicide and it is fairly underreported in the literature. Literature indicates that the majority of plastic bag suffocation is in the context of a suicide attempt carried out by either those with mental illness or the elderly using sedatives.

Case: A case of a 70-year-old woman with mental illness and numerous medical co-morbidities is presented. In addition, she had a history of cocaine abuse and a tortuous relationship with her husband. She presented to the medical and psychiatric emergency room numerous times with reported anxiety and various somatic complaints. She completed suicide by asphyxiation using a plastic bag two days after she was examined in the psychiatric emergency room and cleared for discharge where she presented with complaints of anxiety. Upon completion of an autopsy, homicide was ruled out.

Discussion: Suicide by asphyxiation/suffocation with a plastic bag, while rare, has shown a significant increase in the United States and worldwide. It is poorly understood and infrequently reported in the forensic literature. This presentation intends to alert the forensic community about this rare method of suicide and to educate mental health professionals about its increased incidence in the United States. Further, the goal is to review current methods of psychoeducating our patients about suicide and to expand on modalities currently in practice. In addition, this study will explore the physician's perspective of a patient suicide and the potential forensic implications.

Conclusion: The goal of this case is to add to the growing literature about suicide by suffocation and bring to light the need for future investigations to better understand what specific populations are vulnerable and at risk.

Suffocation, Suicide, Mental Illness



Questioned Documents Section - 2015

J1 Developments and Validations of TD-GC/MS and HPLC Methods for the Identification of Ballpoint Pen Ink Components: Study of Their Decomposition on Aging

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After attending this presentation, attendees will learn how an ink entry on a questioned document relates to the origin date and explains how evidence is subject to aging.

This presentation will impact the forensic science community by validating the method of ink age determination using the dynamic physicochemical properties of the ink entries on a document.

Determination of the age of an ink entry from a questioned document is a difficult and controversial issue in forensic science. Currently, the study of ink age determination obtained from the dynamic properties of ink entries have shown that Phenoxy Ethanol (PE), which is one of the solvent inks included, has different behavior with respect to the varying thermal conditions of Thermal Desorption Gas Chromatography/Mass Spectrometry (TD-GC/MS) during methyl losses of the pigments and presents valuable confirmatory evidence of age using High-Performance Liquid Chromatography (HPLC).^{1,2}

New methods determining PE by TD-GC/MS and Crystal Violet (CV), Methyl Violet (MV), Tetramethyl Pararosaniline (TPR), and Victory Blue (VB) were developed and validated in this study. Validation parameters such as specificity, selectivity, intra-day repeatability, intra-laboratory reproducibility, inter-laboratory reproducibility, Limit of Detection (LOD), Limit of Quantitation (LOQ), and linear operating range and recovery were examined. LOD and LOQ values for PE are 10^{-10} ng/ml and 10^{-9} ng/ml, respectively. Operating ranges for CV, MV, TPR, and VB are 0.05-10 ng/ml, 0.5-10 ng/ml, 1-10 ng/ml, and 1-10 ng/ml, respectively. LOD and LOQ values for CV, MV, TPR, and VB are 0.02 ng/ml and 0.05 ng/ml, 0.3 ng/ml and 0.5 ng/ml, 0.5 ng/ml and 1 ng/ml, and 0.5 ng/ml and 1 ng/ml, respectively. For intra-day repeatability and intra-laboratory reproducibility studies, six repeated analyses were performed with solutions which have 2, 4, and 8 ng/ml concentrations. Standard deviations and relative standard deviation values of these analyses were calculated. These values were appropriate for the acceptance criteria. For inter-laboratory reproducibility studies, ink entries from six different documents were analyzed. 97.7% of MV and 100% of CV and TPR were recovered. This validated method for ink composition and age determination is specific, fast, and applicable.

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Validation, TD-GC/MS HPLC, Ink Aging



Questioned Documents Section - 2015

J2 Carbon Black Nanoparticles and Graphene Oxide-Embedded Thin Sol-Gel Film for Analysis of Dye Molecules in Writing Inks by Laser Desorption/Ionization Mass Spectrometry

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After attending this presentation, attendees will understand how carbon black nanoparticles (Optical Mark Recognition (OMR) card marker ink) and Graphene Oxide (GO) used as matrices for ink analysis of questioned documents can lower the detection limits of dye molecules.

This presentation will impact the forensic science community by demonstrating how the small particles of GO have relatively lower matrix noise than conventional Matrix-Assisted Laser Desorption/Ionization (MALDI) matrixes such as 2,5-dihydroxybenzoic acid and how carbon black nanoparticles (OMR card marker ink) and GO have been widely used as matrices for Laser Desorption/Ionization (LDI) Mass Spectrometry (MS).

Carbon black nanoparticles have been used as a matrix for MALDI analysis of small molecules. For the application of the analysis method of forged document using the Surface-Assisted Laser Desorption/Ionization (SALDI), similar to MALDI, a study was conducted using the carbon black colorant as a tool to enhance the detection sensitivity of dyes such as Methyl Violet, Crystal Violet, and Rhodamine B in handwriting ink. This research focused on the chemical properties of the OMR card marker as a carbon black colorant. Through material analysis of these matrix and real-application tests, the carbon black ink from the most appropriate manufacturer was selected. As a result of testing the ink from multiple manufacturers, the CB1 ink was selected for its appropriate viscosity and excellence in its spraying performance. This study investigated the appropriate concentration needed for optimum conditions when applying the carbon black colorant. Depending on the organic dye, the optimum detection sensitivity appeared within the range of 1 ug/mL-100 ug/mL. In addition to the concentration of the dye, the efficiency of contact between carbon black colorant particles and dye molecules was a critical factor that could have an effect on the optimal condition.

Application of the results of this research to real handwriting ink strokes of unknown samples that have aged for a year found the detection sensitivity to be outstanding and capable of differentiation. Additionally, through the comparison of results, this method was found to be more reliable than the matrix agents such as 2-(4-Hydroxyphenylazo) Benzoid Acid (HABA). On the other hand, GO has more recently been widely used for a matrix of LDI/MS of small molecules because GO has a relatively lower matrix noise than conventional MALDI matrixes such as 2,5-Dihydroxybenzoic Acid (DHBA) and also because GO effectively ionizes some neutral molecules such as oligosaccharides, which are often difficult to ionize by conventional MALDI matrixes. However, use of carbon-based materials is not desirable for Time-of-Flight (TOF) MS since carbon nanomaterials stick to TOF electronics and, therefore, cause instability of the MS operation. In order to overcome this issue, GO-embedded sol-gel film substrates were developed for LDI/MS of small molecules. Results showed that the GO sol-gel film matrix substrate produced a much lower background noise than the aqueous GO particle matrix. This suggests that ablated carbon materials were significantly reduced when using the sol-gel film substrates. In addition, GO-embedded sol-gel film produced very clear and intense profiles of synthetic dye molecules in the region of m/z 100-800.

Graphene Oxide & Carbon Black, Laser Desorption/Ionization, Writing Ink Dye Analysis



Questioned Documents Section - 2015

J3 Frequency Occurrence of Handwriting and Hand Printing Characteristics

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After attending this presentation, attendees will understand the most current statistical data available for demonstrating the uniqueness of handwriting in general and specific features.

This presentation will impact the forensic science community by providing answers to some of the commonly asked questions in courts across the country regarding information on the statistical basis for the uniqueness of handwriting.

A National Institute of Justice (NIJ) -funded research project began at the University of Central Florida in 2010. The purpose of the project is to provide statistical data as to the uniqueness of numerous handwriting and hand printing characteristics. This project was initiated by a group of document examiners who noted several court rulings in which judges admitted the forensic document examiner as an expert but noted that they were troubled by the lack of statistical support for the uniqueness claims by these examiners. This group met informally and outlined a research project that would help answer those questions. Eventually, the NIJ provided funding for this project and a formal research project under the administrative umbrella of the National Center for Forensic Science (NCFS) began. NCFS is a forensic research laboratory at the University of Central Florida that was uniquely qualified to provide the support necessary for such a large-scale project.

A project team was brought together to include forensic document examiners, statisticians, and database experts. It was decided that this project must be a statistical project about handwriting as opposed to a handwriting project about statistics. As such, the statisticians were empowered to develop the methodologies used in this project and had ultimate say in any methodology decisions. It was made quite clear, as a group, that accepted standard methodology practices were to be followed and described in detail in the final product. The team developed a stratified population sampling goal for the collection of handwriting specimens and used a previously developed handwriting form for this project. Numerous collectors were engaged to collect handwriting specimens in a random-based method but with the stratified goals in mind. Very limited direction was given to the collectors for purposes of randomness.

The initial selection of handwriting and hand printing characteristics underwent strenuous testing to establish that answers were neither subjective nor open to personal interpretation. Only features that tested with 100% agreement in a pilot test were retained in the final database. Classifiers categorized the handwriting specimens which were collected and tallied by the database expert, then the handwriting specimens were submitted to the statisticians for analyses. This presentation will reveal the results of those analyses, including frequency-occurrence ratios and the issue of interdependency in handwriting.

Handwriting, Statistics, Frequency Occurrence



Questioned Documents Section - 2015

J4 Natural Variation in Modern Handwriting

Jane A. Lewis, MFS, 544 E Ogden Avenue, Ste 700-289, Milwaukee, WI 53202*

After attending this presentation, attendees will have learned about the extent of natural variation in modern handwritten correspondence.

This presentation will impact the forensic science community by developing an appreciation for the range of natural variation that forensic document examiners can expect to encounter in their casework today.

Motivated by changes in modern handwriting due to more keyboarding at the computer, less cursive handwriting taught in schools, and more hand printing in general use, a study was conducted to determine the extent of the range of natural variation in modern handwriting. Natural variation results from each person's departure from the copybook handwriting system that they were taught in school. Natural variation is one of the basic principles of handwriting identification. No one writes exactly the same way twice. Appreciation for the range of natural variation in questioned and known writings helps forensic document examiners correctly identify or eliminate writers of questioned documents.

Envelopes bearing handwritten names and addresses from ten writers were collected from 2011 to 2014. The number of envelopes from the ten writers varied from two to seven. Most writers had three handwritten envelopes each. Also, the handwriting of three siblings was intercompared for the range of natural variation and the significant differences separating them. The differences proved significant even though the siblings grew up in the same house and learned to write in the same grade school. Handwriting considered from each envelope consisted of a first and last name, city name, state abbreviation, and zip code numerals. The number of times each letter of the name and additional address material appeared in the envelopes written by each writer was recorded along with the range of variation of the forms of each letter or number evaluated. Each of the 25 characters evaluated for each writer was rated N for narrow, M for moderate, or W for wide range of variation. The sample letters examined for their range of natural variation in the envelope writings of each writer revealed that these modern writers maintained a narrow to moderate range of natural variation. Only a few examples of a wide range of variation were observed.

Forensic document examiners consider many more features than basic letter construction in their typical examinations and comparisons than those considered in this study. The goal was to make an efficient assessment of natural variation in modern handwriting using limited features.

The samples are representative of everyday handwriting; however, they are possibly of a more formal writing style since they were written for clarity so that each could be delivered via the United States Postal Service system. Examples of the types of letter forms in the handwritten addresses from the ten writers will be demonstrated. This study will help forensic document examiners appreciate the degree of natural variation found in modern correspondence.

Natural, Variation, Handwriting



Questioned Documents Section - 2015

J5 Different Writer or Alternate Writing Style — A Case Review

Charles L. Haywood, MFS, 15751 Sheridan Street, #208, Fort Lauderdale, FL 33331*

The goal of this presentation is to review fundamental concepts related to features/characteristics observed in handwriting which form the basis for identification or elimination of an individual as the writer of questioned or disputed handwriting.

This presentation will impact the forensic science community by attempting to offer clarity to fundamental concepts used in evaluating and comparing handwriting features/characteristics resulting in a more accurate determination of authorship or non-authorship of disputed/questioned handwriting.

The validity of handwriting identification as a forensic science has been under intense attack by academics and legal critics for the past 30 years. In response, the forensic document examination community embarked on unprecedented research which focused on establishing that handwriting can be individualized and that properly trained individuals can associate handwriting that meets certain criteria to a specific individual with significantly greater accuracy than an untrained person.

One of the axioms of forensic document examinations is that two properly trained Forensic Document Examiners (FDEs) examining and comparing the same evidence should reach the same conclusion; however, in practice, it is not uncommon for two FDEs to examine the same evidence and render opinions that are diametrically opposed to each other. In some cases, the conflicting opinions may be caused by somewhat ambiguous evidence, which may be characterized as limited writing, highly stylized writing (e.g., signatures), or disguised questioned and/or specimen writing. However, if the questioned writing consists of multiple entries with many repetitions of the same words, letters, and/or letter combinations and the observed features/characters are consistent throughout the documents, the ambiguousness is less of a factor. Thus, two qualified FDEs with adequate specimens should arrive at the same conclusion regarding authorship. A case is being presented where this did not occur. The conclusions reached by the two examiners were essentially in direct opposition to each other. It goes without saying that one of the examiners made an error. This study presents one examiner's court presentations, the court's summary of evidence presented, and the court's findings (verdict).

Similarity, Variation, Fundamental Difference



Questioned Documents Section - 2015

J6 Development of Individual Handwriting Characteristics in ~1,500 Students: Statistical Analysis and Likelihood Ratios That Emerge Over an Extended Period of Time

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The goal of this presentation is to inform attendees that by collecting writing samples from school-age students and measuring individual characteristics as they develop (as students begin to stray from the copybook style), a true statistical model can be produced that scientifically proves why forensic handwriting comparisons are possible.

This presentation will impact the forensic science community by establishing that collected handwriting characteristic measurements can be used to statistically show how individual handwriting characteristics develop and how each person's combinations of these individual handwriting characteristics develop his/her own individual handwriting style.

Forensic handwriting examinations are often an important part of criminal and civil investigations and court testimony. Cases involving handwriting may include threatening letters, bomb threats, check fraud, homicides, and controlled substances. The need for research in the pattern-recognition sciences has been emphasized for years in many court decisions, articles, and reports, including the National Academy of Sciences (NAS) Report, *Strengthening Forensic Science in the United States: A Path Forward*, published in February 2009.

The Minnesota Bureau of Criminal Apprehension (MNBCA) is conducting a study through a grant from the National Institute of Justice (NIJ) that will statistically analyze data from handwriting samples collected during the developmental stages of individual handwriting characteristics and well into adulthood. The MNBCA is currently gathering large amounts of handwriting and hand printing samples in order to measure individual handwriting characteristics (departures from the instructed copybook style) using a truthing tool from iFOX software developed at the State University of New York (SUNY), Buffalo, under the direction of Professor Sargur Srihari.

The first part of this study has already covered a three-year span with plans to continue collecting handwriting samples from the same students for up to 11 years in three-year increments.

Writing samples will continue to be collected every year from the same students, using the same words and phrases, from second grade through twelfth grade. The copybook style of writing is Zaner-Bloser, which is presently being taught in many schools across the United States.

Individual characteristics will be analyzed and measured as they develop over time, providing an extensive database of writing characteristics for each of the approximately 1,800+ students. This database size is instrumental in being able to run statistical time-model analyses that would lead to understanding many of the complexities involved during the act of writing. By collecting writing samples from school-age students and measuring individual characteristics as they develop (as the student begins to stray from the copybook style), a true statistical model can be produced that scientifically proves why forensic handwriting comparisons are possible. These measurements will be used to statistically show how individual handwriting characteristics develop and how each person's combinations of these individual handwriting characteristics develop their own individual handwriting style. It is these different combinations of individual handwriting characteristics that are the basis for the "theory" or explanation of why forensic handwriting comparisons are possible. This study will make it possible to support the "theory" with solid likelihood ratios that are scientifically accurate and reliable.

Handwriting, Development, Extended



Questioned Documents Section - 2015

J7 Development of a Supplemental Technique to Increase Visualization of Handwriting Indentations in Crumpled Documents With the Use of an Electrostatic Detection Device (EDD)

Kate Butler, BS, Florida Department of Law Enforcement, 1301 N Palafox Street, Pensacola, FL 32501*

After attending this presentation, attendees will consider using one of the supplemental techniques discussed in the presentation when presented with a crumpled or problematic document in which an indentation examination is necessary.

This presentation will impact the forensic science community by providing additional research in solving one of the common problems in EDD examinations.

Forensic Document Examiners (FDEs) receive questioned documents in a wide variety of conditions: chemically processed, folded, crumpled, wet, frozen, charred, wadded, or a combination thereof. Subsequently, upon receipt of a damaged document, FDEs are given the opportunity and, through much field research, the means to “unfold” the document’s secrets despite whatever state it may be in. Among the many examinations FDEs can employ, the application of an EDD is one of the key analyses performed on original questioned documents. These examinations have elicited superb information in the form of latent handwriting indentations as well as pattern impressions that may indicate a printing process which may otherwise have remained unseen; thus, the potential forensic information would not have been utilized. Crumpled or wadded documents have proven to be a problem in the area of EDD as the toner particles will typically adhere to the uneven and prominent creases and folds as opposed to the more subtle and delicate latent handwriting indentations or printing process impressions.

This research was designed to attempt to create a technique that would minimize the toner from adhering to the creases, thereby allowing the toner to potentially reach any of the latent indentations. The technique used two factors to attempt this: (1) increased humidity; and, (2) the application of a weight. These experimental factors were utilized separately and in combination on crumpled pieces of notebook paper containing latent handwriting impressions to determine their effects on an indentation examination. The crumpled papers were first examined with the EDD by applying the manufacturer’s recommended procedure, then re-examined with the EDD after applying increased humidity, pressure, or a combination of both. Images of the results of each indentation examination were captured with a digital camera for documentation. Once all of the experimental procedures were completed, the images obtained were examined to determine whether or not the visualization of handwriting indentations improved. A score was applied to both the “before” and “after” images based on specific criteria. Applying a higher humidity to the documents prior to EDD processing did allow for visualization of more latent handwriting indentations in the crumpled papers than if one had followed the manufacturer’s recommended procedure. A stretching technique was also attempted to see if this would cause any deleterious effects to the latent indentations. It was determined (using the guidelines followed in this research technique) that once a crumpled piece of paper had been exposed to increased humidity and then stretched by its edges, there was indeed an increased visualization of latent handwriting indentations with less interference from the creases and folds.

Indentation Recovery, Crumpled Document, Electrostatic Detection Device



Questioned Documents Section - 2015

J8 Cut-and-Paste Manipulation of a Quitclaim Deed

Farrell C. Shiver, MS, Shiver & Nelson Document, Investigation Laboratory, Inc, 1903 Lilac Ridge Drive, Woodstock, GA 30189*

After attending this presentation, attendees will have a greater awareness of how cut-and-paste manipulation may be used in the fabrication of a fraudulent document.

This presentation will impact the forensic science community by enhancing forensic document examiners' knowledge of how cut-and-paste techniques may be used in the fabrication of fraudulent documents and by providing a case example of an unusual document fabrication involving extensive use of cut-and-paste manipulation.

A quitclaim deed is a legal instrument that transfers one party's interest in real property (the grantor) to another party (the grantee). Quitclaim deeds are often used between family members to transfer property interests.

In early 2014, a father and son were involved in a civil suit over a piece of real estate. The father presented a copy of a quitclaim deed, allegedly signed by the son, granting the father interest in the property. The father also presented a copy of a second quitclaim deed which granted the son interest in the property. The father claimed the second deed was not genuine.

The son claimed the quitclaim deed presented by the father as genuine was actually fraudulent and that the document had been fabricated through a cut-and-paste manipulation of the genuine quitclaim deed. The son presented an original quitclaim deed for examination along with the copies of the documents presented by the father.

Subsequent examination of the documents produced by the son disclosed that the purportedly genuine quitclaim deed presented by the father had actually been produced by extensive cut-and-paste manipulation of a copy of the original quitclaim deed. A number of sections within a page and between pages were rearranged to change the father from the grantor to the grantee. The son was changed to the grantor rather than the grantee.

The cut-and-paste manipulation of the document was skillfully done. Due to the copy quality of the document, there was very little evidence of cut-and-paste on the document. Only one serious mistake was found. When the son's signature was transferred from the second page to the first page, a vestige of a short line was transferred with it. Other than this error, there was minimal internal evidence of the cut-and-paste manipulation on the document. Fortunately, the availability of the original document made the determination that the document had been fraudulently produced a relatively simple task.

Although it was not difficult to determine that the deed presented by the father was an altered version of the original, the difficulty would have been greatly increased had the original document not been available for examination. Without the original document for comparison, it would have been impossible to determine that a number of entries on the document were manipulated through cut-and-paste.

This presentation provides an example of the use of cut-and-paste techniques in an actual document and is also a cautionary tale for forensic document examiners.

Questioned Documents, Handwriting, Alterations



Questioned Documents Section - 2015

J9 Using Video Spectral Comparator (VSC) to Examine Documents Previously Subjected to Latent Print Examination

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The goal of this presentation is to explain the use of the VSC to possibly uncover evidence from documents that have been subjected to latent print examination prior to being examined by a document examiner.

This presentation will impact the forensic science community by providing attendees with a better understanding of the illustrative view of study done in order to obtain evidence using the VSC on chemically treated papers.

In order to prevent irreparable damage to a document by other disciplines in the course of processing, forensic document examiners often request that they have access to documents prior to them being submitted for other more invasive procedures. Several other forensic disciplines use potentially destructive methods; DNA, serology, and latent print examinations all use methods that can hinder a subsequent document examination. This presentation will focus on latent print evidence examinations prior to submission for document examination. It will further explore one way to counter negative effects and still complete an efficient examination.

A common process in latent prints is the use of ninhydrin for latent print development. The chemicals contained in ninhydrin are destructive to documents in several ways, one of which is that the development of indented writing is highly unlikely once the paper has been submerged in any liquid, including ninhydrin. The paper will absorb the liquid and “puff out,” destroying any indentations that may have been present. Another way latent print processing can hinder document examination is when latent prints are developed, the purplish hue of the ninhydrin dye can hinder the forensic document examiner’s view of intricate details of the writing, such as pen lifts and striations. Ninhydrin can also cause certain types of inks to bleed. This bleeding can cause staining of the text on both sides of the document and could prevent forensic document examiners from being able to effectively and efficiently examine the document.

This presentation will focus on the process used to develop latent prints on paper, with special attention given to ninhydrin and the effects it has on ink. There will be a demonstration of both controlled samples and evidentiary documents examined in the lab with an explanation of what occurred when they were examined under various settings of the VSC. The goal of this presentation is to explore what can be done to recover evidence from a document when a questioned document examination is an afterthought. It will further raise awareness of how to use the VSC to recover evidence that may not otherwise have been observable. The inspiration for this project stems from a case that was received in the lab, after the document had already been processed with ninhydrin. The ink had run severely and it was extremely hard to read what was previously written on the document. After placing the document under the VSC and using various settings, much of the original text was able to be read and intricate details were observed.

VSC, Latents, Documents



Questioned Documents Section - 2015

J10 Conductive Inks and the Electrostatic Detection of Indentations

Kevin P. Kulbacki, MSFS, Osborn & Son, 1273 Bound Brook Road, Ste 15, Middlesex, NJ 08846*

After attending this presentation, attendees will understand the unique factors associated with conductive inks. Attendees will also learn what impact conductive inks can have on the development of indentations with electrostatic detection devices.

This presentation will impact the forensic science community by providing information about the theoretical and actual implications associated with specialty writing instruments that utilize conductive inks.

For more than 30 years, forensic document examiners have used electrostatic detection devices to develop latent indentations in questioned documents. These devices allow forensic document examiners to deposit an electrical charge on the questioned document. Indentations that are present on the document have been shown to have a different charge density than the remainder of the document. Through the application of charged toner particles, the forensic document examiner can develop the indentations in a manner that results in a visual transparency of the indentations.

Conductive inks have been available for several years; however, they have not become popular among the general public. Early conductive inks suffered from the inefficient transporting of electrical currents, hours of required drying time, and the need for a stiff base to which they must be applied. Recently, manufacturers have developed efficient water-based conductive inks that are deposited by a ballpoint pen. These inks are intended as educational tools for students to learn how to create basic circuit diagrams on plain paper. Through application with an ordinary pen and the application of magnetic attachments, it is possible to create a working circuit encompassing switches, resistors, and lights on a piece of paper. This advance in technology could lead to the more widespread use of specialty writing instruments that use conductive inks.

Conductive inks pose unique theoretical implications for the forensic document examiner. The development of indentations with electrostatic detection devices relies upon detecting different charge densities on a questioned document. Conductive inks, unlike ordinary inks, are designed to attract a charge. It is therefore theoretically possible that the charge density of the questioned document may be affected in a manner that is adverse to the development of indentations.

This research is being conducted to develop a deeper understanding of conductive inks. Furthermore, it is the purpose of this research to establish to what degree conductive inks affect the development of indentations with electrostatic detection devices. While it is unlikely that conductive inks will become the subject of a questioned document investigation in the near future, this research will provide a greater understanding of factors that could affect the electrostatic detection of inks.

Conductive Ink, ESDA, Indentations



Questioned Documents Section - 2015

J11 Case Studies of Simulated Security Features in United States Identity Documents

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After attending this presentation, attendees will gain an understanding of current trends in simulated security features in plastic United States identity documents. Simulated laser engraving, embossing, and optically variable devices in the United States Border Crossing Card (BCC) and the United States Passport Card will be the focus of this presentation.

This presentation will impact the forensic science community by cultivating an awareness of how an identity document and its security features evolve as counterfeiters constantly find new ways to compromise current security features. The opportunity for most laboratories to study these advancements may be infrequent; therefore, this presentation will provide visual examples as well as methodologies for examinations leveraging a wide array of tools.

The United States BCC allows Mexican nationals to travel to and from the United States. The BCC cannot be used to gain employment or other benefits while in the United States. In order to obtain a BCC, an applicant must establish that they have ties to Mexico that would compel their return after visiting the United States.

The United States Passport Card permits United States citizens to travel between the United States, Canada, Mexico, the Caribbean, and Bermuda at land border crossings or sea ports of entry. This card may not be used for international air travel.

The United States BCC and United States Passport Card have been subject to countless attempts at unlawful replications and alterations over the years. With readily available access to digital printing methods such as thermal, inkjet, and laser printers, document mills have proliferated around the world. As a result, the United States government is constantly working to reinforce security of these documents to discourage and prevent those who seek to gain entry into the United States through unlawful means. Nevertheless, the Homeland Security Investigations Forensic Laboratory (HSI-FL) examines a multitude of counterfeit and altered United States BCCs and United States Passport Cards each year.

Plastic identity documents sometimes consist of polycarbonate layers receptive to laser engraving. These laser-receptive substrates react to the laser by altering the chemical composition of the card. This makes the removal of the laser-engraved data unlikely. Examples of altered United States BCCs and United States Passport Cards encountered in casework at the HSI-FL will be discussed in this presentation.

Security Features, Simulated, Laser Engraving



Questioned Documents Section - 2015

J12 Factors Affecting Electrostatic Detection Apparatus — 2 (ESDA2) Indented Writing Visualization

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After attending this presentation, attendees will gain a better understanding of factors affecting the ESDA2 visualization of indented writing, including paper type, the writing utensil used on the original document, relative humidity of the sample, and the ESDA2 toner development method.

This presentation will impact the forensic science community by providing a comprehensive guide containing the optimum conditions for recovering indentations on different paper types using the ESDA2. Document examiners will no longer waste time and resources nor will they potentially lose valuable evidence attempting to obtain the best conditions for visualizing indented documents through trial and error.

The ESDA2 develops indented impressions which are created when pressure is applied to the original document. Indentations on papers below the original are due to paper fibers breaking. This “invisible” writing provides valuable information about a case, missing person, etc., and even generates handwriting samples.

Past research has yielded poor results for coated paper, but the potential factors responsible for its poor performance have not been systematically examined.¹ Recycled paper is now commonly used but has not been investigated in previous studies. The goals of this study are to maximize the visibility of indentations with ESDA2 on bond, recycled ruled, glossy, and recycled bond paper controlling for three relative humidity levels (50%, 60%, and 70%) and to test three ESDA2 development techniques: Aerosol, Cascade, and Toner Application Device (TAD).

The relative humidity of indented documents prior to ESDA2 development has been examined in various studies.²⁻⁶ James’ and Noblett’s research determined that the best quality indentations were observed when documents were humidified between 40%-60%.³ Baier stated, the more coated the paper, the more time it takes to humidify the paper to obtain a clear visualization of the indented writing with the ESDA2.² Indented documents’ relative humidity in the ESDA2 development is important. This research will determine the best humidity level for different paper types.

The development method is an additional factor to consider when examining ESDA2-developed indented documents. Research has been conducted on the Aerosol and Cascade Methods.^{3,4} Baier concluded, Aerosol works best on the top sheet (sheet directly below original document) and when the indentation is clear, in contrast, Cascade was more efficient than Aerosol at developing weaker indentations.² James and Noblett determined Aerosol produced a higher quality score than Cascade, independent of paper type or amount of pressure applied.³ Past research on ESDA2 toner-development methods have produced conflicting results. No research has been performed on the efficiency of the TAD technique in developing indented documents. This research will determine which ESDA2 development method produces the clearest visualization of indentations on different paper types.

A total of 1,080 indented documents were prepared using three writing utensils (ballpoint pen, gel pen, and mechanical pencil) on four paper types (bond, recycled ruled, recycled bond, and glossy). The samples were divided into subsamples of 30 and each subsample was subjected to different humidity levels (50%, 60%, or 70%). Each document was placed within the ESDA2 capacitor and charged. One toner development method (Cascade, Aerosol, or TAD) was applied per document (360 samples for each toner development method). A quality score from zero (no words visible) to four (all words and small details visible) was recorded. The Kruskal-Wallis test followed by Wilcoxon Signed-Rank Test with a Bonferroni Correction was applied to the data. The samples composed with the ballpoint pen had the highest quality score ($p = 1.12 \times 10^{-6}$). It is recommended that ESDA2 control indentations are composed with ballpoint pens for the original sheet. The optimum conditions for the paper types were: (1) bond paper (70% and Aerosol ($p < 4.00 \times 10^{-8}$)); (2) recycled ruled (60% and Aerosol ($p < 1.32 \times 10^{-3}$)); (3) recycled bond (60% and Aerosol ($p < 5.60 \times 10^{-3}$)); and, (4) glossy (60% and Aerosol ($p < 6.30 \times 10^{-5}$)). Based on this study, ESDA2 visualized indentations will be the clearest when recycled ruled, recycled bond, and glossy paper are humidified at 60%, while bond should be humidified at 70%; the Aerosol development method should then be applied across all paper types.



Questioned Documents Section - 2015

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 4. Foster DJ, Morantz DJ. An electrostatic imaging technique for the detection of indented impressions in documents. *Forensic Science International* 1999; 13:51-4.
 5. Cavanaugh Jr. MH, Grose WP, Torres BL. ESDA cascade developer awareness. *Journal of the American Society of Questioned Document Examiners, Technical Note* 1999; 102-3.
 6. Ellen DM, Foster DJ, Morantz DJ. The Use of Electrostatic Imaging in the Detection of Indented Impressions. *Forensic Science International* 1980; 15:53-60.
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ESDA2, Indented Writing, Questioned Documents



Questioned Documents Section - 2015

J13 Analysis Techniques of Plastic Identity Documents

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After attending this presentation, attendees will gain a better understanding of new trends in plastic Identity Documents (IDs) and analysis methods by which to visualize and classify the materials used in their production.

This presentation will impact the forensic science community by: (1) increasing awareness of plastic IDs and of non-destructive/destructive analysis testing methodologies as a means of counterfeit deterrence and adversarial analysis; and, (2) identifying and highlighting tamper-resistant and copy-proof security features and printing incorporated within contemporary plastic IDs.

IDs are produced for and used by people around the world for a variety of different purposes, including proof of identity, employment eligibility, evidence of driving privileges, and domestic and international travel. IDs function as the link between an individual's government-verified biographical data (including, for example, date of birth, nationality, and sex) and the individual himself. These documents must be inherently secure and tamper evident to maintain public confidence and trust.

IDs can be manufactured in many different ways with a variety of types of plastic, including polycarbonate, Poly-Vinyl Chloride (PVC), Polyethylene Terephthalate (PET), or a combination of these and others. These plastics are combined with heat, pressure, and/or adhesives to create an overall card body that guards against tampering or alteration. In addition, security inks, Optically Variable Devices (OVDs), and Radio Frequency Identification (RFID) devices are incorporated within plastic documents as a deterrent against counterfeiting because they are difficult to replicate successfully. In addition, commercial availability of similar products and the knowledge of translating these products into counterfeit IDs are imperative for the manufacturer and designer to contemplate before placing a security feature into a document. Security printing is another important consideration in the manufacture of plastic identity cards; this includes personalization techniques such as laser engraving that discourage counterfeiters from replicating or compromising these methods.

Sophisticated non-destructive and destructive techniques that may be utilized to determine whether or not an ID is genuine will be explored. Non-destructive techniques include digital microscopy, which may be used to: (1) visualize plastic identity cards' security features from a 3D, depth-up perspective; (2) measure the relative heights of these features; and, (3) even classify the internal card body structure. Fourier Transform Infrared Spectroscopy (FTIS) is another non-destructive technique that allows the chemical composition of a variety of plastic substrates to be elucidated quickly and efficiently. A destructive technique that has been utilized at the laboratory in counterfeit deterrence analysis is card cross sectioning, which is achieved by cutting the card at a predetermined point of interest, mounting the card in an epoxy-resin mixture, polishing, and analyzing it with the Scanning Electron Microscope (SEM). Elemental techniques such as Energy-Dispersive X-Ray Spectroscopy (EDS) may then be employed to probe specific areas within the card body, such as the RFID, to ascertain an accurate elemental composition that was used in the card body construction.

Plastic, Identity Documents, Counterfeit Deterrence



Questioned Documents Section - 2015

J14 Are the Principles of Forensic Signature Identification Corroborated by an Analysis of Digitally Captured Biometric Data?

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After attending this presentation, attendees will have learned the results of a study of biometric signature data of both authentic and non-authentic signatures. Analysis of this data is being conducted to determine if the tenets of handwriting identification, derived from more than 100 years of study, observation, and experience, can be supported quantifiably by biometric data collected during signature execution.

This presentation will impact the forensic science community by providing objective evidence regarding signature characteristics such as speed, rhythm, and natural variation, characteristics forensic document examiners consider when determining a signature's authenticity. Although a vast body of literature exists stating speed and rhythm are significant indicators of genuineness or lack thereof, the advent of digital signature pads provides an opportunity to compare the quantifiable data of these characteristics in both genuine and non-genuine signatures. Biometric data also allows for analysis of the signatures from one writer to determine the presence and extent of natural variation.

Data from over 100 digitally captured signatures were studied to prove or disprove the following hypotheses: (1) there will be quantifiable differences between signatures written naturally and unnaturally (simulated or traced); (2) there will be quantifiable differences in signatures executed by different writers; and, (3) there will be quantifiable differences in naturally executed signatures of one writer.

Signatures were collected using a Topaz® signature pad. A piece of paper was placed on the pad and subjects were asked to sign multiple times using a pen stylus. This allowed for the simultaneous collection of both wet-ink and digital signatures. Subjects were asked to provide multiple samples of genuine signatures, simulated signatures, and traced signatures.

Data from naturally and unnaturally executed signatures are compared. Both intra- and inter-writer comparisons are also conducted. This presentation will analyze the results of this study.

Biometric Signature, Natural Variation, Handwriting Principle



Questioned Documents Section - 2015

J15 The Universal Coded Character Set (ISO/IEC 10464) and the Unicode Standard as Aids to Forensic Document Examination in an International and Transnational Environment

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The goal of this presentation is to provide attendees with an awareness of developed standards, their potential benefit, and how to acquire and use them.

This presentation will impact the forensic science community by increasing awareness of, and the ability to deal with, non-English material.

Generations of American forensic document examiners have been well aware of the writing habits that successive waves of immigration brought to our shores and how these habits might carry over to the writing of these immigrants and even their descendants. These factors are still relevant today, but given the globalization of commerce, crime, and terrorism as well as the nature of contemporary internet society and social media, the 21st-century examiner can be confronted with documents from any part of the planet, perhaps in languages that use the Latin alphabet but with characters not seen in English, or even in languages with variants of completely different scripts. Such problems can arise in civil actions over a contract or a will; in criminal cases involving smuggled goods, drug records, or human trafficking; or in the context of a civil disaster like a train accident or airplane crash.

Signatures, handwritten text, or printed matter could be involved. What might seem to be an illegible, highly stylized, and personalized symbolic signature could actually be the completely legible and unremarkable writing of a name in an unfamiliar script. While it is sometimes appropriate to undertake the examination of a foreign text with the aid of a translator, the language must first be determined before the appropriate translator can be sought. A document examiner who can use a limited amount of “weird or foreign” text to provide a stymied investigator with information about the language of contraband packaging, the phone numbers in an address book, the message on a smartphone screen, or the charred remains of a receipt or newspaper clipping in a victim’s pocket might make a truly significant contribution to the resolution of the case.

The goal of this presentation is to familiarize examiners with the **International Organization for Standardization/International Electrotechnical Commission (ISO/IEC) 10464**, the Universal Coded Character Set (UCS), and the Unicode Standard. These are standards developed for data exchange that provide unique names and code identifiers for the characters of the world’s written languages (including some long dead) as well as for many additional symbols.^{1,2} The responsible ISO Working Group has coordinated with the Unicode Consortium for more than 20 years and the two standards are essentially compatible, though Unicode includes much more information and some of the additional features are required for full implementation.

These standards provide internationally accepted naming conventions for characters and symbols, including diacritics, accented characters, digraphs, and new forms that extended the Latin alphabet for use with diverse languages worldwide. Searching the extensive Unicode Code Charts can provide useful leads regarding the language or language group using one of these modified characters or a combination of characters. Potentially useful information can also be found for languages using versions of the Cyrillic or Arabic alphabets and other writing systems.

Specific illustrations and internet search strategies will be provided.

References:

1. ISO/IEC 10646: Information Technology—Universal Coded Character Set (UCS). Geneva: International Organization for Standardization, 2012.
2. The Unicode Standard, Version 7.0.0. Mountain View, CA: The Unicode Consortium, 2014.

Unicode, Alphabets, Diacritics



Questioned Documents Section - 2015

J16 Collaborative Radiography (and Other Interdisciplinary Activities) Between Document Analysts and Forensic Anthropologists at the FBI Laboratory

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After attending this presentation, attendees will understand some of the myriad uses of radiography in forensic science, including ways the same technology can be used for different forensic applications within a laboratory.

This presentation will impact the forensic science community by highlighting ways in which different forensic disciplines (specifically, questioned documents and anthropology) can work together to achieve results.

Since the discovery of X-rays in 1895, radiography has become a routine means of non-invasive examination of internal structures and composition of various objects and materials. Wilhelm Conrad Roentgen, who first discovered X-rays, further discovered that X-rays would penetrate most solid matter, but leave shadows of denser materials including bones and metal. Radiography first became widely used in medicine and dentistry, but other industrial uses followed in the early 1900s. Radiography is currently used in various industries including engineering, automobile safety, and food safety, among others. Of particular interest is the fact that radiography is also widely utilized in a variety of forensic science applications. Although often associated with medical imaging and diagnostics of human bodies by forensic radiologists and pathologists, radiography has many other forensic uses. Forensic anthropologists commonly use radiography in the examination of skeletal remains. Radiography is also used in forensic metallurgical examinations to reveal interior construction or the presence of defects, cavities, or foreign material within an object. It can be used to examine the internal components of explosive devices and to reveal the contents of envelopes, bags, and packages without having to physically open them.

In the interest of fiscal responsibility, equipment-sharing between various units at the Federal Bureau of Investigation (FBI) Laboratory is encouraged when possible. One seemingly unlikely, but ultimately highly successful, partnership recently developed between the Questioned Documents Unit and the Forensic Anthropology Program due to their shared forensic radiography needs. Radiography is used in the FBI Laboratory for document examination to view watermarks, detect concealed communications, view/confirm identity document security features, or determine whether collectors' items such as autographed baseballs have markings or construction details that support or refute their authenticity. In anthropological examinations, radiography is used to detect and locate foreign materials such as bullets and dental restorations, to assess skeletal trauma, and to estimate biological parameters such as sex and age. While many radiography units may be best suited to a specific medium of study, one system was found to serve both units very effectively. The system is a cabinet-style, self-contained unit constructed on a mobile platform. It utilizes a high-resolution X-ray source with an energy range of 10-130Kv, with available detector sizes ranging from 2"x2" to 17"x17". User-friendly touch-screen controls allow for post-processing enhancements such as contrast adjustments that allow examiners to further exploit areas of interest. Being a self-contained and self-shielded unit, no additional shielding (either in the location of use or for personnel operating the machine) is required. Digital radiographic images can be easily saved in a variety of formats.

This presentation will provide a pictographic overview of various uses of this radiography technology in the FBI laboratory. In addition, other previous and potential collaborations between document analysts and anthropologists within the FBI Laboratory will be discussed.

Forensic Radiography, Questioned Documents, Anthropology



Questioned Documents Section - 2015

J17 Characterization and Discrimination of Inkjet Printer Inks Using Micro-Raman Spectroscopy

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After attending this presentation, attendees will understand the potential of micro-Raman spectroscopy to detect *in situ* chemical information from micrometric-colored spots of inkjet-printed documents from different sources.

This presentation will impact the forensic science community, with emphasis toward questioned document examiners, by exploring the chemical properties of inkjet printer inks, particularly non-extractable pigment-based samples. An example involving the counterfeiting of banknotes will be developed.

Inkjet printers are ubiquitous common devices used in various everyday activities. Due to their relative low cost and accessibility, it is not surprising that inkjet-printed documents are used for different illicit activities, including the production of counterfeited banknotes.

The printing process involves the production of a constellation of micrometric colored spots. This form of evidence makes Raman spectroscopy a suitable method for their rapid *in situ* detection, thus avoiding any extraction procedure. The Raman technique detects the phenomenon of light scattering from samples that are excited with an intense irradiation source, such as a laser. Colorants (i.e., dyes and pigments) are known to be strong Raman scatterers. While micro-Raman spectroscopy has only recently gained interest in forensic laboratories, this technique has already demonstrated its potential to be a useful method for ink analysis, especially in cases where ink extractions are not successful (i.e., pigment-based inks such as gels).

The goal of this research effort is to implement a non-destructive and relatively rapid analytical procedure for the *in situ* detection of microscopic ink spots produced by colored inkjet printers in order to help identify a (list of) printer source(s) from unknown printed specimens. For this research, the micro-Raman spectrometer is utilized to determine whether inkjet printer inks from different brands and models can be differentiated by means of their detected chemical profile. The inter-source variation of the obtained chemical profiles is studied by considering Raman data from cyan, magenta, and yellow spots. The approach consists of evaluating what colors are the most discriminating ones and if there are dependencies between these three variables, given a particular brand and model.

As a preliminary step for this project, 135 Raman spectra were captured from nine inkjet printer ink samples as well as from a counterfeited banknote, which was obtained from the collection of the Criminal Investigative Division, Treasury Obligations Section of the United States Secret Service. Extraction with methanol was carried out on all ink samples to determine if the ink was dye-based or pigment-based. Extractions were conducted on samples representing the three individual colors separately. Then, all samples were processed *in situ* on a printed document using a Near-Infrared (NIR) laser wavelength at 785nm. Cyan, magenta, and yellow spots were focused for each sample using a 50x objective lens. Five replicates per sample were obtained to verify the repeatability of the spectra. The resulting spectra were pre-processed via baseline correction, normalization, Multiplicative Scatter Correction (MSC), and Standard Normal Variate (SNV) transformation. After pre-processing, identifications of the colorants used in the ink were attempted by comparing them to a house-made reference spectral library. Data analysis of the Raman spectra was conducted using Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) in order to explore the inter-variability of the ink colors. Linear Discriminant Analysis (LDA) was carried out on the counterfeit samples to implement the structure of a system that, once a representative number of spectra is obtained, will classify the spectra with the purpose of inferring the potential brand(s) and model(s) of the inkjet printer(s) based on similar Raman profiles.

Questioned Documents, Raman Spectroscopy, Inkjet Printer



Questioned Documents Section - 2015

J18 A Novel Automated, Searchable Database for the Chemical Characterization and Comparison of Printing Inks

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After attending this presentation, attendees will be informed about a new database that was created for the automated search and comparison of printing ink evidence. The database contains spectra from the following six analytical methods using standardized acquisition parameters for each method: (1) Attenuated Total Reflectance Fourier Transform Infrared (ATR/FTIR) spectroscopy; (2) Raman spectroscopy; (3) Scanning Electron Microscopy with Energy Dispersive Spectroscopy (SEM/EDS); (4) Pyrolysis Gas Chromatography/Mass Spectrometry (Py-GC/MS); (5) nanosecond Laser Ablation Inductively Coupled Plasma/Mass Spectrometry (ns-LA-ICP/MS); and, (6) Direct Analysis in Real Time Mass Spectrometry (DART[®]-MS). The relative discrimination and overall performance of the database search routine, including error rates (false associations and incorrect discrimination), are reported for the first time for each of the six analytical methods that make up the chemical database as well as for combinations of the data (data fusion). Attendees will gain knowledge on the relative utility and performance of these analytical methods for the examination of different types of printing inks.

This presentation will impact the forensic science community by providing a comprehensive evaluation of the performance of a searchable database that was designed and validated as a tool to augment the chemical information mined from ink evidence and to aid in document examinations and related investigations. The software will become commercially available for use and it is expected that the database will expand with the number of users, making the database increasingly useful through the continuous feedback from examiners.

The database contains two main sets of data: the reference database and the training or testing database. The reference database currently contains 4,658 data files acquired from representative samples from selected printing sources to account for some of the global variety in printers, including 319 toner, inkjet, offset, and intaglio inks. The testing database contains over 800 data files acquired from 45 duplicate control samples used to train the searching algorithms. Duplicate controls were analyzed over different days to evaluate correct associations and to assess the potential for instrumental variation, operator variation, and within-sample heterogeneity.

The developed software makes use of machine-learning algorithms for classification and comparison of unknown samples to the database collection, in particular Partial-Least Squares Discriminant Analysis (PLSDA) and K-Nearest Neighbor (KNN) spectral comparisons. The search algorithms generate similarity scores that permit the operator to significantly narrow down the possible sources of ink samples contained within the database. The user may select to use any number of methods depending on the case, type of ink, sample size, or other factors. Data fusion algorithms were also built into the database to allow for data fusion from two of any of the analytical techniques, often providing improved performance for fused data searches over searches using any technique in isolation. The relative discrimination of all the analytical methods has been determined for each ink type. LA-ICP/MS provides the best discrimination (>99%) and py-GC/MS provides the least information, regardless of ink type. The discrimination capability of SEM/EDS was relatively poor for inkjets and offsets but was found to be good for toner and intaglio samples, with discriminations of 97.2% and 98.2%, respectively. Although FTIR and Raman provided lower discrimination capabilities, they are useful for characterization of major organic components and classification or grouping of inks.

A validation study of the database search algorithms, using blind searches of duplicate controls, reveals that LA-ICP/MS and FTIR data searches result in the best performance for associating duplicate samples analyzed by different operators on different days with >90%-100% of duplicate pairs correctly associated. DART[®]-MS data search results in 83%-100% of the duplicate pairs correctly associated, with the other analytical techniques resulting in 33%-100% correct associations for SEM/EDS and 6%-81% for Raman, respectively. The lower correct association performance is often related with a limited number of duplicate controls available in the current testing database for particular sensors and/or particular limitations of certain techniques for some types of ink.



Questioned Documents Section - 2015

Although the current collection set represents a relative small snapshot of the printing inks universe, it serves as a proof of principle of its potential utility and relevance in the field. This novel chemical database now provides a comprehensive inorganic and organic characterization of different printing inks and permits data fusion of the multiple sensors.

Printing Inks, Database, Data Fusion



Questioned Documents Section - 2015

J19 Computer-Scored Test Answer Marks, A.K.A. Bubble Marks: How Individual Are They?

Janet F. Masson, BJ, 908 Town & Country Boulevard, Ste 120, Houston, TX 77024*

After attending this presentation, attendees will better understand what can and cannot be determined from the examination of bubble marks. Attendees will also learn how to make an examination of such marks.

This presentation will impact the forensic science community by presenting the results of research about examinations of marks not previously studied but coming under scrutiny in recent lawsuits.

Over the years, many document examiners have inspected standardized tests on which handwritten answer marks are used. In these cases, there is commonly a concern about whether cheating has occurred. This cheating can take many forms, including, but not limited to: (1) the use of impersonators (a student sending in a proxy or impersonator to take the exam on his/her behalf); (2) swapping of tests and answer sheets by two persons in the same room; or, (3) making after-the-fact changes to specific answers or completing unanswered blanks on an answer sheet in order to increase a student's or school's score. In fact, a survey of newspaper articles written over the last few years reveals numerous reports of alleged large-scale cheating on standardized tests across the country.

Standardized answer sheets are used for obtaining information in a format that allows answers to be counted or scored electronically. While such forms are often used to obtain answers on standardized tests, they are also sometimes used for surveys and ballots. The pencil marks made to indicate answers or choices on these forms are called grid marks, bubble marks, or simply answer marks.

Typically, when a document examiner receives one or more standardized answer sheets for examination, the document contains at least some general handwriting, possibly one or more signatures, and often handwritten numeric calculations. Examination of this writing is a typical, straightforward process discussed in many textbooks on document examination and covered by relevant standards in the field. Furthermore, considerable research has been conducted regarding identification of signatures, hand printing, and numerals. Yet sometimes the questions about a test answer sheet, survey, or ballot can only be resolved by the evidence in the answer marks themselves.

The literature on document examination contains little if any discussion of the limited subject of handwritten answer marks on standardized tests as well as those on ballots and surveys. This project was designed to study these answer marks or "bubble marks" from the perspective of the forensic document examiner.

In this project, samples of answer marks were obtained from more than 100 participants. The questions studied were: (1) how answer marks should be examined; (2) whether any or all writers have a consistency in making answer marks; (3) whether answer marks have individuality; (4) what conditions need to exist in order to reach a meaningful opinion about these marks; and, (5) what opinions can be reached from the evidence found in answer marks.

Handwriting, Bubble Marks, Standardized Test Answer Marks



Questioned Documents Section - 2015

J20 Another Look at Ink and Toner Intersections: A Word of Caution

Laura A. Mancebo, BS, Applied Forensics, LLC, 1975 Hempstead Turnpike, Ste 407, East Meadow, NY 11554; and Dennis J. Ryan, MBA, 1975 Hempstead Turnpike, Ste 407, East Meadow, NY 11554*

After attending this presentation, attendees will understand how to conduct an examination of the intersection of toner and ink on a paper substrate. Attendees will understand techniques that will assist in the intersection examination. Attendees will also understand that caution is needed when drawing conclusions from the examination of the intersection of the toner and ink.

This presentation will impact the forensic science community by serving as a “wake-up” call when drawing conclusions from the examination of ink and toner intersections. This presentation will impact examiners by making them more cautious when drawing conclusions in this type of examination.

Factors considered in an ink/toner intersection problem include the writing instrument, the paper substrate, and the writing surface. Different writing instruments will be discussed, including ballpoint, roller ball, gel pens, and felt tip/porous point pens and their effect on drawing any conclusions.

Is spectral reflectance from the ink a critical factor in arriving at a conclusion? Can spectral reflectance dissipate over an extended period of time, thus inhibiting the examination? Is the spectral reflectance phenomenon a critical factor in drawing any conclusions?

Other facts that are considered are the paper substrate and its effect on the ink/toner examination. Does that substrate affect the possibility of making any determination? A look at the effect of the writing surface is also a factor to be considered in this type of examination. This presentation will look at the factors needed to make a definitive conclusion as to the sequence of the ink over toner or toner over ink. Examiners may be “tricked” by the observations they make. Can the luminescent properties in the ink assist in making the sequence determination?

These types of examinations are critical in cases where a document has been called into question as to whether an addition has been made to the document after it has been signed. If there is no toner/ink intersection, it is very difficult to make any determination. If the toner and ink has intersected, some conclusions may be drawn as to which came first, the ink or the toner. In most cases, where the toner lies over the ink, this may be a significant factor in determining if the document has been altered. If the toner shows evidence of being crushed by the trough of the pen, this may be evidence of the toner being present when the document was signed.

In conclusion, this presentation will look at what evidence should be present to make any definitive conclusion and whether spectral reflectance and any presence of luminescent properties in the ink are needed to make any determination.

Intersections, Sequencing, Toner



Questioned Documents Section - 2015

J21 The Origin of a Tracing

Donald D. Moryan, BS*, 4930 N 31st Street, Forest Park, GA 30297

After attending this presentation, attendees will have a better understanding of how signature tracings are created.

This presentation will impact the forensic science community by showing examination techniques involving traced signatures.

This presentation details a case study regarding a life insurance document in which the beneficiary information was allegedly changed. The authenticity of the principle signature was in question. Examination revealed the signature was spurious and possibly a simulation or a tracing of a genuine signature. At first glance, the questioned signature appeared to be spurious. It contained many features associated with a simulation/tracing of a genuine signature. Unnatural movement and the apparent lack of speed in writing was present. The signature also was of poor line quality and contained pen lifts. Blunt beginning and ending strokes were present as well. Further inspection revealed the questioned signature was pictorially similar to the victim's standard signatures that were provided. The signatures were so similar that detailed scrutiny of each known signature was warranted to ascertain if indeed one of them was used as a possible model for the questioned signature.

The examination revealed that one known signature and the questioned signature looked remarkably alike. Microscopic examination of the questioned signature did not reveal any of the typical signs of a tracing, such as pencil or carbon remnants nor any indented lines. Also, examination of the original standard signature did not reveal any such evidence. Overlaying the questioned and known signatures proved the questioned signature was a tracing of the known signature. Examination of one of the known documents with oblique lighting techniques revealed indented lines adjacent to letters of the first name. These indentations corresponded exactly to the questioned signature. This proved the known signature was used as the model for the questioned signature. The tracing was undoubtedly made with the use of transmitted light. This would account for the stray indented lines on the known document. Conclusions that can be drawn from this examination are: (1) the questioned signature is a tracing; and, (2) the signature appearing on the bottom of the copied emergency data record was the model used to make the questioned signature.

Determining authorship was another matter. Traced signatures contain no evidence of their writer as the following of a written line has nothing to do with natural writing. They do not contain handwriting characteristics of their maker and therefore are not identifiable. Therefore, in this case, no opinion could be made on the maker of the traced signature. Even if no conclusion regarding authorship could be made, the discovery of the model signature is very important, even though forgery can be proven without locating the model signature.

Tracing, Simulation, Model Signature



Questioned Documents Section - 2015

J22 Document Examination For Non-Document Examiners

Gregory A. McNally, BS, 20 Whispering Sands Drive, Apt 501, Sarasota, FL 34242-1667*

After attending this presentation, attendees will be able to give presentations to non-document examiners that engage their attention by presenting an interesting selection of document examinations and by interjecting experiences and anecdotes of unusual and many-times entertaining situations which have occurred.

This presentation will impact the forensic science community by allowing the document examination community to inform the public, in an interesting manner, of analyses which are performed. This may also open the door to additional requests for document examination services.

Everyone has sat through their share of boring presentations. It's mind-numbing to be forced to listen to dry content that's delivered (or worse, read) at one, rather than discussed with one. Often, all this talking is in vain. Research has shown 91% of listeners at presentations admitted to daydreaming and an impressive 39% admitted to actually falling asleep at some point.

This presentation will demonstrate how to inform the audience that you encourage their questions, that you expect interaction, the types of questions you'll entertain, and when these questions should be presented. Also discussed will be when to present from the stage and when to intermingle with the audience as you present. During the presentation, discuss real-life situations surrounding document examinations. This would include circumstances which may have occurred prior to being retained, during the examination and testimony, and additional information that transpired after the matter was resolved. Some examples in this presentation consist of cases including Leona M. Helmsley, the Andy Warhol estate, and the "handwriting on the bathroom stall." These often-humorous anecdotes have previously entertained audiences.

Hopefully, at the conclusion of this presentation, attendees will be aware of tactics that not only inform their future audiences about document examiners' duties but are also entertaining, thus making their presentations memorable experiences.

Document Examination Review, Non-Document Examiners, Entertaining



Questioned Documents Section - 2015

J23 Addressing Admissibility Challenges to Forensic Document Examination From Both a Federal and a State Examiner's Perspective

Carl R. McClary, BA*, 2600 Century Parkway, Ste 410, Atlanta, GA 30345; and Karen J. Nobles, BA*, Forensic Document Examinations, PO Box 411, Pensacola, FL 32591-0411

After attending this presentation, attendees will have gained an understanding of the discovery requirements of witnesses in federal criminal or civil cases with respect to Rules 16 and 26. Examples of required information will be given in addition to material that can be helpful in thwarting an admissibility challenge. *Daubert* and *Frye* states will be identified and recent admissibility rulings will be discussed.

This presentation will impact the forensic science community by providing samples of discipline-specific information that can be appended to required material to preclude an admissibility challenge. This information will also assist in educating the legal community on the questioned documents discipline's recent history of challenges and provide the basis for how the discipline meets the factors found in *Daubert*.

The questioned documents discipline was the focus of many reliability challenges in the mid- to late-1990s and early 2000s. Those same challenges are still being utilized by defendants, although to a lesser extent, even though Federal Appeals Courts in every circuit have ruled in favor of admitting this type of testimony. The presentation will address the latest and most popular wording in motions that have been filed in the hopes of challenging some of these appellate rulings by creating new areas of doubt. It will provide guidance in the prevention of these challenges through preliminary discovery filings as well as providing material for formal responses to those motions that have already been received. Recent rulings will also be given to include, one in the state of Florida which adopted the *Daubert* standard for admissibility in 2013. Language from the ruling in this case, which is the first challenge of this type since the adoption of the standard in Florida, will be discussed including that court's observance that the *Daubert* standard was intended to be more flexible than the *Frye* standard with respect to scientific testimony. Although not required, other states have adopted the *Daubert* standard in recent years and a current list of those will be presented.

This study will present useful tools for potential witnesses to preclude a challenge, including material in Discovery and Inspection Rule 16 that requires copies of written reports and a written summary of any testimony that the government or the defendant intends to present under Federal Evidence Rules 702, 703, or 705. Rule 26 of the Federal Rules of Civil Procedure, General Provisions Regarding Discovery, will also be explained and the differences between the two highlighted. Every witness must be familiar with these rules and other requirements in these rules such as the witness's qualifications and any visual aids that are intended to be used at trial. Too often, prosecutors and witnesses alike fail to provide this information in discovery. An example of an expanded Rule 16, which provides more-than-required discovery material for the opinions and the methodology used, will be offered. Often, the education of opposing counsel as to how the witness intends to address and explain admissibility factors can be enough to preclude challenges of this type. One of the most difficult tasks facing the examiner is to ensure that their counsel is not only educated on the history of these challenges, but also understands the importance of preparation and comprehensive written responses.

Questioned Documents, Admissibility, Rule 16 and Rule 26



Questioned Documents Section - 2015

J24 The American Dreyfus Affair: Parallels Between the Alfred Dreyfus and the Leo Frank Matters in America

Arthur T. Anthony, BS, Forensic Document Examiner, PO Box 620420, Atlanta, GA 30362*

After attending this presentation, attendees will learn about the historical aspects of a notorious murder case that took place in Atlanta, GA, in 1913 and the involvement of questioned documents.

This presentation will impact the forensic science community by discussing how forensic science and the judicial system has evolved over the past 100 years.

In 1913, Mary Phagan, a teenage worker at the National Pencil Factory in Atlanta, GA, was found murdered in the basement of the factory. A handwritten note, in two pieces, was left at the murder scene and was the initial focal point of the investigation. This discussion will highlight the ensuing investigation by the Atlanta Police Department, the Pinkerton Detective Agency, and the defense team for Leo Frank, the factory superintendent who was ultimately charged with Phagan's murder.

Leo Frank, of Jewish descent, was born in Texas but, as a young man, moved to New York City with his family. He ultimately graduated from Cornell University with a degree in Mechanical Engineering. His uncle and two investors owned the National Pencil Factory in Atlanta, GA, and needed someone to take over its daily operation. They convinced Frank to travel to Germany to learn the pencil manufacturing process before taking charge of the factory. He spent approximately a year in Germany learning the business and, after returning to the United States, took over supervision of the Atlanta factory. Frank became a prominent member of the Atlanta Jewish community, at that time the largest in the American southeast.

Leo Frank ultimately became the focus in the murder investigation of Mary Phagan. He was tried and convicted of Phagan's murder and sentenced to death. The governor of Georgia commuted his sentence to life in prison hoping that Frank would be exonerated. Not satisfied with this, a vigilante mob dragged Leo Frank from the prison and took him to a remote area where he was hung. Some news articles of the time termed this tragedy as the American Dreyfus Affair. The parallels are similar as to the anti-Semitic aspects against both the principals, Alfred Dreyfus and Leo Frank, and their hatred by the public.

Questioned Documents, Murder, Anti-Semitism



Questioned Documents Section - 2015

J25 Standardization of Conclusions: Meeting Current Needs

Thomas W. Vastrick, BS, 522 S Hunt Club Boulevard, Ste 217, Apopka, FL 32803*

After attending this presentation, attendees will have a better understanding of how current activities may require modification to standards.

This presentation will impact the forensic science community by bringing attention to the need to address the schism between scientific language and legalese. In addition, this presentation will propose standard modifications in keeping with the National Academy of Sciences (NAS) Report, *Strengthening Forensic Science in the United States: A Path Forward*.

Forensic document examination has utilized standardization of conclusions for many years now. Many would harken back to a popular paper authored by Thomas McAlexander, Jan Beck, and Ron Dick that provided the outline that was adopted by **Scientific Working Group for Forensic Document Examination (SWGDOC)** and eventually became the American Society for Testing and Materials (ASTM) Standard E-1658 which was not only referenced but quoted verbatim in the NAS Report. Subsequent to the aforementioned NAS Report, there have been no significant changes to the standardization of conclusions; this does not mean that we necessarily have to change anything. However, this presentation brings one examiner's thoughts for limited modification to the table for discussion and debate. Prompters to these ideas have been the NAS Report, discussions with other document examiners, and suggestions from the legal profession.

The NAS Report specifically states that forensic examinations should culminate in conclusions. There have been other presentations that have suggested a wholesale termination of providing conclusions, but this is not in line with the recommendations of the NAS Report and contrary to the path that forensic science is taking. The report develops further that reports should, "describe, at a minimum, methods and materials, procedures, results, and conclusions, and they should identify, as appropriate, the sources of uncertainty in the procedures and conclusions along with estimates of their scale (to indicate the level of confidence in the results)."

Examiners often come in contact with questions concerning the relationship between scientific terminology and legal terminology. The most common situation involves the question, "Is your conclusion within a reasonable degree of scientific certainty?" Many examiners shudder at this question, primarily because the legal profession appears to be unable to provide a standardized definition for that term. Another common question is, "How does the confidence level of our conclusions coincide with the legal levels of proof requirement?"

Document examiners have expressed certain concerns with the current status of standardized terms to include some of the language used and that the standard reflects an inaccurate implication that the association side of the conclusion scale and the disassociation side of the conclusion scale are mirror images. It is widely accepted within the practice of forensic document examination that eliminations are inherently more difficult than identification (in general). As such, the level of evidence is often much stronger for elimination than its counterpart in order to reach a level of confidence required to use comparable vocabulary in the conclusion statement.

This presentation addresses these issues and proposes modifications to the current standard that directly address all of these concerns. This presentation is one examiner's opinion and should be considered as such — hopefully initiating conversation and further debate leading to action.

The structure of forensic science standardization appears to be headed toward a major shift. However, as is commonly known by questioned document examiners, all known movement of such monumental proportions are often slow and should be considering the seriousness of the task. As such, it remains with each discipline within the forensic sciences to maintain the best possible standards. Examiners need not wait on anyone. If one profession does not provide a standard for a term or phrase, why can't the subject-matter expert provide a definition that can be used as a standard by those within our profession?

Conclusions, Standards, Handwriting



Questioned Documents Section - 2015

J26 A New-Dominant Hand: Training the Non-Dominant Hand to Perform the Complex Task of Handwriting

Brenda N. Lanners, BS, San Diego County Sheriff's Regional Crime Lab, 5255 Mount Etna Drive, San Diego, CA 92117*

After attending this presentation, attendees will have an understanding of the problems and challenges encountered when writing with the non-dominant hand and will be aware of possible differences and improvements that can be expected in non-dominant-handwriting over time.

This presentation will impact the forensic science community by bringing awareness to a growing source of handwriting that may appear to be disguised or distorted, by assisting Forensic Document Examiners (FDEs) in understanding the process involved when training the new-dominant hand, and by highlighting certain features and characteristics that might be observed in the handwriting produced by the new-dominant hand.

Use of the non-dominant hand is a method sometimes utilized to disguise one's handwriting. FDEs are always mindful of possible disguise in questioned and known handwriting when performing examinations, but it should be remembered that use of the non-dominant hand is not always used as a means of disguise. Sometimes, it is forced on a writer due to amputation, illness, immobility, or other factors. Consider for a moment, veterans of the Iraq and Afghanistan wars. As of December 31, 2013, there have been 1,558 major limb amputations performed on United States soldiers.¹ United States soldiers remain in combat in parts of the world today, so these numbers are likely to increase.

So, it was with our soldiers and wounded veterans in mind that this study began. With guidance from a book called *Handwriting for Heroes: Learn to Write with Your Non-Dominant Hand in Six Weeks*, this study documents a six-week process of training a writer to use the non-dominant hand to perform the complex task of writing.² The project consisted of 12 daily writing assignments, such as repetition of letter combinations, tracing cursive writing outlines, shading in objects with a pencil, and copying sentences. There was also a daily "homework" assignment meant to increase dexterity and coordination. Specific problems and challenges encountered during the exercises were documented. Differences between the dominant-hand writing and non-dominant-hand writing were seen in: slant, letter designs, retracings, beginning strokes, connecting strokes, speed, and fluency. Changes to the handwriting, which improved noticeably over the six weeks, were also documented.

Those who have lost the use of their dominant hand due to injury, illness, or amputation have no choice but to transform their non-dominant hand into their new-dominant hand. If faced with samples of their writing, it must be understood that they are not necessarily attempting to disguise their writing, but perhaps just trying to survive and thrive. This exercise is meant to bring awareness to a growing source of handwriting that may appear to be disguised or distorted, to assist FDEs in understanding the process involved when training the new-dominant hand, and to highlight certain features and characteristics that might be observed in the handwriting produced by the new-dominant hand.

References:

1. Fisher, Hannah. A Guide to U.S. Military Casualty Statistics: Operation New Dawn, Operation Iraqi Freedom, and Operation Enduring Freedom. CRS Report for Congress. Congressional Research Service, 2014.
2. Yancosek, Kathleen E, Gulick, Kristin. *Handwriting for Heroes: Learn to Write with Your Non-Dominant Hand in Six Weeks*. Ann Arbor, MI: Loving Healing Press; 2009.

Non-Dominant Hand, Amputation, Disguise



Questioned Documents Section - 2015

J27 The Reliability of Hand Printing Identification by the Forensic Document Examiner

Linda L. Mitchell, BS*, 243 S Escondido Boulevard, #304, Escondido, CA 92025-4116; and Mara L. Merlino, PhD*, 1066 Tamworth Lane, Frankfort, KY 40601

After attending this presentation, attendees will be aware of the preliminary results of the most recent study involving the ability of the Forensic Document Examiner (FDE) to properly identify the authors of block printing. They will also have reviewed the most current *Daubert* rulings on this aspect of handwriting analysis.

This presentation will impact the forensic science community by explaining how the results of this study will directly affect future *Daubert* challenges by providing blind study data that could potentially support the premise that current protocols for forensic handwriting analysis also apply to hand printing, specifically block printing.

On October 8, 2013, the 7th United States Federal Court for the Western District of Wisconsin conducted a *Daubert* hearing to determine whether testimony by an FDE regarding the identification of hand printing “rests on a reliable foundation.”¹ The court ruled that the theories of handwriting analysis have not been adequately tested and found reliable when applied to hand printing. In this instance, Bolsover’s testimony was excluded.

Alternatively, in May of 2014, there was a similar request for a *Daubert* hearing in the 15th Judicial Circuit Court in Palm Beach, FL, regarding the reliability of testimony of the identification of hand printing.² The court ruled that *Daubert* requisites had been met by FDEs William Flynn and Grant Sperry. Testimony of this kind was supported by the 2002 Kam/Lin study wherein it was indicated that FDEs perform better than laypersons in the identification of both cursive and hand printing.³

It has been widely noted in the media and within the forensic community that hand printing, specifically block letters, has become more commonly used in everyday writing. It is very frequently seen in forms, addresses, and work-related communications as well. Because of these factors, it has become increasingly important to verify for the purposes of *Daubert* that forensic examination of hand printed documents by qualified FDEs is reliable. This study was undertaken in partial response to the previously cited lack of supporting research in the area of hand printing. It is the purpose of this research to determine the FDE’s reliability in identifying block letter hand printing (ALL CAPITALS) using the same methods and protocols as cursive. The virtual absence of lower-case letters in this study is expected to reduce the amount of potential variation and individualizing characteristics present for identification purposes. It likewise mirrors block printing common to FDE casework.

The materials within the study packets were intended to reflect evidence and procedures similar to normal bench work in a condensed form. Twenty-five questioned writings were offered in individual “case packets.” Each was associated with sample writing of three potential “suspects.” Participants were asked to opine (on a nine-point scale) whether or not each suspect was the writer.

The “testing” portion of this study was concluded in October 2014 and the compiled data was analyzed under the auspices of Dr. Mara Merlino. The preliminary results will be presented along with potential interpretations of the analyzed data sets.

References:

1. United States District Court for the Western District of Wisconsin. United States of America v. Gerald Johnsted, Opinion and Order 12-cr-146-wmc.
2. Circuit Court of the Fifteenth Judicial Circuit in and for Palm Beach County, Florida, Criminal Division “S”. State of Florida vs. Jesse Lee Miller. Case Number 2007CF010420AXX
3. Kam, Moshe, Ph.D. and Lin, Erwei, M.S. (December 28, 2002). Writer Identification using Hand-Printed and Non Hand-Printed Questioned Documents

Hand Printing, Daubert Challenge, Handwriting Identification



Questioned Documents Section - 2015

J28 Some Reasons for Qualified Conclusions

Ronald N. Morris, BS*, 307 Springhill Circle, PO Box 905, Bedford, VA 24523

The goal of this presentation is to discuss some of the reasons why a Forensic Document Examiner (FDE) is not always able to reach or express an unqualified conclusion after conducting an examination and comparison between questioned and known writings.

This presentation will impact the forensic science community by providing a better understanding of the reasons for and rationale behind the necessity to express qualified conclusions in those cases where the evidence in the examined writing is less than conclusive. This discussion is very important because currently some believe that in every science it is possible to always reach an unqualified conclusion. This position is blatantly absurd.

The examination and comparison of handwriting and hand printing is a reliable means of identifying individuals based on their writing habits. It is reliable when the FDE properly, accurately, and conservatively applies the established principles of handwriting and hand printing identification that have been proven true and correct over many years. These principles are well documented in numerous texts and technical papers written by qualified, competent, and ethical FDEs around the world.

Reaching any conclusion concerning authorship of writing must be supported by the combined significance of the evidence in the examined writings. In many cases, the evidence does not support an unqualified conclusion and when it does not, the FDEs must determine what degree of belief they have concerning authorship. This presentation addresses some of the reasons for qualified conclusions and the language used to express them.

There are numerous technical reasons for qualified conclusions. Several examples of why it is not always possible to either identify or eliminate a writer will be discussed in the presentation.

Some of the reasons to be presented and discussed are: (1) the writing is insufficient in quantity or quality and therefore has marginal value for comparison purposes, either questioned or known, or both; (2) limited writing, that is the writing may consist of a few general letterforms or marks; (3) sufficient individuality in the writing; (4) unnatural or disguised writing; (5) simulation or tracing; (6) patching and retouching of letters and words that is not explainable or an irreconcilable difference between the questioned and known writing; (7) completely different styles of writing that may have a limited amount of similar characteristics; (8) writing that is not contemporaneous; (9) the presence of transitory and/or accidental features not present in both questioned and known samples; and, (10) the examination of photocopies, faxes, and digitally produced writing.

The principle that is presented is that identification or elimination of a writer is based on the cumulative effect of all the available observable evidence in the examined writing. It should not be based on the presence or absence of any single characteristic, quality, or feature in common or different in the writing. If the cumulative evidence is not sufficient to reach the conclusion that there absolutely was or was not another writer of the questioned writing, then an identification or elimination conclusion is justified.

This same principle applies when there is insufficient evidence to reach an unqualified conclusion. The cumulative evidence, while not sufficient for an identification or elimination, must be sufficient to express a qualified conclusion. The level of certainty expressed by the language used must be accurate in expressing the degree of belief the FDE has concerning the results of his/her examination and comparison.

Handwriting, Qualified, Conclusions



Questioned Documents Section - 2015

J29 Forensic Document Examiner (FDE) Testimony Related to Quality Processes Denied — What a Florida Jury Did Not Hear and More (or Less)

Grant R. Sperry, BS, Forensic Doc Exam Services, 7859 Cross Pike Drive, Germantown, TN 38138-8117*

After attending this presentation, attendees will have been provided information on how certain courts may apply adverse rulings to the various quality processes and methodologies employed by forensic document examiners.

This presentation will impact the forensic science community by providing insight on the judicial demands of the forensic science disciplines and the resulting counterintuitive decisions by the judiciary when those demands are met.

This presentation will discuss the facts and circumstances surrounding forensic document examinations and three subsequent trials of an individual accused of murdering a young assistant manager at a popular chicken restaurant in West Palm Beach, FL, more than 15 years ago.

The specifics of the lengthy and controversial investigation and multiple trials will shed light and promote discussions on forensic document examination processes, testimony, extremely controversial rulings, un rebutted “expert critic” testimony, and the tainting of testimony by association.

In 1999, a young 18-year-old assistant manager at a West Palm Beach fast food restaurant was bound and shot execution-style while closing the restaurant. Almost five years later, the questioned handwriting allegedly written by one of the perpetrators while at the scene and purported known writings of numerous individuals were submitted to the laboratory by investigators with the West Palm Beach Police Department and later from the State Attorney General’s office. Two examiners from different laboratories ultimately identified the same individual as the person responsible for the questioned writing. In each instance, the conclusions reached by the primary examiners were reviewed by another FDE as part of each laboratory’s quality assurance process.

The first trial resulted in a mistrial (eleven to one, guilty). The second trial resulted in a guilty verdict. The Florida Court of Appeals reversed the conviction on the grounds that the FDEs testifying about their peer review process were, in effect, bolstering their testimony. The court ruled the trial judge was in error by allowing each FDE to testify to their quality review process, without providing the opportunity to the defense to cross-examine the FDEs conducting the quality review. Of course, there were depositions of the FDE conducted prior to trial and the defense was well aware of the processes employed with respect to quality review and verification at both FDE laboratories.

This presentation addresses the forensic document examinations, the process methodology employed by two recognized forensic laboratories, testimony by two FDEs at multiple trials, and the controversial rulings by the court on several issues, including the court’s refusal to allow testimony by more than one examiner, the court’s decision to forbid testimony by the FDEs who provided the peer review and verification, and the spill-over impact or tainting of the case based on issues related to problematic investigative techniques.

At the third trial, the defendant was acquitted. In that trial, not only was the FDE forbidden to provide the jury with information on the quality processes employed, but the court ruled the FDE who conducted the quality/peer review would not testify because the testimony would amount to “bolstering.” So, this begs the question...just exactly how is the jury to assess the reliability of our quality processes if we are not allowed to testify to them and/or if other FDEs involved in the process are precluded from testifying as to their role in the process? As a result of another questionable ruling from the bench, a rebuttal witness to the testimony of a pervasive “expert critic” was not allowed to testify.

There are certainly anomalies within the circumstances of this case and rulings by the court which may never appear again as they did in this case. However, the rulings in this case are difficult to understand given the past criticisms of the judiciary and other critics. The ever-critical judicial system has demanded of the forensic science profession methodologies and processes which are well-grounded in science and quality. Yet, in this case, the jury was forbidden to hear about the methodology and quality processes the forensic document examination community has been criticized for failing to employ in years past. As forensic document examiners, this is indeed cause for concern.

Quality Process, Methodology, Bolstering



Questioned Documents Section - 2015

J30 The Forensic Document Examiner Testimony List

Ellen M. Schuetzner, BA, 6348 N Milwaukee Avenue, #161, Chicago, IL 60646-3728*

The goal of this presentation is to make attendees aware of a testimony list that can be used in court challenges to the profession. This list can be used by examiners in criminal and civil cases and provides information about the types of examinations and opinions that are being offered in courts across the United States.

This presentation will impact the forensic science community by making forensic document examiners aware that a testimony list is available so they can be better prepared for a court challenge. A forensic document examiner can also learn how and why he/she should maintain his/her own list and contribute to this continuing list.

Since the *Daubert v. Merrell Dow Pharmaceuticals, Inc.* decision and its requirements for testimony, there were many challenges to the testimony of forensic document examiners. To address some of the court challenges to the testimony of forensic document examiners, a list of the testimony by these practitioners was started and continues to be maintained. This list includes criminal and civil cases, municipal, county, state, and federal cases in the United States and some of its overseas courts. Some administrative and other governmental hearings have been included. The list includes only testimony in which judges have allowed the forensic document examiner to testify to his/her opinion in court.

The list was started in the year 2000 in response to some of the *Daubert* challenges that forensic document examiners faced. It is a collection of testimony by qualified forensic document examiners since the advent of the *Daubert* ruling in 1993. It includes the name of the case, the date of testimony or date of decision in the case, the location of the trial, the type of court, and the type of opinion(s) and examination(s) that were testified to. These examinations and opinions are only those that are related to forensic document examination. Those who have contributed are examiners who were trained by traditional methods such as those described in the American Society for Testing and Materials (ASTM) *Standard Guide for the Minimum Training Requirements for Forensic Document Examiners*.

Forensic document examiners from all over the United States have contributed to this testimony list. Some examiners have submitted their list after many years of testimony and some contribute after each testimony. The list has entries from all fifty states and other judicial jurisdictions. Since its inception, the list has been used in *Daubert* challenges and it has been made available when *Daubert* challenges are threatened. In one recent case, the judge commented that the evidence demonstrated that the testimony of forensic document examiners was regularly admitted in courts throughout the United States.

This presentation will address the contents of the testimony list, including a breakdown of how the list is formulated, some of the entries, the statistics of some of the entries, and the use of the list in some court cases. One of the purposes of this presentation is to encourage forensic document examiners to continue their testimony lists to the ongoing effort to keep this testimony list current and usable for future court cases.

Forensic Document Examination, Testimony List, Court Challenge



Questioned Documents Section - 2015

J31 The Scientific Working Group for Forensic Document Examination (SWGDOC) and the Organization of Scientific Area Committees (OSAC) Process

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The goal of this presentation is to educate forensic document examiners on the future of standards development.

This presentation will impact the forensic science community by educating it on the future of standards development. Courts of law are relying more on the use of standards in an expert's examinations. Lack of this knowledge may impact a practitioner's allowance to testify as an expert witness.

SWGDOC has been in existence, under different names, since 1997. With the genesis of OSAC by the National Institute of Standards and Technology (NIST), the Scientific Working Groups (SWGs) will be both replaced and combined. There will be five Scientific Area Committees (SAC), each with several subcommittees representing what have up to now been the SWGs. The subcommittee for Questioned Documents (QD) will be under the Physics/Pattern SAC. At the time of this abstract, the members of the Physics/Pattern SAC (other than the chair) and the QD subcommittee have not been publicized.

This study will present an up-to-date analysis of the OSAC process and the conversion from the SWGDOC business model to the OSAC business model. This presentation will include the members of the QD subcommittee and their backgrounds, as well as the members of the Physics/Pattern SAC and their backgrounds. The membership of each subcommittee is currently planned for a maximum of 20 voting members, broken down with a distribution goal as follows: 70% (14) practitioner (20% (four) federal, 30% (six) state and local, 20% (four) civil or other; 20% (four) researchers (including statisticians, epidemiologists, etc.); and, 10% (one) Research and Development (R&D) technology partners and providers. There is also a plan to allow up to five invited guests per meeting. The plan is for the subcommittees to meet in person once per year, with virtual meetings occurring more often. It is the understanding of the presenters that membership will be for a specific period of time (perhaps three years) and there will possibly be term limits on the number of times an individual can hold consecutive memberships. As a point of reference, SWGDOC currently has 38 members, all practitioners, with a balance of federal, state and local, and private practitioners. SWGDOC also has two international members. It is unknown at this time if there will be any international members involved in the OSAC process.

The OSAC process for drafting, vetting, and publishing standards, guidelines, and best practices will also be presented. This process, depending on the type of document being considered, also includes reviews for Human Factors, Legal Analysis, and Quality Infrastructure. The Forensic Science Standards Board will give the final blessing on documents that are intended to be labeled as "standards" under the OSAC process. Those that will be labeled as guidelines and best practices need only be approved at the Physics/Pattern SAC level.

SWGDOC, OSAC, Questioned Documents



K1 A Reduced Workflow Solution for the Analysis of Gamma-Hydroxybutyrate (GHB) in Human Hair Samples Via an Automated Bead Mill as a Precursor to High Resolution-Gas Chromatography/Time-of-Flight (GC/TOF) and 2D Gas Chromatography/Time-of-Flight (GCxGC/TOF)

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After attending this presentation, attendees will be able to describe the usefulness of improved automated instrumentation for the determination of GHB in hair. Silylation of GHB is the most common derivatization technique employed with GHB for the analysis of forensic samples. This creates a challenge when testing hair samples due to interference from other endogenous compounds. One approach to cleaning up the samples is the use of mass spectrometric deconvolution software and multidimensional GC. In addition, it is critical to extract as much of a drug from the hair matrix as possible so this study has investigated the use of a Biotage® Bead Ruptor 24 to enhance the recovery of GHB from hair.

This presentation will impact the forensic science community by increasing awareness of alternative instrumental techniques that can be used to optimize the determination of silylated GHB in hair.

Techniques to interrogate hair samples have proven valuable in detecting human host exposure to drugs of abuse over a long sampling window. The hair matrix is challenging to work with and a number of methods have been reported. This investigation details the feasibility of incorporating an automated bead mill to homogenize the matrix and disrupt non-selective analyte binding on a molecular level by destroying the matrix on a macro level. It was determined that the bead mill was effective in improving the efficiency of the subsequent digestion. Proof of concept was determined using adult human head hair samples (n=5) which were prepared in duplicate using the laboratory's standard preparation procedure (cutting the hair into 1mm-2mm segments using scissors) versus automation in the bead mill at the Center for Forensic Science Research and Education in Willow Grove, PA, using a Biotage® Bead Ruptor 24. The homogenized samples were incubated overnight in methanol at 40°C, filtered, and evaporated to dryness. The sample tubes were sent to Leco's application laboratory in Saint Joseph, MI, for derivatization and GC/TOF analysis, followed by post-acquisition deconvolution software. A GCxGC approach was employed to mitigate a co-eluting peak tentatively identified by Pharmacy Benefit Manager (PBM) software as Tetramethylsilane (TMS) -urea. The GCxGC method was successful for the selective analysis of GHB in hair. Preliminary data suggests alternatives to GCxGC may be found in Solid Phase Extraction (SPE) techniques prior to single stage GC/MS. Additional samples from a range of individuals (age and sex) were similarly prepared and tested via the optimized GCxGC/TOF method. This method was found to give better specificity and sensitivity than GC/MS and is effective in separating GHB-2TMS from endogenous interferences.

GHB, Bead Ruptor, GCxGC/TOF



K2 A Novel Extraction Methodology for the Analysis of Lorazepam and Oxazepam Glucuronide Hydrolysis in Meconium

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The goal of this presentation is to present research studies establishing a novel analytical approach to the analysis of benzodiazepines in meconium using an enzymatic hydrolysis step *in situ*, followed by weak anion exchange clean-up in a disposable pipette, prior to analysis by Liquid Chromatography/Mass Spectrometry (LC/MS). Attendees will learn how the proposed methodology reduces biological matrix effects and minimizes sample preparation time.

This presentation will impact the forensic science community by illustrating how the proposed analytical methodology provides practical advantages over existing methods in terms of rapid sample clean-up and the removal of biological matrix effects that could potentially interfere with the determination of benzodiazepines by LC/MS. This method enables routine rapid monitoring of meconium for cases of suspected drug abuse.

Benzodiazepines are a schedule IV class of psychotropic drugs commonly used for their depressant properties.¹ Monitoring benzodiazepines in meconium is critical both for monitoring abuse by pregnant women and to identify potential health risks for newborns. Benzodiazepines are thought to possibly cause oral clefts.² Although analysis of urine can be simple and fast, benzodiazepine metabolites persist in urine only for a few days after use and drug exposure may be underestimated. Meconium is a valuable toxicological matrix because it acts as a repository for xenobiotics including drugs of abuse from the 16th week of gestation until birth; however, the use of meconium as a biological matrix to monitor drug use by liquid chromatography/mass spectrometry is problematic because of the potential for large matrix effects.^{1,3} Reducing matrix effects requires extracting target analytes from the endogenous biological interferences, a task that is often time consuming and labor intensive. The objective of the present research is to minimize both meconium matrix effects and sample preparation time by a hydrolysis step *in situ*, followed by weak anion exchange disposable pipette extraction tips for sample clean-up, prior to analysis of benzodiazepines by liquid chromatography/mass spectrometry.

An aliquot of 250 μ L of water was added to 25mg of blank standards of meconium in an Eppendorf tube. The mixture was vortexed until the meconium became homogeneously distributed/dissolved in the water. A solution consisting of 75mL of pH 7.5 potassium phosphate buffer, 50mL of a β -glucuronidase enzyme, and 10mL of 500ppb internal standard in water, was added. This mixture was then incubated for one hour at 55 $^{\circ}$ C. A 600mL aliquot of acetonitrile was added to the mixture to precipitate proteins. This mixture was vortexed and centrifuged. The supernatant was removed and placed into a clean sample vial (~950ul solution). The solution was aspirated into a WAX-S tip (20mg 55-65um resin/40mg salt) twice. This step separates the acetonitrile and water layers and facilitates transfer of the analytes into the cleaner acetonitrile supernatant. The top acetonitrile layer (~500-600ul) was then transferred to a vial suitable for solvent evaporation. This step separates the acetonitrile and water layers and facilitates removal of the analytes in the acetonitrile supernatant. After evaporation in a fresh vial, the residue was reconstituted in 100mL of 10:90 methanol:water.

All analyses were performed using a triple quadrupole system with an Agilent[®] 1100 HPLC with a C-18 column (3.0 x 50mm, 2.7 μ m). Sample injections of 20 μ L were made using an injection valve incorporated on an autosampler. The mobile phase used 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The initial gradient was 70% A for 0.25min, which ramped to 5% A at 2min. The gradient remained at 5% A for 1min, then back to 70% A for a total run time of 6.5min. The eluent was diverted to waste during the intervals of 0-0.5min and 5-6.5min after injection. The column flow rate was 0.4mL/min. The electrospray voltage was 4,000V and the gas pressure was 60psi.

Initial success of hydrolysis was illustrated through analysis of neat meconium samples spiked with lorazepam and oxazepam glucuronides. Post-hydrolysis, a decrease of the glucuronides and concurrent increase in the parent compounds demonstrated that the method was viable. To test the validity of this method further, a blind study was performed with a collaborative laboratory including 35 meconium patient samples tested for ten benzodiazepines and/or metabolites. The blind study resulted in a correlation of approximately 92%. In conclusion, the combination of fast hydrolysis, coupled with a simple clean-up scheme, offers an effective analytical approach for the analysis of benzodiazepines in meconium.



Toxicology Section - 2015

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 2. Bar-Oz, B. "Comparison of Meconium and Neonatal Hair Analysis for Detection of Gestational Exposure to Drugs of Abuse" *Archives of Disease in Childhood. Fetal and Neonatal Edition*, 2003, 88, F98-F100.
 3. Williamson, S., "Determination of the Prevalence of Drug Misuse by Meconium Analysis" *Archives of Disease in Childhood. Fetal and Neonatal Edition*, 2006, 91, F291-F292.
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Meconium, Benzodiazepines, Chromatography



K3 Analysis of Promethazine, Chlorpromazine, and Selected Metabolites in Decomposed Skeletal Tissues by Microwave-Assisted Extraction/Microplate Solid Phase Extraction/Ultra High-Performance Liquid Chromatography (MAE/MPSPE/UHPLC)

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After attending this presentation, attendees will understand how to develop a microwave-assisted extraction methodology using bone tissue. An example of this methodology's practical application using vertebral bone will be presented.

This presentation will impact the forensic science community by adding to the body of data illustrating the utility of skeletal tissues as a matrix for toxicological analysis and by demonstrating an efficient method for preparation of skeletal tissue samples.

The purpose of this study was to develop and validate a Microwave Assisted Extraction (MAE) followed by a Microplate Solid Phase Extraction (MPSPE) method for the detection and semi-quantitation of Promethazine (PMZ), Chlorpromazine (CPZ), and selected metabolites (promethazine sulphoxide, desmethylpromethazine, chlorpromazine sulphoxide, and desmethylchlorpromazine) in the skeletal remains of rats using Ultra High Performance Liquid Chromatography (UHPLC).

Male Wistar rats (n=10) received either a dose of PMZ (50mg/kg, i.p.) or CPZ (50mg/kg, i.p.). Five rats acted as drug-free controls. The rats were euthanized by CO₂ asphyxiation 20min after exposure and placed outdoors to decompose to skeleton. Vertebral bone was recovered and washed with 3mL Phosphate Buffer (PBS: 0.1M; pH6), 3mL methanol, and 3mL acetone and air-dried. Bones were pulverized and samples underwent MAE in 5mL of methanol at 80°C in a MARS™ 6 microwave oven for a total of 60min, with extraction solvent recovered and replaced with fresh solvent at 15min and 30min. All solvent extracts were recovered, evaporated to dryness, and reconstituted in 1mL of Phosphate Buffer Saline (PBS). Promazine was added as an internal standard (500ng) and extracts then underwent protein precipitation by adding 1mL of PBS along with 3mL of acetonitrile-methanol (1:1) followed by storage at -20°C for 24h. The samples were centrifuged and the supernatant was collected and evaporated to 1mL. PBS (2mL) was utilized to dilute the samples which were then acidified with 100µl of acetic acid. All samples were subjected to SPE using XCEL I (130mg) 48 microwell plates. Wells were conditioned by sequential addition of 3mL methanol, water, and PBS. Samples were loaded by gravity. Wells were washed with PBS (3mL) and 0.1M acetic acid (3mL) and dried for 5min under vacuum (10 in Hg). Wells were washed with methanol (3mL) and dried for 10min (10 in Hg). Analytes were eluted using 3% NH₄OH in 20:80 isopropanol:dichloromethane (3mL). Extracts were evaporated to dryness and reconstituted in 500µL of mobile phase A (0.1 % formic acid in 90:10 water:acetonitrile). Samples were centrifuged for 10min and then 15µl of sample was injected into the UPHLC with Photodiode Array Detection (PDA). The column used was a Raptor™ biphenyl column (150mm x 2.1mm, 1.7µm) with a column temperature set to 50°C. The mobile phase gradient began with 95:5 A:B (B: 0.1%formic acid in 90:10 acetonitrile:water) held for 1min, then increased to 70:30 A:B over 4min, held for 1min, then increased to 20:80 A:B over 3min, and reversion back to 95:5 A:B which was held for 1min, for a total run time of 10min at a constant flow rate of 0.400mL/min. Quantitative measurements were made using the response ratio measured at 240nm for the sulphoxide metabolites and 250nm for the remaining analytes.

Method validation involved preparation of standard analyte samples in drug-free Bone Tissue Extract (BTE). The response ratio was linear from 10ng/mL to 5,000ng/mL ($R^2=0.990-0.999$). The precision was <25% (n=3 on each of three different days). The limit of detection was approximately 10ng/mL for each analyte, and the limit of quantification for the method was approximately 25ng/mL for each analyte. The majority of analytes were recovered after 30min extraction interval. Analytes were stable under the microwave extraction for at least 60min.

Promethazine, Chlorpromazine, Bone



K4 Detection of Trace Buprenorphine and Norbuprenorphine in Human Hair Using Enzyme-Linked Immuno-Sorbent Assay (ELISA)

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After attending this presentation, attendees will be able to develop, validate, and implement an ELISA method in their forensic toxicology laboratories for detecting buprenorphine and its metabolite, norbuprenorphine, in human hair.

This presentation will impact the forensic science community by introducing a sensitive, robust, and short turn-around-time method to detect both the parent drug and the metabolite to support surveillance of compliance with opioid dependence treatments.

Buprenorphine (BUP) is a partial mu opioid agonist with kappa opioid antagonist property that has been used as a substitution drug for opioid dependence treatment; however, the drug has potential for abuse and is more easily obtained than other substitution drugs such as methadone. A few studies have suggested that hair analysis of BUP and norbuprenorphine (norBUP), the major N-dealkylated metabolite, can complement urine drug analysis to monitor the drug intake in a detection window of up to three months. Combined BUP and norBUP were reported to accumulate to more than 20pg/mg in hair at a dose as low as 0.2mg/week maintained for two to three months.

Currently at this study's laboratory, only a Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) method has been implemented to quantitate both BUP and norBUP in human hair with a Lower Limit Of Quantitation (LLOQ) of 8pg/mg for each analyte. Only ~30% of the hair samples in the laboratory reported out with quantitated results had BUP/norBUP ratio greater than 1.0 (BUP 8 — 1,517pg/mg, norBUP none-detectable — 1,295pg/mg), and the rest had norBUP as the predominant analyte, including those in which only norBUP was quantitated (BUP none-detectable — 775pg/mg, norBUP 19 — 2,192pg/mg).

An ELISA method was sought to be utilized as the initial detection method, which ideally should detect both the parent drug and its metabolite at the desired analytical sensitivity.

An ELISA kit targeting BUP was validated according to the Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines. An approximate 20mg aliquot of 1.5-inch hair segment proximal to scalp was washed once with acetone, pulverized, and then sonicated with heat in 1.5mL methanol for two hours. The methanol mixture was centrifuged and 1.0mL of the resulting supernatant was evaporated and reconstituted with the diluent provided by the ELISA kit. Laboratory-determined volume of the final reconstituted hair extract was then pipetted for ELISA development according to the manufacturer's package insert. The assay principle is heterogeneous-competitive ELISA, where the intensity of the developed color is inversely proportional to the sample drug concentration. The absorbance of each sample well was normalized to that of the negative controls (B/B0) within the same batch. The desired cut-off level was 20pg/mg of BUP in hair.

Controls at five concentrations (cut-off, $\pm 25\%$ and $\pm 50\%$ of cut-off) were prepared for analysis of precision and a control at 100pg/mg was included to determine extended assay linearity. Coefficients of variation for the measured B/B0 were 4.6%-11.4% within-run ($n=4$) and 7.6%-11.7% between-run (five runs, $n=20$). The B/B0 mean ± 2 Standard Deviations (SDs) for concentrations at $\pm 50\%$ of cut-off were well separated from the mean B/B0 at cut-off. Correlation coefficient R^2 of B/B0 versus concentrations (expressed in logarithm) was 0.9953, demonstrating satisfactory linearity. The mean B0 — $3.3 \times$ SDs determined limit of detection to be 6.3pg/mg. The ELISA did not present hook effect and carry-over at least at 2,000pg/mg. The assay did not show interference from common over-the-counter or prescription drugs at 25ng/mg.

The previously analyzed hair samples in-house for BUP and norBUP by LC/MS/MS were de-identified and randomly chosen based on their quantitated results for ELISA analysis. The hair samples were categorized into three groups: (1) High BUP group with >30 pg/mg of both BUP (32 — 1,517pg/mg) and norBUP (155 — $>2,000$ pg/mg) ($n=10$); (2) Borderline BUP group with non-detectable — 21.7pg/mg BUP and 27.3 — 123pg/mg norBUP ($n=8$); and, (3) Negative BUP group with non-detectable BUP and norBUP ($n=9$). All High BUP and seven Borderline hair samples were determined positive, and all Negative hair samples were determined negative by the ELISA method. One Borderline hair sample (BUP=17.9, norBUP=54.2pg/mg) was equivocal to cut-off controls. With this limited sampling size, the ELISA demonstrated to detect both parent drug and its metabolite, achieving $\geq 94.4\%$ sensitivity and 100% specificity.



Toxicology Section - 2015

To provide forensically defensible toxicology results, an initial detection of substances should be confirmed whenever possible by a second technique based on a different chemical principle. The ELISA developed and validated herein satisfies the laboratory's needs to be implemented as an appropriate initial test method to complement the currently used confirmatory LC/MS/MS method.

Buprenorphine, Hair Testing, ELISA



K5 The “I’s” Have It: A High-Performance Liquid Chromatography Tandem Mass Spectrometry Method for the Determination of 25I-NBOH, 25I-NBOMe, and 2C-I in Urine

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After attending this presentation, attendees will understand how to analyze for the three 4-iodo-2,5-dimethoxy-phenethylamines designer hallucinogens.

This presentation will impact the forensic science community by offering a method for the identification and quantitation of three designer hallucinogens in human urine which may be used in clinical and forensic toxicology laboratories.

Introduction: In 2010, a novel class of designer hallucinogens, the N-methoxybenzyl-methoxyphenylethylamine (NBOMe) derivatives of Alexander Shulgin’s 2,5-dimethoxy-phenethylamines, became readily available on the internet. These derivatives are potent serotonin 2A (5-HT_{2A}) receptor agonists. Currently, NBOMe derivatives are sold as powders or on blotter paper with 25I-NBOMe (4-iodo-2,5-dimethoxy-N-(2-methoxybenzyl)-phenylethylamine) being the most commonly reported. Clinical presentations of severe NBOMe intoxication include tachycardia, agitation, hypertension, aggressive/violent behavior, hallucinations, and continuous agitation and seizures which can persist for as long as three days. In November of 2013, three designer phenethylamines, 25I-NBOMe, 25C-NBOMe, and 25B-NBOMe (the 4-chloro- and 4-bromo- 2,5-dimethoxy-N-(2-methoxybenzyl)-phenylethylamine), were temporarily declared Schedule I drugs. Recently, unscheduled NBOMe type derivatives have become available, with anecdotal evidence suggesting that 2-((4-iodo-2,5-dimethoxyphenyl)ethylamino)methylphenol (25I-NBOH) is the most prevalent. Shulgin’s designer hallucinogen, 2,5-Dimethoxy-4-iodophenethylamine (2C-I) is a precursor of both 25I-NBOMe and 25I-NBOH, and may be present in specimens as an impurity. Evidence also suggests 2C-I is a metabolite, formed by N-debenzylation, of both 25I-NBOMe and 25I-NBOH.

Objective: To develop a method for the detection and quantification of 25I-NBOMe, 25I-NBOH, and 2C-I in human urine as part of a dose response, disposition, metabolism, and behavioral studies concerning these designer hallucinogens.

Methods: An Ultra Performance Liquid Chromatography/Tandem Mass Spectrometry (UPLC/MS/MS) method was developed for the detection and quantification of 25I-NBOMe, 25I-NBOH, and 2C-I in human urine. Following the addition of the deuterated internal standard (25I-NBOMe-d₃), the hallucinogens were isolated by a previously published solid phase extraction method.¹ Chromatographic separation was performed on a Selectra® PFPP column, 10cm x 2.1mm, 3.0µm. The mobile phase consisted of A: water with 10mM ammonium formate, and B: methanol with 10mM ammonium formate. The following gradient was used: 0.0-3.0min starting at 60% B, with a linear gradient to 95% B, and then returning at 4.5min to 60% B. An injection volume of 5µL was used with a mobile phase flow rate of 0.4mL/min and a total run time of 4.5min. The following transition ions (m/z) were monitored for 25I-NBOMe: 428>121, 428>91, 428>272; 25I-NBOH: 414>107, 414>291, 414>308; 2C-I: 308>91, 308>276, 308>291; and, 25I-NBOMe-d₃: 431>124, 428>92, 428>275. The method was evaluated for absolute recovery, ion suppression, accuracy/bias, inter-day and intra-day precision, interferences, bench top stability, freeze/thaw, and post-preparative stability.

Results: Duplicate calibration curves were determined to be within 20% of the nominal value for each analyte. The linear regression correlation coefficients for each analyte’s calibration r² were 0.99 or greater. 25I-NBOMe and 25I-NBOH were linear from 10pg/mL to 500pg/mL, while 2C-I was linear from 50pg/mL to 500pg/mL. The Limit Of Detection (LOD) was administratively set at 10pg/mL for 25I-NBOMe, 25I-NBOH, and 2C-I. Assay performance was evaluated using a set of five quality-control specimens. Accuracy/bias of the assay was determined to be within +/-20% of the target value for each analyte in each quality control specimen. The CV for inter-day and intra-day precision samples did not exceed 15%, except for the Limit of Quantitation (LOQ) samples which did not exceed 20%. Two urine specimens were analyzed; one contained all three analytes, while the other specimen contained only 25I-NBOH.

Conclusion: This validated method was found to be robust and reliable for the detection and quantification of 25I-NBOMe, 25I-NBOH, and 2C-I in human urine.



Toxicology Section - 2015

Reference:

1. Poklis JL, Charles J, Wolf CE, Poklis A. High-performance liquid chromatography tandem mass spectrometry method for the determination of 2CC-NBOMe and 25I-NBOMe in human serum. *Biomed Chromatogr.* 2013, 12:1794-800.
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Designer Drugs, UPLC/MS/MS, 25I-NBOMe



K6 Identification of Synthetic Cathinones From Electron Impact Mass Spectra

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After attending this presentation, attendees will be able to describe characteristic fragmentation pathways for existing synthetic cathinones and predict new fragments for novel analogs as they arise.

This presentation will impact the forensic science community by highlighting the importance of mass spectral properties, the specificity of ion selection during analysis, and the practical limitations associated with some drugs within this class.

The popularity of synthetic cathinones and the diverse number of drugs within this relatively new class has increased considerably in recent years. Gas Chromatography/Mass Spectrometry (GC/MS) is still the most widely used technique in routine forensic toxicology investigations. Due to the proliferation of structural analogs and limited cross-reactivity toward the entire class of drugs, chromatographic-based screening is of great importance for the synthetic cathinones. Chromatographic separation of analytes can be readily achieved using multi-component mixtures; however, the Electron Impact (EI) mass spectral properties of some of the forensically important synthetic cathinones can present a challenge due to the limited number of diagnostic ions.

The characteristic fragmentation pathways synthetic cathinones are described and discussed for nineteen secondary and tertiary amines within this class. These include buphedrone, ethcathinone, methcathinone, pentedrone, 4-EMC, 4-MEC, flephedrone, mephedrone, methedrone, α -PVP, MPBP, naphyrone, pyrovalerone, butlyone, ethylone, methylone, pentylone, MDPBP, and MDPV. Although protonated molecular ions are readily observed using hyphenated Electrospray Ionization (ESI) techniques, parent ions are hard to obtain using EI/GC/MS. Molecular ions when they are present are odd, due to the "nitrogen rule." The mass spectra of synthetic cathinones are dominated by two characteristic cleavages to form iminium and acylium ions that are associated with the side chain and the core benzene ring (which is often substituted). The presence of the carbonyl bond on the α -carbon and the lone pair of electrons on the oxygen of the ketone moiety plays an important role in EI ionization. In addition to the rationalization of non-derivatized cathinones, acylation, silylation, and two-step reductive silylation and reductive acylation methods will also be presented and discussed in terms of their mass spectral properties. Although fragmentation is largely predictable, it presents some practical limitations in terms of the specificity of some diagnostic ions, necessitating careful attention to chromatographic separation and identification criteria.

Cathinones, Mass Spectra, Electron Impact



K7 Investigation of Unknown Designer Drugs and Metabolites in Urine Collected From Electronic Dance Music (EDM) Attendees

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After attending this presentation, attendees will understand the process of structure elucidation and compound identification when unknown analytes are encountered using chromatographic and spectrometric techniques for the analysis of urine samples. This presentation may also give rise to the determination of novel compounds identified in the attendees of EDM festivals by allowing meeting attendees to contribute their input regarding similar peaks they may have encountered.

This presentation will impact the forensic science community by presenting the identification of several metabolites discovered in authentic urine samples that have not been thoroughly explored in the literature.

The designer drug market has continued to expand over the last several years with structural modifications continually being introduced in an attempt to skirt legal rulings. Blood, urine, and oral fluid samples were collected from volunteers attending an EDM festival in addition to asking the volunteers to answer questions regarding recent drug use, effects, and dosage. This group was targeted in an effort to attain authentic specimens from a population suspected to have a high likelihood of using designer drugs. The urine samples were analyzed via a battery of analytical methods in order to fully investigate the compounds present as well as the anticipation of detecting unknown analytes or metabolites. Results produced by the analysis on both Gas Chromatography/Mass Spectrometry (GC/MS) and Liquid Chromatography/Time-Of-Flight (LC/TOF) were key in the determination of unknown compounds. Samples were extracted using a solid phase extraction which targeted basic drugs to optimize the detection of designer stimulant and hallucinogen related compounds.

The GC/MS results were very promising with a quantity of designer drugs being detected including methylone, ethylone, butylone, dimethylone, 5-APB, fluoroamphetamine, and alpha-PVP. This presentation will describe the process to elucidate unknown peaks, several of which were minor metabolites of common compounds including cocaine, quetiapine, and dextromethorphan or were endogenous in nature. The most interesting unknown peaks were present in combination with alpha-PVP. The mass spectra of each of the two peaks displayed a prominent 126 ion, although the two peaks had separate retention times than all the other designer drugs present in the extensive in-house library. The samples were also analyzed on the LC/TOF and produced peaks with accurate masses and retention times that were unique to these unknown compounds as compared to the instrument database.

These two peaks of interest have yet to be identified and it is the goal of this presentation to allow the scientific community to comment and collaborate to aid in the identification of unpublished metabolites or novel compounds. The combination of structure prediction software, comparison of results from various analytical methods, and investigation of fragments using elemental composition serve as great tools in the elucidation of unknown compounds.

Metabolite Elucidation, Designer Drugs, LC-TOF



K8 Development of a Quantitation Method for Synthetic Cannabinoid Metabolites in Urine Using Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS)

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After attending this presentation, attendees will be able to perform a Liquid-Liquid Extraction (LLE) method followed by an LC/MS/MS method to extract, detect, and quantify synthetic cannabinoid metabolites of JWH-018, UR-144, AKB-48, AB-PINACA, ADB-PINACA, ADBICA, PB-22, 5F-PB-22, and BB-22 from urine.

This presentation will impact the forensic science community by demonstrating a validated method for the extraction and quantitation of metabolites of nine synthetic cannabinoids from urine samples. The parent drugs are currently scheduled by the Drug Enforcement Administration (DEA) as Schedule I compounds or have been recently identified as emerging cannabinoid agents being sold in synthetic cannabis blends. Development of an updated assay to detect and quantify the metabolites is important for forensic and toxicology laboratories for the assessment of prior consumption of the latest generation of synthetic cannabinoids.

Over the past few years, synthetic cannabinoids have become increasingly popular and prevalent in an effort by drug users to bypass current legislation and achieve a “legal” high. At the federal level in the United States, most are classified Schedule I substances by the DEA as they have a high potential for abuse and no medical purpose, so in an attempt to escape legal consequences, “manufacturers” produce compounds that are structurally different from currently scheduled drugs, but still give similar effects to achieve that high. JWH-018, UR-144, ADB-PINACA, AKB-48, PB-22, and 5F-PB-22 have been scheduled by the DEA while AB-PINACA, ADBICA, and BB-22 are currently not scheduled but have recently been identified as being components in synthetic cannabis blends in Japan and the United States. The structures of emerging synthetic cannabinoid compounds are believed to have similar properties to previously recognized compounds. As the number of compounds continues to increase, it is essential to develop a method that is versatile and can keep up with the rapidly emerging compounds as these newly emerged compounds may not register a positive result in common drug screening procedures.

An updated method was developed and validated to extract, identify, and quantify the N-pentanoic acid metabolites of JWH-018, UR-144, AKB-48, AB-PINACA, ADB-PINACA, and ADBICA; and the 3-carboxyindole metabolites of PB-22, 5F-PB-22, and BB-22 from urine, using LLE followed by LC/MS/MS. The metabolites were chosen based on the possibility of being active metabolites based on recent publications and also since there would be no detection issues as there could be with the various hydroxylated metabolites.

The liquid chromatograph and mass spectrometer conditions were optimized for the nine synthetic cannabinoid metabolites, including the mobile phase gradient and Multiple Reaction Monitoring (MRM) transitions. The LC conditions included a ten-minute run with initial conditions of 70:30 ratio of 0.1% formic acid in water (mobile phase A) and 0.1% formic acid in methanol (mobile phase B). Over eight minutes, the ratio switched to 10:90 of A:B and was then held for one minute, before re-equilibrating to the starting conditions at 9.1 minutes. The method achieved baseline separation and identification was based on their MRM transitions. Calibration models were produced in the range of 1ng/mL-100ng/mL for JWH-018, UR-144, AKB-48, PB-22, and 5F-PB-22 metabolites; 1ng/mL-50ng/mL for ADBICA, AB-PINACA, and ADB-PINACA metabolites; and 5ng/mL-100ng/mL for BB-22 metabolite ($R^2 > 0.98$). The limits of detection were at or less than 2ng/mL and limits of quantitation were at or less than 5ng/mL. The method was validated using Scientific Working Group for Toxicology (SWGTOX) guidelines for quantitative methods and once the method was validated, it was applied successfully to authentic urine samples. The range for authentic samples was 2ng/mL-250ng/mL. Specificity was determined by testing possible compounds that could produce a possible interference and then comparing the true negatives to the sum of samples that were true negatives and false positives. This method will be of use to the field of forensic toxicology as it incorporates a method to test for the consumption of currently scheduled compounds and recently emerging synthetic cannabinoids.

Synthetic Cannabinoids, Urine, LC/MS/MS



K9 Recreational Drug Use Trends and Emerging Analytes Identified in Blood, Urine, and/or Oral Fluid From Attendees at an Electronic Dance Music (EDM) Festival

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After attending this presentation, attendees will be able to assess and review the trends of recreational drug use generated by survey data and analytical testing of biological samples and compare user accounts of what they are ingesting with toxicological results within an EDM population.

This presentation will impact the forensic science community by providing data on the extent and nature of emerging analytes. Additionally, attendees will be able to discuss trends in Novel Psychoactive Substance (NPS) use within this population, which have not previously been studied in the United States.

EDM is a popular music genre in Europe and the United States and has a strong association with various specific types of drug use, especially NPSs, which is documented by surveys with EDM attendees and is reflected in online discussion groups associated with EDM culture. EDM festivals within the United States have recently been a focus of media attention due to drug-related deaths and mass hospitalizations or medical aid calls, which have caused cancellations of the events. The use of these novel and potentially toxic drugs within these venues makes EDM festivals an important site to collect information regarding recreational drug use and potentially characterize emerging analytes.

Participants were recruited during an EDM festival in Florida in 2014. The study received institutional review approval for human subject studies. After obtaining informed consent, each participant filled out a brief questionnaire regarding prescription medication and recreational drug use within the last week. Participants were asked to provide a blood, urine, and two oral fluid samples for laboratory-based drug screening and confirmation. One oral fluid sample was collected for presumptive screening using the Alere® DDS2 Mobile System and the other was collected with an Immunalysis Quantisal™ collection device for subsequent laboratory-based confirmatory analysis.

A total of 145 volunteers participated in the sample collection. The average age of the study participants was 23 years old. Not all subjects provided all three biological samples. Sixty-six blood samples, 105 urine samples, 125 oral fluid samples for the Alere® DDS 2, and 136 oral fluid samples for the Quantisal™ were provided by the participants. Blood samples were screened using Liquid Chromatography/Quadrupole/Time-Of-Flight/Mass Spectrometry (LC/Q/TOF). All urine specimens were screened via several analytical techniques including Enzyme Multiplied Immunoassay Technique (EMIT), Gas Chromatography/Mass Spectrometry (GC/MS), and LC/Q/TOF. The oral fluid sample collected for the Alere® DDS 2 Mobile System was screened on-site for the presence of cannabis, cocaine, opiates, methamphetamine, amphetamine, and benzodiazepines. Alcohol was confirmed and quantitated using a headspace GC/Flame Ionization Detector (GC/FID) for all blood and urine samples. Any sample which screened positive was sent for confirmation.

When asked whether or not the individual had taken any medicinal or recreational drugs within the past week, 70.7% of the participants answered “yes.” The most common substance participants indicated they had taken was marijuana (n=60), followed by cocaine (n=17). In terms of NPS, compounds like MDMA (3,4-methylenedioxy-N-methylamphetamine), “Molly,” and/or “ecstasy” were reported to have been used within the past week by 33 participants. Forty blood samples have been screened using LC/Q/TOF. Thirty percent of the blood samples (n=40) screened positive for at least one NPS drug. Of the 104 urine samples screened, only 16% were completely negative for the presence of any drugs and/or alcohol. Twenty-one percent of the urine samples were positive for a single drug, while 35% were positive for polydrug use. Approximately 40 urine samples screened positive for an NPS drug, with more than 80% confirming positive for which there was an analytical assay available. One hundred twenty-two oral fluid samples screened positive using the Alere® DDS 2. The two most common positive results were for cannabis (22%) and cocaine (10%).



Toxicology Section - 2015

The EDM festival culture has been largely understudied in the United States; however, this population has proved to be an invaluable resource in terms of learning about patterns of recreational drug use and emerging NPS. Data on which drugs are being ingested at these events can be useful for educating users about risks associated with NPS, provide opportunities for harm reduction, and enable forensic laboratories to target testing strategies for impairment or death investigations associated with these events.

NPS, Electronic Dance Music, Drug Testing



K10 Evaluation of Cases Admitted to Cukurova University Forensic Toxicology Laboratory From June 2009 to June 2014: A Retrospective Study

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After attending this presentation, attendees will better understand the gender, variety, and number of cases received per year to the Cukurova University Forensic Toxicology Laboratory in southern Turkey, which serves both the clinical and judicial fields.

This presentation will impact the forensic science community by informing attendees of the wide diversity of substances analyzed in the Cukurova University Forensic Toxicology Department.

Cukurova, with its historical name “Mediterranean”, covers Adana, Mersin, Osmaniye, and Hatay provinces and has geographical, economical, and cultural importance. One of the largest population densities of Turkey belongs this area with its seven million people. Throughout history, Cukurova has been an escape point from Europe to the Middle East, and the short transition point from North Middle East to Central Asia. It is also the center of transportation with its two main harbors.

Cukurova University Hospital is located in this region and provides service to surrounding subregions. Cukurova University Forensic Toxicology Laboratory is a unique academic unit that works in the field of forensic and clinic toxicology. Analysis of drugs of abuse, pesticides, volatile substances, and alcohol and carbon monoxide poisoning are performed in biological samples such as blood, urine, hair, and nails by using gas chromatography/mass spectrometry, liquid chromatography/tandem mass spectrometry, headspace gas chromatography, and ultraviolet spectrophotometry. Not only forensic samples but also routine hospital toxicology analyses are performed in this laboratory.

In this study, archives of cases received in this forensic laboratory during the period of June 2009-June 2014 were investigated. Cases were classified according to gender, applied unit, type of substances, and distribution of cases by year. Findings were statistically evaluated by the Statistical Package for the Social Sciences (SPSS) v20.0 software program.

After screening five years of data, it was determined that the number of cases gradually increased every year. A total of 865 cases were evaluated and it was found that 52.3 % of the cases admitted to the toxicology laboratory were clinical, 43.2 % were forensic samples, 2.8% were special request, and 1.7 % were from surrounding hospitals; 74.2 % were male and 25.8 % were female. Furthermore, it was found that 50.2 % of 452 clinical cases were from an emergency department, 22.3 % were from pediatric patients, 12.4 % were from psychiatry patients, 9.7 % from neurology, and the remainder were from other units of the hospital.

Of the 374 forensic cases received, 95.7 % were for drugs of abuse and 4.3 % were for alcohol. Tetrahydrocannabinol (THC) is the most widely determined substance in drug abuse cases and amphetamine and derivatives were commonly used in combination with THC.

Pesticide poisoning is also common due to one of the main sources of agriculture. Spraying for insects is carried out in March, April, and May in the region, so poisoning cases are more frequent in these months.

Carbon monoxide intoxication was more frequent in wintertime than in summertime due to household heating. The number of women who were exposed to carbon monoxide was greater than men, likely because women were much more frequently at home.

This study will present a retrospective study in terms of identification of cases received by Cukurova University Hospital Toxicology Laboratory.

Forensic Toxicology Lab, Demography, Cukurova University



K11 Analysis of the Anticoagulant Brodifacoum in Serum After an Incident of Pesticide Poisoning

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After attending this presentation, attendees will better understand the presentation of pesticide poisoning by brodifacoum from a clinical setting as well as with the analysis of brodifacoum in biological specimens.

This presentation will impact the forensic science community by illustrating how the combination of clinical diagnostics and forensic toxicology appropriately identifies a poisoning agent.

In 2008, the Environmental Protection Agency tightened restrictions on consumer-available rodenticides due to substantial risks posed to humans. Brodifacoum, a second-generation anticoagulant rodenticide, along with bromadiolone, difenacoum, and difethialone, are no longer available in less than one pound packaging marketed to the consumer.¹ Labeled as superwarfarins, these compounds are hazardous in humans due to their long half-life anticoagulation effects which cause excessive bleeding. Commercially, brodifacoum is the most commonly used in commercial rodenticides at a concentration of 0.005% and there have been reports of accidental and intentional poisonings by brodifacoum.

A 47-year-old woman with no history of bleeding disorder developed nausea and vomiting without diarrhea after eating at a restaurant. One week later, she discovered dark blood-like urine which prompted her to go to the emergency room. She underwent an Esophagogastroduodenoscopy (EGD) during her hospitalization with no complications. Post-procedure, she developed gum bleeding and coagulation labs revealed a Prothrombin Time (PT) of >120s with an International Normalized Ratio (INR) of >9.9 and a Partial Thrombin Time (PTT) of 147.8s. PT and PTT corrected with mixing study. The patient was also found to have bleeding into the collecting system of the kidneys. She received four units of Fresh Frozen Plasma (FFP) before being transferred to another hospital for higher level care and workup. She denied any personal or family history of bleeding disorders and had multiple surgical procedures in the past without any bleeding complications. She also noted no gum bleed, epistaxis, or previous history of hematuria or hematochezia. Coagulation studies showed a decrease in vitamin K dependent factors II, VII, XI, and X of 26%, 4%, 14% and 31%, respectively. Initially her INR corrected with FFP and daily vitamin K but became elevated again two days later despite daily vitamin K. Again her INR was corrected with FFP and with an increased dose of both oral and Intravenous (IV) vitamin K. A super warfarin was suspected.

A qualitative anticoagulant poisoning panel was performed on serum that confirmed positive for brodifacoum. Other analytes in this panel include warfarin, dicumarol, diphacinone, chlorophacinone, difenacoum, and bromadiolone. The extraction of brodifacoum from serum was achieved using protein precipitation by acetonitrile after the addition of chloro-warfarin as the internal standard. Then a solvent extraction was completed using Methyl Tert-Butyl Ether (MTBE); the organic layer was dried down and reconstituted with an 80:20 mix of 0.02% ammonium hydroxide in deionized water and 0.02% ammonium hydroxide in methanol. Analysis was achieved by using High-Performance Liquid Chromatography (HPLC) separation on a BEH C18 column with negative-ion Electrospray Tandem Mass Spectrometry (LC/MS/MS) for detection. The transitions monitored for brodifacoum were 523.1>135.1 and 523.1>80.9. Values that exceeded the method cut-off of 10ng/mL were reported as positive. The response of the sample was approximately 30x that of the cut-off calibrator.

The patient denied any exposure to rat poison and did not feel anyone was trying to poison her. She denied any current suicide attempts but did state an attempt five years prior by overdosing on her antidepressants. She was cleared by psychiatry. The case was reported to the state poison control center and health department. Also the patient's local law enforcement was notified of the findings. She was cleared by the hospital's risk management department and social work department and discharged on daily vitamin K with weekly follow-ups as an outpatient for INR levels.

Reference:

1. Bradbury, S. (2008, June 24). Risk Mitigation Decision for Ten Rodenticides Retrieved from <http://www2.epa.gov/rodenticides/restrictions-rodenticide-products>

Brodifacoum, Pesticides, Poisoning



K12 Effect of Sunlight on Methamphetamine in Urine

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After attending this presentation, attendees will understand the effect of sunlight exposure time to the methamphetamine concentration in urine.

This presentation will impact the forensic science community by providing results in an area with very little previous research. This presentation will discuss one of the factors that can affect the concentration of methamphetamine in urine.

Malaysia is a tropical country close to the equator and has hours of abundant natural sunshine almost every day. The strong sunlight could damage goods. Hence, forensic toxicology chemists are often challenged by lawyers in drug abuse trials concerning the accuracy of the analytical result, especially in cases where the specimen of the accused was kept in the enforcement officer's office or in a car exposed to sunlight during the transportation to the toxicology laboratory. The possibility of decomposition or degradation of a drug in the urine specimen by sunlight has become an issue.

In this study, the stability of methamphetamine in urine was evaluated after various exposure periods of sunlight. Nine urine specimens (marked "M1" to "M9") containing 1ug/mL to 5ug/mL methamphetamine were chosen for this study. The presence of methamphetamine in the urine specimens was identified by gas chromatography mass spectrometry prior to the transfer of 1mL of each specimen to a 2mL microtube. Twenty microtubes were prepared for each specimen. All prepared microtubes were capped properly and placed in the sun from 10:00 a.m. to 3:00 p.m. every sunny day. The microtubes were kept in a chiller after the experiment. One microtube from each specimen was collected when the preset interval period of the sunlight exposure had been attained. The concentration of the analyte after exposure to 5-310 hours of sunlight was determined by Enzyme Linked Immunosorbent Assay (ELISA) technique.

Results showed that the concentration of methamphetamine in all urine specimens decreased as the sunlight exposure time increased. No methamphetamine was detected in specimen M1 to M7 after 255 hours of sunlight exposure. The methamphetamine in M8 and M9 decreased in accordance with M1 to M7, and would likely be negative for methamphetamine if the sunlight exposure time was increased further. Although the temperature of the urine increased during the sunlight exposure process, previous research suggested that there was no significant effect of temperature to the loss of methamphetamine in urine.^{1,2} In conclusion, sunlight could reduce the concentration and eliminate the methamphetamine in urine and exposing the urine specimen to sunlight, especially for a long period, should be avoided.

References:

1. C. Jiménez, R. de la Torre, M. Ventura, J. Segura, R. Ventura. Stability studies of amphetamine and ephedrine derivatives in urine. *Journal of Chromatography B*, Volume 843, Issue 1, 20 October 2006, Pages 84-93.
2. D. Wong. Thermal Stability of Methamphetamine in Urine. *Proceedings of the 2nd International Forensic Science Symposium*, 2011, Kuala Lumpur, Malaysia.

Methamphetamine, Sunlight, Urine



K13 Analysis of Buprenorphine, Norbuprenorphine, Naloxone, and Their Glucuronides From the Urine Obtained in Drug and Driving Cases

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After attending this presentation, attendees will better understand choosing the most efficient method for extracting buprenorphine, norbuprenorphine, naloxone, and related glucuronides from urine employing available Solid Phase Extraction (SPE) cartridges and Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS). Buprenorphine is being requested more often as a test in drug and driving cases. Buprenorphine does not cross-react with typical opiate immunoassays and cannot be detected in basic drug screens using Gas Chromatography/Mass Spectrometry (GC/MS) without derivatization. LC/MS/MS does offer an efficient alternative to GC/MS.

This presentation will impact the forensic science community by offering analysts operating in forensic facilities information regarding the extraction and analysis of buprenorphine, norbuprenorphine, naloxone, and the related glucuronides in urine samples obtained in drug and driving cases using SPE and LC/MS/MS. These drugs are now regularly being encountered in drug and driving casework and this method will greatly assist analysts in offering better interpretation to submitting agencies.

Method: 1mL samples of urine (calibrators, controls, and test samples each containing deuterated internal standards) were diluted with 3mL of 0.1M aqueous phosphate buffer (pH 6), vortex mixed and centrifuged. The supernatant liquid was applied to a pre-conditioned SPE mixed mode (C8/SCX) column. The SPE columns were conditioned with methanol, Deionized (DI) water and 0.1M phosphate buffer (3mL, 3mL, 1m, respectively). After loading samples at 1mL/minute, the SPE cartridges were washed with DI water, 1.0M acetic acid, and methanol (3mL of each, respectively). The SPE columns were dried and eluted with 3mL of a solution containing methylene chloride-isopropanol-ammonium hydroxide (78-20-2) and 3mL of a solution of methanol containing 4% ammonium hydroxide. The eluates were collected separately and combined to form one solution. The eluate solutions were evaporated to dryness under nitrogen at 35°C. The dried residues were dissolved in 100µL of mobile phase for LC/MS/MS. LC was performed in gradient mode employing a 50mm x 2.1mm (2.1µm) aromatic phase LC column using mobile phase consisting of acetonitrile and 0.1% aqueous formic acid at a flowrate of 0.5mL/minute.

Tandem mass spectrometry was performed in positive Multiple Reaction Mode (MRM). The following transitions were monitored (quantification transition ions underlined): buprenorphine (468.3 to 396.2, 414.3), buprenorphine-d4 (472.5 to 400.1, 415.3), Norbuprenorphine (414.3 to 340.1, 326.0), norbuprenorphine-d3 (417.3 to 343.4, 326.0), naloxone (328.2 to 253.0, 212.1), respectively. The glucuronides were monitored as follows: buprenorphine glucuronide (644.3 to 468.1, 396.3), norbuprenorphine glucuronide (590.3 to 414.3, 396.2), naloxone glucuronide (335.2 to 299.1, 273.1), naloxone-d5 (332.1 to 258.1, 273.1), respectively. In this presentation, representative chromatograms are shown to illustrate the efficiency of the chromatography and analysis of buprenorphine, norbuprenorphine, naloxone, and related glucuronides from 20 (completed) drug and driving cases

Results: The limits of detection/quantification for this method were determined to be 5ng/mL and 10ng/mL, respectively, for all of the analytes (i.e., buprenorphine/norbuprenorphine/naloxone, and glucuronides). The method was found to be linear from 10ng/mL to 1,000ng/mL ($r^2 > 0.999$). The analyte recoveries were found to be greater than 95% for all of the noted compounds. Interday and Intraday variation of the method were found to <8% and <10%, respectively. Matrix effects were determined to be <6%. Details regarding the concentrations of buprenorphine/norbuprenorphine/naloxone found in 20 genuine urine cases are presented.

Conclusion: This method demonstrates the efficient use of both SPE coupled with the use of LC/MS/MS for the analysis of buprenorphine/norbuprenorphine/naloxone and their related glucuronides in cases of driving under the influence of drugs. The ability to analyze buprenorphine, norbuprenorphine, naloxone, and their glucuronides in drug and driving cases will greatly assist toxicologists in offering the appropriate interpretation to the submitting agencies.

Buprenorphine Glucuronide, Naloxone, SPE



K14 Validation of the Neogen® Enzyme-Linked Immuno-Sorbent Assay (ELISA) Fentanyl Ready-to-Use (RTU) Kit for Whole Blood and Urine Specimens

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After attending this presentation, attendees will understand the performance of the Neogen® ELISA Fentanyl RTU kit for screening whole blood and urine specimens as evaluated by the Scientific Working Group for Forensic Toxicology (SWGTOX) Standard Practices for Method Validation in Forensic Toxicology Laboratories guidelines.

This presentation will impact the forensic science community by demonstrating the validation of an ELISA method following SWGTOX guidelines.

Objective: The validation of a semi-quantitative method for the rapid screening of whole blood and urine specimens by a Dynex DSX® Automated ELISA System using the Neogen® Fentanyl (RTU) Kit.

Method: Neogen® Fentanyl kit assay instructions for incubation times, reagent volumes, and sample volumes were followed. Whole blood samples were diluted 1:5 with buffer before being loaded onto the instrument. Urine samples were diluted 1:2 with buffer by the instrument. Performance of the assay was evaluated at two decision points for each matrix (low control and cutoff control). Urine was evaluated at 1ng/mL and 5ng/mL and blood was evaluated at 0.5ng/mL and 1ng/mL. SWGTOX guidelines were followed for the validation of the assay. The validation included the evaluation of sensitivity, repeatability, specificity, carryover, plate drift, ruggedness/robustness, and a case sample comparison.

Results: Carryover was evaluated by running three replicates of a blank matrix control following a positive matrix control at 500ng/mL for both blood and urine. Carryover was not detected in the assay. The sensitivity for this method was evaluated by replicate analysis of a blank matrix control to determine the theoretical Limit Of Detection (LOD) and by the analysis of standards at successively lower levels to determine an experimental LOD. The theoretical LOD was determined to be 0.18ng/mL for blood and 0.03ng/mL for urine. The experimental LOD was determined to be 0.5ng/mL for both blood and urine assays. Repeatability was evaluated at 0.25ng/mL, 0.5ng/mL, 0.75ng/mL, 1ng/mL, and 1.5ng/mL for blood and 0.5ng/mL, 1ng/mL, 1.5ng/mL, 2.5ng/mL, 5ng/mL, and 7.5ng/mL for urine with three replicates at each level over five separate runs. The mean response ± 2 Standard Deviations (SD) at each decision point for both blood and urine did not overlap with the mean response ± 2 SD of standards prepared at $\pm 50\%$ of the concentration of the decision points. The repeatability was determined by calculating the Coefficient of Variation (CV) for 15 inter-run replicate measurements of each assay at each concentration. The CV was less than or equal to 3% for blood and 6% for urine. Specificity was evaluated in blood and urine by the analysis of negative matrix samples spiked at 250ng/mL, 500ng/mL, and 1,000ng/mL of norfentanyl and 5ng/mL, 10ng/mL, and 25ng/mL of acetyl fentanyl. Observed cross reactivity was similar to that stated by the manufacturer. For acetyl fentanyl, cross reactivity was between 29% and 35% for whole blood and between 50% and 59% for urine. For norfentanyl, cross reactivity was between 0.04 and 0.17% for whole blood and between 0.05 and 0.20% for urine. There were no false positives for fentanyl resulting from screening known samples, which contained morphine, hydromorphone, buprenorphine, hydrocodone, oxycodone, and oxymorphone. Two urine cases and one blood case containing fentanyl were positively identified. Plate drift was evaluated by analyzing 24 replicates at the concentration of the cutoff control for each matrix. The number of replicates analyzed was greater than the number of samples run in routine casework. Plate drift was not observed.



Toxicology Section - 2015

Whole Blood (n = 15)	Level (ng/mL)	Mean O.D.	SD	CV (%)	Mean + 2 SD	Mean – 2 SD
	0.25	1.848	0.029	1.6	1.906	1.790
Low Control	0.5	1.663	0.036	2.2	1.735	1.591
	0.75	1.528	0.026	1.7	1.580	1.476
Cutoff Control	1	1.379	0.029	2.1	1.437	1.321
	1.5	1.196	0.029	2.4	1.254	1.138
Urine (n = 15)	Level (ng/mL)	Mean O.D.	SD	CV	Mean + 2 SD	Mean – 2 SD
	0.5	1.451	0.042	2.9	1.535	1.366
Low Control	1	1.148	0.027	2.3	1.201	1.094
	1.5	0.984	0.020	2.0	1.024	0.943
	2.5	0.719	0.024	3.4	0.768	0.671
Cutoff Control	5	0.488	0.027	5.6	0.542	0.433
	7.5	0.371	0.008	2.1	0.386	0.355

Conclusion: The Neogen® Fentanyl ELISA kit is a highly sensitive, specific, and rapid screening procedure to detect fentanyl in blood and urine.

Fentanyl, Validation, ELISA



K15 Rapid Drug Screening Using a Combination of Flow Injection Tandem Mass Spectrometry (FI/MS/MS) and the Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) Method

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After attending this presentation, attendees will understand the usability of the combination of FI/MS/MS and the QuEChERS extraction method for rapid drug screening.

This presentation will impact the forensic science community by demonstrating a rapid detection method that reduces the time needed for the total drug screening process to approximately ten minutes. Using this method, toxicological findings can be returned to forensic pathologists during the autopsy process. Thus, the QuEChERS-FI/MS/MS combination method will facilitate determination of the cause of death and allow law enforcement to solve cases more quickly.

There is a compelling need for the rapid diagnosis of drug poisoning in both forensic and clinical toxicology. Currently, several drug-screening methods are available. For example, immunoassay techniques such as the Triage[®] kit are favored in forensic and clinical toxicology because results are obtained in a short time and sample pretreatment is not required; however, such methods can only be used to assay urine samples and can identify the class of drugs present, but not the drug of interest itself. Furthermore, these methods cannot measure all types of drugs and poisons and they have low sensitivity. Analytical methods such as Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS) are also commonly used for drug screening; however, although these techniques have high sensitivity and selectivity and can detect a wide range of drugs and poisons, they usually require time-consuming sample pretreatment and column separation. From the start-up of the instruments to the reporting of the result, including analysis of the blank sample, a period of more than two hours is typical. While many studies have reported rapid drug screening methods using LC/MS/MS, in practice most of these methods require considerable time from the start-up of the instruments to the reporting of the results. Additionally, the use of these techniques is somewhat difficult for less-experienced analysts. Therefore, the limiting factors were addressed by developing a reliable and simple analytical technique using a combination of the QuEChERS method and an FI/MS/MS system, which returns results within 10 minutes.

Whole blood samples were collected from practical forensic cases (N=79) and pretreated using the QuEChERS method.¹ Briefly, 0.5mL of whole blood was diluted three-fold with distilled water. The diluted sample was placed in a plastic tube with 0.5g of the pre-packed extraction kit reagent, a stainless steel bead, and 1mL of acetonitrile. The mixture was shaken for 30sec and centrifuged for 1min. The supernatant was transferred to a 2.0mL centrifuge tube containing the solid-phase extraction sorbent for sample cleanup. The tube was mixed for 10sec and centrifuged for 1min. The extract was analyzed by both LC/MS/MS and FI/MS/MS (analysis time=1.5min). All product ion spectra obtained by FI/MS/MS were automatically processed by Library View[™] software, and the results were compared with those of the LC/MS/MS analysis using Dice's coefficient.

The combination of QuEChERS and FI/MS/MS enabled completion of the entire drug screening process, from the start-up of the instruments through the extraction process and data analysis, within 10min. For actual forensic cases (N=79), the qualitative results roughly matched (96% concordance rate) with the results obtained with the standard LC/MS/MS technique. The false-positive rate with the combined QuEChERS-FI/MS/MS method was 3.4% and the false-negative rate was 3.9%. Although some drugs present at low concentrations were not detected in the analysis of forensic cases, the QuEChERS-FI/MS/MS method was able to detect a wide-range of drugs in whole blood in a relatively short time.

Reference:

1. Usui K, Hayashizaki Y, Hashiyada M, Funayama M. Rapid drug extraction from human whole blood using a modified QuEChERS extraction method. *Legal Med* 2012;14: 286-296

Flow Injection MS/MS, QuEChERS Extraction, Rapid Drug Screening



K16 Detection of Amitriptyline and Nortriptyline in Decomposed Skeletal Tissues by Microwave-Assisted Extraction and Ultra High-Performance Liquid Chromatography

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After attending this presentation, attendees will understand how to develop a microwave-assisted extraction methodology using bone tissue and be provided with an example of its practical application using vertebral bone.

This presentation will impact the forensic science community by adding to the body of data illustrating the utility of skeletal tissues as a matrix for toxicological analysis and by demonstrating an efficient method for preparation of skeletal tissue samples.

The use of Microwave Assisted Extraction (MAE) followed by Microplate Solid Phase Extraction (MPSPE) and Ultra High Performance Liquid Chromatography (UHPLC) to detect Amitriptyline (AMI) and Nortriptyline (NORT) from postmortem skeletal tissues is described. Rats (n=4) received 60mg/kg amitriptyline and were euthanized by CO₂ asphyxiation approximately 20min post-dose. The remains decomposed to skeleton outdoors and vertebral bone was collected. Bones were cleaned with phosphate buffer (PBS, 0.1M, pH 6), methanol, and acetone, then dried under ambient conditions and pulverized to a powder. Bone samples (n=3, 0.5g), as well as one drug-free sample, underwent MAE using methanol in a closed vessel system for a total of 60 minutes. The extraction solvent was replaced with fresh methanol after 10, 20, 30, and 60 minutes of irradiation. The methanolic extracts were evaporated and reconstituted in 1mL PBS. Internal standard (Desipramine, DMI, 500ng) and 100µL glacial acetic acid were added to each extract. Acetonitrile:methanol (1:1, 3mL) was added to each extract, followed by storage at -20°C overnight to precipitate proteins and lipids. Following centrifugation, the supernatants were evaporated to 1mL, diluted to 3mL using PBS and acidified with 100µL glacial acetic acid.

Diluted supernatants underwent further clean-up by MPSPE, using CleanScreen® XCEL™ 1 48 well plates. Wells were conditioned with methanol (3mL), distilled water (3mL) and PBS (3mL). Following loading of samples, columns were washed with PBS (3mL) and 0.1M acetic acid (3mL). Columns were dried (~5 in Hg, 5min) and washed with methanol (3mL). Columns were then dried again under vacuum (~10 in Hg, 10min). Basic compounds were eluted with 3% NH₄OH in 20:80 isopropanol: dichloromethane (3mL). Extracts were evaporated to dryness and reconstituted in 0.1% formic acid in 10:90 acetonitrile:water (500µL). Samples were analyzed using an Acquity UHPLC with a Photo-Diode Array (PDA) detector. The column used was a Selectra DA (100mm x 2.1mm, 3.0µm particle size). Samples were run using a binary gradient elution (A: 0.1% (v/v) formic acid, 10% (v/v) acetonitrile, and 90% (v/v) water; B: 0.1% (v/v) formic acid, 10% (v/v) water, and 90% (v/v) acetonitrile). The mobile phase gradient began with 90:10 A:B, held for 3min, followed by a linear increase to 40:60 A:B over 8min, followed by reversion back to 90:10 over 1min for a total run time of 11min at a constant flow rate of 0.300mL/min. The autosampler was maintained at 25°C, with the column temperature set to 50°C. The wavelengths chosen for analysis were 245nm for AMI and NORT as well as 290nm for DMI. Analyte stability to MAE in methanol was assessed and both analytes were stable for at least 60mins irradiation time. Recovery was at least 95% of maximal value within the first 10min of MAE for all samples assayed. The MPSPE/UHPLC method was linear between 25-10,000ng/mL, with precision and accuracy <20% in triplicate analyses, with a limit of detection of 25ng/mL for both AMI and NORT. The vertebral bone analyzed using this method detected AMI (2.8µg/g -15µg/g) and NORT (1.8µg/g-4.3µg/g) in all samples assayed.

Forensic Toxicology, Bone, Microwave-Assisted Extraction



K17 A Validated Method for the Determination of Salvinorin A and Salvinorin B in Forensic Toxicology Samples

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After attending this presentation, attendees will be able to describe a simple and effective way to identify salvinorins in urine using Solid Phase Extraction (SPE) and Gas Chromatography/Mass Spectrometry (GC/MS).

This presentation will impact the forensic science community by providing a scientifically validated method for the determination of Salvinorin A and B in urine.

Salvia divinorum is a perennial plant from the Lamiaceae (mint) family found in the Sierra Mazateca region of Oaxaca, Mexico, and has been used in religious and medicinal rituals for centuries. *Salvia divinorum* is also used as a recreational drug due to its profound hallucinogenic properties. As many as 35 countries have enacted legislation to control *S. divinorum* and/or salvinorin A, its principal psychoactive component. Street names include Diviner's Sage, Maria Pastora, Sally-D, and Magic Mint. Salvinorin A and its major metabolite (Salvinorin B) are of forensic interest, but are rarely reported during routine toxicological testing. Salvinorin A is the only known naturally occurring non-nitrogenous hallucinogen with a high affinity for the Kappa-Opioid Receptor (KOR). Although it is not federally controlled at present, its rapid onset of action and powerful hallucinogenic effect contribute to its abuse potential.

An optimized and scientifically validated method for the determination of salvinorin A and salvinorin B in biological matrices is reported. SPE and GC/MS using Selected Ion Monitoring (SIM) were used throughout. Both the extraction method and GC/MS parameters were optimized to achieve optimal chromatographic separation and detection. In the absence of a commercially available deuterated salvinorin at the time of the study, testosterone-D3 was used as the internal standard. A GC inlet temperature of 250°C and an initial oven temperature of 260°C produced optimal results. The temperature program involved a 0.5min hold at 260°C with a ramp up to 290°C with a 30°C/min rate and a final hold for 17 minutes. The retention times were 6.9min for Salvinorin B and 7.9min for Salvinorin A.

The method was evaluated using recommendations of the Scientific Working Group for Toxicology (Scientific Working Group for Forensic Toxicology (SWGTOX) standard practices for method validation in forensic toxicology, 2013). Analytical recovery, limit of linearity, limit of detection, quantitation, precision, bias, carryover, interference, and dilution integrity were evaluated. Both Salvinorin A and Salvinorin B were linear from 0ng/mL-1,000ng/mL. The limits of detection and quantitation for Salvinorin A were 5ng/mL and 10ng/mL, respectively. The limit of detection for Salvinorin B was 20ng/mL. Precision and bias were evaluated at three concentrations and produced %CVs and bias of <20%. Carryover was not present at 1,000ng/mL and no interferences were observed from common drugs of abuse. Interferences from other salvinorins and divinatorins were also evaluated. Dilution integrity was evaluated using biological matrices that were diluted 1:10 prior to SPE. These results also demonstrated acceptable precision and bias. This validated method provides an efficient and reliable method to quantitatively identify salvinorins of forensic interest in biological matrices using GC/MS.

Salvinorin A, GC/MS, Urine



K18 Identification of Methcathinone in Urine by Gas Chromatography/Mass Spectrometry (GC/MS) Using a One-Step Simultaneous Dispersive Liquid-Liquid Extraction (LLE)/Cyclohexanone Derivatization

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After attending this presentation, attendees will have a better understanding of how cyclohexanone can be used as a simultaneous extraction and derivatizing agent.

This presentation will impact the forensic science community by providing a simple one-step dispersive (LLE)/derivatization method using GC/MS to identify methcathinone in urine.

Over the last decade, abuses of prescription and illegal drugs have caused serious problems in the United States. Recently a new class of drugs, known as synthetic cathinones, has spread worldwide. These synthetic cathinones are marketed as “bath salts,” “legal high drugs,” and “plant food.” Synthetic cathinones are beta-ketone phenylalkylamine derivatives and are often termed “bk-amphetamines” for the beta-ketone moiety. One of the synthetic cathinones that has been abused illegally is methcathinone. Crime laboratories are in need of methods to detect and identify these drugs not only in solid-dosage form but also in biological fluids such as urine from suspects of driving under the influence.

Skymba and Brettell have previously investigated cyclohexanone as a derivatizing reagent for cathinones using GC/MS.¹ A method was developed using cyclohexanone to form the Schiff-base derivative, creating a more complicated mass spectrum and better separation of the synthetic cathinones. This GC/MS method was developed to screen, identify, and differentiate 11 similar cathinone compounds often present in illicit drug submissions to crime laboratories. Primary and secondary amines will react with cyclohexanone via a Schiff-base reaction to form two distinct, different types of derivatives. Cyclohexanone forms an imine derivative with primary amines and enamine derivatives with secondary amines. The method was found to be reproducible and can be used to screen unknown samples and identify multiple cathinones in one sample.

In this presentation, a method will be described which expands the role of the cyclohexanone to an extraction solvent as well as a derivatizing agent. The method uses 300uL of cyclohexanone as the extraction solvent in a one-step dispersive LLE/derivatization in which methcathinone can be detected and identified from 1mL of urine using GC/MS.

In this method, the GS oven temperature parameters were set with an initial temperature of 120°C which then increased 15°C/min to 275°C. The column used was a 30m x 0.25mm x 0.25µm phenylmethylsilicone capillary column (Rxi®-5Sil MS) using helium as a carrier gas with a linear gas velocity of 36cm/sec. Cyclohexanone was used as the solvent and a sample volume of 1µL was injected in the split mode with a split ratio of 22:1. A retention-time optimization study provided the most advantageous separation conditions with methcathinone eluting with a retention time of 14.3 minutes using these conditions.

A simple, one-step dispersive LLE/derivatization method will be presented that uses cyclohexanone as the extraction solvent and GC/MS to identify methcathinone in urine.

Reference:

1. Brandi A. Skymba, M.S.F.S., and Thomas A. Brettell, Ph.D., “Investigation Into Cyclohexanone as a Schiff-Base Derivatizing Agent for the Detection of Cathinones with Gas Chromatography/Mass Spectrometry,” Abstract #A204, Proceedings of the American Academy of Forensic Sciences, 65th Annual Scientific Meeting, Washington, DC. 2013.

Forensic Toxicology, Methcathinone, Dispersive LLE



K19 Quantitative Analysis of Methylphenidate (Ritalin®) and Ritalinic Acid in Oral Fluid by Liquid Chromatography/Triple Quadrupole/Mass Spectrometry (LC/QqQ/MS)

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The goal of this presentation is to introduce attendees to the use of human oral fluid for the quantitation of Methylphenidate (MPH) and its major metabolite, Ritalinic Acid (RA), through chromatographic and mass spectral analysis. Attendees will be familiarized with the advantages of a non-conventional biological matrix when applied to the forensic and clinical monitoring of these drugs.

This presentation will impact the forensic science community by informing attendees about an efficient, simple, and fast analytical procedure utilizing a non-invasive technique for monitoring MPH and RA in human oral fluid via the introduction of a dilute-and-shoot LC/QqQ/MS method of analysis.

MPH is an amphetamine derivative commonly used in the treatment of Attention-Deficit Hyperactivity Disorder (ADHD) in children, adolescents, and adults, a condition affecting 3%-5 % of the United States population. The 2011 National Survey of Children's Health reported that 11% of children aged 4-17 years were diagnosed with ADHD by a health care provider in the United States, with 6.1% of children in that age group having been prescribed medication for treatment of ADHD. In recent years, methylphenidate HCl has become one of the most frequently abused prescription drugs due to its psychostimulant effects. In addition, the drug has become popular with college students as a "study drug." According to the Drug Abuse Warning Network (DAWN) Report, the number of emergency department visits involving the non-medical use of ADHD-stimulant medications increased from 5,212 to 15,585 between 2005 and 2010. When administered orally, MPH is rapidly absorbed and hydrolyzed to its inactive metabolite RA. Current analytical methods for analysis of MPH and RA involve the analysis of blood and urine matrices using liquid-liquid extraction, solid-phase extraction, and Gas Chromatography/Mass Spectrometry (GC/MS). These methods are time consuming and sample collection is invasive and tedious. Due to the rising abuse of MPH, development of quick and non-invasive monitoring methods is important.

This presentation will demonstrate human oral fluid as a suitable matrix for analysis of MPH and RA using Electrospray Ionization (ESI) Liquid Chromatography/Mass Spectrometry (LC/MS) as a sensitive and specific method for screening and quantitation. Oral fluid samples were collected using an oral fluid collection device containing a cotton pad. They were vortexed, centrifuged for 30min at 3,200rpm and filtered into polypropylene serum tubes. The extracts were stored at -20°C until use. An Agilent 6460 triple quadrupole MS-equipped with Jet Stream ESI technology was used in the triggered Multiple Reaction Monitoring (MRM) mode for the characterization of the analytes. The ESI source was operated at 325°C in positive mode, with an optimized fragmentor voltage of 75V. The tMRM transitions were 234→84 and 234→56 for MPH and 220→84 and 220→56 for RA, with a cycle time of 500ms. The advantage of using multiple transitions is suggested as a fingerprint mass spectral tool for unambiguous identification of the target compounds. Gradient elution was performed on a Poroshell 120 EC-C18 column (2.1 x 100mm x 2.7µm) using MeOH+0.1% formic acid as organic phase and 5mM ammonium formate + 0.1% formic acid as phase modifier, with a total run time of 5min. Six-point linear calibrations with a weight of 1/x based on the internal standards (+)-methylphenidate-d9 HCl and (+)-threo-Ritalinic acid-d10 HCl, ($r^{23}0.99$) were obtained for concentrations between 0.50ng/mL and 100.00ng/mL for the drugs of interest, with quality controls showing an RSD<13%. The limits of detection for MPH and RA were 0.20ng/mL and 0.30ng/mL, respectively, indicating the validity of the method for identification and confirmation at low concentrations.

Methylphenidate, LC/QqQ/MS, Oral Fluid



K20 Assessment of Three Time-of-Flight/Mass Spectrometry (TOF/MS) Drug Screening Technologies Using Different Fragmentation Modes

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After attending this presentation, attendees will be able to describe and distinguish between three different fragmentation techniques in Liquid Chromatography/Time-Of-Flight/Mass Spectrometry (LC/TOF/MS) instrumentation and assess their utility for different types of toxicological analyses.

This presentation will impact the forensic science community by describing novel approaches to improving the sensitivity and specificity of emerging TOF/MS instrumentation in the forensic toxicology laboratory and by assisting laboratory managers and technical staff in selecting the appropriate instrumentation for specific analyses.

There is increasing interest in the use of High Resolution Accurate Mass Spectrometry (HRAMS) for toxicological drug screening. LC/TOF is a very powerful technique due to its increased specificity and sensitivity over immunological screening, its ease of sample preparation, and rapid run time compared to traditional Gas Chromatography/Mass Spectrometry (GC/MS) screening, and its capabilities for retrospective data analysis. Analysis by LC/TOF can be performed in multiple modes, all of which use a calculated exact mass based on the chemical formula of the parent compound and an expected retention time to identify analytes. Fragmentation allows for additional data characteristics to be used for identification thus eliminating some of the false positives caused by artifacts, minor metabolites, degradation products, drug analogs, and isomers. These false positives may lead to unnecessary confirmatory testing and/or an excess of candidate compounds requiring thorough data evaluation for a simple presumptive screening identification.

Three ionization modes were evaluated using an Agilent 1290 HPLC/6530 QTOF mass spectrometer with Jet Stream® technology for the screening of spiked postmortem samples and samples collected from an Electronic Dance Music (EDM) festival population for novel psychoactive substances as well as therapeutic drugs and traditional drugs of abuse. The modes were conventional Quadrupole Time-of-Flight (QTOF) and two All-Ions ionization modes: Collision-Induced Dissociation in the Source (CIDS) and Collision-Induced Dissociation in the Collision Cell (CIDCC). The conventional QTOF mode uses targeted MS/MS analysis, while the CIDS and CIDCC All-Ions modes provide fragmentation data through the use of alternating fragmentor voltages in the source or collision energies in the collision cell, respectively. The elution profiles of each of the ions, parents, and fragments are correlated for use in compound identification.

The All-Ions modes proved more advantageous for screening than the QTOF mode, especially for analytes present at lower concentrations. The QTOF mode is a data-dependent acquisition mode in which the MS/MS data collected for a particular sample is dependent on the precursor ions detected through MS data collection within the same injection. This leads to an increase in analysis time, a decrease in sensitivity, and an overall decrease in the amount of information obtained from a single injection; fewer data points are collected for each ion observed. The All-Ions CIDCC mode is similar to the QTOF mode in that the collision cell of the QTOF is utilized to generate mass spectra of precursor and fragment ions while the fragmentor voltage in the source is maintained at a low level preventing fragmentation prior to the collision cell; however, in this mode, the quadrupole is not used to filter for precursors observed; all ions within the mass range are passed to the analyzer for the entire run. The All-Ions CIDS mode cycles through the low and two higher source fragmentor voltages with the collision cell turned off thus allowing for collection of QTOF-like data using a conventional TOF instrument. Overall, both All-Ions modes performed better than the QTOF mode for broad-spectrum toxicological drug screening.

Toxicological Screening, TOF, QTOF



K21 Analysis of Phosphide Zinc and Aluminium Phosphide

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After attending this presentation, attendees will better understand phosphide and how to diagnose it, especially Aluminium Phosphide (ALP). ALP is a potent poison and negligence in handling and transport can cause serious effects.

This presentation will impact the forensic science community by discussing the adverse effects of ALP, including how the number of ALP-related cases are increasing daily, especially in the northern region of India.

This abstract is based on analysis of ALP. Attendees will understand what the term toxicology means and the role of the forensic toxicologist expert. The word “toxicology” is derived from the Greek word “toxicon” which was used as a poisonous substance in arrowheads. Mathieu Orfila is considered to be the modern father of toxicology. The expertise of forensic toxicologists is primarily utilized in establishing the cause of death and elucidating its circumstances in postmortem investigation.

ALP is an inorganic phosphide used to control insects and rodents in a variety of settings. It is mainly used as an indoor fumigant at crop transport, storage, or processing facilities for both food and non-food crops. It may also be used as an outdoor fumigant for burrowing rodent and mole control or in baits for rodent control in crops. ALP is available in pellet and tablet form, in porous blister packs, sachets, or as dusts. ALP is generally known as a suicide poison; sometimes it is used in a homicide and occasionally it is accidentally ingested. It can easily be bought and has no effective antidote. Its toxicity results from the release of phosphine gas as the tablet gets into contact with moisture. Physical properties of ALP and zinc phosphide differ, as ALP is gray in color while zinc phosphide is black.

ALP can be detected in biological and non-biological matrices as well as other items taken from the scene of a crime. Viscera, postmortem blood, and other samples such as gastric lavage, clothes, etc. can be analyzed for ALP.

Diagnosis is made by clinical suspicion symptoms and history obtained from relatives, parents, and others. Sometimes the symptoms of poisoning are vague, even in fatal poisoning. This poison is mainly absorbed in the stomach and small intestine and some part may be passed out through excretion, sweat, and respiration. Chemical tests such as the silver nitrate test, alizarin test, and instrumental examination such as **Gas-Liquid Chromatography** (GLC) and biochemical examination of the gastric aspirate and viscera are carried out to diagnose phosphide poisoning. Treatment includes early gastric lavage with potassium permanganate or a combination of coconut oil and sodium bicarbonate, administration of charcoal, and palliative care. Specific therapy includes intravenous magnesium sulphate and oral coconut oil. Moreover, acidosis can be treated with early intravenous administration of sodium bicarbonate. This presentation will review the epidemiological, toxicological, and clinical/pathological aspects of ALP poisoning and its management.

Potent Poison, Diagnosis, Antidote



K22 A Total Sample Preparation Screening Solution for Acidic, Neutral, and Basic Drugs Using ISOLUTE® Multimode Solid Phase Extraction (SPE) Prior to High-Performance Liquid Chromatography With Tandem Mass Spectrometry (HPLC/MS/MS) Analysis

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The goal of this presentation is to explain the evaluation of a multimode SPE sorbent for screening of acidic, basic, and neutral drugs from biological fluids.

This presentation will impact the forensic science community by demonstrating the utility of a multimode sorbent for effectively screening drugs of abuse from a variety of biological fluids.

Introduction: Sample preparation methodology that extracts and concentrates an analyte prior to analytical analysis is typically necessary to facilitate accurate quantitative and qualitative results. Typically, the sample preparation method is limited to drugs with the same functionalities (i.e., basic or acidic) to efficiently extract multiple drugs from matrices like urine. Here an all-encompassing method that can be used to extract a broad range of drugs independent of their functionality is described. The multimode silica based sorbent utilizes both a cationic and anionic functionality along with a hydrophobic mechanism to retain all of the drugs analytes prior to extraction and concentration.

Method: Urine and whole blood samples were spiked with a broad array of drug classes containing basic, acidic, and neutral functionalities. The drug classes examined were barbiturates, opiates, benzodiazepines, synthetic cannabinoids, amphetamines, and cathinones. The samples were loaded onto ISOLUTE® Multimode sorbent in cartridge format. Sample extraction methodologies for urine and whole blood were developed with optimized wash steps to maximize cleanliness and minimize observed ion suppression. The drugs were extracted in a two-step process to elute the acidic and neutrals and then the basic analytes. The samples were dried down, reconstituted, and analyzed using an Applied Biosystems® 4000 QTRAP® with an Agilent 1200 liquid chromatographic system.

Results: A preliminary suite of drugs was tested on silica-based mixed-mode SPE sorbents. The drugs tested were opiates and benzodiazepines fortified in urine at 10ng/mL concentrations. A total of 14 replicate samples were run. The observed averaged recoveries for the benzodiazepines ranged from 50%-101% with intra-run %RSDs less than 10% for the analytes in urine. The observed averaged recoveries for the opiates ranged from 78%-110% with intra-run %RSDs less than 10% in urine.

Conclusion: A full screening method for the extraction of a broad panel of drugs with good recoveries is presented.

Sample Preparation, Drugs of Abuse, LC/MS



K23 Adulterants and Diluents in Urine Samples After Consumption of Cocaine: What Compounds Are Typically Found by Liquid Chromatography (LC) Combined With High-Resolution Tandem Mass Spectrometry (HRMS/MS)?

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After attending this presentation, attendees will understand that in forensic urine samples which test positive for cocaine, in addition to metabolites of cocaine, pharmacologically active compounds are usually found. These compounds were added as adulterants to the illicit street drug. Acquisition of mass spectra in SWATH® mode on a Quadropole Time-of-Flight (QqToF) instrument allows retrospective searches for adulterants and their metabolites.

This presentation will impact the forensic science community by presenting a rapid and reliable analytical method to detect potentially toxic and hazardous adulterants in urine samples. Since this additional forensic toxicological information can be routinely collected, it can be helpful in expanding police intelligence.

Goals: Cocaine consumption is observed as a wide-spread phenomenon and in most cases the cocaine abusers also consume a considerable amount of adulterants, diluents, or even toxic contaminants without being aware of possible side effects, long-term adverse health effects, or acute toxicity.

In order to assess the exposure of cocaine users to such substances, an High-Performance Liquid Chromatography/Quadropole Time-of-Flight (HPLC/QqToF) method for urine samples was developed.

Methods: Urine samples were diluted with a mixture of water/acetonitrile/formic acid/ammonium formate (97.5/2.5/0.1%/5.0mM) and three internal standards were added (EME-D₃, Tramadol-¹³C₃, THC-D₃). The diluted samples were injected onto a core shell column (C8, 50 x 2.1mm, 2.6µm) and analyzed on a QqToF instrument with typical run times of 15 minutes from injection to injection.

Results: In the past two years, more than 4,000 urine samples (coming from traffic controls or traffic accidents) were analyzed by LC combined with HRMS/MS and then processed against a home-built high-resolution library. This library currently contains approximately 1,000 spectra which are relevant for drug screening and also includes most of the currently observed adulterants, diluents, and contaminants. All spectra were acquired by the data independent scan mode (sequential windowed acquisition of all theoretical mass spectra) which generates a digital archive of each acquired sample. The scan parameters were optimized in order to meet the complexity of these urine samples. The following parameters were found to be most favorable for significant library hits: scan range from 50 to 950 Da, scan windows of 25 Da, scan time of 35 msec for each scan window, and collision energy of 35eV±15eV (collision energy spread).

In most of the acquired samples, if tested positive for cocaine or its metabolites, adulterants and diluents can be observed. Among the typical compounds are levamisole, phenacetin, lidocaine, and procaine. In some cases, diltiazem and benzocaine were found. Acquisition of mass spectra in sequential windowed acquisition of all theoretical mass spectra mode also allows retrospective searches for additional compounds. During the same period as the urine samples were screened, 684 cocaine hydrochloride samples were analyzed with High-Performance Liquid Chromatography/Diode Array Detection (HPLC-DAD). The following adulterants were found: Levamisole+Phenacetin+others (30%), Levamisole+Phenacetin (26.3%), Levamisole only (20.0%), Levamisole+others (10.4%), Phenacetin+others (5.1%), others only (3.2%), Phenacetin only (2.9%), and none (2.0%).

Conclusions: A fast and reliable analysis of urine samples for adulterants commonly found in cocaine was developed. Results from urine samples obtained with the new method confirm the results from the analysis of cocaine powder samples. The analysis of the illicit cocaine hydrochloride samples indicate that the vast majority of the cocaine contains pharmaceutically active adulterants. These adulterants are an additional health hazard for cocaine consumers.

High Resolution Tandem MS, Cocaine, Adulterants



K24 Impact of Novel Accurate Mass MS/MSALL Acquisition and Processing Techniques on Forensic Toxicological Screening

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After attending this presentation, attendees will be better informed about a novel data-independent mass spectrometric technique that allows Tandem Mass Spectrometry (MS/MS) of All possible candidates (MS/MSALL) and provides significant improvements in identification. Attendees will be aware of the ability to retrospectively analyze the data for compounds that were not originally targeted. This presentation will describe how powerful a technique rapid forensic toxicology screening by high-resolution mass spectrometry is, while showing that some compounds cannot be unambiguously identified with high-resolution MS measurements alone.

This presentation will impact the forensic science community by illustrating that data-independent techniques, such as SWATH™ acquisition (the MS/MS of all possible candidates), significantly improve identifications and enable retrospective analysis of the data.

Introduction: MS/MS fragmentation yields confident identifications of these compounds, but how to ensure quality MS/MS of these compounds? Data dependent techniques, although very powerful, cannot guarantee the measurement of all possible MS/MS candidates. Targeted MS/MS ensures acquisition of the target compounds, but limits the number of compounds.

Objectives: To evaluate the impact of improvements to SWATH™ acquisition, including variable precursor window sizes, overlapping windows, and deconvolution of MS/MS from multiple precursors.

Methods: Urine was spiked with more than 120 drugs and compounds often found in forensics screening panels. The data was collected on a Triple TOF® 5600 system using one of the following methods: (1) a TOF/MS survey scan with Information Dependent Acquisition (IDA) -triggering of up to 20 product ion scans; or, (2) SWATH™ acquisition. For SWATH™ acquisition, the precursor isolation window width was varied for each MS/MS experiment, or the windows were overlapped between each cycle. Data was processed in PeakView® software 2.0, using a research prototype of MasterView™ software.

Results: Astemizole and amlodipine both demonstrated the advantage of having narrower SWATH™ isolation windows. The overlap 20 Da SWATH™ window acquisition (after demultiplexing was performed) resulted in MS/MS that were significantly reduced in interferences. The library match purity scores were improved from 2.2% to 97.5% and 38.8% to 92.7%, respectively. Having narrower isolation windows improves the specificity of MS/MS data, but at a cost. Either accumulation times must be decreased (which would make signal to noise worse) or cycle times will get longer (reducing the number of points across a peak). It is shown with the example of berberine that using overlap SWATH™ acquisition that the demultiplexed MS/MS can approach the quality of a true 10 Da SWATH™ acquisition MS/MS, while having an improved cycle time. Deconvolution of SWATH™ MS/MS was also shown to improve library match purity scores. Unprocessed SWATH™ MS/MS had significantly lower purity scores for many compounds. Simple background subtraction resulted in MS/MS of much better quality. Two other deconvolution techniques were tried. Method A was similar to techniques used for deconvolving Gas Chromatography/Mass Spectrometry (GC/MS) signals, and was implemented to run on an NVIDIA 660 graphics card. Method B is a novel technique making use of Principal Components Variable Grouping (PCVG) to obtain a SWATH™ MS/MS. When the techniques were combined, results were equivalent to those achieved using unit resolution IDA. For a few compounds, IDA was not triggered, resulting in no identification. While the SWATH™ acquisition was able to confidently identify these compounds with good purity scores.

Conclusion: SWATH™ acquisition methods acquire MS/MS for all compounds, at every time point, achieve identification results comparable to unit resolution IDA methods and overlap SWATH™ acquisition can improve cycle times and improve identification results.

Unknown Screening, Data Independent Acquisition, Comprehensive Analyte Coverage



K25 Analysis of Acetyl Fentanyl in Postmortem Blood and Urine Specimens by Gas Chromatography/Mass Spectrometry (GC/MS)

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After attending this presentation, attendees will better understand the quantitation of acetyl fentanyl in biological specimens utilizing Solid Phase Extraction (SPE) and GC/MS. In addition, attendees will better understand the postmortem distribution of acetyl fentanyl.

This presentation will impact the forensic science community by providing the first reported analytical method for the quantitative analysis of acetyl fentanyl in postmortem blood and urine specimens.

In 2013, the Centers for Disease Control and Prevention issued an alert regarding a new illicit drug, acetyl fentanyl. Acetyl fentanyl was implicated in the death of 15 decedents in Rhode Island from March 2013 through December 2013.¹ Acetyl fentanyl is an analog of fentanyl, and its pharmacological effects include altered mood, euphoria, respiratory depression, and central nervous system depression. Acetyl fentanyl is a μ -opioid receptor agonist with potency reportedly 15 times greater than morphine, but one-third the potency of fentanyl, based on animal studies.²

Due to the novelty of the analyte, it was important to develop a sensitive and specific method for the extraction of acetyl fentanyl from postmortem specimens. This study followed method validation procedures published by the Scientific Working Group for Forensic Toxicology (SWGTOX) to develop and validate a method to quantify acetyl fentanyl in postmortem blood and urine.

SPE (SPEware CEREX Trace -B) was utilized to isolate acetyl fentanyl from the biological matrices. A 1.0mL sample of blood and urine was aliquoted and fortified with isotope-labeled internal standard solution containing $^{13}\text{C}_6$ -acetylfentanyl. The samples were buffered with pH 6.0, 0.1M phosphate buffer, vortexed, and centrifuged. The samples were transferred to preconditioned SPE columns. The columns were sequentially washed with deionized water, 100mM acetic acid, methanol, and dried for five minutes under N_2 . The analytes were eluted with a 78:20:2 (v:v:v) methylene chloride:isopropanol:ammonium hydroxide elution solvent. The eluents were dried at 37°C under N_2 , reconstituted with ethyl acetate, and submitted for GC/MS analysis.

The GC/MS parameters included splitless injection with an initial temperature of 140°C, hold for 0.5 minutes, ramp at 30°C/minute to the final temperature of 320°C with a final hold time of seven minutes. The total run time was 13.5 minutes. The helium flow rate was 1mL/minute. GC/MS analysis was conducted in Selected Ion Monitoring (SIM) mode utilizing the following ions: acetyl fentanyl, m/z 231, 188, 146 and $^{13}\text{C}_6$ -acetyl fentanyl, m/z 237, 194, 152. The assay was linear from 1.0ng/mL to 50ng/mL. The lower limit of quantitation was defined as the lowest non-zero calibrator, 1.0ng/mL, and the experimental limit of detection was 0.5ng/mL and 0.75ng/mL in blood and urine, respectively.

Specimens were obtained from the Rhode Island Office of State Medical Examiners and University of Florida Health Pathology Laboratories — Forensic Toxicology Laboratory for the analysis of acetyl fentanyl. The deaths occurred from March to December 2013. Acetyl fentanyl was detected in 14 decedents: nine males and five females, aged 23-57 years old. The results of the acetyl fentanyl analyses are shown in Table 1. According to the results indicated, acetyl fentanyl demonstrates postmortem redistribution with heart-to-femoral blood concentration ratios ranging from 0.97-2.84, with a mean of 1.59 in a group of eight fatalities.

Table 1. Acetyl fentanyl concentrations from casework.

	Femoral Blood (ng/mL)	Heart Blood (ng/mL)	Antemortem Blood (ng/mL)	Postmortem Urine (ng/mL)	Ratio of Heart-to-Femoral Blood Concentration
Mean	338.6	386.7	136.6	3705.3	1.59
Range	89-945	17-915	57-178	41-9825	0.97-2.84



Toxicology Section - 2015

SPE followed by GC/MS analysis was shown to be an acceptable method for the quantitation of acetyl fentanyl in postmortem blood and urine specimens, and the assay provided a sensitive and specific method while limiting interferences typically found in postmortem blood and urine.

Acetyl Fentanyl, Method Validation, GC/MS



K26 Postmortem Distribution of Acetyl Fentanyl

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After attending this presentation, attendees will have a better understanding of the analysis of biological specimens for acetyl fentanyl and the postmortem distribution of acetyl fentanyl.

This presentation will impact the forensic science community by providing medical examiners and toxicologists with an analytical method and postmortem concentrations for acetyl fentanyl, a relatively new drug of abuse in the United States.

Acetyl fentanyl (*N*-(1-phenethylpiperidin-4-yl)-*N*-phenylacetamide) is a potent synthetic opioid with structural similarity to fentanyl. Animal studies have estimated acetyl fentanyl to be about one-third as potent as fentanyl.¹ Acetyl fentanyl has recently gained public attention when it was linked to 12 fatal overdoses in Rhode Island.²

The Office of the chief Medical Examiner of the State of Maryland has investigated three deaths related to acetyl fentanyl use. The cause and manner of death was acetyl fentanyl intoxication/undetermined for each of the three following cases.

Case 1: A 23-year-old White male college student was found unresponsive on his bedroom floor. He had attended a banquet the night before, visited a few bars, then returned home where he watched television with a friend for several hours before going to bed. The friend found him later the next day and he was pronounced deceased at the scene. The decedent's bedroom contained pills, powders, and drug paraphernalia. It was reported that the decedent had a history of drug abuse and had been in rehab approximately one year prior.

Case 2: A 36-year-old African American male was found lying on the floor with vomit and blood on the carpet next to him. He was reportedly visiting with friends and had used cocaine with them. It was also reported that he may have used heroin. He was transported to the hospital where resuscitation attempts were unsuccessful. The decedent did have prior arrests for possession of a controlled substance.

Case 3: A 17-year-old Hispanic male was at a friend's house. He was reportedly offered a powder that another individual was snorting. Soon after, the decedent became unresponsive. Treatment with Narcan™ and resuscitative efforts were unsuccessful.

Acetyl fentanyl was identified in an alkaline drug screen, which involved an alkaline extraction of specimens followed by detection with Gas Chromatography/Nitrogen Phosphorous Detection (GC/NPD) and confirmation by Gas Chromatography/Mass Spectrometry (GC/MS). Acetyl fentanyl elutes shortly before fentanyl on an HP-5 column and prominent GC/MS ions are 231, 146, and 188. Further evaluation of the standard liquid-liquid alkaline extraction screening procedure in use in the laboratory indicated adequate sensitivity for the detection of acetyl fentanyl, which was detected at concentrations less than 5ng/mL. Subsequently, a quantitation method was developed for acetyl fentanyl. Briefly, internal standard (d5-fentanyl) was added to specimens which were alkalized and extracted with n-butyl chloride:ether then back extracted into sulfuric acid and finally alkalized and extracted into methylene chloride, evaporated and reconstituted with methanol. The extract was injected into the GC/MS which was operated in the selected ion monitoring mode. The ions monitored included 231, 146, 188 (acetyl fentanyl) and 250, and 194 (d5-fentanyl). The method was linear from 25ng/mL to 800ng/mL. Case specimens with concentrations above 800ng/mL were diluted with distilled water to ensure the results were within the calibration curve. The results (ng/mL or ng/g) are summarized below.

Case	Heart Blood	Femoral Blood	Liver	Kidney	Urine	Vitreous Humor	Additional Toxicology Findings
1	700	460	1800	1200	1600	500	Urine: alpha-pyrrolidinovalerophenone positive
2	180	180	180	250	380	160	Blood Benzoylcegonine 1.3 mg/L
3	500	430	430	220	1500	350	None



Toxicology Section - 2015

Acetyl fentanyl was detected at much higher concentrations relative to what is typically seen for fentanyl intoxications, which is consistent with its lower potency relative to fentanyl. Although there were some differences between heart blood and femoral blood concentrations, the greatest difference was seen in the case with the highest concentration, and the heart blood concentration was within 35% of the femoral blood concentration. The liver and vitreous humor concentrations were close to the blood concentrations. Urine also contained a higher concentration of acetyl fentanyl relative to blood, making it a suitable specimen to screen for acetyl fentanyl use.

References:

1. Higashikawa, Y, Suzuki, S. Studies on 1-(2-phenethyl)-4-(N-propioylanilino) piperidine (fentanyl) and its related compounds. VI. Structure-analgesic activity relationship for fentanyl, methyl-substituted fentanyls and other analogues. *Forensic Toxicol* (2008) 26:1-5
2. Acetyl Fentanyl Overdose Fatalities - Rhode Island, March–May 2013. *Centers for Disease Control and Prevention Morbidity and Mortality Weekly Report*, August 30, 2013, 62(34); 703-704.

Forensic Toxicology, Postmortem Distribution, Acetyl Fentanyl



K27 Fatal Intoxication with Acetyl Fentanyl

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After attending this presentation, attendees will better understand the postmortem toxicology investigation involving the recreational use of acetyl fentanyl, one of a new group of psychoactive compounds being recreationally abused in the United States.

This presentation will impact the forensic science community by providing useful information pertaining to acetyl fentanyl, an opioid drug rarely encountered in forensic casework.

Among the new psychoactive substances encountered in forensic investigations in the United States is the anilidopiperidine class opioid, acetyl fentanyl. Abuse of this compound has resulted in more than 50 fatalities in the United States since 2013.

A 28-year-old male was found unresponsive on the bathroom floor at a residence he shared with a roommate, who had last known him to be alive approximately 12 hours earlier. A tourniquet fashioned from a belt was secured around his arm and a syringe was found nearby. The decedent, who had a history of substance abuse, including the use of anabolic steroids, was pronounced dead at the scene. External examination revealed needle track marks along the inside of the arm and foamy secretions at the mouth. Autopsy revealed marked pulmonary edema and mild diffuse cerebral edema, with no contributory natural disease or physical injury identified.

Immunoassay of urine for a panel of drugs of abuse gave a positive presumptive result for fentanyl alone. The presence of acetyl fentanyl was confirmed through Gas Chromatography/Mass Spectrometry (GC/MS) analysis of urine and Liquid Chromatography/Time-of-Flight/Mass Spectrometry (LC/TOF/MS) analysis of subclavian blood. Acetyl norfentanyl and its putative metabolite N-phenyl-1-(2-phenylethyl)piperidin-4-amine were detected in the blood, liver, and urine. An existing Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS) procedure for fentanyl and norfentanyl confirmation and quantitation was modified in order to determine acetyl fentanyl in autopsy blood, urine, vitreous fluid, and liver. LC was performed using a Waters® ACQUITY® Ultra-Performance Liquid Chromatograph (UPLC); and tandem MS analysis was performed using a Waters® TQ-D detector with ionization in electrospray positive mode. Method validation consisted of examining bias, calibration model, carryover, and matrix effects. Acetyl fentanyl was detected in subclavian blood, liver, vitreous fluid, and urine at concentrations of 235ng/mL, 59.6ng/gm, 131ng/mL, and 234ng/mL, respectively. Tadalafil was present at 79ng/mL. Urine was analyzed for a panel of anabolic steroids revealing the presence of testosterone at 34.7ng/mL and epitestosterone at 2.5ng/mL, with oxandrolone also present in the urine. Acetyl fentanyl was detected by GC/MS analysis in a methanolic rinse of a syringe found at the scene.

Currently, limited information indicates that acetyl fentanyl acts as a mu-opioid receptor agonist with potency less than that of fentanyl, but greater than morphine. Taking into consideration of comparable average acetyl fentanyl blood concentration of 213ng/mL in three other forensic cases evaluated by a private forensic toxicology laboratory, the cause of death in this case report was determined to be acetyl fentanyl intoxication and the manner was deemed to be accidental.

Along with meager recent reports addressing a host of emerging novel opioid analogs, this case underscores the need for awareness and consideration of the potential involvement of such drugs when investigating apparent recreational drug-related deaths.

Forensic Toxicology, Acetyl Fentanyl, Postmortem



K28 Trazodone and M-Chlorophenylpiperazine (m-CPP) Concentrations in Postmortem Blood

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After attending this presentation, attendees will be able to describe expected concentrations of trazodone and m-CPP, a metabolite of trazodone, in postmortem blood.

This presentation will impact the forensic science community by providing data that will assist forensic toxicologists in interpreting trazodone and m-CPP concentrations in postmortem casework.

Introduction: Trazodone has been used as an antidepressant for more than 30 years. Structurally, it is unique and unrelated to the tricyclic and tetracyclic antidepressants, as well as the newer SSRI and SNRI antidepressants. m-CPP is the major metabolite of trazodone; but is also considered a “designer” drug that can be abused by itself. Although postmortem trazodone blood concentrations have been documented, reports of postmortem m-CPP concentrations in blood are lacking.

Method: This study is a compilation of data obtained from 30 cases over a five-month period (femoral or heart blood). Trazodone samples were prepared by analysis using a dual Liquid-Liquid Extraction (LLE). Prior to extraction, each specimen was fortified with internal standards and pH adjusted using ammonium hydroxide. Solvent was evaporated to dryness and reconstituted in 150 μ L 10mM ammonium acetate, 0.1% formic acid High-Performance Liquid Chromatography (HPLC) water (mobile phase). Samples were analyzed via a Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS) instrument comprised of a Shimadzu[®] HPLC and ABSciex[™] API 3200 tandem mass spectrometer. Ionization was attained by electrospray (positive mode) with Multiple Reaction Monitoring (MRM) mode employed for detection and quantification. Gradient chromatographic separation starting at 20% B (0.1% formic acid in acetonitrile) was achieved using a C₁₈ column (100 x 2.1mm, 3 μ m particle). Flow rate was 0.7mL/min with an overall run time of 3.0 minutes. Conservative Limits Of Quantification (LOQ) were 20ng/mL for both analytes. The assay was validated for linearity from 5ng/mL-250ng/mL trazodone and m-CPP ($r^2 > 0.980$ and concentrations within $\pm 20\%$ of target). Precision was characterized by CVs of 4.2% and 3.0% for trazodone and m-CPP, respectively, at 250ng/mL, and 6.3% and 7.9% at 20ng/mL. Accuracy was characterized by deviations of -3.2% and -3.0% for trazodone and m-CPP, respectively, at 250ng/mL, and 5.2% and -1.0% at 20ng/mL. No quantifiable carryover was observed at the method Upper Limit Of Quantification (ULOQ).

Results: Of 19 femoral blood specimens containing trazodone at >20 ng/mL, m-CPP was not detected in five specimens (LOD 5ng/mL), detected at $>$ LOD but <20 ng/mL in six specimens, and quantified at >20 ng/mL in eight specimens (trazodone mean of 596ng/mL, range 225ng/mL-1,383ng/mL, and m-CPP mean of 36.0ng/mL, range 21.4ng/mL-69.5ng/mL). Of 11 heart blood specimens containing trazodone at >20 ng/mL, m-CPP was not detected in three specimens (LOD 5ng/mL), detected at $>$ LOD but <20 ng/mL in two specimens, and quantified at >20 ng/mL in six specimens (trazodone mean of 836ng/mL, range 188ng/mL-2,145ng/mL, and m-CPP mean of 90.4ng/mL, range 26.6ng/mL-125ng/mL).

Conclusions: Trazodone and m-CPP concentrations are presented from 30 postmortem cases obtained over a six-month period. Trazodone concentrations were similar to literature values, with m-CPP detected $>$ LOD in 22 of 30 cases. This data set will assist forensic toxicologists in casework where trazodone or m-CPP intake has occurred.

Trazodone, m-CPP, Postmortem Blood



K29 A Case of Suicide Using Veterinary Drug T-61® With Subsequent Submergence in the Sea

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After attending this presentation, attendees will understand the suicide case of a man who used Tanax® (T-61®), a drug used for euthanasia in veterinary practices, with subsequent submergence in water.

This presentation will impact the forensic science community by presenting the results of the postmortem examination which showed elements that excluded drowning as cause of death. Instead, histological findings, autopsy evidence, and toxicological analysis (a high level of embutramide and mebezonium iodide in the blood) resulted in the conclusion that death was a result of Tanax® injection.

The present case concerns an acute fatality resulting from self-administration of Tana® (T-61®) in a 35-year-old man, who was found near water and at a first look, appeared like an accidental drowning.

T-61®, consisting of a mixture of embutramide, mebezonium, and tetracaine, is a pharmaceutical used for euthanasia of animals because of its narcotic and muscle relaxant (curariformlike) activity. Embutramide is a general anesthetic that possesses a strong narcotic effect that rapidly induces deep anesthesia. Mebezonium iodide is a quaternary ammonium that paralyzes the skeletal muscles, resulting in a respiratory collapse. Finally, tetracaine, a local anesthetic, is used to reduce painful tissue reactions at the injection site.

A man was found dead near the sea wearing underpants, socks, shoes, and a backpack full of stones; beside his clothes four apparently empty used syringes and a bottle were found. He worked in a drug warehouse, had no known mental disorder, and seemed to be in good health.

The prosecutor arranged for an autopsy on the body to clear up the circumstances of his death and distinguish between homicide and suicide by drowning. A complete autopsy was performed 24 hours after death.

At the external examination, the man showed peculiar lesions on both inner arms, two needle puncture marks, and a remarkable cyanosis of the face, lips, and nails.

All internal organs were congested. The pleural cavities contained 12cc of yellowish fluid. The lungs were edematous with areas of hemorrhage mainly seen on the right side. The pericardial cavity contained 2cc of yellowish fluid. The heart showed few epicardial petechia. The abdominal cavity contained 15cc of yellowish fluid. The stomach was empty. The liver was congested and had steatotic appearance on cut sections. Other organs were unremarkable except of edema. Histopathologic examination showed wide foci of early contraction band necrosis in heart samples. The lungs presented alveolar septa mildly thickened by edema and capillary congestion, alveolar edema. Steatosis was confirmed in the liver. The kidneys presented air bubbles in glomerular capillaries and immunohistochemistry performed with antibody anti-CD 61 and fibrinogen showed a vital reaction, platelet aggregates (anti CD 61 antibody) at the edge of air globules and an adsorbed fibrinogen (anti-fibrinogen antibody) layer to the interface of air globules. In addition, the immunohistochemical study was completed using antibody anti-heat shock proteins researching the renal tissue heat shock proteins (HSP 70, 27, 90) which are a group of proteins that are rapidly induced in response to physiological stress, including hyperthermia, infections, tumors, ischemic stimuli, and exposure to toxicants. The reaction revealed a strong positivity for HSP 27, an intermediate positive reaction for HSP 70, and a mild positivity for HSP 90. During the autopsy, femoral blood and urine were collected for toxicological analysis.

Embutramide and mebezonium iodide were found in both biological matrices using a direct and sensitive liquid chromatography/tandem mass spectrometry method for the simultaneous determination of the two drugs. Lidocaine was used as an internal standard. Limits of detection and quantitation were 0.01mg/L and 0.05mg/L, respectively, for both compounds.

Embutramide concentrations in blood and urine were 10.1mg/L and 0.40mg/L, respectively. The mebezonium iodide concentration was 0.65mg/L in blood and 0.07mg/L in urine. The chromatographic method was additionally optimized for determination of diazepam; diazepam was found in both samples (0.015mg/L in blood and traces in urine). The blood sample collected during the postmortem examination was tested by gas chromatography for detection of ethanol which was found at a concentration of 0.40g/L.



Toxicology Section - 2015

The urine was further examined for amphetamine and related compounds, cannabinoids, methadone, opiates, cocaine, and its metabolites, all with negative results.

Tanax® (T-61®) Injection, Suicide, Drowning



K30 Development and Validation of a Method Using Gas Chromatography/Mass Spectrometry (GC/MS) After Liquid-Liquid Extraction (LLE) for the Detection and Quantification of Clotiapine in Blood and Urine and Its Application to a Postmortem Case

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The goal of this presentation is to inform attendees of possibly fatal complications due to clotiapine intake by considering the autopsy results and the toxicological findings, including substance concentration and distribution in biological samples.

This presentation will impact the forensic science community by providing a reliable method for the detection and quantification of clotiapine in blood and urine samples by means of GC/MS after LLE.

Introduction and Goals: Clotiapine is an atypical antipsychotic of the dibenzothiazepine chemical class. It was first introduced in a few European countries in 1970. Despite the high incidence of extrapyramidal side effects, it has demonstrated efficacy in treatment-resistant schizophrenic patients.

Here, a method for the detection and quantification of clotiapine in blood and urine samples by GC/MS after LLE has been developed and fully validated. The method has been applied to a fatal case involving a 45-year-old man found dead in his private apartment.

Methods: For the extraction, blood, urine, and gastric contents were extracted according to the following procedures: to 1mL of each liquid sample were added 1mL of deionized water and 500ng of methadone-d9 as internal standard. Samples were extracted at pH 8-8.5 (50mg of solid $\text{HCO}_3^-/\text{CO}_3^{2-}$ -buffer added) with 4mL of extraction solution (n-hexane/dichloromethane (85/15v/v) for 15min. After centrifugation (4,000 rpm, 3min) the organic layer was evaporated to dryness under nitrogen flow. The residue was reconstituted with 50 μ L of ethyl acetate.

GC analysis was carried out on an Agilent® HP 7028A GC coupled with an Agilent® MSD 5975. The capillary column used was an HP-5MS (17m x 0.25mm I.D. coated with a 0.25 μ m film). The GC conditions were as follows: the column temperature was programmed from 120°C to 290°C with an increase of 10°C/min; the injection port and the transfer line temperature was 270°C; helium was used as carrier gas with flow rate of 1mL/min; the split injection mode had a ratio of 15:1. The mass analyzer operated by electron impact (-70eV) in the Selected Ion Monitoring (SIM) mode. Quantitative analysis was carried out recording ions m/z 209-244-343 for clotiapine and m/z 78-165-303 for methadone-d9. The underlined ions were used for quantitative analysis.¹

Application to a Postmortem Case: A 45-year-old man died in bed in his private apartment. Relatives could offer very little information about the circumstances of death, but positive information about mental illness (schizophrenia) was reported. The autopsy showed the presence of bladder over-distension due to massive urinary retention and contained 3.7L of urine. All other organs were unremarkable, with only a moderate pulmonary edema being found. The toxicological analysis, using the method developed above and validated showed the following concentrations: 1,318ng/mL in peripheral blood, 487ng/mL in urine (the clotiapine concentrations in urine samples were creatinine-normalized) and 1,860ng/mL in the gastric contents. No other drugs nor alcohol were detected in the biological samples.

Conclusions: A reliable method for the detection and quantification of clotiapine in blood and urine samples has been developed and validated. The application of the method to the reported case allowed identification of clotiapine at very high concentrations in blood, urine, and gastric contents, although there is little evidence in literature about its toxic values. In this case, the cause of death, taking into consideration the autopsy and toxicological findings, was due to a post-renal failure due to a severe bladder over-distention induced by clotiapine.

Reference:

1. Peters et al., Validation of New Methods, *Forensic Sci. Int.* Volume 165, 2007.

Clotiapine, GC/MS Method, Postmortem Case

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K31 Pesticide Poisoning Deaths in Istanbul and Nearby Cities in Turkey

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After attending this presentation, attendees will better understand pesticide poisoning, its impact, prevention, and the concerns about safety parameters.

This presentation will impact the forensic science community by providing results from a seven-year retrospective study in the vicinity of Istanbul, Turkey. This presentation will add to research being conducted by forensic medicine, forensic toxicology, and public health organizations by contributing to the understanding of the proper usage of pesticides in agriculture and the different methods of consumption for other purposes, either accidental or suicidal.

Although some kinds of agricultural pesticides are strictly banned in developed countries, they are easily found in local markets and pharmacies in most of the developing countries. Despite the high amount of consumption ratios of the developed countries, pesticide poisoning rates are higher in developing and underdeveloped countries. Despite the regional studies, extensive and comprehensive data about the manners of pesticide poisoning in Turkey are still lacking.

Pesticides are chemicals that are used to reduce the deleterious effects of various species such as insects, rodents, weeds, and fungi which cause the qualitative and quantitative loss of agricultural products during the production, storage, and consumption processes. Pesticides are categorized as fungicide, herbicide, rodenticide, repellent, or insecticide. Mechanism of action varies according to the type of the pesticide. Effects of irresponsible and uncontrolled consumption of pesticides to human health and the environment are also important issues and should not be ignored. Poisoning is generally encountered accidentally during agricultural usage or as a result of suicidal and homicidal exposure.

In the present study, 29,438 forensic autopsy cases performed in the mortuary department of the Council of Forensic Medicine, Turkey, from 2007 to 2013 were evaluated retrospectively for pesticide poisoning cases. Thirty-four pesticide poisoning cases were determined and studied for a detailed epidemiological and medicolegal analysis. Of these cases, 73.5% were male and 26.5% were female. The mean age of the cases was 42.5 years. Most of the cases (59%) were reported from Istanbul. The most common pesticide encountered was dichlorvos (DDVP) (29.4%), followed by endosulphan (8.8%).

Pesticide poisoning-related deaths are still an important public health issue in society. All safety regulation measures must be taken by all related authorities integratively in the prevention of the deleterious effects of irresponsible consumption of pesticides in agriculture and deliberate poisonings. Further study is needed to arrive at more detailed and comprehensive results of pesticide poisoning data from all regions of Turkey to understand pesticide poisoning in its entirety.

Pesticide, Poisoning, Epidemiology



K32 The Role of Cytochrome P450 2B6 (CYP2B6) Genetic Polymorphisms in Unexpected Methadone Fatalities

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After attending this presentation, attendees will understand the potential role of cytochrome P450 2B6 (CYP2B6) genetic polymorphisms in methadone metabolism and overdose. CYP2B6 is one of the key enzymes involved in the metabolism of methadone. The research presented explores the association of specific Single Nucleotide Polymorphisms (SNPs) in the CYP2B6 gene to methadone metabolism and whether the presence of such SNPs increases the likelihood of fatal methadone intoxication in the Caucasian population. This research may provide insight as to why some individuals succumb to methadone intoxication.

This presentation will impact the forensic science community by serving as a key aspect for the development of genetic testing to be used for screening patients placed on methadone therapy. The hypothesis of this study is that one or more SNPs located within the CYP2B6 gene contributes to or is linked to the methadone poor metabolizer phenotype and identifying such individuals before treatment may decrease the number of fatalities due to methadone intoxication.

West Virginia and Kentucky ranked in the top ten states for increases in fatal methadone overdoses in 1999-2005. CYP2B6 plays a significant role in stereo-selective metabolism of (S)-methadone to 2-ethyl-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), an inactive methadone metabolite. Elevated (S)-methadone can cause cardiotoxicity by prolonging the QT interval of the heart's electrical cycle. Large inter-individual variability in the pharmacokinetics of methadone causes ambiguity in the relationship between dose, plasma concentrations, and side effects. While other pharmacogenetic studies have been conducted involving methadone-related intoxications, these studies did not include methadone-only cases. Rather, the studies used cases involving mixed drug intoxications and/or had small sample sizes.

The current study examines 228 cases involving fatal methadone intoxications, 136 of which are attributed to methadone alone. A control group of 268 cases without methadone detected was also studied. Genomic DNA was extracted from blood stain cards prepared during autopsies performed at the West Virginia and Kentucky Offices of the Chief Medical Examiner using the QIAamp® DNA Micro DNA Kit following the manufacturer's protocol for dried blood spots with modification for greater DNA concentration. SNP genotyping was achieved using TaqMan® SNP Genotyping kits from Life Technologies™ following the manufacturer's protocols for real-time polymerase chain reaction and allelic discrimination analyses. Allelic and genotypic frequencies were determined for the six SNPs on the CYP2B6 gene (rs2279344, rs3211371, rs3745274, rs4803419, rs8192709, and rs8192719) in the fatalities attributed to methadone intoxication (n=228) and the control (n=268) cases.

The frequency distributions for each of the six SNPs genotyped were in Hardy-Weinberg equilibrium based on a Chi-squared goodness of fit test with two degrees of freedom. The frequency of minor allele carriers was significantly different between the observed genotypic frequencies and general population ($p < 0.05$) for all the SNPs, except rs8192709, in the West Virginia/Kentucky population for all of the methadone overdose cases and the methadone-only overdoses. A Tukey test showed there was a significant difference in the mean blood methadone concentrations (mg/L) between the variants for SNP rs3211371 in the methadone only fatalities ($p=0.003$). This was not observed in the other SNPs genotyped, but there was an apparent enrichment of the minor allele in the methadone cases.

The results of this study indicate that SNP rs3211371 on the CYP2B6 gene is likely linked with a slow-metabolizer phenotype for methadone and may contribute to unexpected methadone fatality. An individual carrying at least one copy of the minor allele for any of the SNPs studied could have a poor methadone-metabolizer phenotype leading to an increased risk of fatal methadone intoxication.

Methadone, Genetic Polymorphisms, CYP2B6



K33 A Case of Suicide Due to Oral Ingestion of Phorate Revealed by Gas Chromatography/Mass Spectrometry (GC/MS): A Contribution to Clarify the Human Toxicokinetic and the Mechanism of Death

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The goal of this presentation is to examine a case of suicide by organophosphates (phorate) revealed by GC/MS. The analytical method, the postmortem toxicological concentration of phorate revealed in gastric content, and the modality of death are discussed.

This presentation will impact the forensic science community by informing attendees of the importance of both the analytical method developed and used for quantifying postmortem phorate and of the useful information about the pathological pathway leading to the related death.

Text: Organophosphates (OP) are the most frequently used insecticides worldwide. These compounds are esters, amides, or simple derivatives of phosphoric and thiophosphoric acids and cause 80% of the reported toxic exposures to insecticides. Phorate (phosphorodithioic acid O,O-diethyl S-(ethylthio)methyl ester) is a systemic and contact restricted-use pesticide; it is primarily formulated as granules to be applied at planting in a band or directly to the seed furrow. It is also used as a nematocide.

Phorate has been classified as a Class I, high-risk toxic OP compound with an LD50 of 1.1-3.7mg/kg bw for rats. Due to its severe acute risk for human health, the World Health Organization has classified phorate as a technical product of extreme hazard; however, it is continuously being used in several countries. Its mechanism of action acts like inhibition of acetylcholinesterase activity by phosphorylating the serine hydroxyl group of the substrate binding domain, which results in accumulation of acetylcholine and induces a "cholinergic syndrome" that induces overstimulation of nicotinic, muscarinic, and central acetylcholine receptors, including various Central Nervous System (CNS) effects such as headache, drowsiness, dizziness, confusion, blurred vision, slurred speech, ataxia, coma, convulsions, and blockage of the respiratory center.

Case Report: A case of suicide in a 70-year-old gardener is described; he was found by his daughter finishing a drink with white granular powder mixed with water. She immediately saw him become disoriented and sweaty and alerted emergency health workers, but the man suddenly collapsed. During transport to the emergency department, despite resuscitation attempts, the man died.

Autopsy was performed 48hrs after death. External examination was unremarkable; the autopsy revealed hyperinflated, overexpanded, and ballooned lungs occupying the entire thoracic cavity. The stomach contained 100mL of a brown liquid that was sampled. Other organs showed an intense vascular congestion. The organ specimens were fixed in 10% buffered formalin and embedded in paraffin. Histological examination of samples revealed generalized stasis. In particular, the lungs presented an eosinophilic proteinaceous material and some hemosiderin-laden macrophages in the alveolar cavities, associated with blood congestion of the interstitial vascularization.

The etiopathogenetic definition was outlined by a comprehensive toxicological screening performed on cardiac blood, urine, and gastric contents using a combination of immunoassay and chromatographic techniques. A Liquid-Liquid Extraction (LLE) was performed. At the time of autopsy 1mL of blood, urine, and gastric contents were collected and stored at -20°C. In detail, the LLE was performed for five minutes with cyclohexane, after the organic layer was evaporated until dry under a stream of nitrogen at room temperature. The dry residues were then taken up with 100µL of acetone for the GC/MS analysis. Phorate calibrators of 0.5mg/L, 1mg/L, 2mg/L, and 5mg/L were prepared in blank human blood. Phorate concentration, detected in the gastric contents, was 3.29mcg/mL. No others exogenous substances were found. The complete toxicological results with graphics and tables will be presented.

According to current research, a lethal ingestion of phorate is rarely reported in the literature. Human toxicokinetic data are available but limited. Results related to toxicity and pharmacodynamics data were obtained through animals studies. The acute phorate toxicity due to poisoning can manifest in three different phases: acute cholinergic crisis, Intermediate Syndrome (IMS), and delayed polyneuropathy.



Toxicology Section - 2015

In this case study, according to macroscopic and microscopic findings, the cause of death was most likely respiratory failure and pulmonary dysfunction due to acute cholinergic crisis. In conclusion, this presentation provides useful information about the modality of death and a clear indication of a toxic concentration of phorate detected in gastric contents.

Phorate, Cholinergic Crisis, Toxicological Findings



K34 Heroin-Related Deaths in the West of Scotland Between 2008 and 2011

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After attending this presentation, attendees will be able to detail the demography, toxicology findings, and pathology of Heroin-Related Deaths (HRDs) in the west of Scotland between 2008 and 2011.

This presentation will impact the forensic science community by providing an evaluation of the role of morphine in HRDs, Drug-Related Deaths (DRDs), and non-Drug-Related Deaths (non-DRDs) in 767 cases during a three-year period.

In this study, HRDs in the west of Scotland between 2008 and 2011 were analyzed and compared to non-DRDs in the same period. A total of 767 cases were identified in which morphine was detected postmortem. Information on these cases was extracted from the Glasgow University Forensic Medicine (GUFM) database and analyzed using Excel®.

In 2009, the General Register Office for Scotland reported 545 DRDs in Scotland, with 37% occurring in the Greater Glasgow and Clyde area. A large proportion of these deaths occur due to the use of heroin, which was implicated in 59% of all DRDs in Scotland. Heroin continues to be reported in a large number of DRDs in the west of Scotland, often leading this area to be referred to as the heroin capital of Europe.

There were 323 HRDs identified over the study period. The mean age in these cases was 34.9 years, 84% were male, and the majority of people died in their own home (54.9%). In cases which were not classified as DRDs, the average age was 45.9 years, 69% were male, and 46% of people died in a hospital. Areas within Greater Glasgow and Clyde with the highest percentage HRDs were Glasgow West, Glasgow East, and Renfrewshire. In this population, the median blood morphine concentration in HRDs was 0.26mg/L, which was significantly higher than the morphine concentration in non-DRDs: 0.09mg/L. In the minority of HRDs (37%), only heroin was implicated in the cause of death. Alcohol (35%) and methadone (24%) most often contributed to the cause of death. In 23 cases of all non-DRDs, heroin was administered for medical care prior to death. The median blood morphine concentration in those cases was 0.16mg/L, which was lower than blood morphine concentration in HRDs, but not statistically significant.

The majority of heroin-related fatalities are male with an average age of 34.9 years at death, which means a considerable loss of life compared to the average life expectancy in Scotland. Most deaths occurred at home, indicating there was little time from heroin use to death. Next to heroin, alcohol and methadone were involved in the cause of death in almost 60% of cases. Overall, the mean blood morphine concentration in heroin fatalities was more than 50% higher than in non-DRDs (0.37mg/L vs. 0.24mg/L).

Heroin, Demographic, Postmortem



K35 Analysis of N-Bombs: Quantitation of NBOMe-type Novel Psychoactive Substances (NPS) in Biological Fluids by Liquid Chromatography With Tandem Mass Spectrometry (LC/MS/MS)

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After attending this presentation, attendees will be able to describe the characteristics and effects of NBOMe-class compounds, implement methods for their analysis in biological fluids using LC/MS/MS, and learn about the concentrations of these analytes detected in biological fluids in forensic cases.

This presentation will impact the forensic science community by describing a comprehensive assay for the detection of this emerging class of NPS with known adverse effects. The detection and quantitation of these drugs in toxicology casework will also be discussed.

NBOMes, known as N-Bombs by the drug-using community, are a group of psychedelic substituted phenethylamines being seen with increasing frequency in recreational drug products being sold online and through underground suppliers in the United States. They are derivatives of the 2C family of phenethylamine stimulants first described by Alexander Shulgin throughout the 1980s. 25I-NBOMe is the N-benzyl methoxy derivative of 2C-I; the substitution is considered to increase the potency and psychoactivity of the compound. Substitutions of the other members of the 2C class result in additional members of the NBOMe series. These compounds have gained popularity among recreational drug users as an alternative to LSD and are often impregnated onto blotter papers or administered in powder and liquid formulations. Members of the NBOMe series have been linked to fatal intoxications, characterized by violent behavior, psychosis, and extreme sympathetic stimulation. Case reports of NBOMe use have included hospitalizations and deaths. Due to the substantial risk of toxicity from this group of compounds, and the need to confirm their use, it was necessary to develop testing for the detection of NBOMes for forensic toxicology applications.

A method for the detection of four members of the NBOMe class — 25I-NBOMe, 25B-NBOMe, 25C-NBOMe, and 25H-NBOMe — is described. Since the NBOMes have been reported to be active in sub-milligram doses, a positive ion mode LC/MS/MS procedure was developed for the determination of these compounds in serum, plasma, and whole blood.

Samples were prepared for analysis by a simple protein precipitation process using acetonitrile. Ultra Performance Liquid Chromatography (UPLC) conditions for the LC/MS/MS method included 0.1% formic acid in water vs 0.1% formic acid in methanol, at 0.400mL/min on a BEH C18, 2.1mm x 50mm column. The test is routinely performed qualitatively; however, quantitative values were achieved using the standard addition approach. The calibration range for the standard addition method used a calibration curve from 0.5ng/mL-50ng/mL of each drug. A qualitative urine extraction for the detection of NBOMe samples was developed separately. The NBOMes were extracted from urine using a solid-phase extraction after pH adjustment. Conditions for analysis by UPLC are similar to the blood method.

Since September of 2013, this study has encountered 25 positive cases for the NBOMe drugs, including 22 whole blood samples, one serum/plasma sample, and three urines. Of the 25 cases, 14 screened positive for 25I-NBOMe, three for 25C-NBOMe, five for 25B-NBOMe, and three cases contained more than one of the target drugs (25I-NBOMe plus 25B-NBOMe; 25I-NBOMe plus 25H-NBOMe; and, 25C-NBOMe plus 25H-NBOMe). Of the cases with demographic information available (n=18), the median age was 18 years and included 11 males and seven females.

Available samples were subsequently subjected to quantitative analysis using the standard addition method. Of 12 blood cases tested, 25I-NBOMe was detected in seven of these cases with mean and median concentrations of 2.63ng/mL and 1.82ng/mL, respectively (range 0.79ng/mL-6.3ng/mL). 25C-NBOMe was detected in three of the blood samples, with mean and median results of 2.96ng/mL and 2.18ng/mL (range 2.16ng/mL-4.53ng/mL). In one sample, 25H-NBOMe was present at a concentration of 2.10ng/mL in conjunction with 4.53ng/mL of 25C-NBOMe.



Toxicology Section - 2015

Although quantitation of NBOMe drugs have been previously reported in isolated cases, this is the first documented attempt to develop a comprehensive panel for the most popular NBOMe drugs which can be easily revalidated for new members of the drug class when they appear. The method can be used qualitatively as a screen or with standard addition for confirmation and quantitation. This LC/MS/MS approach provides the sensitivity and specificity necessary for the detection and quantitation of NBOMes in forensic toxicology casework.

NBOMe, Novel Psychoactive Substances, Toxicology



K36 Phencyclidine (PCP) in San Francisco: A Review of 50 Postmortem and Human Performance Toxicology Cases Between 1997 and 2013

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After attending this presentation, attendees will understand the frequency of PCP detection as well as the concentration ranges of PCP in Postmortem (PM) and Human Performance (HP) cases in the City and County of San Francisco.

This presentation will impact the forensic science community by expanding the existing body of scientific knowledge of demographic characteristics in PCP-positive PM cases, Driving Under the Influence (DUI) and other police cases (including toxicologic findings in these cases), commonly analyzed specimens, and ranges of blood concentrations typically encountered in such cases.

The Forensic Laboratory Division (FLD) of the Office of the Chief Medical Examiner (OCME) analyzes evidence from postmortem and human performance cases on behalf of 14 law enforcement agencies operating within the City and County of San Francisco. For PCP, commercially-available Enzyme Linked Immunosorbent Assay (ELISA) kits are used to screen blood (cardiac/central blood in PM cases, venous blood in HP cases) and/or urine received by the FLD. The ELISA cutoffs for blood and urine PCP are 10ng/mL and 50ng/mL, respectively. Following a positive ELISA screen, confirmation and/or quantitation is performed in blood (peripheral blood in PM cases; a new aliquot of venous blood in human performance cases) and/or a fresh aliquot of urine by Gas Chromatography/Mass Spectrometry (GC/MS) with a Limit Of Quantitation (LOQ) of 0.01mg/L. The assay uses tripeleamine as Internal Standard (IS). The observed retention times are: 8.16min (PCP) and 9.96min (IS). The Target (underlined) and Qualifier ions (m/z) for PCP are 91, 200, 242 and for IS are 58, 91, 185, and 197.

In order to determine PM and HP cases involving PCP in San Francisco over the period of interest, the in-house database was manually interrogated.

Twenty HP cases were confirmed to have PCP in blood, in which subjects averaged 42.4 years of age (range: 20–57 years), were predominantly male (n=20; 75%), with a racial distribution of Hispanic (50%), White (30%), Other (5%), Unknown (5%), and Not Available (10%). The PCP blood concentrations in these cases were (mg/L): mean 0.06 (median 0.05, range 0.01–0.11, standard deviation 0.03). PCP was encountered by itself in seven of the 20 cases and in combination with other psychoactive compounds in the remaining 13 cases. The most commonly encountered drugs detected in PCP-positive cases were amphetamines (n=5), cocaine/benzoylcocaine (n=3), ethanol (n=2), methadone (n=2), and THC (n=2).

In addition, there were 30 PM cases involving PCP, 24 of which had peripheral blood concentrations. Thirteen of the 24 also had PCP confirmed in urine. Another four cases had PCP only in urine, one had PCP in cardiac/central blood, and one had PCP measured in liver. Decedents averaged 42.6 years of age (range: 21–62 years), were predominantly male (n=30; 83%), with a racial distribution of White (50%), White Hispanic (40%), Black (7%), and Native American (3%). The manners of death were: 16 accidents, six suicides, five natural deaths, two homicides, and one undetermined. The PCP peripheral blood concentrations (n=24) were: mean 0.23, median 0.13, range 0.01–0.59, standard deviation 0.19. PCP was encountered by itself in only two of the 24 cases. In all other cases, it was present in combination with other psychoactive drugs: morphine/codeine (n=10), cocaine (n=9), ethanol (n=9), amphetamines (n=6), and methadone (n=5).

This presentation offers valuable information on the demographic distribution of PCP users and decedents in the City and County of San Francisco and offers PCP reference blood concentrations. The San Francisco data suggests that PCP remains a popular recreational drug found in many types of cases. These data show that 35% of PCP-positive living subjects apprehended for DUI and other related offenses were more likely to have PCP on its own. In comparison, only 7% of PCP-positive decedents had PCP by itself, suggesting PCP serves as only one compound of a typical poly-substance death involving PCP. Comparison of mean blood concentrations indicates statistically-significant differences in blood concentrations with PCP-positive decedents having concentrations averaging more than 3.5 times higher than those of living subjects. The data presented is useful to forensic toxicologists, medical examiners, pathologists, coroners, attorneys, as well as other law enforcement agents, who need to understand and interpret PCP concentrations in HP and PM toxicologic specimens, for the purpose of their medicolegal investigations.

Phencyclidine (PCP), Postmortem Toxicology, Human Performance Toxicology



K37 **Determination of Methamphetamine Concentrations in Thighbones Buried in Soil**

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After attending this presentation, attendees will understand the interpretation of methamphetamine concentrations in the bone tissue of decomposed or skeletal remains.

This presentation will impact the forensic science community by demonstrating the role and importance of toxicological analysis of bone in the diagnosis of death from drug abuse.

Bone samples are used for toxicological analysis of decomposed or skeletal remains. To determine the relationship between the concentrations of methamphetamine in bone and those in blood or muscle, the methamphetamine concentrations in mouse thighbone were determined and these concentrations were compared with those in heart blood and muscles. The methamphetamine concentrations in thighbones buried in soil for 7 to 180 days were also determined.

Male ddY mice were intraperitoneally injected with methamphetamine at doses of 1mg/kg, 5mg/kg, or 10mg/kg or with saline, once a day for seven days (n=25 per group). Heart blood samples were collected under general anesthesia by cardiac puncture and the thigh muscles and thighbones were also removed. Thighbones were buried in soil and left in a chamber maintained at 16.2°C and 62% humidity for 0, 7, 30, 90, or 180 days (n=5 at each time point). Five hundred microliters of heart blood or 0.5g thigh muscle were analyzed using Liquid Chromatography coupled with Tandem Mass Spectrometry (LC/MS/MS). Thighbone samples were sterilized with distilled water and acetone and were dried at 50°C for 24h. The dried thighbones were pulverized with a bead homogenizer and were evaluated using LC/MS/MS.

In all mice groups, methamphetamine concentrations in thighbone samples were higher than those in heart blood and thigh muscle samples. Significantly higher concentrations of methamphetamine were determined in thighbone samples of mice administered a dose of 1mg/kg or 10mg/kg methamphetamine ($p<0.05$). Although methamphetamine was detected in all thighbone samples after a burial period of 7-180 days, methamphetamine concentrations in buried thighbone samples were significantly lower than those in thighbones without burial in all mice groups ($p<0.05$). The results of this study indicate that: (1) methamphetamine accumulates in bone and shows higher concentrations in bone than in blood or muscle; (2) methamphetamine is detectable in bone for up to 180 days after burial; and, (3) methamphetamine shows lower concentrations in buried bone than in blood and muscle; this may be because of its diffusion into the soil.

Methamphetamine, Bone, Decomposition



K38 Supported Liquid Extraction (SLE) as a New Technique for the Clean-Up of Hair Extracts Containing Drugs of Abuse

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After attending this presentation, attendees will understand a novel method for the clean-up of hair extracts in order to detect drugs of abuse using SLE.

This presentation will impact the forensic science community by introducing a faster and more efficient method for detecting drugs in the hair matrix.

Hair analysis has been regarded as a complementary matrix to blood and urine in forensic cases as it is a very stable matrix, thus allowing for a detection window that is days to years post-exposure. Analyzing hair for the presence of drugs of abuse consists of many steps, initially starting with sampling and cutting of the hair into small segments, which is followed by decontamination, extraction, and clean-up of the hair extract prior to qualitative and quantitative analysis. Drug residues in hair have previously been reported at trace levels, which progressively degrade by oxidation and/or hydrolysis, so the optimization of analyte extraction and clean-up steps are critical components to ensure the quality of method performance.

The objective of this research was to apply a reduced workflow preparation technology to a range of basic drugs extracted from hair. SLE has been used for the determination of drugs in conventional matrices, such as blood and urine, both of which showed high recovery. In the SLE process, the sample is pH adjusted, then loaded in an aqueous phase to ensure the analytes of interest load as neutral compounds. To date, no peer-reviewed literature discussing the application of this technique in hair has been published.

In this study, Biotage® ISOLUTE® SLE+ was compared to traditional Solid Phase Extraction (SPE) for the determination of cocaine, methadone, opiates, and amphetamines using control samples. These samples were prepared by spiking washed drug-free hair in deionized water and Dichloromethane (DCM) prior to spiking with the drugs (methamphetamine, amphetamine, morphine, 6-monoacetylmorphine, codeine, methadone, cocaine, and benzoylecgonine) at a concentration of 0.5ng/mg and 2ng/mg.

Validation of the SLE method was carried out according to the Scientific Working Group for Toxicology guidelines using the control hair samples, which were weighed (20mg) and, together with standards and negative controls, were tested according to specific procedure. One mL of phosphate buffer pH=7.4 and 50µL of β-glucuronidase solution was added to controls containing amphetamines. Samples were sonicated for one hour at 40°C and incubated for one hour. Prior to extraction, 100µL of 10% ammonium hydroxide was added. In controls containing opiates and methadone, 1mL of 0.1M hydrochloric acid was added before sonication for one hour at 40°C and overnight incubation at the same temperature. Before extraction, 100µL of 5% ammonium hydroxide was added to the supernatant. For controls with cocaine, 1mL of methanol was added and samples were sonicated for one hour at 40°C and incubated over night at 40°C. Before extraction, 100µL of 5% ammonium hydroxide was added to the supernatant. Deuterated internal standards for all drugs were used. A set of split samples was extracted by both SPE and SLE+ in order to compare results. Before applying samples (1mL) onto ISOLUTE® SLE+ cartridges, samples were pH adjusted to pH>10 in aqueous environment in buffer. Prior to evaporation, 100µL of 1mg/mL tartaric acid in ethyl acetate was added to samples containing amphetamine. The extracts were then evaporated and derivatized using pentafluoropropionic acid (PFPA):ethyl acetate (2:1) for amphetamines and BSTFA + 1%TCMS for other drugs. The samples were analyzed using Gas Chromatography Mass Spectrometry (GC/MS) in selected ion monitoring mode.

In order to verify the SLE+ method, hair samples from rats, containing amphetamine and methamphetamine, and human hair from forensic and clinical cases were tested in similar fashion. SLE+ was determined to be a suitable alternative to SPE for the quantitation of cocaine, benzodiazepines, methadone, morphine, codeine, and 6-monoacetylmorphine in hair. Amphetamines and methamphetamines were detected in rat hair at concentration ranging from <0.1ng/mg to >5ng/mg with comparable results between the two clean-up methods. Similarly, a wide range of concentrations (from lower than low limit of quantitation to higher than upper limit of quantitation) were determined in human hair samples.

SLE+ provides better efficiency and less solvent waste than SPE and is a suitable clean-up method for hair analysis.

SLE, SPE, Hair



K39 Surface-Enhanced Raman Analysis of Synthetic Cannabinoids Using Gold Nanoparticles and Various Aggregating Agents

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After attending this presentation, attendees will understand the principles of Surface-Enhanced Raman Spectroscopy (SERS), how SERS can be used to lower the limit of detection of synthetic cannabinoids, the effect of using different aggregating agents when combined with gold nanoparticles to enhance the limit of detection, and how SERS can be a fast and easy analysis for drug detection in toxicological samples.

This presentation will impact the forensic science community by demonstrating the application of SERS as a useful procedure for detecting trace levels of synthetic cannabinoids in solution that is rapid, sensitive, and applicable to a variety of biological matrices.

The use and abuse of synthetic cannabinoids has increased significantly in recent years due to their easy access and growing popularity in young adults. Initially, these drugs, known as “Spice” or “K2,” were sold in retail outlets or via the internet and labeled as “not for human consumption” to avoid any possible regulation of the products by the Food and Drug Administration. This popularity led to an increase in emergency room visits due to synthetic cannabinoid intoxication in recent years. As more of these drugs become illegal, new synthetic legal versions of these drugs are being made. This presents problems for the hospitals and the forensic investigator as standard methods may not detect the target drug.

The most common method of screening detection for drugs of abuse in biological samples is the immunoassay; however, this method presents some disadvantages, particularly for newly synthesized compounds. Other problems include cross-reactivity between different synthetic cannabinoids, hook effects, and high cut-off values for determining if the drug is present. More advanced methods have also been used, such as Gas Chromatography/Mass Spectrometry (GC/MS), however these procedures involve complex sample preparation and long run times.

A potential solution to this issue is Raman spectroscopy. This procedure is an under-utilized technique for the detection and identification of drugs due to its perceived low sensitivity for analytes in solution using traditional procedures; however, when Raman spectroscopy is performed in the presence of metallic nanoparticles, signal can be enhanced several orders of magnitude, which is known as SERS. The addition of aggregating agents, generally ionic salts, further increase the signal via the creation of hot-spots due to displacement of the stabilizing agent which leads to a change in the surface charge of the metallic nanoparticle and the ionic strength of the solution. This method has already been confirmed to work for the toxicological detection of benzodiazepines with limits of detection ranging from 1ng/mL-200ng/mL and for THC with limits of detection less than 10ng/mL. In addition, this method can be made portable and used for on-site detection allowing for a faster analysis time.

In this project, gold nanoparticles were prepared using a sodium citrate, hydroxylamine, or borohydrate reduction and aggregating agents were used to enhance the Raman signal of five different synthetic cannabinoids: JWH-018, JWH-073, JWH-081, JWH-122, and JWH-250. Seven different aggregating agents including MgCl₂, CaCl₂, KCl, NaCl, MgSO₄, KNO₃, and Na₂SO₄ were examined at varying concentrations to optimize sensitivity of detection. Other factors, including the concentration of nanoparticles and time and temperature, were also examined. Upon analysis, the Raman spectrum of each synthetic cannabinoid could be easily distinguished when compared to the Raman signal of the powder form of the drug. Nanogram-per-milliliter concentrations can be detected of each synthetic cannabinoid.

These results demonstrate that SERS can be utilized to detect trace amounts of synthetic cannabinoids in aqueous solutions. Therefore, following the extraction of the analyte, SERS can be used as a detection method of synthetic cannabinoids in toxicological samples, which can be useful in a hospital setting, workplace drug testing, and in forensic toxicology laboratories.

SERS, Synthetic Cannabinoids, Toxicology



K40 Characterization of Binding of Ricin Toxin to Cultured Human Lung Cell Line A549

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After attending this presentation, attendees will better understand the targets and nature of ricin toxin binding to a human lung cell line and its relationship to potential ricin poisoning incidents. The goals are to understand how ricin-binding proteins on human lung cells can be identified and how some of their properties can be determined. As human lungs are one of the target organs of ricin poisoning, more information on proteins that are involved in enabling ricin toxicity could be helpful in further understanding events in toxicity and in designing or using molecules that reduce ricin toxicity *in vivo*.

This presentation will impact the forensic science community by providing information on ricin target proteins on human lung cells. This is significant since there is more information of ricin binding to animal tissues than there is for human cells. After attending this presentation, attendees will better understand how the interaction of ricin with human lung cell lines can be characterized by electrophoresis and fluorescence microscopy.

The plant protein ricin is a highly toxic protein with no antidote. Toxicity of ricin depends on binding to cell surface galactose and endocytosis. Little work has been done on the characteristics of ricin binding to human lung cells and most studies of the interaction of ricin with cells have involved cells sourced from animals and not humans. The objectives of this study were: (1) to identify lung cell proteins that are bound by ricin; (2) to visualize the binding of ricin to intact lung cells; and, (3) to initiate the development of methods to isolate the lung cell proteins that bind to ricin.

Lung cell ricin-binding was characterized by using lung cell proteins that were separated by sodium dodecyl sulfate polyacrylamide electrophoresis and followed by transfer of the protein profile to nitrocellulose for probing with biotinylated ricin. Major proteins of 150kDa, 100kDa, and 60kDa were bound by ricin in A549 cells and the proteins bound differed from those recognized by ricin in SW-13 adrenal cells, salivary fluid, and serum. SW-13 cells had ricin binding proteins of approximately 100kDa and 60kDa in common with A549 cells but differed in containing a low molecular weight molecule of less than 25kDa which bound ricin. The binding of ricin and RCA-I to intact lung cells was compared by fluorescence microscopy. The protein RCA-I also originates from the plant *Ricinus communis* and has been reported to bind to the same carbohydrate molecules recognized by ricin. Binding to intact A549 lung cells was much stronger for RCA-I than for ricin. Reduction of binding to cells by human serum was also higher for RCA-I than for ricin.

The electrophoretic mobilities of the A549 cell ricin-binding proteins were examined by SDS-polyacrylamide electrophoresis under reducing and non-reducing conditions to see if effects attributable to disulfide bonds could be detected. Three major A549 cell protein bands of approximate molecular masses 150kDa, 100kDa, and 65kDa were bound by ricin after electrophoresis under reducing and electro-transfer to nitrocellulose membranes. The electrophoretic mobility of the 150kDa protein was the same under reducing and non-reducing conditions. The mobility of the approximately 100kDa protein was 8.5mm from the origin under non-reducing conditions compared to 10mm under reducing conditions. The mobility of the approximately 60kDa protein was 15mm from the origin under non-reducing conditions and 17mm from the origin under reducing conditions. Based on these findings, the 100kDa and 60kDa A549 cell proteins that bind ricin may contain disulfide bonds which when intact make these proteins assume a more compact shape.

Batch affinity chromatography was performed on proteins extracted from A549 cells by using the non-ionic detergent Triton™ X-100. The proteins bound by the different matrices, Cibacron Blue-agarose, heparin-agarose, Concanavalin A-agarose, and RCA-I agarose were tested for binding to ricin after SDS gel electrophoresis and transfer of the separated proteins profile to nitrocellulose membranes. The profile of protein that bound to Concanavalin A contained the 100kDa and approximately 60kDa and two proteins in the region of approximately 120kDa to 150kDa as major proteins that were enriched generally compared to proteins that were associated with the other matrices. Therefore, the main ricin-binding proteins in A549 cells also appear to bind to the carbohydrate-binding protein Concanavalin A which binds to carbohydrate structures distinct from those that bind to ricin as well as to different aspects of some structures that bind to ricin.



Toxicology Section - 2015

The conclusions from this study are that: (1) A549 lung cells contain three major ricin-binding proteins; (2) RCA-I, a lectin that is related to ricin, binds to A549 lung cells much more efficiently than ricin; (3) human serum is more effective at competing with RCA-I for binding to A549 cells than it is for competing with ricin for binding to A549 lung cell; (4) two of the major ricin-binding proteins from A549 cells may contain disulfide bonds; and, (5) the major ricin-binding proteins in A549 cells have carbohydrates that are also bound by the lectin Concanavalin A. These observations may help in the eventual isolation of these proteins to determine details of their roles in the toxicity of ricin for human lung cells.

Ricin, Lung, Glycoproteins



Toxicology Section - 2015

K41 The Psychological Autopsy: Psychiatry & Behavioral Science and Toxicology in a Working Relationship — Suicide—A General Overview

Dean De Crisce, MD, Special Treatment Unit-Annex, 15 Paddock Street, Avenel, NJ 07001*

After attending this presentation, attendees will understand and appreciate the recent trends in suicide-related deaths in the United States, groups of individuals at high risk for completed suicides, preventative factors in reducing suicide, and the common means and methods utilized in completed suicides.

This presentation will impact the forensic science community by demonstrating the scope and factors involved in suicide-related deaths in the United States in an effort to assist postmortem analysis and allow the identification of intervention and prevention strategies.

Suicide is a top-ten cause of death in the United States and the second leading cause of violent death following accidental injury. According to the Center for Disease Control and Prevention, the rate of completed suicides has increased in the last ten or more years, and has varied based on demographic factors such as race, region of residence, age, and gender. It is estimated there are at least ten times the number of self-harm attempts as there are completed suicides. The highest rates of completed suicide have been in White males, aged 45 to 64 years, living in western states; however, there are substantial numbers of suicide deaths across all demographics and states.

Much of the literature regarding suicide deaths has been the result of research incorporating postmortem analysis and psychological autopsies. Firearms have been the most common method of completed suicide in recent years, followed by suffocation and toxic ingestion. According to the American Foundation for Suicide Prevention, the majority of those completing suicide suffer from mood disorders, substance use disorders, schizophrenia, and personality disorders; approximately two-thirds of those individuals expressed their intent prior to their death.

Public and mental health literature has investigated risk factors for suicide. Risk factors for suicide include major mental disorders, substance use, prior suicide history, family history of suicide, trauma, serious medical illness, environmental and interpersonal losses, access to lethal methods, barriers to accessing treatment, and exposure to suicide. Protective factors that may decrease risk of suicide include strong social support, mental health and substance abuse treatment, restricted access to lethal methods, and life-affirming cultural and religious participation.

Suicide is a preventable cause of death. Far beyond the individual, the effects impact society emotionally, legally, and financially. More research is needed to identify clinical and sociologic interventions to reduce this tragic loss. Forensic science, through postmortem analysis and psychological autopsy, serves as a major contributor to understanding this phenomenon.

Suicide, Psychological Autopsy, Toxicology



Toxicology Section - 2015

K42 The Psychological Autopsy: Psychiatry & Behavioral Science and Toxicology in a Working Relationship — Medicines of the Mind

Dan T. Anderson, MS, Los Angeles County Dept of ME-Coroner, 1104 N Mission Road, Los Angeles, CA 90033*

After attending this presentation, attendees will understand the different classes of antidepressant and antipsychotic medications in society, analytical profiles of recently approved Food and Drug Administration drugs, how these medications are evaluated in context with the cause and manner of death, and how these drugs are interpreted when the manner of suicide is disputed by a family, leading to a psychological autopsy being performed.

This presentation will impact the forensic science community by serving as a reminder of the importance of toxicology testing, along with its scope in assessing cause and manner of death.

Antidepressant and antipsychotic medications are widely prescribed in our society to treat signs and symptoms of depression as well as psychosis. Antidepressants can be categorized into several subcategories to include older generation drugs such as the tricyclic and monoamine oxidase inhibitors. Newer generations of the antidepressant medication include Selective Serotonin Reuptake Inhibitors (SSRIs) and Serotonin-Norepinephrine Reuptake Inhibitors (SNRIs) and combinations thereof. Antipsychotic medication is generally segregated into two types: “typical” antipsychotic drugs that have the potential of severe side effects to the patient and “atypical” medications that are considered a newer generation class of drugs with lesser side effects. The public has definitely been educated about these drugs and, even with the emerging drugs, by the bombardment of advertisements in magazines, newspapers, and television segments. With such prevalence and public knowledge, prescription medication needs to be assessed in routine medical examiner/coroner cases where the cause and manner of death may or may not be known.

The role of a postmortem toxicologist is varied. The first is to assist the medical examiner/coroner/pathologist in assessing the case and determine the level of analytical testing necessary based on the case history, death scene investigation, incoming mode and manner of death, drug or prescription medications collected, autopsy findings, family accusations, and any other possible question that would have to be answered tomorrow with today’s analysis. The second role is actually analyzing the biological specimen for the presence or absence of drugs/prescription medications with the appropriate quality control measures in place to ensure accurate and precise results. The last role is to provide the results to the medical examiner/coroner/pathologist and possibly assist with the interpretation of the drugs and their concentrations in respect to their autopsy findings and investigations.

For cases where the cause and manner of death is not evident, a general comprehensive toxicology screen is performed and generally accounts for a wide array of drugs and prescription medication available in society; however, for cases where the cause and manner of death appears to be obvious, such as non-drug related suicide involving a hanging or gunshot wound to the head, toxicology testing may not be all that complete. Its important to note that in order to evaluate the psyche of the decedent, a more comprehensive panel of drug tests, including antidepressant and antipsychotic medications, may need to be assessed to understand their level of contribution toward the actual cause and manner of death.

Toxicology, Antipsychotics, Antidepressants



Toxicology Section - 2015

K43 The Psychological Autopsy: Psychiatry & Behavioral Science and Toxicology in a Working Relationship — Psychological Autopsy and the Assessment of Motive

Michael Welner, MD, 224 W 30th Street, Ste 806, New York, NY 10001; and Dan T. Anderson, MS, Los Angeles County Dept of ME-Coroner, 1104 N Mission Road, Los Angeles, CA 90033*

After attending this presentation, attendees will understand the purpose of psychological autopsy and the methodology necessary, including the requisite sources of information, for informing the probabilities involved in death investigation. Attendees will also be informed about confounders to the interpretation of data and legal thresholds for testimony.

This presentation will impact the forensic science community by demonstrating how medicine, toxicology, and other forensic data are properly integrated into death investigation at the psychological level. Attention to the assessment of motives is an essential component of death investigation as well, and this presentation enables the participant to learn its judicious integration into death investigation. Finally, this presentation inventories those cases in which special caution is reserved for the potential ambiguity in motive and contributions to errors.

Death investigation confronts the challenge of resolving the likelihood of suicide vs. homicide vs. accident or natural causes. It is the ideal platform upon which psychiatric and psychological understanding can be infused into the work of the coroner and vice versa. It is also an overlooked aspect of criminal responsibility and pre-sentencing determinations in murder and manslaughter cases. Death investigation is often a vivid illustration of the necessity to embed toxicology analysis into otherwise plentiful but equivocal data. Highly publicized deaths of celebrity actors and musicians revisit these challenges of accidental death vs. intentional overdose and even murder.

Forensic psychiatric assessment draws from human evidence, namely, interviews of collateral informants. Accounting for the conflicts and agendas of these witnesses is important but does not diminish the vital contribution of those informants to reconstructing the choices and movements of the deceased and potential nefarious actors antecedent to death. This includes patterns and sequence of medications and substances ingested, accounting for pertinent medical history and timing.

Like other forensic behavioral science assessment, death investigation is a contemporaneous examination that assesses factors specific to the time of the death event and its immediate past. Unlike other forensic assessment, psychological autopsy is typically routed to consulting forensic pathologists for their own consideration but is more restricted in open court testimony to an inventory of suicidal risk factors.

The assessment of motive is essential to any consideration of homicide and as such is a component of psychological autopsy when murder is under consideration. Different source materials inform a similar endpoint of “why” and “why then,” reconstructing both timing and progression. The complexity of this exercise requires openness to evidence of primary, secondary, and tertiary motive; conscious and unconscious motive; and, material and psychological gain. Reconstructive evidence focusing on the circumstances of the death event advances a range of forensic psychiatric questions and forensic medicine contributions to justice.

Psychological Autopsy, Death Investigation, Motive



K44 The Psychological Autopsy in Practice: Applying Behavioral Science to Mode-of-Death Investigations and a Case Study

Lauren Reba-Harrelson, PhD*, PO Box 1404, Columbus GA 31902

After attending this presentation, attendees will be able to identify basic methodology and types of data considered in a comprehensive approach to conducting a psychological autopsy and apply information obtained through this practice to a mode-of-death determination.

This presentation will impact the forensic science community by demonstrating the possible utility of the psychological autopsy as a collaborative clinical practice tool for establishing mode of death in complex determinations related to alleged suicide.

This presentation provides an overview of the structure and function of the psychological autopsy, a comprehensive, objective retrospective analysis a decedent's state of mind, and actions at the time of death. The history of the psychological autopsy will also be described and a case example will be provided to engage attendees in the process of conceptualizing data obtained in the process of the psychological autopsy and making associated decisions associated with determining the mode of death.

The classification of the mode of death (natural, accident, suicide, or homicide) hinges on the intention of the decedent in relationship to the death. Since its conception in the late 1950s, the psychological autopsy has been used in some medical examiner and coroner's offices as a tool by which final death determinations can be made, particularly in unclear cases of alleged self-inflicted death.¹ In certain contexts, the mode of death may be undermined or equivocal or an outside party protests the death determination. In such circumstances, the psychological autopsy allows investigative principals of behavioral science and specialized knowledge of mental health professionals to be applied to the process of classifying deaths through a comprehensive analysis of many aspects of a decedent's mind around the time of death.² It accomplishes this through a thorough analysis of a wide variety of collateral sources (e.g., interviews and personal, educational, professional, and investigative documents) to identify lifestyle and behavioral history, as well as a breadth of cognitive and personality factors that contribute to that history, and more specifically, the decedent's role in the death.

While relatively rare in the practice of most medical examiners' or coroners' offices, the psychological autopsy can provide a unique opportunity for collaboration between forensic mental health professionals and personnel in disciplines integral to death investigations. The majority of death determinations may not necessitate the assistance of mental health professionals, namely because a medical examiner or coroner typically uses the determined method of death as a guideline for the mode of death. Moreover, based on time, cost, and expert availability, a psychological autopsy may not be possible or necessary. Further, some criticism of the utility of the psychological autopsy, both as a research tool and in practice, may contribute to limiting its use.^{3,4} Limitations of the practice will be addressed, as well as the utility of a rigorously and objectively approached analysis of aspects of the decedent's life in the context of cases in which the mode of death is in question. Ultimately, it is opined that a comprehensively conducted psychological autopsy may help to elucidate factors contributing to a decedent's intentions surrounding death not otherwise clarified through more traditional means of medicolegal death investigation. Approaches to facilitating collaborations between mental health professionals qualified to conduct psychological autopsies and offices of medical death investigations will also be discussed.

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Psychological Autopsy, Mode of Death, Suicide



K45 The Psychological Autopsy as an Aid to Scientific Investigation: The Application of the Italian Model to a Cold Case Murder

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The goal of this presentation is to inform attendees of the Italian model of the psychological autopsy which is adopted in murder cases and cold cases, in addition to problematic death cases.

This presentation will impact the forensic science community by comparing the traditional application of the psychological autopsy with the new Italian model.

Concerning the psychological autopsy in Italy, De Leo et al., beginning with and integrating models of Farberow and Shneidman, Ebert, and Canter, defines it as the analysis of the victim's characteristics and the interactive processes that link the victim and offender.¹⁻⁴ The model proposes guidelines for the analysis of the psychological connection between the victim and the offender and focuses on behavioral risks, relational vulnerabilities, and possible levels of victim-offender connection.

The goal of this study is to determine what kind of relationship there was between the victim and the offender and, consequently, which hypotheses can be utilized for the possible motive and clues about the likely murderer. This study proposes an analysis of possible communicative goals of violent action, reconstructed on the basis of the analysis of the crime scene and other scientific evidence. The investigative hypotheses that emerge from this analysis are then cross-referenced with other findings of investigation.

The psychological autopsy, together with the analysis of violent and communicative action, represents the Italian model for the analysis of unsolved murders and cold cases.⁵

This presentation will describe the application of the model by De Leo in the scientific investigation in a cold case which is well known to the Italian news media. In 1990, the murder of a young 19-year-old girl occurred in the office where she worked. Analysis consisted of review of legal documents, videotapes of television programs in which her sister and parents participated at the time, letters written by the victim, and psychological interviews conducted with her sister and mother.

In the first phase of the investigation, an analysis was made of the contents of documentary material, which was "questioned" with the psychological autopsy guidelines. At this same time, scientific investigations identified the alleged murderer's DNA on the girl's shirt and bra and, via old autopsy photos, identified an alleged bite on the girl's left breast.

In the second phase of the investigation, letters of the victim and psychological interviews of her mother and sister were analyzed to determine the murderer's possible motive and to document the laundry habits at the victim's home.

The identification of the murderer was determined to be the victim's boyfriend. The substantial findings of the interdisciplinary approach adopted in this case led to the boyfriend's conviction with a 24-year prison sentence for the first case, followed by "not guilty" decisions in the second and third case.

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Psychological Autopsy, Scientific Investigation, Interdisciplinary Group



K46 Utility of Oral Fluid in Driving Under the Influence of Drugs (DUID) Investigations

Christine Moore, PhD, DSc, Immunalysis Corporation, 829 Towne Center Drive, Pomona, CA 91767*

After attending this presentation, attendees will better understand the potential role of oral fluid testing for investigations into DUID. Attendees will learn that drugs as well as alcohol are responsible for, or at least a factor in, traffic accidents.

This presentation will impact the forensic science community by emphasizing the advantages and viability of testing oral fluid in DUID situations.

Currently, blood or urine is collected for toxicological analysis in suspected cases of DUID; these take time to obtain from a driver, allowing drugs to dissipate from the body. Specimens collected proximate to the traffic stop have a higher probability of containing recently ingested substances which may be potentially impairing. Oral fluid has significant advantages over currently used biological samples, specifically fast, easy collection at the roadside or at a detention facility; rapid collection allows drugs currently circulating in the body to be preserved for analysis. Technological improvements have promoted the use of oral fluid analysis for various applications, including DUID. Additionally, collection devices have been improved, laboratory procedures have become routine, and drug test panels have been expanded.

Over the last decade, numerous roadside surveys in the United States and Canada have established the validity and viability of oral fluid collection for the determination of DUID; drug prevalence rates of 14%-16% have been reported, with marijuana accounting for approximately half of the positive results. An overview of various survey results, drug prevalence rates in drivers, as well as field studies involving both roadside tests and confirmatory specimen analysis will be presented.

Because more and more field studies involving oral fluid are being performed, guidelines for the implementation of data-collection projects have been developed by a subcommittee of the Joint American Academy of Forensic Sciences/Society of Forensic Toxicology (AAFS/SOFT) Drugs and Driving committee. The guidelines are intended for the use of researchers planning to collect information on drug intake from stopped drivers. Focal points of field studies include ways to identify drivers under the influence of drugs in a more efficient and effective manner and to deter drug intake prior to driving by demonstrating reliable drug detection.

In a separate consensus meeting of experts recruited from survey respondents and members of the National Safety Council Alcohol, Drugs and Impairment Division, previously recommended drug concentrations for blood and urine analysis were updated to include oral fluid as a sample matrix.¹ Both of these initiatives will be discussed in the presentation.

Reference:

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Oral Fluid, Drugged Driving, Roadside Testing



Toxicology Section - 2015

K47 An Evaluation of Oral Fluid Testing Devices During Drug Influence Evaluations

Kyle J. Clark, MA, Institute of Police Technology and Management, 12000 Alumni Drive, Jacksonville, FL 32224*

After attending this presentation, attendees will understand the effectiveness of oral fluid testing when used in a law enforcement capacity and compare its effectiveness to a current accepted test.

This presentation will impact the forensic science community by providing an understanding of the needs and limitations available to Driving Under the Influence (DUI) enforcement regarding drug-impaired driving as well as the evaluation of the effectiveness of an oral fluid testing device in this context.

Oral fluid testing is rapidly advancing in accuracy and efficacy within the DUI enforcement community. The biggest hurdle for the acceptance of evidential oral fluid testing deals with the accuracy of the test. A secondary hurdle is the length of time required for the testing process. Both of these hurdles have a significant impact on law enforcement roadside investigations. A testing process that is inaccurate can result in the improper release of a drug-impaired driver, leading to drug-using drivers becoming more confident in their ability to escape detection, and can reinforce the behavior. Inaccurate results can also result in the improper arrest of an innocent motorist, resulting in the deprivation of civil rights. Law enforcement officers have limitations in the detention of motorists in the absence of evidence of a crime and courts have limited the length of detention of a motorist in non-criminal traffic infractions. A roadside testing process that consumes too much time can violate constitutional protections provided in the United States Constitution against unreasonable seizure. For roadside testing to be a viable tool for law enforcement, it must meet both of these requirements, coupled with legislative provisions permitting its use.

A recent certification training conducted as part of a drug evaluation and classification program provided a pool of 95 subjects who were suspected of drug impairment. These subjects were examined for physiological indicators of drug usage as part of a drug-influence evaluation using methods proven effective in both laboratory and field studies. During the evaluation, candidate Drug Recognition Experts (DREs), under the supervision of an experienced DRE instructor, identified physiological signs in the form of pupil size and nystagmus, pupillary reaction to light, psychophysical indicators of divided attention impairment, and collected vital signs to opine if the subject was impaired and the most likely category (categories) of drug responsible. Toxicological samples were collected from each subject in the form of oral fluid and urine and tested onsite. The results of the oral fluid tests were compared with the results of the urine testing and both were compared with the opinions reached during the drug-influence evaluation. This presentation will provide an evaluation of the Alere™ DDS2® device for both its accuracy when compared with the drug-recognition expert opinions and the results obtained from MEDTOX® VERDICT® urine field screening devices, as well as the time element required for the testing process.

Drug, Impairment, Oral



K48 The Perilous Practice of Predicting the Past: Does Retrograde Extrapolation Accurately Predict Prior Blood Alcohol Level?

Ronald L. Moore, Esq., JD, 15635 Alton Parkway, Ste 120, Irvine, CA 92618*

After attending this presentation, attendees will better understand the accuracy of retrograde extrapolation in predicting prior blood alcohol levels in arrested subjects.

This presentation will impact the forensic science community by challenging the accuracy of predictions of Blood Alcohol Concentration (BAC) often made by forensic scientists in court using data from arrested subjects.

Retrograde extrapolation is often used in criminal prosecutions to predict the alcohol level at the time of driving for people arrested for Driving Under the Influence (DUI). The calculations are based on either a blood or breath test taken at a later time. The accuracy of these predictions is often vigorously challenged in legal proceedings. A considerable body of literature exists on alcohol metabolism that is often used to substantiate or undermine such calculations. Much of this data is from laboratory studies rather than under real-world testing conditions.

In the present study, data was collected from the case files of two southern California law firms with significant DUI defense case loads. Cases selected for the study were those where arrest reports indicated that the subject submitted to a pre-arrest breath test at roadside, which was then followed by either a blood or breath test after arrest. A total of 234 cases qualified: 160 males and 74 females, with an average age of 33 years (range 17 to 79 years). A comparison was made between the average of the pre-arrest results to the average of the post-arrest results to determine the direction of alcohol metabolism (rising or falling) during the intervening period and the magnitude of the change over the interval. On average, it took 28 minutes from driving to the first field breath test and, on average, an additional 45 minutes to complete the post-arrest chemical test. Of the 234 subjects, the blood alcohol level went up or remained the same in 108 subjects and went down in the remaining 126 subjects. In addition, calculations were made using typical retrograde extrapolation procedures to estimate the BAC at the time of the first chemical tests based on the time and results of the second set of chemical tests (using an elimination rate of 0.015g% per hour, applied to the time difference between the pre-arrest tests and the post-arrest tests). Fully 75% of the predicted BACs were higher than the actual BAC measured by field breath test. There was a significant difference between the likelihood of overestimating the field BAC based on whether the post-arrest test was blood or breath. If the second test was blood, 86% of the BAC estimates were higher than the field breath test, while if the second test was breath, only 65% of the estimates were higher than the field breath test. This presentation will graphically present this data and give further statistical treatment.

Retrograde, Extrapolation, BAC



K49 Evaluation of the Impact of Expanding Enzyme-Linked Immuno-Sorbent Assay (ELISA) Screening in Driving Under the Influence of Drugs (DUID) Investigations

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After attending this presentation, attendees will better understand the prevalence of the therapeutic drugs carisoprodol, meprobamate, and zolpidem in blood samples from DUID arrests. Attendees will be able to use the data to justify the use of ELISA testing for these compounds as part of a standardized drug screening approach in DUID investigations.

This presentation will impact the forensic science community by increasing knowledge of rates of these therapeutic drugs among the population of drivers arrested for DUID. The results should encourage other laboratories to test for these impairing drugs in DUID cases.

Historically, investigating agencies in North America have employed different standards and approaches to DUID testing. Many laboratories or agencies requesting testing in DUID cases rely on immunoassay (ELISA) screening methods which frequently do not employ zolpidem (Ambien®) or carisoprodol (Soma®) assays as part of their scope. A recently published set of standardized guidelines by Logan et al. added carisoprodol and zolpidem to the recommended scope of drug testing in DUID arrests.¹ These central nervous system depressant drugs have been reported to appear frequently in DUID cases in a 2012 survey of toxicology laboratories across the United States. There is still significant variability in terms of the scope of testing employed by different toxicology laboratories. This results in incomplete datasets in which drug incidence patterns are difficult to ascertain.

The purpose of this study was to determine what the incidence of carisoprodol and zolpidem are in a DUID population, in order to assess the frequency with which potentially impairing drugs might go undetected.

A large dataset (n=1,672) of drug screen results from DUID investigations between June 2013 and June 2014 was provided by NMS Labs in Willow Grove, PA. Toxicology results were obtained on blood samples using a Liquid Chromatography/Time-Of-Flight/Mass Spectrometry (LC/TOF/MS) method that was validated according to Scientific Working Group for Toxicology (SWGTOX) guidelines. The method tests for approximately 280 common therapeutic and abused drugs and their metabolites. Positive cases were confirmed and quantified by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) or Gas Chromatography/Mass Spectrometry (GC/MS). Results specifically for zolpidem and carisoprodol and its metabolite meprobamate were evaluated, as well as data on other drugs and alcohol present in these cases.

Zolpidem was found to be positive in 5.3% of cases (n=89). Among the cases which tested positive for zolpidem, many were positive for other drugs among which opiates (20%, n=18), benzodiazepines (19%, n=17), and alcohol (18%, n=6 (33 of 89 tested)) were most prevalent. Carisoprodol and/or meprobamate were positive in 5.9% of cases (n=99). Among the cases which tested positive for carisoprodol and/or meprobamate, other drugs present included opiates (62%, n=61) and benzodiazepines (53%, n=53).

Following assessment of these results, ELISA screening kits from Neogen® for carisoprodol, meprobamate, and zolpidem were validated, and a further 300 random blood samples in DUID investigations that had been tested according to a more limited protocol were subjected to ELISA testing for these three drugs. Positive results were confirmed using a Solid Phase Extraction (SPE) extraction method followed by LC/MS/MS or GC/MS.

Reference:

1. Logan BK, Lowrie KJ, Turri JL, Yeakel JK, Limoges JF, Miles AK, et al. Recommendations for Toxicological Investigation of Drug-Impaired Driving and Motor Vehicle Fatalities. *J Anal Toxicol* 2013;37(8):552-8.

DUID, Carisoprodol, Zolpidem



K50 Sleep-Driving Is a Nightmare: Driving Under the Influence of Zolpidem Cases in Colorado

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The goal of this presentation is to assist attendees in identifying signs and symptoms of zolpidem impairment and evaluate whether the zolpidem levels correlate with observed impairment, based on a study of all zolpidem-positive cases from July 2009 to July 2014 which were tested by ChemaTox Laboratory. The study focused on identifying the following: behaviors that appear in multiple reports, the most frequent multiple-drug intoxication combinations with zolpidem, and the concentrations of zolpidem in both single- and multiple-drug intoxication cases. Selected cases are examined in detail to highlight specific traits.

This presentation will impact the forensic science community by informing attendees that if a laboratory is not looking for zolpidem in their routine testing, it is likely missing these cases. Relying on law enforcement to request zolpidem testing is not sufficient to detect many of these cases, as people often do not inform officers they are taking zolpidem. The popularity of zolpidem, along with the sensationalized publicity surrounding the possible side effects, have led to common themes in cases. These themes include involuntary driving/intoxication, sleep driving, tolerance to the drug effects, and residual levels from the night before. All of these possible issues should still be considered; however, knowing and understanding the common features of these cases and the available research on the topic are critical for the most accurate evaluation of a case. It is imperative that the forensic science community and the public be educated about the dangers associated with driving under the influence of zolpidem.

Introduction: Zolpidem, commonly known as Ambien®, is a z-drug. Z-drugs comprise a class of non-benzodiazepine sedative-hypnotics typically prescribed for the treatment of insomnia. Zolpidem has a relatively short half-life of 2-5 hours and is rapidly eliminated. Zolpidem is intended to be taken immediately prior to going to sleep when the individual taking it is able to devote eight full hours to resting. Incidents of individuals driving under the influence of zolpidem, often to a substantially impaired degree, are becoming more frequent. In the past few years, the proportion of zolpidem-involved Driving Under the Influence of Drugs (DUID) cases tested by ChemaTox has increased. In 2011, zolpidem was the sixth most common drug/drug class in DUID cases tested by ChemaTox; by 2013, it had become the fourth most common drug. The zolpidem medication guide infamously cautions the user against “sleep-driving.” Concern about potential residual levels recently led the FDA to significantly decrease its dosage guidelines. Although more research and education have been provided in the forensic community, the most typical defense used in DUID-zolpidem cases in Colorado is that of involuntary intoxication. Of the cases submitted to ChemaTox, incidence of true involuntary intoxication is rare. Although individuals suspected of DUID-zolpidem often experience confusion, blood levels within expected therapeutic range, and lack of memory of the incident, this does not negate individual responsibility. Analysis was conducted of several typical and remarkable zolpidem-involved cases undertaken by ChemaTox within the years 2009-2014.

Objective: To identify signs and symptoms of zolpidem impairment and evaluate whether zolpidem levels correlate with observed impairment in all zolpidem-positive cases received by ChemaTox between July 2009 and July 2014. The study focuses on identifying the following: behaviors that appear in multiple reports, the most frequent polydrug intoxication combinations with zolpidem, and the concentrations of zolpidem in both single drug and polydrug intoxication cases. Selected cases are examined in detail to highlight specific traits.

Methods: Zolpidem-positive blood samples from DUID cases submitted to ChemaTox within the timeframe studied were evaluated. Samples were screened via Enzyme-Linked Immunosorbent Assay (ELISA). Confirmation testing was performed via Gas Chromatography/Mass Spectrometry (GC/MS) or Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS), both with limits of detection of 20ng/mL and limits of quantitation of 50 g/mL for zolpidem. Samples were tested via ELISA and GC/MS or LC/MS/MS for any other requested drugs. Law enforcement case reports are not routinely provided to the laboratory. Reports were requested for all cases and, when available, were analyzed for documented indicia of impairment, subject demographics, and whether zolpidem or other drug use was admitted by the subject.



Toxicology Section - 2015

Results: In DUID cases undertaken by ChemaTox between 2009 and 2014, the most common drugs/drug classes detected were, in descending order, D-9-tetrahydrocannabinol, benzodiazepines, amphetamines, zolpidem, opiates, cocaine, oxycodone, and tramadol. Zolpidem was confirmed in 203 cases (concentration range: <50ng/mL-2,400ng/mL). In available police reports, documented indicia of impairment included erratic weaving and lane travel, “ping-ponging” or colliding with multiple stationary objects, difficulty keeping eyes open, hallucinations, poor memory of recent events, difficulty following instructions and answering questions, and poor reaction time.

Conclusion/Discussion: If a laboratory does not routinely test for zolpidem, it is likely failing to detect impairment in zolpidem-involved cases. Relying on law enforcement to request zolpidem testing is not sufficient as subjects frequently do not inform officers that they are taking zolpidem. The popularity of zolpidem along with sensationalized publicity surrounding the possible side effects have led to trending defense strategies, including involuntary driving/intoxication, sleep driving, tolerance to drug effects, and residual concentrations. All of these potential explanations should be considered; however, familiarity with common themes of these cases and available research are critical for accurate case evaluation. It is imperative that the forensic community and the public be educated about the dangers associated with driving under the influence of zolpidem.

Zolpidem, DUID, Driving Impairment



K51 Synthetic Cannabinoids in Operating While Intoxicated (OWI) Casework: Field Observations and Outsourced Testing

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After attending this presentation, attendees will better understand how synthetic cannabinoid impairment presents at roadside. This presentation will also enhance existing case evaluation skills which are used in pursuing initial negative results that might be augmented by additional testing.

This presentation will impact the forensic science community by providing insight into the prevalence of synthetic cannabinoids in recent Wisconsin OWI casework and will include some cases utilizing Drug Recognition Experts (DREs). Casework examples will demonstrate the utility of targeted external analyses for potentially impairing substances not included in a laboratory's routine scope of testing.

Synthetic cannabinoids usage has been increasing at a rate faster than most forensic laboratories can develop and validate analytical procedures. In cooperation with the Wisconsin Bureau of Traffic Safety, select casework including 16 DRE cases listing potential synthetic cannabinoids use were forwarded to NMS Labs for synthetic cannabinoids testing since March 2012, based on information provided by law enforcement agencies. This work encompasses a total of 48 cases (n=43 males, n=5 females) at least two of which involve a motor vehicle death. Where narratives or DRE evaluations were available, subjects often self-reported cannabis or synthetic cannabinoids use, including brand and frequency. The results of routine ethanol and drug testing were fairly unremarkable. Twenty-six of 48 cases (54%) were negative for synthetic cannabinoids. Twenty-two of 48 cases (46%) had at least one synthetic cannabinoid compound detected and five of 48 cases (10%) had between three and seven synthetic cannabinoids detected. The synthetic cannabinoids compounds identified and their prevalence include: JWH-018 5-chloropentyl (n=1); JWH-018 (n=4); JWH-022 (n=3); JWH-081 (n=2); JWH-122 (n=5); JWH-210 (n=3); AM-2201 (n=12); UR-144 (n=5); and, XLR-11 (n=11).

While acknowledging standardized field sobriety tests were not specifically validated for synthetic cannabinoids or equally applied across these specimens, the types of impairment noted were mostly consistent with the cannabis category. It is also worth noting individuals under the influence of synthetic cannabinoids can demonstrate impairments consistent with other drug categories. Another challenge of testing for synthetic cannabinoids is the stability of these compounds in whole blood which has not been fully represented in the literature. While the challenge of analytical testing for these and other synthetic compounds will remain for some time, laboratories should be aware of outside resources and funding that may provide that testing as casework warrants.

Synthetic, Cannabinoid, Impairment



K52 Development of an Analytical Method for Nootropic “Smart” Drugs in Biological Fluids

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After attending this presentation, attendees will better understand the class of nootropic (“smart”) drugs, be able to describe the relative merits of different models of analysis, and implement a method to identify, confirm, and quantitate the drugs in biological fluids.

This presentation impacts the forensic science community by describing the development of an analytical method for newly emerging drugs that are subject to misuse and abuse and can cause mind-altering effects. The drugs are not currently regulated and are emerging online and in illicit supply chains.

Smart drugs, also known as nootropics, are an emerging area of growth in recreational drug use with implications for forensic toxicology. The drugs have stimulant properties and are alleged to boost brain function and cognition. The media attention on these drugs has increased within the last few years. The drugs have developed an underground following and are commonly sold online and in illicit supply chains. Most have not been approved or scheduled in the United States and are therefore of concern to regulators such as the Food and Drug Administration and Drug Enforcement Administration. There are ongoing investigations in the applications of smart drugs in the treatment of Alzheimer’s disease, Huntington’s disease, and attention-deficit hyperactivity disorder. The stimulant properties of the drugs have led to their use in academic doping and as drugs of abuse. Some drugs are also prohibited by the World Anti-Doping Agency.

The goal of this project was to develop a single analytical method for screening, confirmation, and quantification of a series of the more widely known smart drugs in blood and urine. Gas Chromatography/Mass Spectrometry (GC/MS) and Liquid Chromatography/Mass Spectrometry (LC/MS) were investigated to determine the optimum approach for sensitivity and ability to detect a broad range of compounds, specifically piracetam, pramiracetam, aniracetam, modafinil, adrafinil, ciproxifan, and noopept.

Analytical methods included the use of GC/MS. Analysis was performed using an Agilent® 6890/5973 GC/MS system with an Agilent® DB-5MS column (15m x 0.25mm; 0.25µm). Temperature programs starting at 70°C and injection port temperatures as low as 175°C were evaluated. The racetam class of drugs which contain a pyrrolidineacetamide structure (e.g., piracetam, pramiracetam, and aniracetam) were thermally unstable and degraded into three to five derivative structures. The structures of the degradation products were tentatively identified based on molecular weight and their mass spectra were added to a database to assist with identification of breakdown artifacts in future toxicological analysis. Adrafinil, a modafinil prodrug, was found to degrade to modafinil during GC/MS analysis by water loss, resulting in an inability to distinguish between the two substances. Even at low injection port temperatures, degradation persisted. Derivatization of the compounds using BSTFA with 1% TMCS to form TMS derivatives as a means of stabilizing the compounds was also investigated, but also failed to produce single stable chromatographable analytes.

As a result of the demonstrated thermal instability of these drugs, LC/MS was investigated. A successful analytical method was developed using an Agilent® 1100 series system with an Eclipse Plus C18 column (4.6mm x 100mm; 3.5µm), a gradient consisting of 10mM ammonium acetate buffer (pH 4) and 50:50 acetonitrile:isopropanol (v/v), a flow rate of 0.6mL/min, and positive (aniracetam, piracetam, pramiracetam, noopept, ciproxifan) and negative (modafinil and adrafinil) ionization. The ions that were monitored are as follows: aniracetam 220.1m/z; piracetam 143.1m/z; pramiracetam 270.1m/z; noopept 319.1m/z; ciproxifan 271.1m/z; modafinil 272.1m/z; and, adrafinil 288.2m/z. All of the peaks were baseline resolved.

Following characterization of the above conditions, extracts of blood and urine samples were prepared using supported liquid extraction procedures and evaluated for recovery cleanliness, reproducibility, limits of detection and quantitation, accuracy, and precision. In conclusion, LC/MS provided a superior means of identification for the target compounds due to the stability of the compounds under these analytical conditions. The methods evaluated were effective for the detection of the targeted compounds in biological fluids, such as whole blood and urine, at toxicologically meaningful concentrations.

Smart Drugs, Nootropics, LC/MS



K53 Adverse Effects of Synthetic Cannabinoids: A Case-Oriented Review

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After attending this presentation, attendees will be able to describe the adverse effects reported in humans who have taken synthetic cannabinoids.

This presentation will impact the forensic science community by providing a review of internationally reported cases. It will highlight the importance of performing quantitative analysis on synthetic cannabinoid samples in order to gain a full understanding of the effects of this class of drug and of the individual compounds.

The term “synthetic cannabinoids” refers to artificial substances which are intended to mimic the physiological effect of cannabis. A wide variety of such compounds have been developed, partly in an attempt to avoid the legal penalties associated with the manufacture, distribution, and use of cannabis. These drugs differ in their chemical composition and, therefore, also differ in their effects on humans and their likelihood of detection by toxicological methods. The scientific understanding of synthetic cannabinoid compounds is hampered by a lack of awareness of this variety and, in practical terms, it is important to be able to correlate observed clinical effects with their likely toxicological causes. The reports presented will include data from small (<11 people) and large (>168 people) populations without toxicology confirmation, in addition to reports with qualitative confirmation and reports with quantitative analysis.

Synthetic cannabinoid drugs were first produced for research purposes in 1980. Since then, synthetic cannabinoid drugs have been manufactured in clandestine laboratories internationally, and sold in the United States in the form of smoking mixtures. Common brands of synthetic cannabinoids have been marketed, including Spice and K2. The first report of synthetic cannabinoid drugs in the United States was in 2008, when a synthetic cannabinoid compound was found in botanical material. Since then, the popularity of synthetic cannabinoids use has increased, as has the number of compounds which have been developed. With these different compounds and new compounds emerging onto the market, it has been challenging for forensic toxicologists to identify the individual drugs and metabolites in samples. Without quantitation of these drugs, it is difficult to understand the true effects these drugs are having on the user. This is why reports which include this data and dose response information are beneficial to the understanding of the adverse effects of these drugs. The adverse-effect profile of these drugs has not been studied in humans, and only infrequently in animal models; thus, much of the information about their toxicity comes from emergency department treatment reports and forensic case studies.

Case reports have been published describing adverse effects including data collected from emergency department admissions, mental health admissions, and clinical and forensic case reports. The current state of knowledge of adverse effects, both clinical and forensic in humans, includes non-serious physiological effects similar to smoking marijuana, and adverse effects including kidney damage, pulmonary dysfunction, cardiovascular effects, central nervous system effects, seizures, and psychosis. Reports to date include samples which have single synthetic cannabinoid compounds and mixtures of multiple compounds. With so many different synthetic cannabinoid compounds emerging and on the market, it is challenging to determine if these adverse effects are associated with specific compounds or with the class of drug. There is growing toxicological and pharmacological evidence of impairment, psychosis, tissue injury, and isolated deaths attributable to this emerging class of drugs.

Designer Drugs, Synthetic Cannabinoids, Adverse Effects



K54 4-Methoxy- α -PVP: *In Silico* Metabolite Prediction, Assessment of Metabolic Stability With Human Liver Microsomes, and Metabolite Identification After Human Hepatocyte Incubation With High Resolution-Mass Spectrometry

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After attending this presentation, attendees will better understand different approaches of investigating metabolic pathways and of selecting potential 4-methoxy- α -PVP metabolites to be targeted in human specimens.

This presentation will impact the forensic science community by demonstrating the applicability of utilizing *in silico* software predictions and *in vitro* metabolism experiments to elucidate the metabolic profiles of these emerging novel psychoactive substances.

Introduction: Synthetic cathinones emerged on the designer drug market as popular “legal” alternatives to illicit drugs during the late 2000s and are marketed as “legal highs” not for human consumption to avoid regulation. These novel psychoactive substances are continuously developed to circumvent legislative and regulatory efforts. As such, limited pharmacological and toxicological information is available. Recently, 4-methoxy- α -PVP was identified for the first time in illegal products purchased in Japan in 2013.¹ No metabolism studies have been performed on this compound. Complete metabolic profiles are needed for these novel psychoactive substances to enable identification of their intake and to link adverse effects and educate the public about the dangers of these “designer drugs.”

Objectives: To evaluate *in silico* metabolism predictions, perform metabolic stability assessment with Human Liver Microsomes (HLM), and to identify metabolites after human hepatocyte incubation with High-Resolution Mass Spectrometry (HRMS) for 4-methoxy- α -PVP.

Methods: *In silico* predictions were performed with MetaSite software, which predicts metabolites based on enzyme-substrate recognition and site reactivity of the molecule. To investigate the kinetics of 4-methoxy- α -PVP, 1 μ M drug was incubated with 50-donor-pooled human liver microsomes at 0, 3, 8, 13, 20, 30, 45, and 60min. For the human hepatocyte experiments, 10 μ M drug was incubated at 37°C with pooled cryopreserved human hepatocytes at 0, 1, and 3h based on HLM half-life. Diclofenac also was incubated as a control for human hepatocyte functional viability. Chromatographic separation of HLM samples, diluted 1:100 with mobile phase A (0.1% formic acid in water), was achieved with an Accucore™ C18 column (2.6 μ m, 100mm x 2.1mm) with mobile phase A and B (0.1% formic acid in acetonitrile) within 20min. Hepatocyte samples were diluted 1:5 with mobile phase A, and separated utilizing a Synergi 4 Hydro-RP column (80A, 150mm x 2mm) within 30min. Data from HLM and human hepatocyte samples were collected with a Thermo Scientific™ QExactive™ high-resolution mass spectrometer, and analyzed by WebMetabase software-assisted data mining. HLM and hepatocyte data were acquired using a high-resolution, full-scan, data-dependent mass spectrometry method. In addition, hepatocytes were acquired with and without an inclusion list of predicted metabolites generated by MetaSite, and with an All-Ion-Fragmentation (AIF) mass spectrometry method to identify potential unexpected metabolites. Scans were thoroughly data mined with different data processing algorithms utilizing WebMetabase.

Results: 4-methoxy- α -PVP exhibited a long half-life of 79.7min in HLM, with an intrinsic clearance of 8.7 μ L/min x mg. In addition, this compound is predicted to be a low-clearance drug with an estimated human hepatic clearance of 8.2mL/min/kg. Based on the structure of this synthetic cathinone and the results obtained, the following biotransformations were proposed: O-demethylation, aliphatic ring hydroxylation, ketone reduction, iminium formation, pyrrolidine ring hydroxylation followed by dehydrogenation of the corresponding lactam, pyrrolidine ring opening followed by oxidation to carboxylic acid, iminium formation and aliphatic hydroxylation, dihydroxylation, ketone reduction and O-demethylation, and ketone reduction and hydroxylation. The most dominant metabolite in the HLM and human hepatocyte samples was 4-hydroxy- α -PVP, also predicted *in silico*.

Conclusions: These are the first data identifying 4-methoxy- α -PVP metabolites that could document drug intake for forensic and clinical investigations. It is necessary to elucidate the metabolic pathways of these new psychoactive substances to enable linkage to adverse effects and document 4-hydroxy- α -PVP intake. This presentation will impact the forensic science community by demonstrating the applicability of utilizing *in silico* software predictions, and *in vitro* metabolism experiments to elucidate the metabolic profiles of these emerging novel psychoactive substances.



Toxicology Section - 2015

Supported by the National Institutes of Health, Intramural Research Program, NIDA.

Reference:

1. N. Uchiyama et al., *Forensic Toxicology*, 32, 105–115 (2014)
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4-Methoxy-A-PVP, In Silico Prediction, In Vitro Metabolism



K55 Identification of Major Metabolites in Human Blood and Urine Associated With the Ingestion of Alpha-Pyrrolidinopentiophenone (Alpha PVP)

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After attending this presentation, attendees will be able to identify the metabolic profile associated with use of alpha-PVP and identify its major metabolites. Attendees will be able to describe the process of using human liver microsomal incubations to verify the identity of novel drug metabolites.

This presentation will impact the forensic science community by providing an example of the use of various laboratory-based analytical and *in vitro* tools for metabolite identification of Novel Psychoactive Substances (NPS).

The purpose of this project was to collect biological samples from volunteers attending Electronic Dance Music (EDM) festival and evaluate samples for emerging NPS. Blood samples screening positive for alpha-PVP were further investigated for the presence of potential metabolites that had been produced *in vitro* using Human Liver Microsomes (HLM).

Alpha-PVP is an emerging novel psychoactive substance of the pyrrolidinophenone family, which has been identified in forensic casework samples over the last three years. Limited data exists on the mechanisms of action of alpha-PVP; however, it is believed to produce similar effects to Methylenedioxypyrovalerone (MDPV), which acts as a norepinephrine-dopamine reuptake inhibitor, producing stimulant-like effects and euphoria. Its metabolic profile has been investigated in a rat model with analysis of the urine by Gas Chromatography/Mass Spectrometry (GC/MS); however, there have been no reports of its metabolism in humans.

The analysis of several urine specimens found to contain alpha-PVP also disclosed the presence of other compounds with similar mass spectral characteristics. This prompted the investigation of the identity of these compounds to verify their origin as alpha-PVP metabolites. Several tools including High Resolution Accurate Mass Spectrometry (HRAMS) on a Liquid Chromatograph/Quadrupole/Time-Of-Flight/Mass Spectrometer (LC/Q/TOF), Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS), GC-MS, and comparison of the profile of authentic human urine specimens from drug users with analysis of the products of incubation of the parent compound with a human liver microsome preparation were used to identify the metabolites.

Metabolites of alpha-PVP were produced by incubating a solution of alpha-PVP with pooled HLM. Phosphate buffer (pH 7.4) was spiked with 5,000ng of alpha-PVP. NADPH, a co-factor for the enzymatic reaction, was added to the buffer solution and allowed to incubate for two hours at 37°C. Following filtration, the samples were analyzed using LC/Q/TOF to generate exact mass data. Incubated samples were compared with controls that were incubated without the essential NADPH co-factor to help identify products of metabolism.

Authentic human blood samples from cases in which paired urine samples had tested positive for alpha-PVP were extracted using a basic liquid-liquid extraction using 0.1M borate buffer (pH=10.4) into n-butyl chloride and ethyl acetate. The organic phase was evaporated to dryness and reconstituted in 90:10 5mM ammonium formate and 0.1% formic acid in acetonitrile and analyzed using LC/Q/TOF.

Human blood and urine samples that had screened positive for alpha-PVP by LC/Q/TOF were further examined using extracted ion chromatograms for the exact masses of potential metabolites produced using the HLM incubation procedure described above.

One of the major alpha-PVP metabolite candidates from the HLM experiments had a retention time and accurate mass (246.1645g/mol) equivalent to $C_{15}H_{21}NO_2$, which is consistent with methoxetamine, an unrelated NPS compound with dissociative anesthetic properties; however, the fragment ions in the high energy mass spectra were not consistent with those present in the methoxetamine standard.

The identity of the apparent alpha-PVP metabolite was established as resulting from hydroxylation of the side chain on alpha-PVP (Figure 1). Using structural elucidation tools with the metabolite structure, possible fragmentation patterns were suggested by the software, based upon the ions seen in the high energy mass spectra. Three of the fragments proposed from the software were found in the high energy mass spectra, confirming its identity as a hydroxylated metabolite of alpha-PVP.

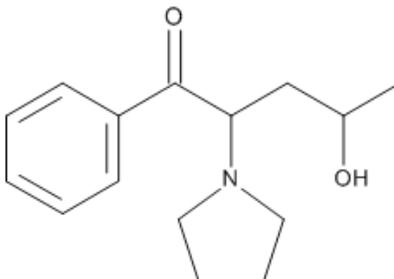


Figure 1

The combined results from analysis of mass spectrometric data, HLM incubations, and analysis of urine and blood from authentic cases of alpha-PVP ingestion allowed identification of several major metabolites in humans, several of which were consistent with results from published rat metabolic profiles; in addition other previously unknown metabolites were identified.

Alpha-PVP, Metabolism, Novel Psychoactive Substances



K56 Characterization of AB-FUBINACA Metabolites in Rat Urine by Liquid Chromatography/Time-of-Flight/Mass Spectrometry (LC/TOF/MS)

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After attending this presentation, attendees will better understand the synthetic cannabinoid receptor agonist AB-FUBINACA and its metabolites in the rat model. Since it is a relatively new and potent drug that remains largely uncharacterized, this study is an important step in elucidating oxidative metabolites as targets for revised screening assays in forensic casework.

This presentation impacts the forensic science community by increasing awareness of the *in vivo* metabolites of AB-FUBINACA as determined by LC/TOF/MS analysis. The knowledge gained will help to improve detection capabilities and practices.

Synthetic cannabinoids were first produced by academic and pharmaceutical laboratories with the hope of providing therapeutic pain relief without the toxicity that accompanies chronic opiate use. While many of these drug derivatives were published in the medical literature, others were merely patented. These stale patented recipes have become the basis for a new generation of illicit drug manufacture, replacing the first generation JWH species. AB-FUBINACA is one of the newest synthetic cannabinoids introduced in the past several years, first detected in blended synthetic products in 2012.¹ Because of its potency and obscurity, little is known about the metabolism or pharmacology of these substances.

In order to study the metabolic fate of AB-FUBINACA, the Wistar rat model was utilized to deliver drugs by intraperitoneal injections and then urine was collected for analysis. Rats weighing from 160g to 200g were housed in a temperature- and humidity-controlled room with a 12-hour light/dark cycle. After acclimatization, the rats were transferred to individual metabolic cages for the course of the experiment, to collect urine. Injections consisted of 5mg/kg of AB-FUBINACA dissolved in dimethyl sulfoxide, which were repeated daily for five days. The control group (n=3) received dimethyl sulfoxide injections, while the experimental group (n=5) received the dissolved drug. Urine samples were collected every day at the same time during injection and refrigerated until preparation for analysis.

Extractions were performed by combining 0.2mL of urine with an organic mixture of 1:1 isopropanol/1-chlorobutane, fortified with saturated magnesium sulfate and adjusted to pH 10 with ammonium hydroxide. After vortexing and centrifuging, the organic layer was removed to a fresh vial where the extract was evaporated to a residue with compressed nitrogen gas. The residue was reconstituted with mobile phase buffer and injected for LC/TOF/MS scanning. Examination of the time-of-flight data showed possible hydroxyl metabolites at 385.1687m/z, in comparison to the parent mass of 369.1721m/z. While the position of the hydroxyl addition on the molecule is unclear, the accurate mass and retention is now known for successful library matching.

Other published articles have studied metabolites of AB-FUBINACA in human liver microsomes, but this work is the first *in vivo* study to determine the metabolites of AB-FUBINACA in urine.² A putative hydroxyl metabolite of AB-FUBINACA was identified; however, a carboxyl metabolite has not been defined by this method.

References:

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Synthetic Cannabinoids, AB-FUBINACA, LC/TOF/MS



K57 Broad Detection of Synthetic Cannabinoids in Whole Blood Using Ultra High-Performance Liquid Chromatography-Quadrupole-Time-of-Flight (UHPLC-Q-TOF)

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After attending this presentation, attendees will understand the challenges associated with the analysis of synthetic cannabinoids and be able to describe the current panorama of these drugs.

This presentation will impact the forensic science community by increasing awareness of the need to analyze for synthetic cannabinoids in cases of both the living and the deceased.

Synthetic cannabinoids are the most prevalent group of new psychoactive substances emerging on the illicit market. The changes in availability from internet sites are partly a result of legislation where substances becoming scheduled are replaced by new compounds. Therefore, it is a challenge to keep methods up to date. This study was performed to create a strategy for the analysis of a broad range of synthetic cannabinoids that was flexible and to which one could easily add new compounds as they appear on the market and subsequently in the toxicological samples received.

Blood samples (1.0g) were prepared by liquid-liquid extraction with diethylether at pH 10.2. An in-house library comprising full Tandem Mass Spectrometry (MS/MS) spectra of 75 synthetic cannabinoids was built by analyzing solutions from certified standards. Identification was based on retention time, accurate mass, isotopic pattern and MS/MS spectra. Analyses was performed on an Agilent® 6550 iFunnel Q-TOF Liquid Chromatography/Mass Spectrometry (LC/MS) system combined with a 1290 Infinity® LC system. Ions were generated in positive electrospray ionization mode and were detected by data dependent acquisition MS/MS. Separation was achieved by gradient chromatography on an YMC Triart-C18 column within 8.5 minutes. Mobile phase A consisted of 0.05 % formic acid in 10mM ammonium formate and phase B of 100 % methanol.

The method was validated accordingly to guidelines for qualitative methods including selectivity, matrix effects, extraction recovery, response variation and spectra score variation at the threshold concentration, and stability in matrix. The selectivity was excellent; however, the analyte response was affected by the matrix with 28 of the 75 analytes presenting with matrix effects above 25%. Still, the variation in response and spectra scores was less than 15% at the thresholds chosen. Sixty-six analytes obtained a threshold of 100pg/g and seven analytes 200pg/g whole blood. All analytes except AM-2201 were stable when refrigerated for two weeks. After four weeks refrigeration, JWH-122 and UR-144 also showed a decrease of more than 20%.

The method was applied to cases including petty drug offences, DUID, violent crimes, and autopsies where analysis of synthetic cannabinoids was requested. In total, 442 samples were run during the first two months in routine with a positive rate of 24% resulting in the detection of 14 different parent compounds. The most prevalent compound was AB-FUBINACA (N=78) followed by AB-PINACA (N=12), BB-22 (N=10), 5F-NNEI (N=7), FUB-PB22 (N=5), 5F-PB22 (N=4), THJ2201 (N=4), 5F-AKB48 (N=3), 5F-AB-PINACA (N=2), NNEI (N=2), and one finding each of STS-135, PB22, FDU-PB22, and UR-144.

Blood as a matrix was chosen because of the better availability of parent compounds rather than metabolites from certified suppliers. A qualitative approach was chosen to simplify validation and inclusion of new analytes. High-resolution mass spectrometry with UHPLC was chosen to increase both the chromatographic and mass spectral selectivity. In conclusion, the strategy provided a sensitive, flexible, end-point method that detects parent compounds, simplifying prosecution in cases where the intake of a scheduled drug is the charge. A disadvantage of the qualitative approach is that it does not allow for a detailed interpretation; however, the pharmacodynamics of synthetic cannabinoids is largely unknown and a concentration does not necessarily increase the interpretation power.

Synthetic Cannabinoids, Whole Blood, TOF/MS



K58 Sleeping With the Enemy: Zolpidem in Driving Under the Influence (DUI) and Postmortem Cases

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After attending this presentation, attendees will better understand the pharmacokinetic properties that contribute to the therapeutic benefits of zolpidem (Ambien®), the toxicological findings in blood specimens, and zolpidem's potential impact on DUI and medicolegal investigations encountered by the Harris County Institute of Forensic Sciences. In addition to the reported findings and influence of zolpidem use in Harris County, its prevalence in the mainstream media will also be discussed.

This presentation will impact the forensic science community by describing the mechanistic properties, therapeutic benefits, and Food and Drug Administration (FDA) -established regimen guidelines of zolpidem, as well as disclosing the potentially deleterious effects of improper usage as determined by toxicological findings from casework investigations.

Zolpidem was approved by the FDA in 1992 and has become a common drug to aid with insomnia but it has recently gained attention for potential dangerous side effects of sleep walking, abnormal thinking, sleep driving, and residual effects into the morning. Zolpidem is a short-acting sedative-hypnotic prescribed for the short-term treatment of insomnia and works by activating the neurotransmitter GABA by a mechanistically similar manner as the benzodiazepines such as Xanax® and Valium®. It is typically prescribed as 5mg or 10mg capsules for immediate release and 6.25mg to 12.5mg for extended-release products (Ambien® CR). This medication is known to contribute to sleepiness the day after ingestion and is dependent on drug dose and individual characteristics. Therefore, it is recommended that the patient has at least eight hours of sleep to ensure that the drug is fully metabolized due to morning impairment. To further address the concern, the United States Food and Drug Administration approved a dose reduction in 2013, reducing the recommended dose of long-lasting impairment from 10mg to 5mg, depending on each individual.

Zolpidem has been depicted as a controversial sleeping aid since its long-lasting effects on patients have surfaced revealing abnormal behaviors due to its consumption. Previously, these abnormal behaviors were attributed to the combination effects with alcohol; however, there has been a growing revelation that these behaviors are induced with just zolpidem taken alone. Furthermore, there has been a growing concern on the evaluation of driving impairment with the use of zolpidem and motor vehicle accidents due to the increase of drugged driving cases. Finally, there has also been a significant increase in deaths from ingesting zolpidem in combination with other drugs and/or ethanol.

Harris County has received 136 cases from 2012-2014 that were positive for zolpidem. Several cases will be discussed related to zolpidem in DUIs and deaths that have resulted, including cases that have been highlighted in the media, as well as cases analyzed by the Harris County Toxicology Laboratory. In this presentation, several cases will be discussed related to DUI and postmortem cases.

Case 1: A 26-year old Asian male was driving under the influence. Upon analysis, zolpidem was found in the blood at a concentration of 1.0mg/L.

Case 2: A 63-year-old White male was driving under the influence with a blood level of 0.99mg/L for zolpidem. Ethanol was also detected at 0.01g/100mL which is below the level of impairment. Although norcarboxytetrahydrocannabinol was detected at 16ug/L, (the inactive metabolite of THC) it is not considered to have contributed to any level of impairment.

Case 3: A 54-year-old Black male was found dead in his home, apparently the victim of a suicide. Zolpidem was analyzed at a concentration of 1.4mg/L. Tramadol and desmethyltramadol were found in his blood sample at concentration levels of 17mg/L and 0.54mg/L, respectively.

Other cases that have gained national media attention will also be presented.

Zolpidem, DUI, Postmortem



K59 Pyrimethamine Toxicity: A Case Report

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After attending this presentation, attendees will understand the pharmacology and toxicology of pyrimethamine.

This presentation will impact the forensic science community by offering a description of the analysis of biological and non-biological material for pyrimethamine, a drug not normally encountered in routine forensic casework. In addition, a brief review of published cases involving pyrimethamine toxicity and a case report of an infant showing symptoms of pyrimethamine toxicity will be provided.

Pyrimethamine (PYR) is an antiparasitic medication used for treatment of protozoal infections including toxoplasmosis. The recommended dose for treatment of congenital toxoplasmosis is 1mg/kg/day for two to four days, then 0.5mg/kg/day for one month. Peak plasma concentrations following doses of 0.5-2mg/kg/day were 0.29-2.2mcg/mL with half-lives of 2.7-5.2 days. Plasma concentrations in previously reported cases involving toxicity in infants were 1.5, 6.2, and 13.0mcg/mL. Adverse effects associated with pyrimethamine treatment include anorexia, rash, and hematological disorders including bone marrow depression. Concurrent administration of folic acid has been used to minimize the risk of reversal neutropenia that has been noted in infants undergoing pyrimethamine treatment. PYR toxicity characterized by neurological hyper-excitability, seizure, and gastrointestinal symptoms has rarely been reported.

In this case, a five-month-old male child, on therapy for congenital toxoplasmosis complicated by chorioretinitis, developed irritability and was seen in an emergency room and discharged after receiving a single dose of PYR. Shortly after a second dose, he developed irritability and suffered a tonic-clonic seizure. The seizure responded to lorazepam administration and he was admitted to the hospital. PYR was stopped for 24 hours and no seizures were noted. Upon re-introduction of the medication from the same dispensed bottle, the child suffered another seizure. Serum samples collected 18 hours, 4 days, and 11 days after last exposure were analyzed for pyrimethamine along with the liquid medication used to treat the infant.

PYR was quantified in serum using a validated assay. Samples underwent a three-step liquid-liquid extraction, and then were dried, reconstituted in toluene, and derivatized with butyric anhydride. Samples were then injected on the gas chromatograph with a nitrogen phosphorus detector and separated on a DB-17 capillary column (15m x 0.32mm I.D., 0.15µm film) using a temperature gradient and constant gas flow. The serum specimen, collected ~18 hours after the last dose, contained 3.8mcg/mL PYR. Four days later, PYR concentration had dropped to 1.2mcg/mL and 11 days post-exposure the PYR was below the reporting limit of the assay (0.2mcg/mL). The liquid medication, which was supposed to contain 2mg/mL PYR, was determined to have a concentration of 94mg/mL. Based on the calculated concentration in the medication the infant received, approximately 280mg of PYR instead of 6mg ((based on the prescribed dose of 3mL of a 2mg/mL solution). Despite the long half-life of PYR, it can still be assumed that the peak concentration of PYR would have been achieved well before this serum was collected so it would have been greater than 3.8mcg/mL and in the range of previously reported toxicities. The concentration of 1.2mcg/mL four days later is consistent with the reported half-life of PYR.

Pyrimethamine, Pediatric, Toxicology



K60 Ethyl Glucuronide, Ethyl Sulfate, and Nicotine and Metabolites Quantified in Human Fetal Liver From Electively Terminated Pregnancies

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After attending this presentation, attendees will be able to describe a simultaneous human fetal liver sample preparation and two Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS) quantification methods for hepatic alcohol and tobacco markers. Method validation data and results for eight alcohol and nicotine markers in 118 fetal livers will be presented.

This presentation will impact the forensic science community by providing fetal liver alcohol and, for the first time, tobacco concentrations to document *in utero* exposure and potential related exposure toxicities.

Introduction: Adult health is partly programmed during fetal development. Fetal exposure to tobacco, alcohol, and other drugs alters normal hormone regulation and early development, possibly leading to maladaptations and contributing to adult metabolic syndromes. Mechanisms through which fetal drug exposures result in reduced adult health are poorly understood. The objective was to develop quantitative alcohol and tobacco marker assays for human fetal liver to further laboratory research on fetal endocrine disruption.

Methods: Blank liver (0.25g) was fortified with deuterated internal standards. Samples were bead beater homogenized in methanol 0.01% formic acid and passed through a 10 μ m reservoir filter. Two filtered liver supernatant portions were aliquoted into separate tubes for supported liquid extraction of nicotine and metabolites and anion-exchange solid phase extraction for Ethyl Glucuronide (EtG) and Ethyl Sulfate (EtS). An AB SCIEX™ 5500 Qtrap® mass spectrometer was interfaced with a Shimadzu® UFLCXR system for analysis with gradient chromatographic separation of nicotine, cotinine, 3-*trans*-hydroxycotinine (OHCOT), nicotine-N-glucuronide (NG), cotinine-N-glucuronide (CG), and OHCOT-O-glucuronide (OHCOT-G) on an Agilent® Poroshell 120 EC-C8 column (150 x 2.1mm, 2.7 μ m). Liver EtG and EtS were separated on a Phenomenex® Kinetex® XB-C18 (100 x 2.1mm, 2.6 μ m) column. Sensitivity, specificity, linearity, accuracy, imprecision, extraction efficiency, matrix effect, carryover, dilution integrity, and stability were evaluated during method validation. Human fetal liver samples (n=118) from normally progressing, electively terminated pregnancies (11-21 weeks gestation) were analyzed.

Results: Linear ranges were 1-300 (cotinine), 5-1500 (CG, nicotine, EtS), 2.5-750 (NG and OHCOT), 10-2,000 (OHCOT-G), and 20-3,000ng/g (EtG). Calibration employed 1/x² weighting (correlation coefficients ≥ 0.989). Extraction efficiencies were 72%-87% (EtG and EtS), 80%-94% (nicotine, cotinine, and OHCOT), and 53%-71% (NG, CG, and OHCOT-G). Matrix suppression for all analytes was 17.5%-57.3%, except CG (11%-12.3% enhancement). Overall accuracy was 84.2%-115.4% for all analytes at three Quality Control (QC) concentrations across the linear range; between-run imprecision (%CV) was 3.5%-9.4% (n=20). Five unique negative liver samples had no interfering peaks. None of 90 potential exogenous interferences fortified at 4,000ng/g into low QC samples interfered. No carryover was detected at 1.6 times the upper LOQ. Analytes were stable ($\leq 19\%$ change) after 72h on a 4°C autosampler, for 16h at room temperature, 72 h at 4°C, and three freeze-thaw cycles, and in filtered liver supernatants for five days at 4°C. Of 118 authentic human fetal liver samples, 11% were EtG-positive, 72% EtS-positive, 80% cotinine-positive, 64% OHCOT-positive, 59% nicotine-positive, and 31%-68% positive for nicotine glucuronide metabolites. All EtG-positive samples were also EtS-positive, with EtG concentrations always greater (1.6-4.9 times) than EtS; of 13 samples positive for both, 10 were positive for tobacco markers. In EtS-only positive samples, 78% were positive for tobacco markers. In 33 alcohol-negative samples, 28 were positive for tobacco markers.



Toxicology Section - 2015

Conclusions: The novel bead beater homogenization method with separate sample clean-up and chromatographic methods for acidic EtG and EtS and basic nicotine and metabolites provided accurate quantification in a single 0.25g liver sample. Investigators needing to quantify *in utero* alcohol and tobacco exposure in human fetal liver will find this method simple, effective, and highly reproducible. Fetal liver EtG is most likely of maternal origin, as EtG readily crosses the placenta and fetal glucuronidation capacity is limited. Fetal liver EtS may be from maternal and fetal origin, as this analyte likely crosses the placenta and fetal sulfotransferase activity is variable and significant. These data impact the forensic science community by providing fetal liver alcohol and, for the first time, tobacco concentrations, to document *in utero* exposure, and potential related exposure 1 toxicities.

Supported by the Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health, and Medical Research Council (UK) grant MR/L01 0011/1. The collection of fetal liver material was approved by the National Health Service Grampian Research Ethics Committees (REC04/S0802/21).

Fetal Liver, Ethyl Glucuronide, Nicotine



K61 The Egg Roll Driving Under the Influence (DUI) Defense: A Unique DUI Defense Incorporating Slow Gastric Emptying Due to the Presence of Egg Rolls, Followed by Rapid Gastric Emptying Due to Vehicle-Collision Impact

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After attending this presentation, attendees will understand a unique DUI defense put forth in a California Supreme Court, and how the criminalist and prosecution team contended with the issues. Attendees will learn more about how the concepts of tolerance and perception of impairment can be used to influence the jury. Attendees will also better understand some unusual legal aspects and challenges at a DUI trial, including: (1) a granted motion to compel the defense to discover to the prosecution; (2) the presence of the criminalist throughout the duration of the trial; (3) an attempt to impeach the criminalist on the stand; and, (4) information about the verdict, sentencing, and “reconsideration” of the sentence by the court.

This presentation will impact the forensic science community by creating an awareness of a unique DUI defense; it will also educate the forensic science community with regard to challenges and lessons in DUI courtroom testimony.

After attending a holiday social gathering on December 21, 2012, where he had been eating and drinking, the defendant, Coulter Mann, was traveling northbound on Highway 101 in far northern California. At approximately 8:54 p.m., and at the moment of receiving a cell phone call, the defendant drifted into the southbound lane of Highway 101, causing a fatal head-on collision with the driver traveling south. Due to the condition of the defendant’s vehicle after the collision, he had to be extricated from the vehicle and transported to a local hospital for emergency treatment. He arrived at the hospital at approximately 10:00 p.m. His legal blood draw occurred at 10:55 p.m. and the results were 0.20 grams/100 milliliters.

The defendant was an assistant principal at a local middle school and was a very well-liked and well-respected member of the community, as was his entire family. The defense team included two attorneys, one of which was the defendant’s father, and a toxicology expert. During trial in January of 2014, the defense’s theory was that the defendant was not impaired at the time of driving, and that the cell phone call was the sole cause of the collision. The defendant had admitted to drinking 35-40 ounces of an 8.8% beer plus two to three 12-ounce bottles of a 6% beer over the course of approximately four hours, except that he had finished two-thirds of his last bottle of beer shortly before he left the gathering. While drinking the beer, he also consumed some appetizer-type food. Of specific interest to the defense was that the defendant had consumed six to seven egg rolls just prior to arriving at his social gathering. According to the defense, the multitude of ingredients in the egg rolls delayed the defendant’s absorption of ethanol to the point that he was not at or above a 0.08 grams/100 milliliters blood alcohol concentration at the time of the collision; therefore, he was not impaired for driving purposes. While administering first aid at the scene of the collision, an ambulance driver did not note that the defendant had signs of impairment nor did they note an odor of alcohol from his breath. The California Highway Patrol officer that responded to the scene did not make note of an odor of alcohol on the defendant’s breath. The emergency room physician did not note any signs of impairment, including no observed horizontal gaze nystagmus, while she treated the defendant. A blood chemistry laboratory panel performed at the hospital approximately one-half hour prior to the legal blood draw indicated the presence of ethanol. When the California Highway Patrol was able to leave the scene of the collision and go to the hospital to obtain a statement from the defendant, he noticed a strong odor of alcohol emanating from the defendant and eventually obtained a legal blood draw for DUI purposes. The only injuries sustained by the defendant were a broken ankle and an approximate 5mm abrasion to his spleen. All other systems, including gastrointestinal, were within normal limits. While the defense did not offer specific absorption or elimination rates, they opined that the impact of the collision, as demonstrated by the laceration to the spleen, forced unabsorbed food and ethanol into the small intestine where the ethanol became rapidly absorbed by the time the legal blood draw occurred at the hospital.

DUI Impairment, Alcohol Pharmacokinetics, Courtroom Testimony



K62 Determination of Gamma-Hydroxybutyric Acid (GHB) in Hair Using Alternative Derivatization Techniques

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After attending this presentation, attendees will better understand a new method of derivatization for improved separation of GHB and urea in hair samples, following methanolic incubation, by Gas Chromatography/Mass Spectrometry (GC/MS). Attendees will also be provided with an overview of the effects of external factors such as diet, drug use, etc., on the levels of endogenous GHB in human hair.

This presentation will impact the forensic science community by providing information about an alternative derivatization technique which allows for better separation of silylated GHB and urea in hair than what is currently achieved by using N,O-Bis(trimethylsilyl) Trifluoroacetamide (BSTFA) as a derivatizing agent.

GHB is an important forensic drug due to its potential for abuse and its implication in drug-facilitated crimes; however, it is also an endogenous compound and is therefore found in low concentrations in human hair. GHB is only detectable in blood and urine samples for up to 12 hours. Therefore, in cases of suspected GHB use, hair can be used as an alternative matrix. When analyzing hair samples for GHB, the most commonly used derivatization reagent is BSTFA with 1% Trimethylchlorosilane (TMCS). One issue that is commonly encountered using BSTFA is the incomplete separation of GHB and urea by GC/MS.

Derivatization with BSTFA is a suitable method when analyzing hair samples by Two-Dimensional Gas Chromatography/Time-of-Flight/Mass Spectrometry (GCxGC/TOF/MS) or by Gas Chromatography/Tandem Mass Spectrometry (GC/MS/MS), which are both able to sufficiently separate GHB and urea, but most forensic laboratories do not have access to this instrumentation. Therefore, an alternative method of derivatization is needed which will allow for better separation by GC/MS. The following derivatization reagents were analyzed for their ability to separate GHB and urea: BSTFA with 1% TMCS, N-methyl-N(tert-butyl)dimethylsilyl trifluoroacetamide (MTBSTFA) with 1% tert-butyl)dimethylchlorosilane (TBDMCS), boron trifluoride (BF₃) in methanol, pentafluorobenzyl bromide (PFBBBr), and pentafluoropropionic acid anhydride (PFPA). MTBSTFA was found to yield enhanced separation compared to BSTFA. The alkylation and acylation reagents, BF₃, PFBBBr, and PFPA, were not found to sufficiently derivatize GHB or urea for GC/MS analysis.

The determined method of derivatizing with MTBSTFA was used to analyze hair samples collected from volunteers in a university community (students, faculty, and staff). Each participant answered questions about their diet, hair treatments, personal life, and drug and alcohol use. For each sample, the provided answers were compared to the detected GHB concentrations in order to determine any effect of the external factors on endogenous GHB concentrations. Concentrations were between 0.2ng/mg and 1.0ng/mg. Some outliers were detected, indicating possible links between diet, lifestyle, hair treatment, and GHB concentration.

GHB, Derivatization, GC/MS



K63 Cannabinoid Disposition in Oral Fluid After Controlled Cannabis Vaporizer Administration

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After attending this presentation, attendees will better understand cannabinoid disposition in Oral Fluid (OF) following cannabis vaporization.

This presentation will impact the forensic science community by providing a framework for interpreting OF cannabinoid concentrations after vaporization.

Background: Cannabis is the most prevalent illicit drug worldwide. OF is an advantageous sampling matrix for drug screening due to ease of observed collection, non-invasiveness, and ability to collect and analyze onsite. Limited data exist for cannabinoid disposition following vaporization, a common alternative to smoking.

Hypothesis: OF THC, CBD, and CBN maximum concentrations will occur immediately post-inhalation and decrease rapidly. CBD and CBN will appear in lower concentrations than THC. When THCCOOH is detected, it will be in low concentrations, with maximum concentration later in the time course.

Methods: Current occasional ($\geq 1x$ /last 3 months, ≤ 3 days/wk) cannabis smokers provided written informed consent and OF specimens for this Institutional Review Board-approved controlled cannabis administration study. Participants inhaled 500mg placebo, low (2.9%)- Δ^9 -tetrahydrocannabinol (THC), or high (6.7%)-THC cannabis in separate sessions in a randomized within-subject design. OF specimens were collected with the Quantisal™ collection device prior to and 0.17, 1.4, 2.3, 3.3, 4.3, 5.3, 6.3, 7.3, and 8.3h post-dose. Specimens were quantified for THC, 11-nor-9-carboxy-THC (THCCOOH), cannabidiol, and cannabinol (limits of quantification 0.5 μ g/L, 15ng/L, 1 μ g/L, and μ g/L, respectively). Maximum concentration (C_{max}), time to C_{max} (t_{max}), and time of last detection (t_{last}) were determined and area under the curve from baseline to 8.3h ($AUC_{0-8.3h}$) calculated by the linear trapezoidal method. Within-subjects medians were compared with the Wilcoxon Matched-Pairs Test.

Results: Median (range) C_{max} , t_{max} , t_{last} , and $AUC_{0-8.3h}$ from 28 participants (19M, 9F, ages 21-40 years) are presented in the table. Significant differences ($p < 0.05$) were detected ^aplacebo vs. low, ^bplacebo vs. high, and ^clow vs. high, as indicated.

		THC	THCCOOH	CBD	CBN
Placebo	C_{max} , μ g/L (ng/L, THCCOOH)	4.7 ^{a,b} (0-25.9)	0 ^{a,b} (0-361)	0 ^{a,b} (0-1.7)	0 ^{a,b} (0-1.9)
	t_{max} , h	0.17 (0.17-1.4)	2.3 (0.17- ≥ 8.3)	0.17 (0.17-0.17)	0.17 (0.17-0.17)
	t_{last} , h	6.3 ^{a,b} (1.4- ≥ 8.3)	≥ 8.3 (5.3- ≥ 8.3)	0.17 (0.17-0.17)	0.17 (0.17-0.17)
	$AUC_{0-8.3h}$, h* μ g/L (h*ng/L, THCCOOH)	6.6 ^{a,b} (0-56.1)	0 ^{a,b} (0-1941)	0 ^{a,b} (0-1.4)	0 ^{a,b} (0-1.39)



Toxicology Section - 2015

Low	C_{max} , $\mu\text{g/L}$ (ng/L, THCCOOH)	848 ^a (16.3-18230)	24.1 ^a (0-686)	6.0 ^{a,c} (0-100)	54.4 ^a (1.2-941)
	t_{max} , h	0.17 (0.17-0.17)	3.3 (0.17- \geq 8.3)	0.17 (0.17-0.17)	0.17 (0.17-0.17)
	t_{last} , h	\geq 8.3 ^a (2.3- \geq 8.3)	\geq 8.3 (0.17- \geq 8.3)	0.17 ^c (0.17-2.3)	2.3 (0.17-7.3)
	AUC _{0-8.3h} , h* $\mu\text{g/L}$ (h*ng/L, THCCOOH)	723 ^a (13.9-3865)	42.9 ^a (0-2935)	3.1 ^{a,c} (0-79.0)	44.1 ^a (0.85-246)
High	C_{max} , $\mu\text{g/L}$ (ng/L, THCCOOH)	862 ^b (25.1-23680)	18.6 ^b (0-464)	34.7 ^{b,c} (1-1106)	32 ^b (0-766)
	t_{max} , h	0.17 (0.17-3.3)	2.8 (0.17-6.3)	0.17 (0.17-3.3)	0.17 (0.17-3.3)
	t_{last} , h	\geq 8.3 ^b (6.3- \geq 8.3)	\geq 8.3 (0.17- \geq 8.3)	2.3 ^c (0.17- \geq 8.3)	2.3 (0.17- \geq 8.3)
	AUC _{0-8.3h} , h* $\mu\text{g/L}$ (h*ng/L, THCCOOH)	934 ^b (38.4-19090)	66.2 ^b (0-2181)	32.0 ^{b,c} (0.72-912)	28.9 ^b (0-617)

Conclusion: OF THC concentrations after vaporization are comparable to previously-published smoking data. THC, THCCOOH, and CBN did not show any statistically significant low vs. high dose differences, suggesting participants were able to titrate dose by adjusting inhalation topography, similar to smoking behavior. When present, OF THCCOOH has low concentrations compared to the other analytes; in some occasional smokers, THCCOOH was not detected in OF even after the high dose. OF THCCOOH (if detected) was suggested to help differentiate active vs. passive cannabis exposure. This presentation will impact the forensic community by providing a framework for interpreting OF cannabinoid concentrations after vaporization.

Research was supported by the NIDA/NIH Intramural Research Program, NHTSA, and ONDCP.

Cannabis, Vaporizer, Oral Fluid



K64 Case Report of a Death Involving the Designer Opioid MT-45 Raises the Spectre of Synthetic Opioids in Forensic Casework

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After attending this presentation, attendees will understand the increasing relevance of synthetic opiates in forensic toxicology and death investigation casework and learn about the use of Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) for the detection and quantification of the synthetic opioid “MT-45.”

This presentation will impact the forensic science community by introducing the first known case of death due to use of the designer synthetic opioid MT-45, as well as by placing the toxicology results in the context of the autopsy findings, and by explaining the detection and quantification methods used.

Opiates always figure prominently among the most frequently encountered drugs in toxic deaths. Interpretation is often complex due to issues including therapeutic misadventure, tolerance, palliative care, drug-drug interactions, and recreational versus medical use. While the most frequently encountered opioids are morphine (as a therapeutic agent or heroin metabolite), oxycodone, hydromorphone, hydrocodone, and fentanyl, recently illicit “designer opioids” have begun to appear in forensic casework. These include deaths attributed to acetyl fentanyl, a synthetic analog of fentanyl which resulted in a series of deaths in Rhode Island, Louisiana, and North Carolina, and a report of the unrelated synthetic opiate agonist AH-7921, a research chemical linked to deaths in Sweden and the United States. Kratom, a Southeast Asian plant containing the drug mitragynine with opioid-like effects which is currently uncontrolled in the United States, has also been seen in deaths in combination with tramadol. Most recently, much media attention has been paid to “Krokodil” (desomorphine), a synthetic derivative of codeine, although no toxicologically confirmed cases have been documented in the United States. Reported is the first documented incident of a death following use of a novel designer opioid, MT-45 (1-cyclohexyl-4-[(1R)-1,2-diphenylethyl]-piperazine. MT-45 is a substituted 4-(1,2-diphenylethyl)piperazine which is chemically unrelated to other known opioid agonists. It has been demonstrated to have approximately 80% of the potency of morphine in animal studies and is currently uncontrolled in the United States.

The case involved a 35-year-old White male, with a known history of substance abuse, who had not been seen for two days prior to being found deceased. He appeared to have collapsed adjacent to his drug preparation area where a scale, spoon, pipe, lighter, and two packets of white powder were found (syringe not present). One packet tested positive for MT-45 and the other tested positive for etizolam, an illicit thienodiazepine with benzodiazepine-like properties. Neither drug is prescribed in the United States; further investigation determined the decedent had purchased these online from a Canadian company and had been doing so on a monthly basis for quite some time. Autopsy revealed cerebral edema, congested lungs, and a possible old injection site on the dorsum of the foot. The postmortem toxicology panel routinely requested came back positive for diphenhydramine 220ng/mL in whole blood (over-the-counter medication present at scene), while the urine was presumptively positive for benzodiazepines and cannabinoids.

The postmortem blood was subsequently analyzed for MT-45 using LC/MS/MS. Sample preparation involved liquid-liquid extraction following addition of ammonium hydroxide and an extraction solvent of n-butyl chloride and acetonitrile (4:1,v/v). The instrument was operated in positive electrospray, Multiple Reaction Monitoring (MRM) mode. The column used for separation was a BEH C18, 2.1 x 5.0mm with mobile phases of 0.1% formic acid in methanol and 0.1% formic acid in water. The internal standard reference was acetyl fentanyl D5. The transitions for acetyl fentanyl D5 were 328.3>105.1 and 328.3>188.1. The transitions used for MT-45 were 349.3>181.1 and 349.3>169.2. The retention times for acetyl fentanyl D5 internal standard and MT-45 were 1.72min and 2.26min. The calibration curve was 1-100ng/mL, with higher concentrations diluted to bring them within the linear range. Targeted analysis of the MT-45 in the case described above was measured at a concentration of 520ng/mL. The cause of death was determined to be acute opioid (MT-45) intoxication. Target analysis for etizolam in the blood also measured a concentration of 35ng/mL. No specificity or matrix effects experiments were performed due to the unique nature of the targeted analysis for uncommon analytes.

Synthetic Opiates, MT-45, Etizolam



K65 The Art of Embalming vs. the Science of Forensic Toxicology

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After attending this presentation, attendees will better understand the challenges unique to interpretation of toxicological findings in postmortem cases. Attendees will be provided with an overview of toxicology aspects of postmortem changes associated with the postmortem artifacts.

This presentation will impact the forensic science community by further delineating the interpretive aspects of toxicological findings in embalmed cases and the need for analysis of the embalming fluid whenever an unexpected analyte is detected to eliminate or confirm postmortem contamination.

Postmortem forensic toxicology is an important integral part of the medicolegal investigation of death. The complexity of postmortem toxicology testing and the nature of specimens submitted for analysis make the interpretation of forensic toxicology results a continuing challenge. In addition to evaluating laboratory methodologies for drug analyses, the condition of the body, drug characteristics, matrix, and site of specimen collection are among the factors that need to be considered in the proper interpretation of an autopsy specimen result. Chemical fixatives such as formalin or embalming fluids cause interference with toxicological analyses. Some funeral homes also add ethylene glycol and/or propylene glycol to the embalming fluid to increase the effectiveness of the arterial embalming process. Consequently, these substances added to commercially available embalming solutions are not included as components on material safety data sheets provided by the manufacturer.

The case involved an 81-year-old man who died at home under hospice care. His death was reported for cremation clearance with cause of death attributed to end stage renal disease due to diabetes mellitus. Following embalming and the wake, the family requested that an autopsy be performed based on their suspicion that the decedent was possibly poisoned with “antifreeze.” Ethylene glycol is used as a major component of antifreeze fluids. An oral dose of 100mL is believed to be fatal to most adults. Propylene glycol, a relatively non-toxic substance, is used extensively as a substitute for ethylene glycol in automotive antifreeze. Its pharmaceutical applications include use as a preservative, emollient, and vehicle for both oral and intravenous medications.

In addition to routine toxicological analysis, specimens were tested for the presence of ethylene glycol and propylene glycol. Analysis was performed using a gas chromatograph with a 5973 mass spectrometer equipped with a RTX-BAC1 column (30m x 0.32mm x 1.8µm film thickness). Acetonitrile was used as the protein precipitation solvent and 1,3-propanediol as the internal standard. The reporting limit was 50mg/L.

Ethylene glycol was not detected in any of the specimens. Results for propylene glycol were as follows: brain, 567mg/kg; liver, 122mg/kg; gastric content, 381mg/kg; vitreous humor, 432mg/L; and, urine, less than 100mg/L. Further investigation revealed the presence of propylene glycol in one of the embalming fluids used by the funeral home.

Specimens from an additional 27 embalmed cases were subsequently analyzed for the presence of ethylene glycol and propylene glycol. Ethylene glycol was detected in six cases, propylene glycol in 12 cases, and both were detected in three cases.

Propylene Glycol, Embalming Fluid, Postmortem Artifacts



K66 Case Study: A Suicide Death by Sotalol Overdose

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After attending this presentation, attendees will understand the dual mechanistic actions of sotalol that make it a preferential treatment option for individuals with symptoms of cardiac arrhythmias, as well as the potentiation of its adverse effects in a fatal overdose.

This presentation impacts the forensic science community by detailing the investigative and toxicological analyses that assisted in resolving the first report of a suicide by sotalol in Harris County, TX.

With the capacity to function as both a beta-adrenoreceptor antagonist and potassium channel inhibitor, sotalol is the preferentially prescribed b-blocker for the treatment of ventricular tachycardia and ventricular fibrillation. As a b-blocker, sotalol is a non-selective competitor for binding sites on the b_1 and b_2 adrenergic receptors that regulate the production of cAMP, and, subsequently, intracellular levels of calcium that are required in generating electrical signals for muscle contraction, as well as generating the force for contraction; however, later studies have also established sotalol as an inhibitor of potassium ion channels, an action that has been demonstrated to cause a concomitant decrease in the efflux of potassium ions. In this manner, sotalol can further delay the generation of electrical signals for contraction and serve a dual therapeutic application for the correction for type III cardiac arrhythmias. Taken together, sotalol can function to decrease intracellular calcium concentrations while also inhibiting the efflux of potassium channels and these dual mechanistic actions can cause prolongation of both the PR and QT intervals that have been implicated in the treatment of rhythm disturbances of the heart and hypertension.

This study describes the case of a 61-year-old woman with a history of depression, who was found deceased in a secured and locked hotel room. A handwritten "last will and testament" were also found on the bed, near the decedent, as well as several prescription medications and loose pills. The decedent had a history of depression and prescription drug abuse, which, in combination with the scene description, indicated a likely suicide with toxicological relevance. Initial findings from the laboratory confirmed only modest amounts of nortriptyline, clonazepam, and tramadol consistent with the prescribed and therapeutic regimen for the decedent; however, these findings were inconsistent with the suicidal ideation described in the case details, thus prompting a re-evaluation of the blood specimen by Liquid Chromatography/Time-of-Flight/Mass Spectrometry (LC/TOF/MS) analysis. One advantage of LC/TOF/MS over other applications of chromatography with mass spectroscopy is the ability to screen, simultaneously, for a spectrum of drugs as comprehensive as the content of the drug library data. A review of the LC/TOF/MS results indicated an unidentified peak consistent with sotalol, suggesting its possible contribution in the cause of death for this case. Additional testing confirmed the presence of sotalol, with reported amounts of 38mg/L in blood and 83mg/L in the stomach contents. For comparison, the therapeutic target range of sotalol is 0.5-3mg/L in blood.

In conclusion, the acute poisoning potential of a combined b-blocker and potassium-channel inhibitor with an emphasis on the toxicological findings and an application for screening by LC/TOF/MS for the implication of sotalol in an intentional overdose death will be discussed. This case highlights a rare fatality since scant information exists on this subject. As sotalol gains widespread use, these events may become more prominent. The lessons learned from this presentation will raise awareness about the methods to identify sotalol, including interpretive support for postmortem toxicology consultations.

Sotalol, LC/TOF/MS, Overdose



K67 Manners of Death in Drug-Related Fatalities in Florida

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The goal of this presentation is to inform attendees about drug distribution and demographic characteristics associated with manner of death in drug-related fatalities in Florida so as to identify potential risk factors by drug or drug class, age, sex, and race with regard to manner of death.

This presentation will impact the forensic science community by helping to understand drug trends in potentially preventable deaths, identifying populations at greater risk of drug-related deaths, and hence, possibly leading to a more informed development of preventive measures of such deaths.

All drug-related deaths reported to the Florida Medical Examiners Commission through toxicology reports from 2001 to 2012 (n=92,596) were included. A death was considered “drug-related” if at least one drug was identified in the decedent, whether the drug contributed to the death or was merely present. For the purpose of this study, no distinction was made for drug-caused and drug-present deaths. Deaths related to the following drugs or drug groups were reported: amphetamines, benzodiazepines, cannabis, carisoprodol, cocaine, ethanol, γ -hydroxybutyric acid, heroin, inhalants, opioids, phencyclidine, and zolpidem. Manner of death was categorized into five groups: accidental, homicide, natural, suicide, and undetermined. Age cohorts included <18 years old, 18-34 years old, 35-54 years old, and ≥ 55 years old. Race was examined by African American, Hispanic, and White; other races were not included owing to <1% occurrence frequency. Relative risk refers to the category-specific probability of a particular manner of death compared to overall drug-related deaths, using Fisher’s Exact Test for significance testing.

Of all drug-related fatalities from 2001-2012, the most prevalent manner of death was accidental, comprising more than half (52.3%), followed by suicide (19.3%), natural (18.5%), and homicide (7.9%); 2.0% were undetermined. Expectedly, accidents contributed most frequently to drug-related deaths in all age groups (36.7% for ≥ 55 years old to 63.5% for 18-34 years old). Homicide was the second most common manner of death among the decedents under 35 years of age ($\geq 15.0\%$), whereas it was the least frequent for those ≥ 35 years of age ($\leq 6.0\%$). The reverse was true in natural deaths: 9.4%, 4.7%, 19.5%, and 32.6% of deaths of <18 years old, 18-34 years old, 35-54 years old, and ≥ 55 years old, respectively. Similar age disproportion was observed in suicides albeit to a lesser extent: 8.0%, 15.0%, 18.8%, and 26.0% deaths of <18 years old, 18-34 years old, 35-54 years old, and ≥ 55 years old, respectively. Males were significantly overrepresented in drug-related deaths on the whole; however, the proportion of mortality occurrence by manner of death was similar between females and males, except for homicide (5.5% of female deaths vs. 8.8% of male deaths). While accidental death was the most common drug-related death in African Americans (41.9%), Whites (53.5%), and Hispanics (62.3%), the race distribution of other deaths differed; the second most frequent manner of death was homicide for African Americans and Hispanics, but it was suicide for Whites. Proportions of natural deaths were more evenly distributed. Relative risk compared to overall drug-related deaths was >1.2 ($P<0.001$) in accidental deaths for 18-34 years of age; in homicides for <18-34 years of age, African Americans and Hispanics; and in natural deaths and suicides for ≥ 55 -years of age. Yearly proportional changes in the manners of death were generally marginal from 2001 to 2012 in the age, sex, and race groups, except a noticeable decrease in accidental deaths of <18-year-olds from 71.0% in 2001 to 44.7% in 2012. Drug distribution within the overall drug-related deaths showed frequent presence of ethanol, benzodiazepines, cannabis, cocaine, and opioids. Over 1.2 relative risk ($P<0.001$) was observed with alprazolam, carisoprodol, cocaine, heroin, methadone, and oxycodone for accidental death; amphetamines, cannabis, and cocaine for homicide; and diazepam and zolpidem for suicide. While the proportion of opioids in accidental deaths declined in 2011-2012, the opioids still remained as the most prevalent drug group and their proportions in other manners of death were not decreased during the study period.

A high relative risk was associated with age under 35 years for accidental death; age under 35 years, male, and African American/Hispanic for homicide; age ≥ 55 years for natural death; and age ≥ 55 years and White for suicide. Cannabis was significantly associated with homicide. Central Nervous System (CNS) stimulants including amphetamines and cocaine showed high relative risks for accidental death and homicide, whereas CNS depressants including benzodiazepines, carisoprodol, opioids, and zolpidem were more strongly associated with accidental death and/or suicide.

Drug-Related Deaths, Manner of Death, Toxicology

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K68 A Survey of Human Performance and Postmortem Cases Involving Ketamine in San Francisco Between 1997 and 2013

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After attending this presentation, participants will better understand the frequency of ketamine detection, as well as the concentration ranges of ketamine and its primary metabolite, norketamine, in Human Performance (HP) and Postmortem (PM) forensic toxicology cases in the City and County of San Francisco.

This presentation will impact the forensic science community by adding to the existing body of scientific knowledge of demographic characteristics of ketamine-positive PM cases, Driving Under the Influence (DUI) cases, and drug-facilitated crime cases and their toxicologic findings (including types of analyzed specimens), and ranges of concentrations typically encountered in such cases.

The Forensic Laboratory Division (FLD) of the Office of the Chief Medical Examiner (OCME) examines evidence from HP and PM cases on behalf of 14 law enforcement agencies operating within the City and County of San Francisco. For ketamine, commercially available Enzyme Linked Immunosorbent Assay (ELISA) kits are employed to screen blood (central/cardiac blood in PM cases; venous blood in HP cases) and/or urine received by the FLD. The ELISA blood and urine cutoffs for ketamine are 20ng/mL and 300ng/mL, respectively. Following a positive ELISA screen, confirmation and/or quantitation is performed in blood (peripheral blood in PM cases; a new aliquot of venous blood in HP cases) and/or a fresh aliquot of urine by Gas Chromatography/Mass Spectrometry (GC/MS) with a limit of quantitation of 0.01mg/L and 0.05mg/L for ketamine and norketamine, respectively. The assay uses tripeleamine as internal standard. The Retention Times, Target (underlined), and Qualifier ions for ketamine, norketamine and tripeleamine are presented in the table below.

Analyte	Rt (min)	Ions Monitored (m/z)
Norketamine	5.00	138, <u>166</u> , 168, 195
Ketamine	9.10	152, <u>180</u> , 182, 209
	9.96	58, <u>91</u> , 185, 197

In order to determine those HP and PM cases which involved ketamine and norketamine in San Francisco over the time period of interest, the in-house database was manually interrogated.

Seven HP cases were identified with confirmed ketamine and norketamine in blood, in which subjects averaged 31.7 years (range: 22-40 years), and were predominantly male (n=7; 71%), with a racial distribution of White (71%) and Asian (29%). The ketamine concentrations in these cases were (in mg/L): mean 0.30, median 0.18, range 0.05-0.65, standard deviation 0.24. The norketamine concentrations in these cases were (in mg/L): mean 0.61, median 0.29, range 0.00-1.52, standard deviation 0.56). In HP cases, ketamine was never encountered alone, but was always in the presence of other psychoactive drugs, most commonly ethanol (n=3), cannabis (n=3), benzodiazepines (n=2), and cocaethylene/benzoylecgonine (n=1).

In addition, 25 PM cases were identified involving ketamine. Twenty-one of these had the drug in the decedent's blood and 12 of the 21 also had ketamine confirmed in urine. Two PM cases only had ketamine confirmed in urine while one had ketamine confirmed in muscle, and one in liver. Decedents averaged 40.1 years (range: 19-64 years), were predominantly male (n=25, 88%), with a racial distribution of White (75%), Black (15%), White Hispanic (5%), and Asian (5%). The Manners of Death were: 17 Accidents, four Natural Deaths, two Undetermined, one Homicide, and one Suicide. The ketamine concentrations in these cases measured in peripheral blood (n=14) were: mean 0.91, median 0.29, range 0.01-3.71, standard deviation 1.24). Norketamine was only found in five case bloods (ranging from 0.05-0.16mg/L) and confirmed in four urines. Ketamine was encountered by itself in less than 10% of all PM cases in which it was detected. The most commonly encountered drugs with ketamine were amphetamines (n=8), cocaine (n=7), morphine/codeine (n=6), ethanol (n=4), methadone (n=3), oxycodone (n=3), GHB (n=3), diphenhydramine (n=3), and diazepam (n=3).

This study provides valuable information on the demographic distribution of ketamine users and decedents in the City and County of San Francisco and offers ketamine and norketamine reference blood concentrations. Comparison of the mean concentrations suggests that decedents with ketamine in their peripheral blood have concentrations three times higher than those measured in living individuals; however, comparison of the median values reduces the difference to approximately 60%, suggesting that ketamine blood concentrations should not be considered in isolation, but should instead be reviewed together with all other information available for Copyright 2015 by the AAFS. Unless stated otherwise, noncommercial *photocopying* of editorial published in this periodical is permitted by AAFS. Permission to reprint, publish, or otherwise reproduce such material in any form other than photocopying must be obtained by AAFS.



Toxicology Section - 2015

a better understanding of the totality of each case. Norketamine in living subjects was detected in concentrations almost twice that of ketamine in the same cases, suggesting a sufficient time interval for metabolism to occur. In postmortem cases, norketamine was rarely detected, suggesting that rapid deaths occurred shortly after exposure allowing little or no time for ketamine to be metabolized. The data presented in this study is useful to forensic toxicologists, pathologists, medical examiners, coroners, attorneys, and other law enforcement agents who need to understand and interpret ketamine and norketamine concentrations measured in HP and PM toxicologic specimens for the purpose of their medicolegal investigations.

Ketamine, Postmortem Toxicology, Human Performance Toxicology



Toxicology Section - 2015

K69 The Detection and Preservation of Arsenite (AsIII) in Pig (*Sus Scrofa*) Skeletal Muscle Tissue Decomposition in Incubated Soil

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WITHDRAWN



K70 A Rare Case of Criminal Poisoning by Means of Butane Gas: N-Butane Quantification in Biological Fluids and Tissues

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After attending this presentation, attendees will understand that the proof of a criminal poisoning with butane gas intoxication depends on the rapidity of collecting blood and tissues samples and butane determination using Headspace/Gas Chromatography-Mass Spectrometry (HS/GC/MS).

This presentation will impact the forensic science community by underlying the necessity of close collaboration between forensic physicians and toxicologists so that samples are analyzed as soon as possible after death. Moreover, forensic physicians will learn that this type of analysis must be carried out in a laboratory with an adequate method of detection and quantification by HS/GC/MS. Finally, this case broadens the literature concerning n-butane concentration in postmortem samples.

This study will present a rare case of criminal poisoning with n-butane as an example of the practical application of this necessary interdisciplinary communication and collaboration. It is recommended that medicolegal death investigators become familiar with the specificity of research of n-butane in postmortem samples.

There are some reports of fatal cases related after accidental or deliberate n-butane inhalation, but criminal poisoning with n-butane remains exceptional. N-butane determinations in biological samples can sometimes be of interest to confirm intentional or accidental fatal intoxications with this volatile aliphatic hydrocarbon.

This study reports the case of a 52-year-old woman found dead in a car next to her husband who was conscious, showed no sign of distress, and alleged an unsuccessful suicide pact. He explained that the couple had chosen to inhale n-butane together in their vehicle parked in the garage as the method of committing suicide. A gas bottle with an 8.5cm pipe was found in the car. Death examination at the scene revealed petechial haemorrhages to the upper eyelids and slight abrasions on the neck. An autopsy was requested. Before this was performed, the forensic physician contacted the toxicologist. Because of the volatile nature of n-butane, the toxicologist explained blood and tissues samples had to be extracted as soon as possible after the body was incised.

The autopsy showed a congestive aspect on the head, peri-ocular petechial hemorrhages, conjunctival hemorrhages, abrasions on the lips, and small semi-circular abrasions with bruises on the neck. The first step of internal examination consisted of cardiac blood sampling. Samples of blood, brain, lung, liver, and heart were removed 20h after the discovery of the body. Internal examination showed pulmonary and cerebral edema, congestion of the organs associated with bruises on the tongue and on the left side of the thyroid cartilage. No important injury was seen in the cervical area. The cause of death was noted as asphyxiation.

A large drug and toxic compound screening was performed on cardiac blood, brain, lungs, liver, and heart and a specific research for volatile substances was performed with HS/GC/MS. After having determined the presence of n-butane, quantitative determination was performed. Briefly, an external calibration (from 0.078 μ g to 3.9 μ g of n-butane) by means of volumetric dilutions from a supplied calibration gas mixture was achieved. A gas-tight sample lock syringe and a specific connecting device enabling direct sampling in the gas cartridge were used. Chromatographic separation was then performed using an RT-Q-Bond Column (30 m x 0.32 mm i. d.) and the detection of n-butane occurred in single ion monitoring mode: m/z 41 for quantitation; m/z 43 and 58 for qualification. In 1mL blood sample, the method is linear from 78 μ g/L Limit of Quantitation (LOQ) to 3,900 μ g/L. Precision was checked by inter-day CVs and associated relative bias (n=5), which were lower than 25%, and 20%, respectively. N-butane concentrations were 610 μ g/L (blood), 50ng/g (brain), 134ng/g (lungs), 285ng/g (liver), and 4,090ng/g (heart).

A rare criminal poisoning case of asphyxia associated with n-butane inhalation has been described in this study. Even if the exact time of death is unknown, due to close cooperation between the forensic physician and the toxicologist, rapid collection of blood and tissue samples during the autopsy and an adapted analytical method, the proof of intoxication as the cause of death was found. Collecting samples in properly sealed containers designated for volatile substances and analyzing the samples for the presence of volatiles solutions as soon as possible after collection is recommended.

N-Butane, Criminal Poisoning, HS/GC/MS

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K71 Asphyxia Due to Inhalation of Hydrogen Gas

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After attending this presentation, attendees will understand a unique case in which suicide by asphyxiation using hydrogen was suspected. The detection of hydrogen in postmortem specimens was performed using Headspace/Gas Chromatography/Thermal Conductivity Detection (HS/GC/TCD).

This presentation will impact the forensic science community by providing a method for the detection of hydrogen in postmortem specimens, allowing a toxicological confirmation for cause of death.

Suicide via asphyxia using inert gases, most commonly helium or nitrogen, has become more common in recent years. Asphyxiation under these conditions results from the inhalation of inert gas instead of oxygen; unconsciousness can occur in as little as one minute. For these kinds of cases, the typical signs of asphyxia include cerebral and pulmonary edema, congestion of internal organs, petechial hemorrhages, and frothy edema in the respiratory tract. These signs are sometimes present, although there are often no significant postmortem abnormalities.

Hydrogen is the most abundant element and is also the lightest element on the periodic table. It exists as a diatomic gas, H₂, at standard temperature and pressure. Hydrogen gas is used in a variety of different areas, including fossil fuel processing and ammonia production.

A 50-year-old man was found dead by his wife in his home office with a large blue recycling bag over his head with tubing coming from the bag and attached to a pressurized tank of hydrogen. The carbon monoxide alarm was going off in the house. He was a mechanical engineer for the National Aeronautics and Space Administration and had access to various gases and chemicals. His wife stated that the decedent often took pressurized tanks home and left them in his office.

Alcohol and drug screens, as well as carboxyhemoglobin analysis, performed on this case were negative. Postmortem specimens were analyzed for the presence of hydrogen using a method that had been developed originally for cases in which helium was used as an asphyxiant to commit suicide. At the time of autopsy, samples of lung, brain, blood, and fat were collected and sealed in 22mL headspace vials by the forensic pathologist. Each specimen was analyzed using HS/GC/TCD, with separation performed at 50oC (isothermal) on an HP-Molesieve column using argon as the carrier gas. This method is capable of separating several gases, including nitrogen, oxygen, hydrogen, and helium. The vials were incubated at 38oC for two minutes, then 100µL of headspace was removed from the vial and injected into the GC.

Hydrogen was found to be present in all samples analyzed. The Limit Of Detection (LOD) for this case was determined using the peak areas present in the negative controls; the average of these was 8.75×10^4 . Each of the samples analyzed had peak areas of an order of magnitude greater than the LOD. Additional assays were performed on cases in which hydrogen was not suspected to rule out presence in postmortem specimens. This method of analysis will not be useful in cases of moderate to advanced decomposition due to the number of gases produced during decomposition.

In conclusion, this analysis provides a method for detection of hydrogen that aids medical examiners in the determination of cause and manner of death. Additionally, these assays are easily conducted, both in specimen acquisition and toxicological analysis.

Hydrogen, Gas Chromatography, Postmortem



K72 Characterizations of AB-PINACA, AB-FUBINACA, and Metabolites Identified in Driving Under the Influence (DUI) and Postmortem Cases by Liquid Chromatography/Time-of-Flight Mass Spectrometry (LC/TOF/MS) and Liquid Chromatography With Tandem Mass Spectrometry (LC/MS/MS)

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After attending this presentation, attendees will better understand the putative structural information of AB-FUBINACA and AB-PINACA metabolites in humans and their identification by mass spectroscopy.

This presentation will impact the forensic science community by raising awareness of a newly emerging synthetic cannabinoid category and the interpretation of toxicological results.

Following the previous wave of JWH Spice/K2 compounds, a new series of carboxamide-based synthetic cannabinoid receptor agonists with an indole or indazole motif have been identified. This new generation of synthetic cannabinoids, including but not limited to, AB-PINACA, ADB-PINACA, ADBICA, 5-fluoro-AB-PINACA, AB-FUBINACA and ADB-FUBINACA, were designated as Schedule I controlled substances in the United States since January 2014.

AB-FUBINACA was first synthesized by Pfizer® as a potent CB1 receptor modulator, with 10-fold greater affinity for the CB1 receptor ($K_i=0.9\text{nM}$) than that of JWH 018, for potential therapeutic use. AB-FUBINACA was found in illegal herbal products along with AB-PINACA in Japan in 2012. To date the biochemical, physiological, and toxicological properties of these synthetic cannabinoids in human have not been determined. The pharmacokinetics properties *in vitro* or *in vivo* via rat model have been reported by other laboratories. Here, synthetic cannabinoids and their metabolites were investigated by the analysis of DUI and postmortem casework samples.

Specimens were prepared by liquid-liquid extraction using 1:1 isopropanol/1-chlorobutane solvent mixture, followed by LC/TOF/MS screen in a water/methanol mobile phase system. The individual drugs were identified by a PCDL library with an identification criteria window of $\pm 15\text{ppm}$ mass error and ± 0.1 minute retention time of target analyte to yield scores greater than 55. The retro-analysis of LC/TOF/MS results revealed the presence of hydroxyl, dihydroxyl, and carboxylic adducts of AB-PINACA and ADB-PINACA, as well as hydroxyl modification to AB-FUBINACA, but without the observance of the carboxylic acid metabolite. The hydroxyl transformation was also found for other indazole-based synthetic cannabinoids.

The confirmation was later carried out by LC Triple Quad by the multiple reaction monitoring method, in which the parent compounds and their selected oxidized forms (i.e., ADB-PINACA-N-4-hydroxypentyl, ADB-PINACA-N-5-hydroxypentyl and ADB-PINACA pentanoic acid) were included. In consideration of ion interference, the isobaric and isomeric compounds were distinguished by HPLC separation. The metabolites were confirmed by the comparisons of retention times, fragment ions, and ion ratios from known standards.

By monitoring synthetic cannabinoids from the end of 2013 through 2014 in Harris County, frequent use of carboxamide-indazole-based cannabinoid receptors agonists was observed versus the fading, near absence of indole-based JWH compounds, except UR-144 and XLR11. Among them, AB-PINACA is the most commonly observed, followed by AB-FUBINACA and ADBICA.

Synthetic Cannabinoids, AB-PINACA, LC/TOF/MS



K73 Determination of Presence and Quantification of Ketamine, Norketamine, and Dehydronorketamine in Dosed and Buried Rat Remains at Different Stages of Decomposition

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After attending this presentation, attendees will understand the potential to detect and quantify ketamine in buried and decomposing remains using a three-step homogenization, Solid Phase Extraction (SPE), and a Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS) method. Attendees will also become aware of the effects of postmortem interval and body parts on detection.

This presentation will impact the forensic science community by demonstrating the effects of burial on the distribution of drugs and enabling the toxicological analysis of organs in buried and decomposing bodies.

To date, several studies have been carried out to identify drugs in decomposed/decomposing remains, yet few studies on buried remains have been published. Studies on buried remains have tested only skeletal tissue in controlled testing environments; no other tissue matrices sampled from buried remains have been tested.

The goal of this study was to determine if ketamine could be detected in soft tissues collected from buried rats, and if so, in what quantity compared to the dosage given. Rats were dosed at three levels: 20mg/kg (n=13); 30mg/kg (n=13); and, 40mg/kg (n=13). Control/untreated animals were also tested (n=4). Injections were made daily for ten days. Rats were then euthanized, two sets (n=21) at an hour after the last injection and one set (n=15) ten days after the last injection. One rat at each dosage (0mg/kg, 20mg/kg, 30mg/kg, and 40mg/kg) was analyzed without burial for comparison. The remaining rats were buried and then exhumed at different stages of decomposition (77, 188, 293, 793, and 3,104 Accumulated Degree Days (ADDs) after burial) to determine the effect of burial length and stage of decomposition on drug distribution and detection. The rats were dissected and samples were taken from the brain, heart, and liver. When those were no longer discernible, the general viscera in the areas of the brain, heart, and liver were sampled. At 77 and 188 ADDs, organs were still intact. By 293 ADDs, some organs had started to liquefy and by 793 ADDs, most organs were liquefied. Brain samples were most affected by decomposition and were not available from some animals in the later stages of the research. Samples were weighed, diluted with saline (1:1 for brain and liver, 1:2 for heart), and homogenized using a Biotage® Bead Ruptor 24. The samples were then centrifuged and the drugs were extracted from the supernatant using SPE. The amount of drug present, ketamine, norketamine, and dehydronorketamine, was quantified in soft tissues using LC/MS/MS with ketamine-D4 as the internal standard.

As expected, ketamine, norketamine, and dehydronorketamine were not detected in the tissue samples from the rats that were euthanized ten days after the last dose. Ketamine and its metabolites were detected in samples from the dosed rats that were not buried and exhibited a dose-response relationship. The drugs were also detected in most of the buried rat tissue samples, with higher concentrations in the higher dosed rats. Where discernable tissue was available, liver concentrations were found to be higher than the heart and brain concentrations.

Ketamine, Buried Remains, LC/MS/MS



K74 Analytical Method Development and Robustness Evaluation for Gas Chromatographic Analysis of Piperazine Designer Drugs

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After attending this presentation, attendees will have a better understanding of an analytical screening method for piperazine designer drugs by Gas Chromatography (GC) coupled to both Mass Spectrometry (MS), Flame Ionization Detector (FID), and Nitrogen Phosphorus Detector (NPD).

This presentation will impact the forensic science community by demonstrating thorough development of a GC analytical method for the detection of piperazine designer drugs. This method would provide crime laboratories with a more robust GC analytical method for screening piperazine-derived drugs and improve overall accuracy of base-type drug analysis.

Synthetic designer drugs are among the most commonly abused drugs on the market.¹ Designer drugs are compounds that are synthesized to simulate the structures and effects of illegal drugs of abuse. To evade the risk of being charged with using drugs, there has been a trend toward consumption of designer drugs instead of the illegal drugs of abuse. Synthetic piperazine-derived drugs are one of the many emerging types of synthetic designer drugs on the market. Piperazine-derived drugs are not only being consumed directly, but also being added to multiple different street drugs. Developing a method to analyze and detect piperazine compounds (i.e., BZP and TFMPP) will aid the screening for emerging synthetic piperazine drugs.

The objective of this research is to optimize the conditions and parameters for a method using simultaneous GC/MS/FID/NPD in order to screen for and detect piperazine-derived compounds. An NPD has higher sensitivity than an FID and a single quadruple MS system, but it is less specific than MS. Due to the amine group of the piperazines, NPD was used to quantitate, providing a lower level of detection than the MS. Additionally, the NPD is likely to exhibit less instrumental drift than the MS, making it a potentially better choice for quantification. In addition, piperazines may exhibit chemical reactivity in the GC sample pathway. A series of commonly used piperazine drugs were evaluated as part of a study to investigate the chemical reactivity. During the course of this research, the impact of the deactivation chemistry of the inlets and columns was evaluated, with results ranging from poor to excellent. Based on chromatographic probes, including tailing factor, the best combination of column and inlet liner was chosen.

The rapidly changing synthetic piperazine market and regulation of these compounds has created a need for developing a more reliable screening technique, which allows crime laboratories to handle various kinds of piperazine-derived compounds. This method increased the accuracy of the analysis by comparing the chemical reactivity in the GC pathway and increased the sensitivity and selectivity by coupling both MS and NPD. This complete investigation of the chromatographic variables as directed to both native piperazines, and their relevant metabolites, in both recreational drug samples and human urine will allow for a consolidated analytical methodology that is more efficient in a commercial laboratory.

Chart 1 Column Comparison Based on Average Tailing Factor Using a Restek-Based Deactivated Gooseneck Inlet Liner

Column	Avg. Tailing Factor
Rtx-5MS	1.728±0.127
Rxi-5SilMS	2.395±0.164
Rtx-5 Amine	2.021±0.062
Rtx-35	1.892±0.106
Rxi-35 SilMS	2.094±0.27
Rtx-1301	2.611±0.13
Rxi-1301Sil	1.949±0.132

Chart 2 Gooseneck Inlet Liner Comparison Based on Average Tailing Factor Using a Rxi-35 SilMS Column

Liner	Avg. Tailing Factor
Base Deactivated	2.09±0.273
Siltek Deactivated	1.99±0.137



Toxicology Section - 2015

IP Deactivated	2.163±0.148
Sky Liner	2.652±0.215
Ultra Inert Liners	2.01±0.083

Reference:

1. Arbo, M.D., Bastos, M.L., Carmo, H.F. Piperazine Compounds as Drugs of Abuse. *Drug Alcohol Depend.* 2012, 122, 174–85.
-

Piperazine, GC/MS/NPD, Inertness



K75 Comparison of Solid Phase Extraction (SPE) and Supported Liquid Extraction (SLE) Columns for the Extraction of 23 Novel Psychoactive Substances From Blood and Urine

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After attending this presentation, attendees will be able to compare SLE and SPE columns and identify the correct choice for optimum recovery of cathinones and NBOMes from a variety of biological matrices.

This presentation will impact the forensic science community by increasing awareness of extraction options for the determination of novel psychoactive substances in forensic toxicological samples.

SLE columns are now commercially available and pose an alternative to SPE. SLE has commercial benefits over SPE in that it does not produce the same amount of solvent waste and can be carried out in fewer steps depending on the type of SPE column being compared.

Recently, there has been an influx of new synthetic substances to the recreational drug market with laboratories struggling to keep pace in this “cat and mouse” game. To improve detection rates, it is vital that laboratories are using the optimum sample preparation technique to allow maximum analyte recovery and sample throughput.

The goal of this research was to determine whether SLE+ columns can be used as a possible alternative sample extraction method for the detection of synthetic cathinones and NBOMes and to evaluate which clean-up method produces the maximum recovery across a range of 25 drugs.

Blank methanol, urine and blood samples (1mL) were spiked with 100µL of 10µg/mL solutions of various different NPS's (methiopropamine, flephedrone, mephedrone, MDPV, 2-DPMP, butylone, ethylone, naphyrone, 5-APB, 6-APB, 3-MeO-PCE, methoxetamine, benzedrone, 25B-NBOMe, 25C-NBOMe, 25D-NBOMe, 25E-NBOMe, 25H-NBOMe, 25I-NBOMe, Mescaline-NBOMe, 25N-NBOMe, 25P-NBOMe, 25T2-NBOMe, 25T4-NBOMe, and 25T7-NBOMe). Urine samples were pH adjusted to 10.8 using 1% ammonium hydroxide (NH₄OH). To each sample prepared for SPE, 1mL of 0.1M phosphate buffer (pH6) was added before centrifugation for ten minutes at 4,000rpm. UCT's ZDSAU020 columns were conditioned using methanol, deionized water, and phosphate buffer before loading samples. Columns were washed using deionized water, 0.1M acetic acid, and methanol. Samples were eluted using methylene chloride; iso-propanol; NH₄OH (78:20:2). For SLE, following pH adjustment with 1% ammonium hydroxide, samples were loaded directly to Biotage's® SLE+ columns. The sample was held on the column for five minutes before being eluted with 2x4mL of ethyl acetate. Internal standards (mephedrone-D₃, methylone-D₃, ethylone-D₃, MDPV-D₈, and 25I-NBOMe-D₃) were added to the collection tubes prior to elution. Post-extraction, samples were evaporated using a stream of nitrogen, derivatized using 50µL of PFPA:ethyl acetate at 70oC for 40 minutes, before being evaporated again and reconstituted in 100µL of ethyl acetate. Samples were analyzed by GC/MS with the SLE and SPE results being compared directly to unextracted methanolic standards at the same concentration.

All drugs were successfully extracted from each matrix using both SPE and SLE columns. For blood, SLE+ columns provided a higher recovery rate of drug than the SPE columns, with an average increase of 10% (recovery ranging from -47% to 80%). SPE-extracted urine samples more efficiently providing an average of 5% increase in recovery rates (recovery ranging from 47% to 92%).

In conclusion, when analyzing blood samples, SLE+ should be used whereas SPE is more efficient for the extraction of these analytes from urine.

SPE, SLE, Novel Psychoactive Substances



K76 Application of Time-of-Flight/Mass Spectrometry (TOF/MS) With Three Different Fragmentation Modes to the Toxicological Screening of Urine Samples Collected From an Electronic Dance Music (EDM) Population

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After attending this presentation, attendees will better understand the pattern of use of Novel Psychoactive Substances (NPS) drugs for a population at risk of abuse of the newest compounds on the illicit market. Attendees will also have a better understanding of current trends describing the compounds that are most prevalent and those emerging on the scene.

This presentation will impact the forensic science community by providing information that can be used for harm reduction, education, and increased certainty of detection following the use of the newest compounds on the market. Manufacturers of standards, law enforcement, emergency medical care professionals, and those involved with user education and drug treatment programs can all utilize this information.

A variety of analytical methodologies including immunoassay (**Enzyme-Linked Immuno-Sorbent Assay (ELISA)**), Gas Chromatography Mass Spectrometry (GC/MS), and Liquid Chromatography/Mass Spectrometry (LC/MS) screening were applied to samples collected from attendees at an electronic dance music festival where there was a high level of self-reported use of NPS drugs. Samples were additionally analyzed using three different modes of Liquid Chromatography/Time-Of-Flight/Mass Spectrometry (LC/TOF/MS).

The range of NPS drugs is constantly changing and many similar compounds, analogs, and isobaric compounds are being sold and distributed; the ability to distinguish between closely related compounds is critical for forensic toxicology testing. Considering High Resolution Accurate Mass Spectrometry (HRAMS) data of both the parent compound and fragment ions provides more specific structural information that can be used for identification of drugs and their metabolites, eliminating some of the false positives caused by artifacts, minor metabolites, degradation products, drug analogs, and isomers in complex forensic specimens. Incorrect presumptive identifications from a screening method may lead to unnecessary confirmatory testing and/or an excess of candidate compounds requiring thorough manual data evaluation for a simple presumptive screening identification.

An Agilent® 1290 HPLC/6530 Q/TOF mass spectrometer with Jet Stream® Technology was used to analyze samples; LC conditions were kept constant while evaluating three different ionization modes. The modes were conventional Quadrupole Time-of-Flight (Q-TOF) and two All-Ions ionization modes: Collision-Induced Dissociation in the Source (CIDS) and Collision-Induced Dissociation in the Collision Cell (CIDCC). The conventional Q-TOF mode uses targeted MS/MS analysis, while the CIDS and CIDCC All-Ions modes provide fragmentation data through the use of alternating fragmentor voltages in the source or collision energies in the collision cell, respectively. CIDS mode also allows for the acquisition of Q-TOF-like data on a conventional TOF mass spectrometer. The elution profiles of each of the ions, parents, and fragments are correlated for use in compound identification.

Samples were analyzed against a database/library containing approximately 140 emerging drugs of abuse or NPS. Each entry was complete with molecular formula, providing the accurate mass up to four decimal places, and the retention time; MS/MS spectra was available for some compounds. Presumptive identifications were made based on retention time, mass accuracy, isotope ratios and spacing, abundance thresholds, the presence of fragment ions, and the comparison of ion ratios to the database reference entry.

Of the samples analyzed, the most common compounds identified and confirmed consisted of methylone, alpha-PVP, MDA, MDMA, amphetamine, and ethylone. O-desmethyltramadol has not been confirmed but was commonly found by all three modes and the additional screening methods. Some of the other compounds that have been confirmed in at least one sample were fluoroamphetamine, buprenorphine, butylone, dextromethorphan, methamphetamine, norketamine, and psilocin. Of the more prevalent compounds, ethylone was the most commonly missed compound by all three methods, with the Q-TOF not identifying it in any of the samples for which



Toxicology Section - 2015

it was confirmed. MDMA was another relatively common false negative missed mostly by the Q-TOF mode; however, there was no Q-TOF reference spectra for MDMA in the database. Amphetamine and alpha-PVP yielded few false negatives. In addition to the false negatives observed, the Q-TOF mode produced the most false positive findings. Generally, the All Ions methods performed better than the Q-TOF method in terms of false positive and false negative findings.

Novel Psychoactive Substances, Time-of-Flight (TOF), Toxicological Screening



K77 Optimization of Extraction Parameters Using IMCSzyme™ β -Glucuronidase for Opiate Analysis of Various Toxicological Matrices

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After attending this presentation, attendees will be able to evaluate the optimal parameters of IMCSzyme™ β -glucuronidase and its use for opiate analysis in whole blood. Attendees will also be able to evaluate the use of those parameters in the analysis of opiates in various toxicological matrices (Antemortem (AM) blood and Postmortem (PM) blood, urine, and liver.)

This presentation will impact the forensic science community by offering analysts information on an enzyme that could shorten the analysis required for total opiate analysis.

Samples of whole porcine blood were spiked with various concentrations, representing the lower and upper ends of the calibration standards used, with morphine- β -glucuronide, morphine-6 β -glucuronide, codeine-6 β -glucuronide, hydromorphone-3 β -glucuronide, and oxycodone-3 β -glucuronide. Samples (1mL) were hydrolyzed at 55°C using a 1:1 mixture of IMCSzyme™ β -glucuronidase:pH 6.8 phosphate buffer. The enzyme volume was optimized by examining the recovery using 100 μ L or 200 μ L IMCSzyme™ β -glucuronidase (52,000 U stock). The hydrolysis time was also optimized by testing 30-minute or 1-hour hydrolysis time with the different enzyme amounts. After hydrolysis was complete, deuterated internal standard (100 μ L) was added to each sample and standard. Samples were then sonicated, centrifuged, and the supernatant was derivitized using 100 μ L of 5% methoxylamine and cleaned up via solid phase extraction. Samples were then dried, derivitized again using 100 μ L of a 1:1 mixture of propionic anhydride and pyridine, then analyzed via gas chromatography/mass spectrometer with selective ion monitoring.

Once optimal parameters were established, those parameters were used to analyze the IMCSzyme™ β -glucuronidase efficiency in various toxicological matrices commonly tested for opiates. All matrices were tested to obtain results for the free opiate content and the total opiate content using IMCSzyme™ β -glucuronidase and the currently used *H. pomatia* β -glucuronidase.

It was determined that 100 μ L of IMCSzyme™ β -glucuronidase with 30-minute hydrolysis time produced sufficient hydrolysis in the spiked porcine samples. The enzyme produced comparable or better hydrolysis efficiency with all five glucuronide forms studied than the currently used *H. pomatia* β -glucuronidase in the initial optimization experiments done on porcine blood. Based on the matrix studies, the IMCSzyme™ β -glucuronidase returned comparable or better hydrolysis efficiency in AM blood, PM blood, and urine than the *H. pomatia* β -glucuronidase; however, IMCSzyme™ β -glucuronidase appeared to underperform in liver samples, with codeine-6 β -glucuronide proving to be the most difficult glucuronide to hydrolyze in all matrices.

Based on manufacturer recommendations, a 2:3 ratio of IMCSzyme™ β -glucuronidase: pH 7.4 phosphate buffer, with 30-minute hydrolysis time at 55°C was used for hydrolysis in urine to increase overall extraction efficiency, especially for codeine-6 β -glucuronide.

Future work will be conducted to attempt to increase hydrolysis efficiency in liver samples. Due to the consistency of the sample matrix, longer hydrolysis times may be tested to allow the enzyme more time to effectively hydrolyze the opiates. Other tests will consist of sonicating the samples prior to hydrolysis or agitating the samples during the hydrolysis step.

Toxicology, Opiate, Glucuronide



Toxicology Section - 2015

K78 Postmortem Pediatric Toxicology

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After attending this presentation, attendees will gain an appreciation for the challenges unique to toxicological findings in postmortem pediatric cases. Attendees will learn interpretive guidelines for pediatric cases involving forensic toxicology in both a general and a case-specific sense.

This presentation will impact the forensic science community by further delineating the interpretive aspects of toxicological findings in the pediatric population.

In this 15th Annual Special Session within the Toxicology section, pediatric cases involving toxicological findings are discussed. As a relative dearth of interpretive information involving toxicological findings in the pediatric population exists, this session is a forum to help elucidate and clarify such issues. The format is a short case presentation including pharmacokinetic data and other relevant ancillary information followed by audience participation to provide interpretive clarity around the case-specific impact of the toxicological findings. This session, attended by various sections of the Academy, allows for various perspectives of case issues that lead to integrative consensus, or differing opinions, as to cause of death in children.

Four cases will be presented that highlight the difficulty in assessing the role of toxicants in each case.

James Gill will be reviewing a case from his vast experience as a forensic pathologist. Dr. Gill has a particular interest in toxicological issues and his presentation will highlight the pathologist's view on toxicological findings and mitigating factors in the interpretation of findings in this population.

Erik Christensen will discuss a case of an 8-month-old female found unresponsive face down in her bed in a blanket. The scene was indicative of potential toxicological involvement, yet the substance ultimately detected in the child, trazodone, was not one of the substances obvious at the scene. Trazodone, an atypically-structured antidepressant is not a drug typically prescribed for an 8-month-old child. The potential role of trazodone in the death of this child will be discussed in respect to a final diagnosis.

Marina Stajic will discuss a high-profile case involving the brutal murder of an 8-year-old boy who was dismembered. The toxicological findings in the case will be reviewed with the potential role of detected agents in the cause of death of the child versus being "red-herring" findings.

Ellen Moffat will present a case of the sudden death of a child and a finding of isopropyl alcohol in postmortem samples. The genesis of the isopropyl alcohol in this child and the potential role of the substance in the fatal outcome will be described. Additionally, the potential sources of postmortem isopropyl alcohol findings in the absence of known ingestion will be described.

The case studies presented reflect current-day findings in medicolegal investigations of childhood deaths. In years past, discussions of these type cases have been educational and demonstrative of the issues in this special population. Only through these continued case studies and audience participation can there be shared perspectives on the meaning of the toxicological findings.

Pediatric, Postmortem, Toxicology A Title Sample



LW1 Asperger's Disorder: Does It Make Someone Violent?

Katherine Ramsland, PhD, DeSales University, 2755 Station Avenue, Center Valley, PA 18034*

After attending this presentation, attendees will know what the current research reveals about a potential link between Asperger's disorder and explosive violence.

This presentation will impact the forensic science community by illustrating the importance of knowing research results before making legal decisions that accept or reject causal links between Asperger's disorder and violent acts.

Elliott Rodgers went on a "day of retribution" rampage in May 2014 in Santa Barbara, CA. He killed six people and injured thirteen before shooting himself. He wanted to punish "you girls" for rejecting him. The media focused on his prior diagnosis of Asperger's disorder.

Adam Lanza killed his mother on December 14, 2012, before going to Sandy Hook Elementary School in Newtown, CT, to gun down six staff members and twenty students. He then shot himself, leaving no explanation. His computer was destroyed, but inside his room were memoirs about Asperger's disorder. There were also reports that Lanza had once received this diagnosis. Assumptions were quickly made in the national media that Lanza's aggression was Asperger's-related violence.

Knowledge about Asperger's disorder (part of Autism Spectrum Disorder (ASD)) has developed significantly since it was named in 1944, but confusion persists, especially regarding its relationship to extreme antisocial behavior. When violent incidents occur that superficially appear to have a link with ASD, there is a risk of making quick assumptions that unfairly stigmatize those who present with this disorder.¹ Yet if research designs have failed to identify a link that actually exists, then threat assessment personnel and attorneys who represent clients with this disorder need this information. However, the research thus far includes small or unrepresentative samples, a lack of targeted research on those with the Asperger's variant of ASD, a lack of specificity in the type of violence studied (as opposed to general aggression), and a lack of community sampling.²

There exist few cases of stranger-based destructive violence, and for those in which ASD might be a factor, other factors are also evident that may play an equal or more significant role.³ Comorbid with ASD, Adam Lanza had severe Obsessive-Compulsive Disorder (OCD), hypersensitivity to touch, a fixation on mass murder, and bouts of depression and paranoia. In most of the ASD cases in which violence has occurred, they generally had a reactive component rather than being planned and prepared.⁴ However, the high-profile violence committed by individuals like Rodgers and Lanza has generated notions that Asperger's is a variant of psychopathy. Some reports imply that, like psychopaths, people with ASD have no empathy and thus no emotional incentive to be prosocial. Hence, they're prone to becoming violent. Such statements rely on erroneous perceptions of ASD.

With the increased media attention on ASD during violent incidents, assumptions are made about its role in violence that can adversely influence treatment of such individuals in the criminal justice system.

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Asperger's, Violence, Mental Illness



LW2 The Gunshot Residue Evidence of *The People of the State of California v. Robert Blake, the Actor*

Bryan R. Burnett, MS*, Meixa Tech, PO Box 844, Cardiff, CA 92007-0844

After attending this presentation, attendees will be aware of the details of the Gunshot Residue (GSR) evidence in the trial of *The People of the State of California v. Robert Blake*.

This presentation will impact the forensic science community by raising awareness of how GSR evidence can be misused in a case.

The shooting of Ms. Bonny Bakley occurred on May 4, 2001, at approximately 9:30 p.m. while she was sitting in the front passenger side of Mr. Blake's 1991 Dodge® Stealth®. Ms. Bakley was waiting for her husband, Robert Blake, to return from the restaurant where they had just dined. Mr. Blake had allegedly returned to the restaurant to retrieve his forgotten .38 caliber revolver. There were no witnesses to the shooting.

The GSR expert in the case of *The People of the State of California v. Robert Blake* was retained by attorney Thomas Mesereau several weeks prior to his withdrawing from the case and his retention by Michael Jackson. Attorney Gerald Schwartzbach was hired by Mr. Blake and the GSR expert's role was as a consultant throughout the case. Schwartzbach had already found and retained a GSR expert by the time he discovered the previous GSR expert's involvement. He did, however, send the discovery to the previous GSR expert. The previous GSR expert's report was submitted to the defense prior to trial, but was ignored.

A criminalist of the Los Angeles County Department of the Coroner performed the majority of the GSR analyses for the prosecution. Even though the jury likely came to an appropriate decision, this case should never have gone to trial with its flawed evidence.

The issues were: (1) the submitted spectra of the alleged consistent GSR from Mr. Blake's hands did not match the testified composition of the particles. It appears Blake's GSR data was mixed with another case; (2) Blake was carrying a .38 caliber revolver the night of the shooting; (3) upon the shooting of Bakley in the passenger seat of the car, Blake sat in the driver's side seat allegedly not realizing she had been shot. He reported touching her; (4) Blake was taken to the police station without hand protection and his hands were sampled for GSR at the police station; (5) the clothing Blake was apparently wearing the night of the shooting was collected by a police officer the following morning after Blake was returned to his home via a police car; and, (6) Blake's clothing was stored in an open box in the trunk of a police car for 48 hours. The clothing was handled by a police officer without hand protection.

Blake's Hand Samples: For issue 1, even though the defense was informed of the mismatch prior to trial, the GSR expert was not impeached in cross nor was this information presented to the jury when the defense got the case. For issues 2, 3, and 4, even if particles were identified as characteristic of GSR on these samplers, it would be meaningless. Contamination from any of these sources cannot be dismissed.

Blake's Clothing Analyses: For issues 3, 4, 5, and 6, even if particles were identified as characteristic of GSR on these samplers, it would be meaningless. Contamination from any of these sources cannot be dismissed.

An Issue of the Chain of Evidence: For issue 5, the chain of evidence was broken. There was no way of verifying the clothing picked up at Blake's home by the police officer was the same as that worn by Blake when the shooting occurred.

The Defense: The defense GSR expert erred by not considering any of the above issues. In addition, the pistol used in this shooting was twice fired with ammunition different from that used in the shooting. The shooter's hands were immediately sampled and a total analysis of the two samplers by scanning electron microscopy was performed. The defense expert's invalid assumptions will be discussed. This work performed by the defense should never have been allowed to be presented to the trier of fact.

Robert Blake, Gunshot Residue, Junk Science



LW3 The Exhumation and Identification of the Remains of St. Marianne Cope of Molokai

Vincent J. Sava, MA, JPAC-CIL, 310 Worcester Avenue, Bldg 45, Joint Base Pearl Harbor-Hickam, HI 96853*

After attending this presentation, attendees will appreciate St. Marianne Cope of Molokai as a historical figure and the role forensic science played in her canonization as a saint in the Catholic Church.

This presentation will impact the forensic science community by showing applications of forensic anthropology to a case involving the remains of a historical figure.

Health and disease influence the histories and cultures of any region. The legacy of leprosy in Hawaii is one of horrific and heartbreaking suffering, unimaginable hardships as well as inspirational and heroic sacrifice, all leading to the sainthood of two individuals. One of these saints, Marianne Cope, is the subject of this presentation.

A hundred years after Western contact, introduced diseases had scythed the Native Hawaiian population from 300,000 to 50,000 people. Leprosy afflicted up to 5% of the Native Hawaiians by the 1870s. To halt its spread, in 1866 Hawaiian authorities exiled several hundred patients to a “colony” on the isolated Kalaupapa peninsula on the island of Molokai. The peninsula is a geographic prison. Ravaged patients were unable to climb the 2,000-foot cliffs comprising the colony’s southern border or swim the rough shark-infested seas ringing its other three sides.

The early days of the colony were grim. Law and order were absent; there was inadequate shelter, little food, filthy water, scant medical care, and no hope. Half of the 800 patients arriving between 1866 and 1873 died within two years. Father Damien DeVeuster, a Belgian priest, arrived in 1873 and worked tirelessly to establish a quality of life for his patients.

A contemporary of Father Damien was Mother Marianne Cope, a Franciscan nun. Mother Marianne was already an experienced and respected health-care provider in New York when she responded to Hawaii’s pleas for assistance. She was ahead of her time, recognized for initiating the first patients’ bill of rights, as the founder of the hospice movement in the United States, and as an early adherent to Lister’s germ theory. Arriving in Honolulu in 1883, she worked tirelessly to improve the care of leprosy patients throughout Hawaii, founding hospitals and homes for children orphaned by leprosy.

Mother Marianne arrived at Kalaupapa in 1888 to care for the patients, including Father Damien who was dying of leprosy. Upon his death in April 1889, she assumed full responsibility for the care and welfare of the Kalaupapa patients. By this time, conditions had improved to the point where providing adequate and state-of-the-art health care for the patients was the predominate issue facing the colony. Originally planning on staying only six months in Hawaii, Mother Marianne remained in the service of her patients until her death from natural causes in 1918.

Mother Marianne was buried on Kalaupapa. As a candidate for sainthood, Mother Marianne’s resting place had to be more accessible to the faithful. Consequently, a decision was made to move her remains from her remote grave to a shrine in her hometown of Syracuse, NY. Additionally, her sainthood cause requested that her remains be identified using forensic techniques. Planning and preparation took nine months, including researching that the grave in question was undoubtedly Mother Marianne’s.

Using forensic recovery methods, Mother Marianne was exhumed from her grave in late January 2005. She was taken to a makeshift laboratory at a nearby convent and circumstantially identified. The lines of evidence used toward the identification were: (1) verification of the grave through historical research; (2) the archaeological findings relevant to the grave and the remains; (3) the biological profile of the remains; and, (4) the material evidence or artifacts recovered from the grave.

Mother Marianne was returned to Syracuse soon after her exhumation. She was canonized a saint by Pope Benedict on October 21, 2012. In July 2014, Mother Marianne returned to Hawaii. Her remains are now enshrined in Our Lady of Peace Cathedral in Honolulu. Leprosy continues to be a problem in Hawaii. On average, 200 new cases are diagnosed in the Hawaiian islands each year. Even though segregation laws were abolished in 1969, about a dozen leprosy patients still reside on Kalaupapa. All are over the age of 65 years old.

Leprosy, Human Remains, Religious Relics



LW4 Romance and Reality: Horses and Experimentation

Jennie Meade, JD, MLS, 716 20th Street, NW, Washington, DC 20052*

After attending this presentation, attendees will understand: (1) how horses are used for experimentation; (2) that the categories and goals of experimentation upon horses vary widely; (3) the effects of different types of experiments on the subject horse's welfare; (4) the necessity for performing certain types of experiments on horses; and, (5) to what extent United States law protects subject horses during experimentation.

This presentation will impact the forensic science community by focusing attention on the little-known uses of horses in experimentation. By describing the types of experiments horses are subjected to, the effects on the subject horses, and legal oversight of the process, this presentation will serve to heighten awareness of the issues associated with the important and growing equine segment of animal experimentation.

The romance and lore of the horse persist in obscuring hard realities associated with the lives of certain horses, both historically and in modern times. Finding that horses are used for medical and other experimentation, along with rats and monkeys, comes as a surprise to those whose comprehension of equines ends with the horse as noble helpmate and sporting partner. Horse experimentation may be on the rise; recent European Union statistics reveal an increase in the use of equines in animal experimentation, while at the same time logging a decrease both in the numbers of animal experiments and in the overall numbers of animals used in experiments.

In the 19th century, horses routinely were used in experiments involving vivisection, and this practice continues today, alongside less invasive experimentation. Horses are used in biological studies, research and development of human medicines, testing of veterinary vaccines, disease diagnosis, toxicology, nutritional studies, and behavioral studies. Certain experimentation is driven by potential human medical benefit and other research is directed toward improving knowledge of the horse. Some horse experimentation has been identified as not only unnecessary but painful for the equine experiment subjects. Certain other experiments are not only painless but may be enjoyable for the horse. "Horse experimentation" covers a spectrum of activities, goals, and results.

Legal regulation of animal experimentation has a long history, beginning in England in the 19th century. True to its tradition of animal welfare activism, today the United Kingdom remains at the legislative vanguard of protecting animal experimentation subjects. United States law, on the other hand, is developing and to date remains less comprehensive than its United Kingdom counterpart, with many lacunae. In areas where United States legal coverage is adequate, inadequate enforcement can render the laws ineffective.

Horses, Experimentation, Law



LW5 Once Upon . . . Forensic Sciences

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After attending this presentation, attendees will understand how it is possible to communicate and teach forensic sciences to younger generations of students without favoring television trends that highlight morbid and macabre aspects but rather by emphasizing the role of the science and forensic scientists.

This presentation will impact the forensic science community by suggesting a new method of teaching forensic sciences based on a clear communication, not the simplistic language of fairy tales.

The CSI effect is a phenomenon according to which the expectations of jurors and the general public for forensic evidence are affected by the popularization of forensic sciences in the so called “reality-based” crime-fiction television drama. On the other side, the proliferation of television programs and movies that involve the role of charming forensic scientists can also create a new and different CSI effect, which would be the extreme interest of young children and teenagers in forensic sciences. This in turn would lead to an increased demand for undergraduate and graduate programs in this area of study. Unfortunately, the fiction cannot present the real contribution that each discipline can give to the investigations, creating wrong expectations in young students who are planning their education. In addition, the tendency of these television dramas is to emphasize the gory aspects of murder investigation and present the bloody scene with amusement.

Against the trend of TV series, this study proposes the use of Disney’s® fairy tales as teaching aids to focus the attention on the role of forensic scientists. The very nature of these fairy tales is completely non-macabre; therefore, it is suitable for young students. In addition, they are appropriate for an adult public which has grown up with these stories. For these reasons, this presentation’s purpose is to shift the emphasis from cruel and meaningless scenes to technical aspects of the investigation presented in an easy-to-understand language.

Starting from the first Disney® animated feature film, *Snow White and the Seven Dwarfs*® (1937), it is possible to show how the Evil Queen could be able to detect that the huntsman returned with a pig heart instead of Snow White’s by conducting a histological test. This very popular film can also teach the meticulous method and caution required in crime scene investigation: Snow White, in fact, misinterpreted the identity of the owners of the Dwarfs’ untidy cottage when she first inspected it.

At the same time, the search for the young princess by Maleficent, in *Sleeping Beauty*® (1959), is a good example of how forensic art could be applied in the aging process of missing children. In addition, in this tale the poison on the spike of the spinning wheel is useful to explain the potential of toxicological analysis.

Other aspects of forensic sciences and investigation can be presented by using other Disney® films, such as *Bianca and Bernie*® (1977), for geographic profiling and the search for kidnapped children. *Lady and the Tramp’s*® (1955) characters provide an insight into the topic of tracking dogs. Still related to the canine world, *One Hundred and One Dalmatians*® (1961) serves as an example of wildlife forensics.

In addition, *Beauty and the Beast*® (1991) offers support to present two important concepts in forensic science: (1) the “fruit of the poisonous tree”; and, (2) the goal of justice. In this animated musical film, the arrogant hunter, Gaston, steals a spellbound mirror from Belle and uses its magic power to spy on the Beast, misconstruing his attitude and using the images to make him appear evil. This movie also serves as a good teaching support to explain the importance of a correct collection and interpretation of the evidence, according to the most recent guidelines of the scientific community. At the end of the film, Gaston tries to incite the mob against the Beast who he wants to have killed. The happy ending demonstrates the fallacies of self-made justice and asserts the actual, primary goals of forensic science and the judiciary system, which are to provide impartial justice based on objective evidence.

This study will describe how it is possible to teach forensic sciences in a fascinating and attractive way by going against the grain of fashion dictated by macabre and morbid television series, converting Disney’s® motto, “Where Dreams Come True™” to “AAFS, where forensics come true.”

Forensic Science, Teaching, Education



LW6 Death in Police Custody: Negligence or Body Packing and Self Administration?

David M. Benjamin, PhD*, 77 Florence Street, Ste 107N, Chestnut Hill, MA 02467-1918

The goals of this presentation are to: (1) review the metabolism of heroin; (2) recognize how the route of the administration of heroin leads to differences in the formation of 6-monoacetylmorphine (6-MAM); and, (3) examine how the onset of the signs of heroin overdose can lead to determining the approximate time at which heroin was used.

This presentation will impact the forensic science community by explaining how the onset of a toxic drug effect may provide information about the time of use of the toxic agent.

Police officers arrested Mr. X for possession of illicit drugs with intent to distribute. At the time of the arrest, police officers alleged that they saw Mr. X swallow several marble-sized bags of contraband containing an unidentified white powder. Police officers believed this powder to be cocaine but no field tests or laboratory tests were ever performed on the samples in question.

Mr. X was taken into custody and assigned to a single cell which was equipped with video monitoring. In addition, police officers were supposed to come by Mr. X's cell and monitor him for safety every 15 minutes. A review of the monitoring checklist indicated that monitoring visits were not conducted according to the 15-minute schedule.

On the day of Mr. X's death, Mr. X appeared in good health between 8:00 a.m.-9:00 a.m., when breakfast was served. However, when police officers came by to serve lunch between 11:00 a.m. and noon, Mr. X was found to be unresponsive with a great deal of blood-tinged, frothy, white fluid emanating from his mouth and nose.

His family filed a wrongful death civil suit against the police department and the city that employed the police officers, alleging negligence and failure to adequately monitor Mr. X's condition while in their care. A forensic expert on the toxicity of heroin was retained on behalf of the police department and the municipality in question.

The metabolism of heroin involves several steps and differs depending upon the route of administration. Following Intranasal (IN), Intramuscular (IM), or Intravenous (IV) administration of heroin, heroin is almost immediately hydrolyzed to 6-MAM in the blood and then travels to the liver where the second acetyl group is removed, liberating morphine. 6-MAM and morphine are the active ingredients which actually manifest the psychopharmacological effects of heroin in the brain. In contrast to IN, IM, or IV administration, orally administered heroin is rapidly converted to morphine in the stomach and virtually no 6-MAM is formed or found in blood or urine.

Findings at Autopsy: At autopsy, three small bags (less than one inch in diameter) of white powder were removed from the decedent's stomach. The contents of the bags were never analyzed, but the presence of Benzoylcegonine (BE) and 6-MAM in the decedent's urine indicated that both cocaine and heroin were present to some extent. The codeine is an impurity extracted from the opium poppy during the processing of the morphine into heroin.

Major laboratory toxicology findings (specimen: postmortem fluoridated femoral blood):

- Free codeine: 56 ng/ml; Free morphine: 987 ng/ml.

In urine:

- Cocaine metabolite, BE: 666 ng/ml.
- 6-MAM: present — less than 40 ng/ml.
- Free morphine: greater than 2,000 ng/ml; free codeine: greater than 2,000 ng/ml.

Plaintiff's Theory of the Case: Plaintiffs alleged that the decedent died as a result of swallowing several bags of heroin and that the heroin slowly leached out of the bags over several days, accumulating to a toxic level, ultimately causing the decedent to experience respiratory depression and pulmonary edema, which led to his death.

Defendant's Theory of the Case: Mr. X appeared in good health during the first few days of his incarceration and never showed any signs of drug ingestion until he was found in pulmonary edema around noontime on the day of his death. Following a heroin overdose, pulmonary edema occurs either immediately or within four hours of the overdose. Since Mr. X appeared healthy at breakfast time (8:00 a.m.-9:00 a.m.), the heroin must have been ingested between 8:00 a.m. and noon. Because 6-MAM was detected in urine at autopsy, the heroin must have been ingested by a route other than absorption from the stomach, implying that the heroin was probably "body packed" into the facility and self-administered by "snorting."

Heroin, 6-Monoacetyl Morphine, Pulmonary Edema

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LW7 **The Investigation Into the Identification and Death of Ukrainian Journalist Georgiy Gongadze: An International Case of Revolution, High Politics, and Toxic Consequences**

William C. Rodriguez III, PhD, OCME, 900 W Baltimore Street, Baltimore, MD 21201*

After attending this presentation, attendees will gain a heightened perspective and awareness of the political and social ramifications involved in conducting international forensic investigations in which the case outcome can result in serious consequences to others as well as a danger to oneself.

This presentation will impact the forensic science community by serving as a strong reminder of the importance of forensic science to society and that it should be utilized in a truthful, careful, and unbiased manner to ensure that justice is served.

This case involves the murder of Ukrainian journalist Georgiy Gongadze, who was kidnaped and murdered in the Taraschanskyi district outside of Kiev, Ukraine, in September of 2000. Gongadze was a well-known Ukrainian journalist and co-founder of the news website *Ukryinska Pravda*. Much of Gongadze's journalistic work involved exposing the massive corruption within the political establishment of the Ukraine, with his primary target of contention being Ukraine President, Lenoid Kuchma. It was reported that during the presidential election in 1999, Kuchma's headquarters made contact with Gongadze, informing him that he had been blacklisted and was to be dealt with after the election. In early 2000, while visiting New York with other Ukrainian journalists, Gongadze warned of "the strangulation of the freedom of speech and information in our state."

Two months later, after Gongadze's disappearance, his badly decomposed and decapitated body was discovered in a forest approximately 43 miles outside of Kiev. Initial examination of the remains revealed they had been doused in dioxin as well as gasoline, which failed to burn properly. In a bizarre turn of events, the headless corpse was confiscated by police and hidden away in a remote barn. The remains later resurfaced in a morgue in Kiev as the result of investigations by other journalists. Several months passed without any official word concerning the remains.

In early 2001, the United States government became concerned regarding the outcry by international journalists and government opposition leaders concerning the Gongadze case and the increasingly authoritarian government of President Kuchma. As a result of political and public pressures against the Kuchma regime, an official request was made to the United States by the Ukrainian government for forensic assistance in the case. The United States responded by sending a small forensic team from the Federal Bureau of Investigation (FBI) and the Armed Forces Institute of Pathology to the Ukraine in March of 2001. During this visit, the United States team was plagued by many obstacles that appear to have been purposely initiated in order to prevent the examination of the remains. As the result of a stalemate, the United States team left the Ukraine but returned a month later to conduct the investigation as originally planned. During this same period, a close bodyguard of President Kuchma requested political asylum in the United States after making public previous secret recordings of the President and top cabinet members giving orders to have Gongadze dispatched.

As a result of the second visitation, the remains in the Kiev morgue were positively identified as those of Georgiy Gongadze via radiographic and DNA comparisons. Even though the second attempt to examine the remains of Gongadze was successful, it was not without incident. At the completion of the examination, and just prior to the joint writing of the report findings, a team member became seriously ill, requiring medical attention, resulting in extreme concern by the United States Embassy staff. A rapid return to the United States by this team member, followed by a medical examination, including toxicology, revealed the presence of high levels of mercury. The high mercury levels which are presumed to have resulted in the illness was considered to be the result of poisoning that occurred via ingestion of water from a bottle that had been tampered with. Other cases of poisoning within the former Soviet Union of individuals who had posed a political threat occurred in 2004 with the poisoning of the pro-western Ukrainian President Viktor Yushchenko by dioxin and in 2006 of the former Russian Komitet Gosudarstvennoy Bezopasnosti (KGB) Agent Alexander Litvinenko, who defected to England and died as the result of lethal polonium 210 poisoning. The obstacles encountered will be presented in order to prepare others as to the political, social, and personal hazards that can be encountered in similar investigations.

Assassination, Human Identification, Poisonings

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Financial Disclosure - 2015

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Key Word Index- 2015

	1
16S RRNA-H104	
	2
.22 Fired Casings-E59	
25I-NBOMe-K5	
2C-B190	
2nd Circuit Ruling-C13	
	3
3D Animation-D58	
3D Body Scanning-H82	
3D Comparison-A12	
3D CT Scan-A2	
3D Data Collection-D59	
3D Database-C6	
3D Reconstruction-H68	
3D Superimposition-C10	
3D Surface Data-C6	
3D Surface Topography-B118	
	4
4-Methoxy-A-PVP-K54	
	6
6-Monoacetyl Morphine-LW6	
	A
AAFS Abstracts-G51	
AAS-B83	
ABC-B46	
AB-FUBINACA-K56	
AB-PINACA-K72	
Abuse-G57	
Abusive Head Injury-F33	
Abusive Head Trauma-H47	
Accident Evidence-D52	
Accident Reconstruction-D5, D6, D8, D48	
Accidental-H153	
Accidental Death-E37	
Accidental Injuries-H146	
Accidents-H124	

Accumulated Degree Hours-H99
Aceclofenac-B51
Aceclofenac Identification-B51
Acetyl Fentanyl-K25, K26, K27
Acids-A15
Acute Cocaine Cardiotoxicity-H77
ADD-H98
Adipocere-A22
Admissibility-F19, F46, J23
Adolescents-I32
Adrenal Gland-H13
Adrenaline Addiction-F52
Adult-A67
Adult Pelvis-A4
Adulterants-K23
Adults-H6
Adversarialism-F51
Adverse Effects-K53
Aerosol-B189
Affymetrix® Chip-B3
Afghanistan-A47, BS4
AFIS-B111
AFMES-W1
Age-A86, E47, G44
Age Assessment-G43
Age-at-Attainment (AaA)-G45, G47
Age-at-Death-A5, A53, A61, A87
Age Dating-D34, D36
Age Determination-A79, A80
Age Estimation-A4, A24, A29, A77, A82, A85, A88, A89, A115, A131, B6, C5, E3, G3, G46, G47
Aging-H101
Aging Population-I14
Agreement-G14
Alcohol Pharmacokinetics-K61
Alcoholic-H151
Alcoholic Cardiomyopathy-H2
Alcoholism-H2
Aldicarb-H80
Alibi-H75



Key Word Index- 2015

Allele Frequencies-B86
Allergen-Specific GE-H7
All-Terrain Vehicle-H125
Alphabets-J15
Alpha-PVP-K55
Alq3-B81
Alterations-J8
Altered Crime Scene-H64
Aluminum Powder-B158
Amalgam Tattooing-G1
Amputation-J26
Anabolic Steroids-H138
Analog-B66
Analysis-ES1, G15
Analysis of Covariance-A81
Analytical Technique-G2, G20
Anaphylactic Shock-H7
Anaphylaxis-H76
Anatomical Teaching Specimens-G39
Anatomy-A115
Ancestry-A1, A26, A28, A92, W5
Ancestry Estimation-A91, A108, A113
Ancestry Prediction-B140
Animal Bite-G41
Animal Models-A45
Animation-D49
Anomalous Congenital Band-H9
Anonymity-C11
Antacid-H74
Antemortem-A102
Antemortem Records-G53
Anthropological Consultation-H149
Anthropological Methods-A87
Anthropological Theory-A111
Anthropology-A6, A75, B101, C10, E3, J16
Anticipated Contradictory-F23
Antidepressants-K42
Antidote-K21
Antipsychotics-K42
Anti-Semitism-J24
Aorto-esophageal Fistula-H45
Apoptosis-H119
Aqueduct of Sylvius-H156
Archaeological-G31
Archaeology-A121, K69
Archived Fingerprints-B180
Arrhythmia-H10
Arson Mythology-F5
Arterial Dissection-H16
Artillery Shell-D24
Asperger's-LW1
Asphyxia-H56
Asphyxial Deaths-H96
Aspirin-H122
Assassination-LW7
Assembly Bill 109-I11
Assessment-I21, I31
Assisted-D17
ASTM F2508-D16
Asylum Seekers-E41
At Crime Scene-B191
Atheroembolus-H143
Atkins-F53
Atomic Absorption Spectroscopy-B197
ATR-FTIR-B174
ATR-FTIR Spectroscopy-E2
Attack-G41
Attempted Murder-F4
Attempted Rape-E17
Attention-E69
At-Work Deaths-H146
Atypical Ammunition-E22
Atypical Wounds-A97
Auricular Surface-A24
Author Identification-D55, D56
Author Verification-D55
Autistic Spectrum-I6
Autobiographical Memory Trace-I2
Autoerotic-H56
Automated DNA Workflow-B8
Automated Image Analysis-B158
Automatic Characterization-D61



Key Word Index- 2015

Autonomic Failure-H140
Autopsy-H6, H11, H41, H46, H65, H85, H142, H147, H150
Autopsy Technique-H9

B

BAC-F48, K48
Backlog-B45
Bacteria-A33
Bacterial Profiling-B92
Bacterial Translocation-H118
Bad Practices-BS6
Ballistic Tool Marks-B116
Ballistics-H69, W12
Ballistics Identification-B117
Balloon Crash-G25
Ballpoint Pen Inks-E35
Bank Robbery-BS3
Barcode Traceability-B8
Barefoot-D16
Barefoot Friction-D20
Barotrauma-H57
Bathtub-E28, E66
Bathtub Drowning-W21
Bayesian Likelihood-D55
Bayesian Networks-B34
Bead Cord Malformation-H8
Bead Ruptor-K1
Beetles-H34
Behavior-W18
Behavioral Biometric-D56
Behavioral Genetics-I3
Benzodiazepines-K2
Best Practices-C15, E53
Bias-F16
Bicycle-D49
Bilateral Symmetric Fractures-A134
Binding Dyes-H111
Bioarchaeology-H115
Biogeoreferencing-A20
Bioko Primates-E29
Biological Profile-A10, A28, A31, A61, A71, A115, W5
Biological Stain-B2

Biological Stain Detection-F55
Biomechanics of Rib Fractures-H42
Biomechanics-D7, D9, D11, D12, D13, D15
Biometric-C4
Biometric Security-C12
Biometric Signature-J14
Biometrics-A65, E61
Bioreactor-A64
Biothreat-E11
Bitemark-G11, G12, G14, G21
Bitemark Analysis-G13, G42
Bitemark Overlay-G11
Bitemarks-G7, G9, G15
Bleach-A14
Blended Learning-E40
Blockage-H156
Blood-B50, B125, E31
Blood Alcohol Concentration-H58
Blood Oxygen Level-I25
Blood Pattern Analysis-E38
Blood Spatter-B112
Bloodhound-E17
Bloodstain-B183, E45, E47
Bloodstain Age-B123
Bloodstains-E38
Bloody Hammer-E9
Bloom's Taxonomy-E33
Blow Flies-H100, H101
Blow Fly-E49, H102
Blunt Abdominal Trauma-H52
Blunt Force-A100
Blunt Force Trauma-A96
Blunt Trauma-A67, G21, H88
Body Covering-I37
Body Farms-A35
Body Fluid-B124
Body Fluid Identification-B52, F38, H117
Body Height-A54
Body Mass-A31
Body Stuffer-H77
Body-Wrapping-H56



Key Word Index- 2015

Bolstering-F29, J29
Bombardment Aircraft-E58
Bone-A14, H33, K3, K16, K37
Bone Biomechanics-A97, A99
Bone Health-A117
Bone Histology-A18
Bone Identification-A46
Bone Moisture-A17
BPA-E39
Briefing-E63
Brodifacoum-K11
Bronchoscopy-H5
Bronchospasm-H15
Brugada Syndrome-H131
Bubble Marks-J19
Bullet Comparisons-B119
Bullet Hole-E64
Bullet Images-B119
Bullet Replication-B114
Buprenorphine-K4
Buprenorphine Glucuronide-K13
Burial-A17
Buried Remains-K73
Burn Patterns-A44, D39, D42
Burned Bone-A41
Burnt Bone-A16
Burnt Human Remains-A39
Burnt Teeth-B5
Bus Crash-H51
Butane and Propane Mixture-H31
Butterfly Fractures-A95

C

Cadaver-H104
Cadaver Destroying-A15
Cadaver Detection-E26
Calliphora Vomitoria-H24
Camera Identification-C1
Camping Stove-H31
Canadian Armed Forces-G55
Canine Detection-B59, B161, E11
Cannabinoid-K51

Cannabis-K63
Cannibalism-H73
Capillary Electrophoresis-H117
Carbon Monoxide Intoxication-E7
Carbon Monoxide Poisoning-E7
Carboxyhemoglobin-H51
Cardiac MRI-H92
Cardiac Myocytes-H2
Cardiac Oxidative Stress-H77
Cardiac Tamponade-H3
Cardiopulmonary Resuscitation-H141
Cardiovascular-H133
Cardiovascular Pathology-H140
Carisoprodol-K49
Carrion-A43
Cartridge Case-B117
Cartridge Cases-B136
Cartridge Casings-B68, B69, B135
Case-F56
Case Linkage-E23
Case Management Skills-B39
Case Recoveries-A70
Case Report-BS5
Case Study-F15
Case-Based Training-G49
Casework-B7, B10
Casework Reviews-F29
Catastrophic Failure-D22
Catecholamine Toxicity-H13
Catheterization-H79
Cathinones-B175, K6
Cause of Death-F21
CE/MS-B195
Cementum-A105
Cemetery-G31
Censoring Stage H-G47
Certification-B46
Cervical-A86
Cervical Spine-D14
Cervical Vertebrae-A90
Cesarean-L1



Key Word Index- 2015

Cesare Lombroso-F2
CF FORT-G55
Challenging Samples-W4
Change-F26
Characteristics-I33
Charred Body-A2
Charred Corpses-H60
Cheiloscopy-G16
Chemical Tests-F22
Chemistry-A34, D33
Chemometric-B49
Chemometrics-B2, B153, E2
Chicago-BS3
Child-H15, I7
Child Abuse-A98, F33, F34, H22, H38, H41, H42, H43, H44, I6
Child Abuse/Torture-E24
Child Neglect-I30
Child Pornography-B6, I24
Child Psychological Abuse-F35
Child Sex Abuse Images-C3
Children-H46
Chiral Separation-B64, B195
Chlorpromazine-K3
Choke Hold-H72
Cholesterol Embolus-H143
Cholinergic Crisis-K33
Chromatography-K2
Cigarette Ash-B197
Citalopram-H10
Civil Liberties-F36
Clandestine Laboratories-B171, B176
Classification-E1, H39, W3
Classification Trees-A55
Clearances-E25
Clothing-H75
Clotiapine-K30
Cloud Computing-C17
Cluster Analysis-B33
Cocaine-H154, K23
Code Fingerprinting-C23
CODIS-B145, F42
CODIS STR-A1
Cognitive Bias-A112, F14, W20
Cognitive Profile-E72
Coin Battery-H45
Cold Case-E3, E6, W9
Cold Case Serial Homicide-L2
Cold Cases-A20, E25
Collaboration-E54
Colloids-B172
Colonization-H28
Colorimetric Test-B24
Columnar Thin Film (CTF)-B81, B110
Commercial Cremation-A39
Commingled-A136
Commingling-A27
Communication-E52, F24, H147
Comparison-B80
Competitive Ionization-E48
Complex Cases-B39
Complex DNA Interpretation-B96
Complex Mixture-B11, B100
Composite Bones-A95
Comprehensive Analyte Coverage-K24
Computed Tomography-A13, G4, H85
Computer Animation-E43
Computer Simulation-A119
Computerized-A136
Concealment-H95
Conclusions-J25, J28
Concordance-B76
Concrete-A22
Concurrence-G37
Condoms-B168
Conduction System-H136
Conductive Ink-J10
Conduit-D42
Confidence Limits-B116
Confocal Microscopy-E36
Congestive Heart Failure-H139
Congruent Matching Cells-B108, B117
Consensus-E67



Key Word Index- 2015

Conservation of Momentum-D51
Constructive Alignment-E33
Contaminant Transport-D35
Contaminated Corpses-H28
Contamination-B32, F40
Contemporary Research-S1
Context-E55
Controlled Substances-B55, B169, B172, B174
Controls-F40
Conviction Integrity-W8
Copper/Aluminum-D42
Corrections-I22
Corticosteroid-H76
Cosmetic Foundation-B18
Cougar-G41
Counterfeit Deterrence-J13
Court-F10
Court Challenge-J30
Courtroom Preparation-E73
Courtroom Testimony-K61
CPVT-H66
Cranial Fracture-A100
Cranial Fracture Patterns-A99
Cranial Fractures-A101
Cranial Morphology-A57
Cranial Non-Metrics-A56
Cranioetrics-A13, A30, A83
Crash and Sled Testing-D5
Crash Reconstruction-D11
Crash Testing-D6
Crashworthiness Design-D6
Cremation-A135
Crime-I7
Crime Assessment-W18
Crime Scene-B113, E16
Crime Scene Behaviors-I37
Crime Scene Investigation-F28, I1
Crime Scene Investigator-F17
Criminal Behavior-I3
Criminal Justice-F15, G10
Criminal Law-F1, F2

Criminal Poisoning-K70
Criminal Responsibility-W2
Criminalistics-B46, B101, B105
Critical Angle-B120
Critical Incidents-BS2
Cross-Examination-F43
Crucifixion-E39
Crumpled Document-J7
Crush Asphyxia-H81
Crush Box-D50
CT Imaging-H57
CT Scan-H94, H129
CT Scans-F20
Cubans-A26
Cukurova University-K10
Cultural Problems-I34
Cut Marks-A15, A118
Cyber Security-C22
CYP2B6-K32

D

Dangerous Mail-B44
DART@-MS-B173, B199, E48
Data Fusion-J18
Data Independent Acquisition-K24
Database-B12, B152, J18
Databases-E61
Dating Game Killer-L2
Dating Human-H78
Daubert-A128, BS8, D19, D27, F7
Daubert Challenge-J27
Daubert Guidelines-A58
Death-E7, E13, E63, H21
Death Certification-H37
Death in Custody-H31
Death Investigation-E21, E37, K43
Death Penalty-F53
Death Scenes-B38
Deception-I28
Deception Detection-D53
Decomposition-A22, A36, A45, B125, H106, H109, K37
Decomposition Odor-E8



Key Word Index- 2015

- Decompression Illness-H62
Decontamination-E30
Deconvolution-B48
Deductive Reasoning-D21
Defense-I27
Defense Counsel-F42
Defensive Medicine-H5
Defibrillator-H134
Defleshing-A14
Degradation-B133
Degraded-W4
Degraded DNA-B184, H110
Dehydration-H14
Delayed Reporting-E27
Demographic-K34
Demography-K10
Demonstrative Evidence-E50
Dental-A86, A105, G25, G35, G36, G44
Dental CFAO-G9
Dental Charting-G6
Dental ID-G34
Dental Identification-G8, G19, G27, G38, G39
Dental Identifications-G6
Dental Law-G22
Dental Materials-G24
Dental Radiography-G48
Dental Treatment Narrative-G33
Dentition-G12
Depravity-F54
Derivatization-K62
Designer Drugs-B17, B170, K5, K7, K53
Desmopressin-H30
Detection-I28
Detector Canines-B60
Determination in Soil-B1
Development-J6
Development Time-H98
Developmental Disorders-I6
Developmental Osteology-A131
Diabetic-H151
Diacritics-J15
Diagnoses-H21
Diagnosis-H90, K21
Diatoms-H75
Differential Decomposition-A42
Differential Extraction-B79, H112
Digital-C24, F56, G35
Digital Autopsy-C14
Digital Dental Evidence-G53
Digital Evidence-C8
Digital Evidence Examination-C7
Digital Forensics-C12, C13, C15, C17, C18
Digital Investigation-C9
Digital Measurement-E59
Digital Radiography-E34, E64
Digital Security-C22
Diomics X-Swab™-B77
Dipyrrone-B192
Direct Amplification-B70
Direct Analysis in Real Time-B26
Direct PCR-B178
Disaster Victim Identification-B143, G19, G50
Discovery Motion-F41
Discriminant Analysis-A93
Discriminant Function-A6
Discriminant Function Analysis-A72, A76
Disguise-J26
Disinterred-G31
Dismemberment-H70
Dispersive LLE-K18
Distance Determinations-D26
Diurnal Oviposition-H102
Diversion-B173
DNA-B12, B74, B95, B104, B110, B113, B129, B133, B142, B177, E14, E17, E54, F16, F25, F39, F42, F43, F45, G7, W4
DNA Collection-B77
DNA Evidence-B37
DNA Extraction-B73, B85, B131, B180, W23
DNA From Touched Objects-B69
DNA Isolation-B5
DNA Laboratory Validation-B72
DNA Methylation-B124, B127
DNA Mixtures-B4, B94, B97, F37



Key Word Index- 2015

DNA on Pistol-E5
DNA Phenotyping-B186
DNA Polymerase-B67
DNA Preservation-B73
DNA Profiles-B141
DNA Profiling-B178
DNA Profiling Context-F38
DNA Profiling of Soil-B91
DNA Purification-B9
DNA Quality and Quantity-B130
DNA Quantitation-B80
DNA Recovery-B77, B130
DNA Recovery From Casings-B68
DNA RRNA 16S-B92
DNA Sensitivity-F37
DNA Typing-B181
DNA Yield-H29
Document Examination-W13
Document Examination Review-J22
Documents-J9, W19
Dog Bites-G42
Domestic Violence-E24
DOMEX-B181
Donor's Death-F3
Doppler-D27
Double-Base-B160
Drew Peterson-W21
Drew's Law-W21
Driving Impairment-K50
Drones-C16
Droplet Digital PCR-B132
Drowning-BS7, E28, E57, E66, H61, K29
Drug-B195, H29, K47
Drug Analysis-B65, B66, B169, B191
Drug Intoxication-H72
Drug Testing-K9
Drugged Driving-K46
Drug-Related Deaths-K67
Drugs-B27
Dry Cleaner-D36
DSA-TOF/MS-B190

DSC-A16
Duct Tape-B149, B150
Due Process-F28
DUI-F48, K58
DUI Impairment-K61
DUID-F49, K49, K50
Drugs of Abuse-K22
Dust Bunny-B90
DVI-B73, G25
Dyed Fibers-B36
Dynamic Mechanical Properties-D10
Dynamic Model DNA Laboratory-B72

E

Earprint-E1
Edentulous-G40
Edible-H120
Education-B74, LW5, S2
Education and Training-E33
Elder Abuse-H53, I14
Elder Maltreatment-E20
Elderly-E13, I18
Electric Heating Coils-D43
Electrical-BS1, D39
Electrical Tape-B20, B149
Electrocution-D39, H124
Electron Impact-K6
Electronic Dance Music-K9
Electrostatic Detection Device-J7
Elemental Analysis-A40, B20, B131
Eligibility-E53
ELISA-K4, K14
Elliptical Fourier Analysis-A53, A106
Embalming Fluid-K65
Embolization-H79
Emergency Stop-D45
Emerging Technologies-B106
Endodontic "Poof"-G1
Engineering Evaluation-D44
Enhanced Injury-D2
ENPPI-H84
Entertaining-J22



Key Word Index- 2015

Entomology-H107
Entomotoxicology-H35, H107
Envelope-B85
Environmental-D32
Environmental Chemistry-D34
Enzymatic Assay-F48
Epidemiology-H61, H127, H155, K31
Epigenetics-I10
Epiglottitis-H6
Epinecrotic Communities-W23
Epiphyseal Union-A29
Equivocal Death-W6
Error-A88, D29, D30, F24
Error/Bias-A111
Error Rate-B108, B115, D28
Errors-F25
ESDA-J10
ESDA2-J12
Estimation-G44
Ethical Payback-B47
Ethics-B37, B47, D50, ES1, F32, G22
Ethyl Glucuronide-K60
Etizolam-K64
European Legislation-F3
Evaluation-E51
Evaporation-B57
Event-Related Potentials-W2
Evidence-C15, D30, E1, E45, F10, F39
Evidence Collection-B38, F15
Evidence Evaluation-F21
Evidence Packaging-A40
Evidence Processing Order-C7
Evidentiary-F46, F56
Evil-F54
Excited Delirium-E44
Exclusion-G32
Experience-A66
Experiment-C21
Experimentation-LW4
Expert Credentials-F17
Expert Errors-F5

Expert Testimony-A128, G13
Expert vs. Fact Witnesses-D21
Expert Witness-E73, F17, W24
Explosive-B1
Explosives-B78, B157, B158, B159
Explosives Fatalities-D24
Extended-J6
Extraction-B142, H33
Extrajudicial Killings-A129
Extrapolation-K48
Extreme Emotional Disturbance-I27
Eye Lens-H78
Eyewitness-E14

F

Fabric Degradation-A21
Fabricated Testimony-F55
Facial Comparison-C2, C6
Facial Identification-C2
Facial Images-B6
Facial Mimicry-D60
Fall-H46
Falls-D9
False Confession-E9
False Exclusions in Soil-B82
False Results-F25
Fantasy-C9
Fast PCR-B146
Fat Embolism-H91
Fatal Fire Modeling-A44
Fatal Thrombo-Embolism-H18
FBI-BS3
FDR-B23
Fear-I10
Feature Matching-E69
Federal Advisory Committee-F9
Federal Court-D37
Federated Testing-C18
Feet Comparison-C4
Female-L1
Femicide-H71
Femoral Neck Axis Length-A91



Key Word Index- 2015

- Femur-A104
Femur Measurement-A54
Fentanyl-B192, H128, K14
FEPAC-B41
Fetal Hypoxic-Ischemic Injury-H135
Fetal Kidnapping-L1
Fetal Liver-K60
Fetal Trauma-H52
FGS-B16
Fiber Analysis-B153
Fibers-B151
Fibula-A23
Field Portable Instrumentation-F22
Field Tests-F22
File Carving-C24
Filicide-I17, I35
Filter Photography-E6
Fingermarks-H111
Fingerprint-B28, B111, D30
Fingerprint Development-B81
Fingerprint Identification-B108
Fingerprint Reconstruction-C12
Fingerprints-B50, B107, B109, B110, B179, E61
Fire Debris-B15, B162, B164, B165, B166, B167
Fire Debris Analysis-B14, B57, W7
Fire Logs-B163
Fire Patterns-F6
Fire/Explosion-D44
Firearm Forensics-B118
Firearm Identification-B115
Firearms-B34, D22
Firearms Comparison-B113
Firearms Deaths-E22
Firearms Examination-E59
Firing Pin-B61
First Derivative-B151
Flash Fire-H51
Flawed Forensics-W20
Flexible Discriminant Analysis-A94
Flipped Classroom-E40
Florida-A70, F49
Florida vs. Zimmerman-D54
Flow Cytometry-B13
Flow Injection MS/MS-K15
Fluid-Dynamics-E45
Fluorescence-A41, B50
Fluorescent Cyanoacrylate-B19
Food-Induced Allergy-H7
Foot and Footprint Characters-E42
Foot Length Measurements-A50
Footwear-B82
Foraging-H23
Force-B22
FORDISC®-A13, A26, A57, A84
Foreign Body Ingestion-H45
Forensic-A49, F32
Forensic Analysis-B51, C11
Forensic Anthropologist-G48
Forensic Anthropology-A3, A4, A25, A27, A35, A37, A40, A51, A57, A58, A59, A61, A62, A73, A77, A82, A89, A90, A110, A112, A114, A116, A120, A124, A126, A128, A135, B103, G3, W9, W17
Forensic Archaeology-A126
Forensic Art-E52
Forensic Botany-B93
Forensic Capacity Building-A127
Forensic Casework-A126
Forensic Chemist-B44
Forensic Chemistry-B199
Forensic Dentistry-G5
Forensic Discrimination-E35
Forensic DNA-B10, B94, B146, F37
Forensic DNA Analysis-B76
Forensic DNA Typing-B139
Forensic Document Examination-J30
Forensic Drugs-B194
Forensic Entomology-B198, E49, H32, H34, H35, H102
Forensic Epidemiology-H154
Forensic Evidence-B188
Forensic Failures-F18
Forensic Family-B43, B88
Forensic Image Analysis-H148
Forensic Linguistic Evidence-F19



Key Word Index- 2015

Forensic Medicine-H55
Forensic Odontologist-G48
Forensic Odontology-G2, G3, G4, G10, G11, G12, G13, G19,
G20, G22, G24, G26, G28, G29, G40, G49, G51, G55
Forensic Odontology Protocols-G50
Forensic Pathology-H61, H83, H122, H132, H148, I16
Forensic Photography-G21
Forensic Podiatry-E42
Forensic Psychiatry-I16, I22
Forensic Radiography-E34, J16
Forensic Radiology-H87, W11
Forensic Science-B115, B197, E64, G29, H73, I18, LW5, W6,
W13
Forensic Science Education-B40, B41
Forensic Science Research-B41
Forensic Services-G4
Forensic Strategies-A127
Forensic Taphonomy-A34, H106, H109
Forensic Team Building-E52
Forensic Toxicology-F21, K16, K18, K26, K27
Forensic Toxicology Lab-K10
Forensics-E15, G38
Forensics Challenges-C17
Forgery-B154
Fork Bead Cord-H8
Fourier Transform-B54
FPSE-B188
Fracture-A102, D23
Fracture Classification-A101
Fracture Propagation-A95
Fractures-A117
Fragmentary Remains-A136
Fragmentary Remains-A23
Frail Elderly-D17
Fraud-G38
Frequency Occurrence-J3
Freshwater Environment-H25
Frontal Bone-A7
Frontal Sinuses-A12
Frye-BS8, D19
Frye Jurisdiction-F27
FSIQ-F53

Functional Genes-H26
Fundamental Difference-J5
Funding-F47
Funding Opportunities-E53
Fungal-H1
Future Developments-W14
Future of Forensic Science-S1

G

Gas Chromatography-K71
Gas Embolism-H62
Gasoline-B14, B62
GBL-B196
GC/MS-B64, B162, B163, K17, K25, K62
GC/MS Data Processing-B48
GC/MS Method-K30
GC/MS/NPD-K74
GCxGC/TOF-K1
Generalist Development-B39
Genetic Investigations-H18
Genetic Polymorphisms-K32
Genetic Testing-H49
Genetics-B105, H142
Genome Sequencing-E29
Geographic Information Systems-A133
Geography-A122
Geometric Morphometrics-A7, A53, A55, A72
Geometric Probability Dist.-D20
Geospatial Technology-A124
Geriatric-E20
GHB-B196, K1, K62
GIS-A70, A118
Glass-D23
Glassing-D23
GlobalFiler™ Express-B70
Glock® Pistol-B61
Glucuronide-K77
Glycans-E47
Glycoproteins-K40
Grants-F47
Graphene Oxide & Carbon Black-J2
Gregariousness-H108



Key Word Index- 2015

Growth and Development-A52
GSR-B25, B32
Guilty Verdict-G58
Gunshot-B112, E56, H50
Gunshot Injuries-H68
Gunshot Primer Residue-B53
Gunshot Residue-B30, B121, B122, LW2
Gunshot Wounds-H55

H

Hair-B22, E2, H115, K38
Hair Analysis-B198
Hair Microscopy-B148
Hair Testing-K4
Halogen Light-D41
Hand Printing-J27
Hand Swabbing-B25
Handedness-A66
Hands-Off Offense-E18
Hands-On Sexual Offense-I24
Handwriting-C8, J3, J4, J6, J8, J19, J25, J28
Handwriting Identification-J27
Handwriting Principle-J14
Hanging-H64
Harassment-I12
Head and Brain Injury-D7
Head Injury-D3, H38
Headspace-Mass Spectrometry-B15
Healing-A102
Heart Rupture-H141
Helicopter-D50
Helicopter Crash-G28
Helmet Testing-D3
Helping Professions-I12
Hemopericardium-H3, H141
Hemorrhage-H48
Hermaphroditism-BS5
Heroin-H128, H155, K34, LW6
High-Definition Survey (HDS)-D59
High Resolution MS-B194
High Resolution Tandem MS-K23
Higher Education Policy-E51
Histology-A33, A103, A116
Histomorphology-A19
Histopathological Factors-H18
Histopathology-H92
Historical-A69
Historical Uses-D32
Hoarding Disorder-F36
Hoax Powders-B156
Homemade Explosives-E48
Homicide-BS7, E21, E24, E28, E66, F34, H38, H64, H71, W9
Homicide-Mimic-E37
Homicide Suicide-H55
Homicides-E25
Homopolymer-H27
Honor-I34
Honor Killing-BS7
Horses-LW4
Hospital-E12
Hot Air Balloon Fires-D40
Household Dust-B89, B90
HS Solid-Phase Microextraction-B65
HS/GC/MS-K70
Human-E31
Human Decomposition-A35, A63, A65
Human DNA-B89
Human Ear-E4
Human Geolocation-E62
Human Identification-B103, LW7, W17
Human Identity-B139
Human Injury-D25
Human Injury Analyses-D8
Human Osteology-A10
Human Performance Toxicology-K36, K68
Human Remains-LW3
Human Remains Detection Dogs-E8
Human Rights-A68
Human Skin-B19
Human Soft Tissue-D25
Human Variation-A93, W5
Human vs. Non-Human-A46
Humanitarian Forensics-A127



Key Word Index- 2015

Humanitarian Identification-G56
Hydrocephalus-H156
Hydrogen-K71
Hydrogen Sulfide-H54
Hydrophilic Polymer-H79
Hypergammaglobulinemia-H11
Hypergolic Mixtures-B63
Hyperspectral Imaging-H101
Hyperspectral Remote Sensing-E26

I

IACI-H84
IBIS®-B61
ICP/OES-B63
Identification-A2, A18, A60, A68, A107, G23, G30, G32, G35, G36, G37, H60, W11
Identification Criteria-B14
Identification Process-B106
Identity Documents-J13
Ignitable Liquid-B62, B171
Ignitable Liquid Residues-W7
Ignitable Liquids-B15, B35, B162, B164, B166
Ignition Temperature-D43
Iliac Artery-H16
Illicit Drugs-B189
Illicit Substance-B60
Image-C4
Image Analysis-B78, B150
Image Comparison-C1
Image-Guided Examination-H82
Image Processing-D61
Image Resolution-E34
Imaging-W19
Immigration-G46
Immunohistochemical Study-H91
Immunohistochemistry-H4, H129
Impact-W14
Impact Analysis-D3
Impact Velocity-D25
Impairment-K47, K51
Implicit Association Test-I2
Impulsive Aggression-W2

IMS-B23, B25
In Silico Prediction-K54
In Vitro Metabolism-K54
Inadmissible Testimony-F29
Inadvertent Transfer-F16
Incarceration Modalities-I25
Incidence-H126
Incidence Rates-G42
Incised Wounds-H59
Inconsistencies-G6
Inconsistent Findings-E21
Increment-A105
Indentation Recovery-J7
Indentations-J10
Indented Writing-J12
Individual Characteristics-W12
Individualization-C8
Inertness-K74
Infant-H22, H41
Infant Abuse-A98
Infant Death-H44, H96
Infanticide-I17
Infants-H42
Inferior Vena Cava-H144
Informed Consent-I15
Infrared Microspectroscopy-B56
Inhibition-B67
Inhibitor-H110
Injuries-D22, E27
Injury Interpretation-F20
Injury Risk-D14
Ink Aging-J1
Inkjet Printer-J17
Innominate-A73
Inpatient-I8
Inquisitorialism-F51
Insects-E30
Interdisciplinary Group-K45
Interdisciplinary Research-E69, F30
Interdisciplinary Theory-A120
Internet Child Pornography-C3



Key Word Index- 2015

Inter-Observer Agreement-A56
Inter-Observer Error-A37
Interpersonal Violence-A132
Interpretation-B37, B95, B155
Interpretation of Chromatogram-W7
Interpupillary Distance-C5
Inter-Rater Reliability-G16
Interrogation-E23
Intersections-J20
Intravascular Coagulation-H74
Intravenous Drug Abuse-H152
Intuition-I28
Investigation-C20, E16, E57, E63
Investigative-F46
Involuntary Commitment-F36
IOFOS-G50
Ion Mobility Spectrometry-B29
Ion Torrent™-H113
IPL-H11
Iraq-B86
Isoscape-A123
Isoscapes-A114
Isothermal Amplification-B52
Isotope Analysis-A20
Isotope Dilution-B16
Isotope Ratios-B198
Istanbul Forensic Medicine-I20
Italian Criminal Procedure-F23
Italian Law-H71

J

Jeffery Ashton-BS8
Judicial System-F26
Junk Science-D34, D36, LW2
Jurisprudence-F43
Jurors-E50
Jury-F44
Juvenile-A38

K

Kawasaki Disease-H21
Ketamine-K68, K73

Ketoacidosis-H151
Killing-H32
Kinship Algorithm-E10
Kinship Inference-B186
Knee Joint-A52
Korean-A11
Kwashiorkor-H17

L

Laboratory Quality-F11
LabRetriever-B187
LA-ICP/MS-E35
Landslide-G27
Language-F44
Larval Development-H28
Larval-Trail Following-H23
Laryngeal Fracture-H86
Laser Desorption/Ionization-J2
Laser Engraving-J11
Laser Scanning-D58, D59
Latent DNA-H111
Latent Fingermarks-B19
Latent Fingerprint-B28
Latent Print Processing-C7
Latent Prints-E71, E72
Latents-J9
Law-LW4
LC/MS-B192, K22, K52
LC/MS/MS-B18, B121, K8, K73
LC/QqQ/MS-K19
LC/TOF-K7
LC/TOF/MS-K56, K66, K72
LC/APCI-MS/MS-B1
LCN DNA-B138
LCN STR Amplifications-B87
LDI-B27
Left-of-Center-D51
Legal Databases-F19
Legal High-B66
Legal Implications-F8
Legal Medicine-F4
Leprosy-LW3



Key Word Index- 2015

Levamisole-H12
Levine-G18
LIBS-B176
Ligation-B71
Likelihood Ratio-B100, B167, B187, E68
Lime-A42
LIMS-B8
Linguistic Evidence-D56
Lip Prints-G16
Lips-G17
Litigation-BS1
Live-In Friend-E9
Liver Dease-H44
Location-E55
Log-Likelihood Ratio Values-A58
Long Bone Fracture-A96
Long QT Syndrome-H10
Loperamide-H121
Low-Copy Number-B185
Low-Energy Bone Fracture-D8
Low-Template DNA-B96
LRmix-B98
Lung-K40
Lung Transplantation-H152
LVAD-H139

M

MALDI-TOF-B58
Malingering Detection-I2
Malpractice-G5, G54, H5, H74
Maltreatment-I30
Management-F47
Mandible-A60, A85, G34
Mangled Body-G23
Manitoba-H126
Manner of Death-A134, K67
Manual Strangulation-H86
Manubrium-A76
Maps-D57
Margin of Error-F27
Maricopa County OME-A107
Marijuana-B65, H120

Marine Taphonomy-A62
Masquerading-C21
Mass Disaster-B143
Mass Disaster Management-G26
Mass Fatality-W1
Mass Fatality Incident-G27
Mass Grave-A125, E26
Mass Graves-A47
Mass Spectra-K6
Mass Spectrometry-B21, B36, B154, B168, B191
Massively Parallel Sequencing-B139, F8
Material Exculpatory Evidence-F31
Maternal Mortality-H20
Mathematical Model-B57
Matricide-H73, I13
Maxillary Sinuses-A9, G40
M-CPP-K28
Measurement Error-A30
Measurement Standards-A83
Measurement Uncertainty-B55
Meconium-K2
Medea Complex-I35
Medical Examiner-H149
Medical Examiner/Coroner-H145
Medical Examiners-H103
Medical Imaging-A30, A78
Medical Malpractice-H19
Medical Testimony-E73
Medical Toxicology-W15
Medicolegal Death Inquiry-H109
Medicolegal Investigation-E22
Melt Curve Analysis-B127
Memory Forensics-C23
Mental Health-BS2, I11
Mental Illness-I9, I38, LW1
Mental Maps-F14
Mentoring-B88
Mentors-G18
Metabolism-K55
Metabolite Elucidation-K7
Methadone-K32



Key Word Index- 2015

Methamphetamine-B171, B176, K12, K37
Methcathinone-K18
Method-A121
Method Validation-K25
Methodology-J29
Methylphenidate-K19
Methylprednisolone-H76
Metrology-D29
Mexican Migrant-A1
Microascus-H1
Microbial Community Profiling-H26
Microbial Degradation-B35
Microbial Profile-H36
Microbiology-H118, H150
Microbiome-H97
Micro-CT-H86
Microfluidics-B142
Micro-FTIR-B56
MicroRNA-H117
Microscopic Method-B89
Microscopy-A34, B147, D32
Microspectrophotometry-B152, B153
Microvariant-B12
Microwave-Assisted Extraction-K16
Minimum Postmortem Interval-H99
Minutiae-B107
Miscarriage of Justice-F51
Misdiagnosis-G54
Missing-W10
Missing Persons-A109, A124
Mitochondrial-H114
Mitochondrial DNA-H27
Mitochondrial DNA Mutations-A87
Mitral Valve Prolapse-H130
Mixture-B13, B95
Mixture Detection Assay-B99
Mixture Interpretation-B94, B100
Mixture Software-B11
Mobile-B111, B183, C21
Mode of Death-K44
Model Signature-J21

Modeling-D35
Molecular Autopsy-H49, H66
Molecular Photofitting-B105
Morphologically Directed Raman-B156
Morphometric Outline Analysis-A59
Morphoscopic Traits-A92
Mortality-H155
Mortar Accident-D21
Motive-K43
Motor Vehicle Deaths-H125
Motorcycle Reconstruction-D46
Movement-A122
MPMCTA-H88
MPMI-H35
MSP-B149
MT-45-K64
MtDNA-B134, H33
Multi-Car-D48
Multidisciplinary-E15
Multidisciplinary Approach-A3, H60, H95
Multi-Phase Postmortem CT-Angiography-H87, H91
Multiple-E56
Multiple Fatality Incident-G29
Multiple Knives-H63
Multiple Linear Regression-B174
Multiplex-B132
Mummification-H95
Mummified Corpses-H94
Mummy-B102
Munitions Decommissioning-D24
Murder-I27, J24
Myocardial Infarction-H93, H136
Myxomatous Degeneration-H130

N

Nail Polish-B56
NAION-H30
Naloxone-K13
Nanoparticles-B27
Nanotrace-B147
NAS Report-B42, F7, F9, F10, F11, G10
National Commission-F9



Key Word Index- 2015

Natural-J4
Natural Death-H9
Natural Variation-J14
NBOMe-K35
N-Butane-K70
Necrobiome-H105
Neglect-I18
Neolite® Test Liner-D19
Neonate-H14
Nerve-H48
Neurodegenerative Diseases-I15
Neuropsychological Testing-I26
Neuropsychopharmacology-F55
Neuroscience-F1
Neurosciences-F2
Neutrality-W24
New Approaches-B199
New Psychoactive Substance-B31
New Science-F5
New York City-F13
Next Generation Sequencing-B4, B76, H27, H113, H114, H131, H137
Next Generation SNP Sequencing-B184
NFPA 921-F7
NGS-B134
Nicotine-K60
NIST Bullet Comparisons-B119
Nitroglycerin-B157
NMR-B17, B175
Non-Accidental Injuries-H40
Non-Accidental Injury-A117
Non-Controlled-F49
Non-Destructive Collection-B181
Non-Detects-D33
Non-Document Examiners-J22
Non-Dominant Hand-J26
Non-Insane Automatism-I29
Non-Linear Systems Theory-A119
Non-Medical Witness Testimony-F50
Non-Metric Traits-A59
Nootropics-K52
Normality in Digital Forensics-C14

Normalization-B34
Not Criminally Responsible-I29
Novel Psychoactive Substances-K35, K55, K75, K76
Novelty Helmets-D46
NPS-K9
NTFS-C19
Number of Contributors-B96

O

Obscene Phone Call-E18
Observer Error-A84
Ocean-A69
Odontogram-G33
Odontology-G30, G32, G34, G46, G52, G56
Odontology Training-G49
Odontometrics-A108
OEM Refinishes-B155
Off-Label Prescription-H30
Off-Label Treatment-I15
Old Adults-A82
Oligodendroglioma-F4
Online Predator-C9
Operation Identification-G56
Opiate-K77
Opioid Abuse-H121
Optical Microscopy-G43
Optic-H48
Optimal Temperature-H100
Oral-K47
Oral Cancer-G54
Oral Communication-W16
Oral Fluid-K19, K46, K63
Organic-B122
Origin-A122
Origin Determinations-F6
Orthographic Views-D47
OSAC-D54, E67, E70, F12, J31
OSSA-A28
Osseous Injuries-H17
Osteometric Data-A84
Osteometrics-A85
Osteometry-A83

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Key Word Index- 2015

Overdose-H153, K66, W15

P

Pacemaker-H134

Paint Data Query-B155

Pairwise Comparisons-B86

Palate Shape-A108

Palo Mayombe-A49

Palynology-B102

Paper Microfluidic Devices-B24

Paper Microfluidics-B30

Paraphilia-I4

Parental Alienation-F35

Parental Estrangement-F35

Parkinson's Disease-I29

Parricide-I13, I37

Particle Collection-B29

Particle Size Distribution-B82, B156

Parturition Pits-A48

Passenger Vehicle-D48

Past Presidents of AAFS-S1

Pathology-H54

Patricide-I13, I16

Pattern Recognition-A101

Patterned Evidence-B40

Patterned Injuries-H148

PCR-B145

PCR Array-H119

PCR-Free-B185

Pearl Harbor-G30

Pectoral Girdle-A25

Pedagogy-E40

Pedal Evidence-E42

Pedestrian-H58

Pediatric-K59, K78

Pediatric Injuries-H85

Pediatrics-D7, D9, D13, D15

Pedophilia-I25

Peer Review-D38

Pelvic Asymmetry-A8

Pelvis-A71, A89

Perception of Police-I32

Peri-Mortem-A133

Peri-Mortem Trauma-A132

Persistence-B128, B129

Personal Identification-A12, A50, C10, D60, E4

Personality-C3

Personality Disorder-I4

Peru-A129

Pesticide-K31

Pesticides-K11

Pharmaceutical Industry-BS6

Pharmaceutical Preparations-B173

Pharmacodynamics-F50

Pharmacokinetics-F50

Pharmacotrauma-BS6

Phencyclidine (PCP)-K36

Phenethylamines-B190

Phenology-A125

Pheochromocytoma-H13

Phone Scatologia-E18

Phorate-K33

Photoanthropometry-C2

Photogrammetry-H82

Photograph-G36

Photographs-F13

Photometric Stereo-E36

Photoshop®-W19

Physical Assessment-E41

Physical Evidence-E14

Physical Matching-B150

Physical Restraint-H72

Physical Security-C22

Physicians for Human Rights-A47

Pig or Human-A44

Piperazine-K74

Pitfall-H50

Plasma Biomarkers-H131

Plastic-J13

PLOT-Cryoabsorption-B166

PMCT-H88, H89

PMI-E49

PMI Estimates-H103



Key Word Index- 2015

- PMI Estimation-H98
Pneumothorax-H81
Poisoning-K11, K31, W15
Poisonings-LW7
Polarized Light Microscopy-B54
Police-E60
Police Officers-I36
Police Restraint-H66
Policy-F11, F39
Pollen-B102
Polycystic Kidney Disease-H144
Polymer Films-B54
Polymer-Tipped Bullets-H69
Polyurethane Casting-B114
Ponding-D18
Pooling-B87
Pooling Technique-E38
Popliteal-H123
Population Genetics-B97
Portable-B144
Portable X-Ray Fluorescence-A27
Positional Asphyxia-E44
Positive Identification-A9, A65, A104, G1, G26
Post-Conviction Relief-G58
Postcranial Measurement-A74
Posteraniometric-A93
Postmortem-G9, G43, H57, K27, K34, K58, K71, K78
Postmortem Artifact-H70
Postmortem Artifacts-H25, H83, K65
Postmortem Blood-K28
Postmortem Case-K30
Postmortem Computed Tomography-A54, H62, H65, H81, W11
Postmortem CT-H83, H84
Postmortem CT Angiography-H90
Postmortem Descriptors-B106
Postmortem Distribution-K26
Postmortem Estimation-H34
Postmortem Fecundation-F3
Postmortem Hair Root Band-B148
Postmortem Imaging-H50
Postmortem Interference-G39
Postmortem Interval-A17, A21, A36, A38, A63, A64, A119, A120, H119
Postmortem Microbiology-A33, H97, H106
Postmortem Microbiome-H103
Postmortem TC-H93
Postmortem Toxicology-K36, K68
Postpartum Angiopathy-H20
Postpartum Stroke-H20
Potent Poison-K21
Power Lines-D40
PowerPlex® 16HS-B138
PowerPlex® Fusion-B70
Pregabalin-B64
Pregnancy-A78, H16, H52, H135
Preservatives-B18
Presumptive Test-B 175
Print-G17
Printing Inks-J18
PRNU-C1
Probabilistic Genotyping-B98
Probability-E68
Probability Analysis-A109
Probability of Drop-Out-B187
Probability Values at 18-G45
Probing-E8
Process Improvement-B104
Product Liability-D40
Productivity-B45
Profiling-G15, I17
Promethazine-K3
Prone-E60
Propellant Morphology-D26
Propellant Stippling-D26
Propylene Glycol-K65
Prosecutorial Misconduct-F31
Protective Factors-I9
Proteomics-B21, H78, H115
Proximity-B71
Psychological Autopsy-K41, K43, K44, K45
Psychopath-I33
PTSD-F52
Pubic Bone-A77



Key Word Index- 2015

Pubic Symphysis-A8, A24, A78, A80
Public Health-H128
Public Speaking-W16
Publication-D38
Puerto Rico-G8
Pulmonary Edema-LW6
Pulmonary Embolism-H144
Pulmonary Fat Embolism-H90
Pulmonary Granulomatosis-H152
Pupae-H24
Pyrimethamine-K59
Pyrosequencing-B124

Q

QIAcube®-B79, H112
QPCR-B99, B134, H110
QTOF-K20
Qualified-J28
Quality-B45, B145, F18, H147
Quality Assurance-H145, H150, W17
Quality Control-H145
Quality Process-J29
Quantification-B16, B85, B184
Quantiplex-B99
Quantitative Odor Measurement-B161
Quantitative PCR-B132
Quantitative Real-Time PCR-B93
Quarino-B43
Qubit®-B80
QuEChERS Extraction-K15
Questioned Documents-J8, J12, J16, J17, J23, J24, J31
Quicker 3130xl Detection-B146

R

Radiant Heat-D43
Radioactive-E30
Radiograph Comparison-A9
Radiographic-A98
Radiographic Data Bank-A52
Radiographs-A104, A131, G33
Radiography-A106
Raffaelea Lauricola-E11

Raman-B122
Raman Mapping-B49
Raman Spectroscopy-B2, B84, E31, J17
Rape-E54
Rape Kits-E46
Rape Myth-E27
Rapid-B144
Rapid Direct PCR-B67
Rapid DNA-B10, B143
Rapid Drug Screening-K15
RapidHIT™ System-B141
Rapidly Mutating-B182, H116
RbcL-B93
Reaction Time-E43
Realignment-I11
Rear Impact-D1, D5, D14
Rear Impact Collision-D11
Rebleeding-H43
Recidivism-I19, I23
Reconstruction-BS4, D31
Records-G37
Recovery Efforts-A109
Redistribution-H123
Reference Database-B116
Reid-B43
Reliability-D28, G14
Reliability and Validity-F30
Religious Relics-LW3
Relocation Tables-C23
Remains-A3
Remains Recovery-E58
Remodeling-A103
Remote Sensing-A125
Report to the State Prosecutor-H47
Reproducibility of Marks-E36
Reroofing-D18
Research-G51, S2
Research and Development-B42
Research Gaps-B40
Resequencing-B3
Residential Origin-A123



Key Word Index- 2015

Residue-B189
Resistance-F26
Restraint-E44
Restrains-E60
Resuscitation Injury-H22
Retention-E32
Retention Time Locking-B48
Retentive Characteristics-B188
Retrograde-K48
Retrospective-A110
Retrotransposon-B133
Revenge-I35
Reye's Syndrome-H122
Rhantus Validus-H25
Rheometer-D10
Rheumatic Heart Disease-H132
Rib Fractures-H53
Ribosomal Inactivating Protein-B58
Ricin-K40
Ricochet-B120
Ridge Flow-B107
Rim Gouges-D51
Risk-I24
Risk Assessment-I5, I19
Ritual-A49
RNA Profiling-B52
Roadside Testing-K46
Robert Blake-LW2
Robots-C16
Role of Nurses-I14
Roof Collapse-D18
Root Cause Analysis-F18
Roots-B22
Rule 16 and Rule 26-J23
Run Over-H58

S

SAC-E70
Sacrum-A88
Sadism-W18
Safety-D57, I8
Saliva-G7

Salvinorin A-K17
Sample Archiving-B75
Sample Collection-B9
Sample Preparation-K22
Scene-H153
Scene Evidence-H53
Scene Investigation-E20, H146
Science of Digital Forensics-C14
Scientific & Technical Advisor-B44
Scientific Data-F41
Scientific Evidence-F44
Scientific Investigation-F28, K45
Scientific Literature-D38
Scientific Method-A113, BS4
Scientific Standards-F41
Scientifically Valid-F27
Scopulariopsis-H1
Screwdriver and Knives In Situ-H63
Seat-Adjuster Failure-D1
Seat and Belt Failure-D2
Seat Belt Failure-D4
Seat Failure-D4
Security-D57
Security Features-J11
Seized Drugs-B24
Selective Suppression-C13
Self-Organization-H108
Self-Strangulation-H67
SEM-B32, G20
SEM/EDS-G24, G52
SEM/EDX-B53, B121
Semen-B125, B128, B129
Semen Identification-B84, B127
Sengal-G17
Sensitivity-B177
Sentencing-F54
Sequencing-J20
Serial Murder-E23
Serology-B21
SERS-B84, K39
Sewer Gas-H54



Key Word Index- 2015

Sex-A103
Sex Assault-E16
Sex Assessment-A48, A75
Sex Determination-A6
Sex Estimation-A5, A7, A10, A25, A51, A56, A72, A73, A74, A76, A90, A91
Sex Offense-I19, I21
Sexual Abuse-I20
Sexual Assault Kits-E46
Sexual Dimorphism-A55, A94
Sexual Offender-I4
Sexual Offending-I23
Sexual Predator-F13, L2
Shaken Baby-D13
Shaken Baby Syndrome-F33, H47, W20
Shaking Impact-D15
Shaped Impacters-A99
Shark Predation/Scavenging-A32
Sharp Force Injuries-E12
Sharp Force Injury-H70
Shifted Science-W8
Shooting Reconstruction-B112, B120
Shootings-E43
Short-Listing Capability-A106
Shroud of Turin-E39
Siblings-B88
Sideswipe Accident-D52
Sideswipe Case Studies-D52
SIDS-H137
SIDS/SUID-H37
Signature Comparison-F30
Signature Examination-W13
Silk-B154
Siloxanes-B168
Similarity-J5
Simulated-J11
Simulation-J21
Single-Base-B160
Single Molecule-B185
Single Nucleotide Polymorphism-B3, I3
Single Tooth-G8
Skeletal Abrasion-A62
Skeletal Aging Methods-A8
Skeletal Gunshot Trauma-A134
Skeletal Identification-A39
Skeletal Morphology-A46
Skeletal Remains-B87, B131, H149
Skeletal Sample-A79
Skeletal Trauma-A32
Skeletal Variation-B130
Skeletonized Remains-B104
Ski Collision-D47
Ski Patrol-D47
Skull Face Fragments-G23
Skull Fracture-A67
SLE-K38, K75
Small Particle Reagent (SPR)-B109
Smart Drugs-K52
Smile-G57
Smoke Damage-D45
Smokeless Powder-B30
Smokeless Powders-B33, B78
Smokeless Powders Database-B160
Snow-G18
Snowmobile-H125
SNP-B186
SNPs-B75, B97, B140, B144, E10
Social Dangerousness-F1
Soft Tissue-D10
Software Implementation-D61
Soil Bacteria-H105
Soil Bacterial Profiling-B91
Soil Evidence-B92
Soil Provenance-H36
Soils-H26
Solder-B83
Sotalol-K66
Source/Activity Level-F38
Sourcing-B159
South Korea-B31
Spa Pool-D41
Spatial Refinement-A123
Speaker Recognition-D54



Key Word Index- 2015

- Speaking Skills-W16
Special Abilities-E72
Species Differentiation-A19
Spectra-B151
Spectral Comparison-B49
Spectral Imaging-B123
Spectral Library-B26
Spectrometer-B183
SPE-K13, K38, K75
Sperm-B126, B128
Spermatozoa-B71, E32
Spheno-Occipital Synchronosis-A130
Sphygmomanometer-H67
Spinal Injuries-D12
Spiral Fractures-H40
Splenic Aneurysm-H19
Splenic Artery Aneurysm-H135
SPME-B157
Sputter Coating-B114
Stab and Sharp Blunt Injuries-H63
Stab Wounds-H59
Stable Isotope Analysis-A114, B103
Stable Isotopes-E62
Stage H Errors-G45
Stalking-I12
Stamp Bag-H29
Standardized Protocols-H105
Standardized Test Answer Marks-J19
Standards-D17, E67, E70, F12, J25
Static Collection-B59
Statistical Analysis-B91
Statistics-B33, B167, J3
Stature-A23, A60
Stature Estimation-A11, A50, A81
Steganalysis-C20
Steganography-C20
Stereophotogrammetry-D60
Sternal Fourth Rib-A5
Sternum-A74
Stillbirth-H154
Stoichiometry-B196
Storage-H32
STR-B7
STR Analysis-B69, B90
STR Multiplex-B74, H116
STR Profiles-B179
STR Profiling-B5, B13
STR Typing-B136, B137, B138
Strangulation-H65
Strategic Preparation-F8
Strengthening Forensic Science-B42
Stress Management-BS2
Stroke-H143
STRs-B140, E10
STRs and MtDNA-B4
Student Achievement-E51
Subadult Age Estimation-A130
Subadult Sex Estimation-A71
Subdural Neomembrane-H43
Subjectivity/Objectivity-A111
Submerged Items-B109
Submergence-H24
Substrate Contribution-B164
Sudden Cardiac Death-H93, H113, H130, H134, H142
Sudden Death-E65, H12, H15, H19, H92, H132, H133, H138, H139, H140
Sudden Death in the Young-H37
Sudden Infant Death Syndrome-H39
Sudden Intrauterine Death-H8
Sudden Unexplained Death-H39, H49
SUDEP-H133, H136
SUDS-H137
Sufficiency-E71
Suffocation-I38
Suicide-E12, E19, E55, E56, H59, H80, H126, H127, I1, I36, I38, K29, K41, K44
Suicide Method-E19
Suicide Note-E19
Suitability-E71
Sunlight-K12
Support Vector Machines-A92
Surface Scanning-E50
Surveillance Video Analysis-D46



Key Word Index- 2015

Suspicious Death-I1
Swab From Firearm-E5
SWGDOC-J31
SWGDRUG-B169, B193
Sympercents-A94
Synthetic-K51
Synthetic Cannabinoids-B26, B193, B194, K8, K39, K53, K56, K57, K72
Synthetic Drugs-B170
Synthetic Opiates-K64
System Thinking-B72

T

Taggants-B159
Tanax® (T-61®) Injection-K29
Tap Water-E62
Tape Lift-B28
Tape Lifting-B135
Taphonomy-A19, A21, A32, A42, A43, A64, H97, H118, K69
Target Analytes-D33
Tattoos-E6
TBS-A37
TD-GC/MS HPLC-J1
Teachers-I20
Teaching-F32, LW5
Technical Investigations-F23
Techniques-H123
Technology-G53
Temporal Variability-H36
Testamentary Capacity-I26
Testimony-D37, ES1, F12, W24
Testimony List-J30
Testing-C24
Text Classification-D53
Textile Fibers-B152
TGA-A16
Thanatology/Postmortem Changes-H89
Thanatobiome-H104, W23
THC-H120
The Brady Doctrine-F31
Theory-A110, A112, A116, A118, A121
Thermal Burn-D41
Thermoregulation-H108
Thermoregulation Behavior-H100
Threats-I5, I10
Three-Point Bending-A96
Threshold Diagnosis-F14
Thromboses-H14
Time of Colonization-H99
Time Series Analysis-A38, A45
Timeline-B123
Time-of-Death Estimation-H129
Time-of-Flight (TOF)-K76
Time Since Death-A36
Tissue Decomposition-H4
Titanic-A69
TLC-SERS-B172
TOCSY-B17
TOF-K20
TOF/MS-K57
Toner-J20
Tool Mark Analysis-B118
Tool Marks-A66
Tool Validation-C18
Topography-W12
Tor Browser-C11
Torture/Scars-E41
Total Body Scoring-A63
Touch DNA-B9, B11, B38, B68, B135, B136, B137, B178, B180, E5
Touched Items-B179
Toxicological Findings-H138, K33
Toxicological Screening-K20, K76
Toxicology-H80, H107, H121, K35, K39, K41, K42, K59, K67, K69, K77, K78
Trace Detection-B29
Trace Elements-B83
Trace Evidence-B20, B36, B101, B147, B148
Trace Metal Signatures-B63
Tracing-J21
Tracking-H23
Traffic Radar-D27
Training-F45
Training Aid-B59, B161



Key Word Index- 2015

Transfer-B126
Transgender-A48, I22
Trauma-A100, A129, A133, A135, H96
Trauma Anthropology-H40
Trauma Classification-A97
Trazodone-K28
Treatment-I21
Trend-B31
Trends-B170
Trials-BS5, F45
Tribometry-D16, D20
Triglyceride Analysis by LC/MS-B165
Trotter and Gleser-A11
Troubleshooting-H112
Turkey-G5, I32, I36
Turkish Criminal Law-I7
TVA Kingston-D37
TWGPAM-H87
Typestyle-W3
Typewriter-W3

U

UHPLC-B193
Ultraviolet Light-A41
Uncertainties-D31
Uncertainty-D28, D29, F24
Unclaimed-W10
Uncommon Suicide-H67
Undocumented Border Crossers-A18, A68, A132
Undue Influence-I26
Unexpected Death-E65
Unicode-J15
Unidentified-W10
Unidentified Border Crosser-A107
Unidentified Persons-G28
Unintentional Injuries-I30
Unique Samples-B177
United Arab Emirates-B141
United States Service Member-H124
Unknown Ancestry-A81
Unknown Screening-K24
Unmanned Systems-C16

Unpadded Vehicle Structure-D2
Untested Rape Kits-E46
UPLC/MS/MS-K5
Urban-H127
Urine-K8, K12, K17
Utility Lines-D44

V

Vacuum Swabbing-B137
Vadose Zone-D35
Valid Data Length-C19
Validation-A31, B23, B58, B98, F40, H4, J1, K14
Validity-A113
Valsalva Sinus Aneurysm-H3
Vapor-B62
Vaporizer-K63
Variation-J4, J5
Variations and Uniqueness-E4
Vascular System-H89
Vasculitis-H12
VDL-C19
Vegetable Oils-B163
Vegetable Oils and Fats-B165
Vehicle Occupant Ejection-D4
Vehicle Occupant Injury-D1
Vehicles-B53
Ventilation-W8
Ventilation-Limited Fires-F6
Verdicts-D31
Vertebra-A75
Vertebral Maturation-A29
Veterans-F52
Victim Identification-W1
Victimology-W6
Violence-E13, G57, I5, I8, I34, LW1
Violence Prevention-I31, W22
Violent Behavior-I9
Violent Children-I31
Violent Crimes-E15
Virtopsy-H68, H94
Visibility-D49
Visual Biometrics-C5



Key Word Index- 2015

Visualization-D58
Vitamin D-Related Rickets-H17
Volatile Organic Compounds-B60
Volumetric Analysis-A130
VSC-J9
Vulture Scavenging-A43

W

War Dead-A79, A80
Washing-E32
Washing Machine-B126
Water-E57
Water Damage-D45
Wearable Sensors-W14
Weathering-B35
Weight Measurements-B55
Weight of Evidence-E68
WGA-B75
Whiplash-D12
Whistle Blowing-B47
White-Collar Crime-I33
Whole Blood-K57
Whole-Genome Sequencing-H114
Wildfire-BS1
Wildlife Forensics-E29
Witness Statements-D53
Workflow-B79
Wound Interpretation-F34
Writing Ink Dye Analysis-J2
Wrongful Conviction-G58
WWII-E58

X

XPS-G2
X-Ray-F20, G52

Y

Y Chromosome-B182, H116
YFSF-S2
Young Adults-E65
Youth Gangs-W22
Youth Violence-W22
Y-STR-B7, B182

Z

Zolpidem-K49, K50, K58
Zombie-H69
Zoophilia-I23
Zygomatic Bone-A51



Presenting Author Index- 2015

The presenting author index can provide a quick reference to find when and in what section presenting authors are scheduled to present at the 2014 Annual Scientific Meeting. The reference table below assists you in finding the section in which the

presentation will be given. Letters correspond to the scientific discipline/section in which the presentation is being made while the number corresponds to the numerical sequence of the presentation within the section.

A	Anthropology	J	Questioned Documents
B	Criminalistics	K	Toxicology
C	Digital & Multimedia Sciences	LW	Last Word Society
D	Engineering Sciences	BS	Breakfast Seminar
E	General	ES	Evening Session
F	Jurisprudence	L	Luncheon
G	Odontology	S	Special Session
H	Pathology/Biology	W	Workshop
I	Psychiatry & Behavioral Science		

A

Abdel Fatah, Emam E.-A136
Adams, Donovan-A108
Adams, Nathaniel D.-B86
Adcock, James M.-E25
Adserias, Joe-A39, B5
Afsin, Huseyin-G5
Ah Sam, Stephanie R.-W17
Ahmad, Taha-K32
Ahmed, Irfan-C23
Akiyama, Cliff-W22
Al Na'imi, Khudooma S.-E38
Aleksander, A.K.-D41, D47
Alexander, Peter-D31
Alfter, Alicia-B150
Alghafri, Rashed-H116
Alhattali, Zaina-E38
Ali, Amina-I38
Ali, Leah-B121
Ali, Zabiullah-H84
AlQahtani, Sakher J.-G44
Alsadi, Alaa-E65
Alshehhi, Aamer-B141
Altes, Kristina B.-A48
Amadasi, Alberto-A15, A34

Amick, Gray-B10
Anderson, Bruce E.-B106, W5, W10
Anderson, Dan T.-K42
Anderson, Robert L.-D6
Anderson, Russell L.-D52
Andrews, Sam W.-H145, W11
Andronowski, Janna M.-A56
Annunziata Nicolaidis, Kathleen-J14
Anthony, Arthur T.-J24
Antinick, Timothy C.-B130
Appleton, Avery J.-A62
Aquila, Isabella-H18, H73, I18
Arboe II, David K.-H13
Argo, Antonina-H21
Ashton, Jeffrey L.-BS8
Astin, Justin C.-H119
Atit, Priyanka-A58
Auberon, Cindy-H28, H100

B

Baber, KariAnna-C5
Baerncopf, Jamie M.-B162
Baez, Jose A.-F52
Bagley, Chandra-B138
Bahng, Seung-Hoon-J2
Bailey, James A.-E64

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Presenting Author Index- 2015

- Bailey, Kristen M.-K27
Baker, Charlotte A.-K58
Baker, Joan E.-A80
Balachandra, Thambirajah-H126
Ballantyne, John-B90, F38, W4
Baraybar, Jose P.-A129, W9
Barsley, Robert E.-S1
Bartelink, Eric J.-B103
Bashford, Martha-F13, L2
Baumgarten, Sarah-A73
Bayer, Lindsey A.-E22
Beaver, Aaron-H29
Becker, Ericka A.-H143
Becker, Stefan-B16
Begert, Matthew-D57
Bell, Suzanne-D29
Bellis, Maggie-H6, H57
Beltempo, Pasquale-H72
Bemelmans, Elena A.-B125
Benbow, M. Eric-W23
Benjamin, David M.-F31, LW6
Berends-Montero, Shirly-E33
Beresheim, Amy C.-A8
Berg, Gregory E.-W9
Bernet, William-F35
Bernhard, Werner-K23
Besser, Marcus P.-D16, D19
Best, Kaleigh C.-A26
Bijhold, Jurrien-W14
Billick, Stephen B.-I30
Bintz, Britannia J.-B132
Bird, Cate E.-A132
Bishop, Michael-E9
Bitter, Julie L.-B189
Bjerkhoel, Alissa L.-F42
Black, Valda-A72
Bleakley, Jacqueline E.-E57
Bless, Bethany L.-E12, E21
Blessing, Melissa M.-H125
Block, Sean T.-B83
Boeyer, Melanie E.-A52
Bohan, Thomas L.-D28, S1
Bongiovanni, Rosanne-A89
Bono, Joseph P.-F41
Borrini, Matteo-E39, G3, LW5
Bors, Dana-B157
Bosse, Michael J.-E15
Bouchardet, Fernanda Capurucho Horta-G22
Boulay, Julien-H23, H108
Boyd, Charles C.-A119
Boyd, Derek A.-A63
Boyd, Donna C.-A110
Brady, Thomas V.-I25
Bran, Danna N.-A25
Brandhagen, Michael D.-B134
Bready, Jennifer C.-E19
Bready, Robert J.-E19
Brehe, Rebecca J.-B57
Brehmer, Jeremy C.-F50
Brent, Jeffrey-W15
Bresler, Scott-F36, I5
Brettell, Thomas A.-B196
Brosz, Helmut G.-BS1, D40
Brown, Carrie A.-A88
Brown, Gary R.-D37
Brunelli, Ronald-W10
Bruno, Thomas J.-B166
Bryant, Craig M.-B165
Bugajski, Kristi-H102
Bugelli, Valentina-H30, H32
Burgee, Lisa-B80
Burgess, Ann Wolbert-L1
Burkes, Ted M.-J31
Burnett, Annessa L.-B176
Burnett, Bryan R.-LW2
Burns, Tesa L.-A71
Busardo, Francesco P.-H138
Bush, Peter J.-G52
Bussiere, Genevieve D.S.-G55
Butler, John M.-F9, F37
Butler, Kate-J7
Buzzell III, Lewis H.-W24



Presenting Author Index- 2015

Buzzini, Patrick-J17
Byrd, John E.-W17
Byrnes, Bradford-W17
Byron, Douglas E.-W7

C

Campbell, Courtney A.-K3
Can, Ismail-H104, W23
Cansino, Gabriella-I3
Cappella, Annalisa-A82
Carabellese, Felice F.-I9, I15, I16
Carlson-Greer, Sean Y.-A75
Carman, Steven W.-F6, W8
Carr, Mary-E27
Carter, David O.-H106
Case, Mary E.S.-E28, W21
Celik, Safa-H46
Cengiz, Salih-B1, J1
Chabaud, Kathryn R.-B30
Chancellor, Arthur S.-E16
Chang, Joseph P.-B184
Chapin, Megan-A74
Charabidze, Damien-H98
Chaski, Carole E.-D53, D55, D56, F19
Chavira, Cynthia-I11
Chen, Michael-K72
Chesson, Lesley A.-A114
Chezem, Linda L.-F32
Chila, Melissa M.-B11
Chmaj, Julia D.-A77
Cho, James P.-F36
Choi, Julia-H16
Christensen, Alexander F.-A79
Christensen, Angi M.-J16
Christensen, Erik D.-K78
Christman, Daniel V.-E45
Christophe, Deion P.-W12
Chu, Fanny-B174
Chu, Sarah-F11
Chu, Wei-B108, B115
Churchill, Jennifer D.-B139
Chute, Dennis J.-H10

Clark, Brian S.-BS3
Clark, Kyle J.-K47
Clark, Steven C.-W10
Class, Pamela-B109
Clément, Renaud-E41
Clermont Beaudoin, Valerie-B75, B140
Close, Sandra L.-B97
Coble, Michael D.-B94, B182
Collini, Federica-A135, I35
Collins Gaines, Michelle L.-B79
Collins, Kelsey-A9
Collins, Kim A.-E20
Combs, Laura G.-B131
Congram, Derek-A124
Conigliaro, Aime-G43
Conlogue, Gerald J.-E34
Connon, Catherine C.-B146
Cornett, Charles R.-B62
Cornthwaite, Heather M.-K16
Costello, Carrie-E24
Craig, Emily A.-W10
Crawford, Karyn-B122
Crooks, C. Richard-K28
Crowder, Christian-A116, W1
Cunningham, David-B28
Cunningham, Susan M.-K27

D

D'Alonzo, Susan Steele-A106
D'Anjou, Corinne-G40
Dabbs, Gretchen R.-A37
Dadour, Ian-H101
Daimaru, Shohei-D25
Damaso, Natalie-H36
Daniel, Kelly-E30
Dautartas, Angela M.-A45
David, Thomas J.-G31
Davis, Christie T.-F40
Davis, Gregory B.-F45
Dawson Cruz, Tracey-B180
De Carlo, Dania-H77
De Crisce, Dean -K41



Presenting Author Index- 2015

De Forest, Peter R.-B39, B112, F28
Deaton, Sherri-H33
Decker, Lauren A.-H65
Deibler, Amy-H2
deJong, Joyce L.-H96
Delannoy, Yann-A17
dell'Aquila, Massimiliano-H9
DelMundo-Galicia, Marisa-H133
Demas, John P.-G58
deMenna, Quinn-D17
Dempsey, Sara-K5
Dennis, Dana-Marie K.-B160
Deppen, Caitlyn-B127
Derrick, Sharon M.-W11
Desiderio, Jr. Vincent J.-F22
Desranleau, Sylvain-G26
Di Battista, Benedetta-H71
di Luca, Alessandro-F4, H31, H129
Di Nunzio, Ciro-H73
Di Vella, Giancarlo-G21, H11
Diaczuk, Peter J.-B120
Diaz, Francisco J.-E56
Dieng, Khalifa-G17
Dierksen, Jennifer-H121
DiFrancesco, James M.-B128
Dolan, Julia A.-W7
Dominguez, Victoria M.-A103
Domitrovich, Stephanie-E73, F27, F35
Donoghue, Edmund R.-S1
Dorion, Robert B.J.-G12, G26, G40
Doty, Kyle C.-E31
Dowdy, Liotta N.-A20, W9
Downey, Lotte-B4
Downs, J.C. Upshaw-ES1
Downs, Jennifer L.P.-ES1
Doyle, James M.-W8
Dross, Ryan-B25
Druid, Henrik-H74
Dubois, Timothy J.-H153
Dudley, Mary H.-H54
Dudzick, Beatrix-A57

Duecker, Hailey A.-A68
Dunn, Tyler E.-A31
Dunphy, Melissa-A64
Duval, Janet B.-W22
Dvorscak, Lauren E.-H39
Dwyer, R. Gregg-BS2, I21
Dye, Emily-B170

E

Edelman, Gerda-B123, W14
Edson, Suni M.-B104
Efeoglu, Pinar-K10
Egger, Coraline-H89
Eisenberg, Arthur J.-W4
Elian, Albert A.-K13
Elifritz, Jamie-W11
Ellefsen, Kayla N.-K54
Ellingham, Sarah-A16
Endicott-Popovsky, Barbara E.-C13
Enslow, Sandra R.-E52
Errickson, David A.-E50
Estrada, Irene A.-E14
Evans-Nguyen, Kenyon M.-B191

F

Fabrice, Dedouit-H50
Fabyanic, Emily B.-B34
Fahnestock, Katherine A. S.-B168
Fais, Paolo-H86
Fancher, James P.-G56
Farrell, Amanda L.-W18
Farrugia, Audrey-H113
Fedoroff, J. Paul-I21
Felthous, Alan R.-W2
Fenton, Todd W.-A100
Ferguson, David G.-C19
Ferrazzano II, Stephen J.-W2
Ferreiro-Gonzalez, Marta-B15
Fesolovich, Jillian C.-B187
Fikiet, Marisia A.-B172
Filippi, John E.-W10
Findley, Keith A.-W20



Presenting Author Index- 2015

Finkelstein, Marissa J.-K25
Finlayson, Janet E.-A27
Finley, Sheree J.-H105, W23
Fischer, Christopher-I28
Fisher, Barry A.J.-B47, S2
Flach, Patricia M.-W11
Fleming, Diana-B129
Flores, Alejandra-B153
Floyd, McKenzie-B154
Focardi, Martina-H60
Fojas, Christina L.-A61
Foley, Dustin-B98
Foonberg, Alan M.-D57
Foran, Brendan J.-D57
Forbes, Shari-E8
Fornaro, Stefania-H19, H30
Forrest, Alexander S.-G2, G4, G11, G20
Fowler, Faith-B17
Fowler, Gillian M.-A47
Fox, Matthew F.-E65
Franck, Darren-D43, D51
Franck, Harold-D12
Franklin, Frank A.-H154
Franza, Annarita-BS5
Fraser, Diane B.-S2
Fredette, Sara M.-A28
Freeman, Michael-D11
Freeman, Tierra M.-E69, F30
Friedman, Emily-I30
Fries, Craig T.-D58
Frischia, Melissa-K55
Funk, Christine-S2
Furnari, Winnie-G16

G

Gable, Kelly N.-W2
Galarza, Brenda-W10
Gallo, Nicole-E32
Gantz, Daniel-B107
Gantz, Donald T.-B107
Gardner, Elizabeth A.-B66
Gardner, Taylor L.-G39
Garofano, Luciano-K45
Garvin, Heather M.-A98
Gastineau, Tracy-K17
Gauthier, Quentin T.-B85
Geberth, Vernon J.-W6
Geniuk, Steven-E16
Georget, Charles E.-G9
Geraci, Alberto L.-D61
Geradts, Zeno J.-C1, C4, S1, W14
Gerns, John E.-S1
Gerrard, Chandra-W11
Gibelli, Daniele M.-D60
Gill, James R.-K78
Gilson, Thomas P.-H155
Gische, Melissa-D30
Gittelsohn, Simone-E68
Gitto, Lorenzo-H38, H68, H95
Glasgow, James-W21
Goff, Mark-W19
Goldberger, Bruce A.-S1
Goldsmith, Eric-I27
Goodson, Mark E.-D27, W8
Gopalakrishnan, Anupama-H110
Goss, Jessica M.-B58
Graham, Michael A.-H96
Grandhi, Natasha L.-H128
Grattagliano, Ignazio-I12, I13, I14
Green, Ashley-A49
Green, Jordan B.-C20
Green, Matthew K.-B171
Greenberg, Tasha Z.-H45
Greytak, Ellen M.-B186
Grgicak, Catherine M.-B72, B96
Griffin, Ashlee R.-A33
Grimm, Jochen-H87
Grimmer, Nani M.-B144
Gross, Ann Marie-B88
Gross, Susan-B88, B149
Gruler, Andy-BS2
Guerrera, Gina-B164
Guidi, Benedetta-H30, H92



Presenting Author Index- 2015

Guido, Mark D.-C21
Gulmen, Mete K.-I7, I34
Gunther, Wendy M.-H37
Gupta, Avneesh-E56, H156
Gurney, Susan M.-K53

H

Hackett, Jeffery-K13
Haden-Pinneri, Kathryn H.-H146, W5
Hadka, Michael C.-D34
Hafer, Kaitlin E.-B197
Haines, Alicia M.-H111
Hainsworth, Sarah V.-D23
Hale, Amanda R.-A38
Hall, Adam B.-B199
Haller, Deborah K.-B129
Hammond, Heather M.-B53
Han, Joseph-D57
Hanson, Erin K.-B52
Hanson, Lisa M.-J6
Hanzlick, Randy L.-W10
Hardin, Glenn G.-F21
Harman, Stefany E.-B45
Harnarine, Nina A.-J12
Harris, Howard A.-W14
Hart, Gina O.-H40
Hart, Walter T.-C22
Hartman, Rebecca L.-K63
Haskell, Christine-F15
Haskell, Neal H.-E49
Hatch, Gary M.-W11
Hatza, Ani N.-A81
Hayat, Ghazala-W2
Hayes, Jonathan-H96, L2
Haywood, Charles L.-J5
Hefner, Joseph T.-A92, W5
Hellwinkel, Donna J.-G29
Henderson, Carol-S1
Hendrikse, Jeanet-B14
Hentschel, Kelsee-A133
Herman, Martin-C17
Heurich, Charles M.-E53, W10

Hewitt, Terry-Dawn-F7, W8
Hicklin, R. Austin-E70
Hietpas, Jack-B78, B148, B158
Higdon, Sarah A.-H15
Hinchliffe, Judith A.-G25
Hinman, Dayle L.-E23
Hiquet, Jean-K70
Hitchcock, Mike-B64
Hoffman, Amanda J.-B12
Holland, Thomas D.-W17
Holmes, Dawn B.-H120
Holmes, James-W9
Holoyda, Brian J.-I23
Holsworth, Kayla-H112
Honig, Daniel M.-D18
Hoover, Hillary M.-J11
Houck, Max M.-F18
Howe, Julie A.-E20
Howell, Todd M.-W1
Huang, Chia-Hung-H27
Huertas, Adhly M.-B60
Huestis, Marilyn A.-K60
Huffman, Lurena A.-W18
Hughes, Cris E.-A1
Humphries, Ashley L.-H53
Hunt, David R.-A46
Hunt, Ted R.-F8
Hunter, Cheryl D.-S2
Hutchins, Chad W.-BS4

I

Ifimov, Gabriela-E26
Igari, Yui-H62
Ingvoldstad, Megan E.-A53
Inman, Keith-B100
Inoue, Christopher G.-H109
Introna, Francesco-A2, G23
Irvine, Jessica-B84
Irvine, Rebecca-H20
Isa, Mariyam I.-A96
Ismath, Muzeen-H126
Ivory, Matthew A.-D46



Presenting Author Index- 2015

J

Jackson, Glen P.-B198
Jackson, Kimberly-B142
Jacques, Rebekah-H17
James, Rohaizah-B7
Jantz, Richard-W5
Jarvis, Hannah C.-H80
Javan, Gulnaz T.-W23
Jefferys, Roger-B34
Jentzen, Jeffrey M.-E73, F20, F33, W10, W21
Jeong, Yangseung-A11
Jesmok, Ellen M.-B91, B92
Johnson, Mark-J3
Johnson, William R.-K51
Johnston, Cheryl A.-A121
Jones II, John Paul-E67
Jones, Marc-H61
Joshi-Kumar, Monica-B56
Junkins, Emily-H97

K

Kacinko, Sherri L.-K59
Kahn, Roger-H137
Kamenov, George D.-W9
Kassin, Saul-W20
Kauffman, Jr., Mark A.-D35
Kavanaugh, Stephanie-G27
Kedzierski, Nancy A.-K77
Keeling, Sarah A.-B48
Kelbaugh, Cristina L.-A76
Kelly, Anna-K71
Kendell, Ashley E.-A107
Kennedy, Roderick T.-F26
Kenney, John P.-G18, G33, S2
Kessler, Gary C.-C15
Khalil, Angela-A95
Kibayashi, Kazuhiko-K37
Kikkawa, Hitomi S.-B93
Kim, Jieun-A61
Kimmerle, Erin H.-W9
Kindell, Jessica H.-B35

King, Pamela A.W.-F10
King, Patricia-E20
Kingsbury, Stephanie A.-J13
Kishida, Ryotaro-D10
Kisler-Rao, Anne-B22
Klales, Alexandra R.-A71, A126
Klein, Nicole S.-A35
Klim-Lemann, Janice W.-G34
Klobut, Jakub-K38
Kobylanski, Kelly-H127
Koch, Sandra-B101
Koel-Abt, Katrin-A19
Kolpan, Katharine E.-A70
Korenis-Rios, Panagiota-I38
Koutrakos, Andrew C.-B156
Kovari, Ivett-A109
Krishan, Kewal-A50, E4, E42
Kronstrand, Robert-K57
Kruger, Gabriele C.-A94
Krywanczyk, Alison-K64
Kubista, Nicole-F43
Kuehnert, Kristiana M.-B99
Kulbacki, Kevin P.-J3, J10
Kulick, Kelley-F16
Kushner, Danielle-I27
Kushwaha, Priyanka-H26
Kwon, Ye Jin-B13

L

Laforte, Sylvain-G26
Lal, Anita-H151
Langenburg, Glenn M.-W20
Lanners, Brenda N.-J26, W13
LaPorte, Gerald M.-B42
Lathrop, Sarah-H85
Law, Eric-B34
Leach, Jennifer-K18
Lednev, Igor K.-B2, E31
Lee, Dayong-K67
Lee, Jaesin-B31
Lee, Josh D.-F48, F50
Lee, Jr., F.L. Jim-J31



Presenting Author Index- 2015

Lee, Soong Deok-B3
Lee, Steven B.-B67
Leeper, Bobbie J.-A14
LeGarde, Carrie B.-A23
Legg, Kevin M.-B21
Lemaire, Eric-H123
Lemoine, Maxime-B19
Lentini, John J.-F5, W8
Leopold, Craig-K8
Lesciotto, Kate M.-A128
Lesnikova, Iana-H4
Lesta, Maria Del Mar-H90
LeVaughn, Mark M.-H149
Lewis, Jane A.-J4, S2
Lewis, Jr., John A.-G30
Lewis, Jr., Paul R.-D2, D4
Lewis, Lyniece-A40
Liebenberg, Leandi-A93
Lilien, Ryan-B118
Linville, Jason G.-B74
Livelsberger, Benjamin R.-C18
Lloyd, John D.-D7, D9, D13, D15
Lo Pinto, Sara-H67
Logan, Barry K.-S1
Loomis, Peter W.-G41
Lopez, Maria A.-A134
Lopez-Gobernado, Carlos J.-E1
Lopez Morell, Lorraine-H49
Lösch, Sandra-A54
Lottering, Nicolene-A130, S2
Love, Jennifer C.-A117
Lu, Aileen-K49
Lubelli, Sergio-C10
Lucas, Victoria Sorrell-G45
Luckasevic, Todd M.-H69
Luo, Kathleen K.-K74
Lurie, Ira S.-B193
Lusa, Vincenzo-BS5, F1, F2, I10
Lynch, Robert D.-D17

M

MacCrehan, William A.-B161

MacDonald, Lauren-B135
Magni, Paola A.-H24, H25, H56, H75
Maiese, Aniello-H59, H68
Maldonado, Betzaida L.-B70
Mancebo, Laura A.-J20
Manheim, Jeremy M.-E2
Manica, Scheila-A86
Mannocchi, Giulio-K30
Mares, Mollie-K52
Marginean, Ioan-B194
Marlow, Tanya L.-E63
Marpert, Mark I.-D20
Marshall, Judy Y.-G48
Marshall, Pamela L.-B77
Martell, Daniel A.-I26, S1, S2, W20
Martin, Corinne E.-B50
Martinez, Claudia-B20
Massaro, Luca-F1, I1, I10
Massiello, Autumn-K66
Masson, Janet F.-J19
Mauriello, Thomas P.-E40, W16
Mavroudas, Sophia-A18
Maxwell, Ashley B.-A104
Maxwell, Julie-F46
Mazoori, Dallas-A47
Mazoori, Zabi-A47
Mazuchowski II, Edward-W1
McAndrew, Thomas C.-W6
McCarthy, Michael J.-I6
McClarín, Derrick-W12
McClary, Carl R.-J23
McCord, Bruce R.-W4
McCormick, Kyle A.-A24
McCormick, Lara E.-A102
McDonald, Kaylee R.-K2
McDowell, Michael D.-D44
McDown, Robert-C12
McGibbon, Steven E.-E9
McGonigal, Maura K.-B190
McLeod-Henning, Danielle L.-E53
McMahon, Timothy P.-W4



Presenting Author Index- 2015

McNally, Gregory A.-J22
McShane, Justin J.-F48
Meade, Jennie-LW4
Meeker, Alexis-B8
Meeusen, Rebecca-A91
Melito, Stephen J.-K11
Merlino, Mara L.-E69, F30, J27
Mesa, Rodolfo-B188
Mesli, Vadim-H55, H118
Metcalf, Roger D.-G34
Meyers, Patrisha L.-A21
Middleberg, Robert A.-W15
Mikellide, Maria-A127
Milani, Chantal-A60
Miles, Harry L.-F19
Miller, Elizabeth A.-A22
Miller, Raymond G.-G53
Miller, Ross James-H1
Millette, James-D32
Milroy, Chris-BS7
Min, Jisook-E35
Minsky-Rowland, Jocelyn D.-A61
Miranda, Shirley-G50, G51
Miranker, Molly-A4
Mitchell, Erik K.-H48
Mitchell, Linda L.-J27
Mitchell, Randolph L.-G37
Miziara, Carmen Silvia M.-E13
Miziara, Ivan D.-E7
Moffatt, Ellen-H134, K78
Mohammed, Linton-W13
Mohr, Amanda L.A.-K9
Mohs, Amanda J.-B173
Moody, Heather T.-B82
Moore, Christine-K46
Moore, Ronald L.-K48
Morgan, Stephen L.-B151
Morris, Keith B.-B34, B61
Morris, Ronald N.-J28
Morris, Susie-I19
Moryan, Donald D.-J21

Moses Smalley, Laura P.-G1
Mostowtt, Thaddeus-K39
Mottar, Ashley M.-B69
Mourges, Melissa-F13, G10, L2
Moyer, Nea D.-H43
Mozayani, Ashraf-K56
Mroczkowski, Megan M.-I27
Mulet, Carmen T.-K19
Müller, Daniel-B145
Murphy, Rachel E.-A59
Murray, Elizabeth A.-W10
Murray, Thomas L.-C8
Murrie, Daniel C.-W20
Muscatello, Laura-I2
Muzzy III, William H.-D50
Myers, Emily A.-B18
Myers, Wade C.-I37

N

Naisbitt, Gary H.-B49
Nakao, Ken-ichiro-K37
Napoletano, Simona-F3
Needell, Barbara L.-G36
Neff, Jadee L.-H136
Nerkowski, Yolanda-G39
Neudecker, Klaus C.-W18
Newman, Reta-W7
Niessner, Janet-K69
Nisbet, Lorna A.-K75
Nixon, John-D21, D24, D26, S2
Nobles, Karen J.-J23, W3
Nolte, Kurt B.-W11
Norris, Darren-W9
Norton, Emily A.-A125
Noureddine, Maher-E5
Noziglia, Carla M.-ES1
Nuzzolese, Emilio-G13, G21

O

O'Connell, Kerry J.-F34
O'Connor, Craig O.-W4
O'Connor, Wendi G.-H152



Presenting Author Index- 2015

O'Leary, Owen L.-E58
Olckers, Antonel-B37
Oliva, Antonio-H47, H131, H142
Oliver, William R.-H148
Olivier, Martin S.-C14
Onyechi, Ivy-B95
Osbourne, Sasha-H122
Osculati, Antonio M.M.-H8, I4
Ostojic, Lana-B179
Ousley, Stephen D.-A83, A113, W5
Özasçilar, Mine-I32, I36
Özdemir, Erdinç-K31

P

Palenik, Christopher S.-B147
Palenik, Skip-B147
Pantano, Flaminia-K30
Papsun, Donna M.-K35, K64
Parente, Giuseppe Ruggiero-H76
Parker, Glendon-H115
Parmelee, Kevin J.-F17
Parr, Nicolette-A85
Pascale, Natascha-H91
Passalacqua, Nicholas V.-A36
Patete, Furio Martino-H64
Pechal, Jennifer L.-H103, W23
Pentone, Alessandra-H94
Pentz, Robert-D57
Penumetcha, Varma-I8
Perilli, Gabriela-H7
Peters, Dixie-B133
Petraco, Nicholas-B89
Pharr, Lauren R.-A43
Philip, Abraham T.-BS6, H139
Phillips, Balvina Z.-B38
Piccirilli, Kevin J.-B137
Pickup, Michael J.-H83
Pienkowski, David-D8
Pinckard, J. Keith-H96
Pinheiro, Joao E.S.-H63
Pinto, Giorgia-H52
Piper, Helen-K20, K76

Plaza, Dane T.-B181
Plazibat, Stephanie-B81, B110
Plotkin, Sharon L.-W23
Plourd, Christopher J.-W24
Plu, Isabelle-H141
Podini, Daniele S.-B183
Polak, Mark-D57
Pollitt, Mark-S2
Ponsini, Rebecca J.-K6
Pontoriero, Francesco-H130
Pope, Elayne J.-A44
Powell, Rees A.-B152
Pozzi, Mark C.-D1, D2, D4, D5
Pozzoli, Annabella A.-I2
Prahlow, Joseph A.-H96
Prahlow, Samuel-E37
Prat, Sebastien -E18, I24, I29
Pretty, Iain A.-G14
Price, Quashanna-B33
Prickett, Cassandra L.-K73
Primorac, Dragan-E47
Prins, Ronald-W14
Props, Abigail J.-H107

Q

Quarino, Lawrence-B43

R

Racine, Christopher-I27
Rada, Yvette-B143
Rajagopalan, Ashwyn-H66
Rajshekar, Mithun-G42
Ramsland, Katherine-F14, LW1
Ranadive, Anjali A.-ES1, S2
Raponi, Sara-F1
Ray, Rebecca-B68
Reba-Harrelson, Lauren-K44
Redd, Harrison-B87
Reedy, Edward A.-W1
Reedy, Paul-F56
Reffner, John A.-B159
Reich, Karl-B9



Presenting Author Index- 2015

- Reid, Desiree A.-B43
Reid, Jacqueline S.-G38
Reinhard, Karl J.-B102
Reinstein, Ronald-W24
Renegar, Thomas B.-B114
Resk, Thomas K.-H51
Reyes-Rodriguez, Jenise-C24
Richard, Adam H.-A30
Richards, Elizabeth-S2
Richards, Stephanie M.-H79
Richards, Victoria J.-B119
Richer, Sarah M.-A12
Riley, Amber D.-G35
Risinger, Michael-W20
Risso, Enrico A.-H140
Rivera, Xiomara N.-G8
Roas, Laura-A3
Roberts, Graham J.-G47
Rodriguez III, William C.-LW7
Rodriguez-Cruz, Sandra E.-B55, B169
Rogers, Christopher B.-H150
Rolleri, Lucas N.-A69
Rollman, Christopher M.-B195
Rorke-Adams, Lucy B.-W20
Rosenbaum, Karen B.-I27
Rosenquist, Scott D.-D14
Rosner, Richard-S1
Ross, Ann H.-S2
Ross, Darrell L.-E60
Rossi, Riccardo-H93
Rowe, Walter F.-B41, B54
Rozendaal, Andrew S.-A90
Rubin, Katie M.-E3
Rudy, Donald J.-D57
Ruest, Andre-G26
Rushton, Catherine G.-E51
Rutherford, Cynthia-I38
- S**
-
- Sablatura, Joshua D.-C12
Sachdeva, Geetanjli-K21
Saczalski, Kenneth J.-D1, D2, D3, D6
Saczalski, Todd-D5
Safarik, Mark E.-L2
Saint-Martin, Pauline-H132
Salerno, Monica-H135
San Nicolas, Alexander C.-K36, K68
Sanchez, Claudia L.-B59
Sanford, Jon A.-D17
Sanford, Michelle R.-H99
Sang, John L.-S1
Sanger, Robert M.-F53
Santos, Nelson-F9
Sathyavagiswaran, Lakshmanan-H147
Sauerwein, Kelly-A65
Saul, Tiffany B.-A65
Saunders, Lindsey E.-F47
Sava, Vincent J.-LW3, W17
Savito, Marco-K33
Schaefer, Maureen-A131
Schauben, Jay L.-W15
Schaye, Michala K.S.-A32
Scheck, Barry C.-W20
Schmidt, Gavin M.-E55
Schmitt, Stefan-A47
Schneeweis, Lynn A.-B45
Schorr, Steven M.-D59
Schotsmans, Eline M.J.-A42
Schuetzner, Ellen M.-J3, J30
Schwartz, Janet M.-I33
Scott, Ronald R.-D22
Sebetan, Ismail M.-E14
Segovia, Adrienne-H144
Seidel, Andrew C.-A5
Seigfried-Spellar, Kathryn C.-C3
Senn, David R.-G46
Serinelli, Serenella-H38, H81
Setia, Puneet-E56
Shakir, Abdulrezak M.-H12
Shefchick, Thomas P.-D39, D42, D45
Shek, Chin Hong-B71
Shelton, Donald E.-W20
Sherwood, Kate D.-A29



Presenting Author Index- 2015

Shirley, Natalie R.-A84, A115
Shiver, Farrell C.-J8
Shokry, Dina A.-H88
Shu, Irene-K4
Sigman, Michael E.-B167
Silva, Deborah-B124
Silva, Ricardo H.A.-G7
Simmons-Ehrhardt, Terrie-A13
Simms, Tanya M.-B185
Simon, Alison-E11
Simon, Dan S.-W20
Simpson, Whitney A.-B76
Sims, Brooke-H3
Singer, Ronald L.-S1
Sinha, Sudhir K.-W4
Sisco, Edward-E48
Skandalis, Lisa-H114
Skidmore, Eric-BS2
Smith, Erich D.-E36, W12
Smith, James S.-D33, D36, D38
Smith, John J.-D48
Smith, Stephanie L.-B44
Song, Junfeng J.-B115
Song, Kristine D.-H14
Sonkin, Roy H.-G28
Soomer Lincoln, Helena-G57
Soon, Alistair-G4, G19, G24
Sorensen, Amy E.-B73
Souviron, Richard R.-G15
Spamer, B.J.-W10
Spanbauer, Alan C.-E53
Spatola, Brian F.-A97
Sperling, Marnie L.-G6
Sperry, Grant R.-J29
Spitz, Werner U.-E44
Spradley, Kate-W5
Spurgeon, Tammy-W24
Stajic, Marina-K65, K78
Stanciu, Cristina E.-B13
Stanford, Matthew S.-W2
Stauffer, Eric-W7

Staymates, Jessica L.-B29
Stein, Paul-E14
Stephens, Peter J.-H44
Stephenson, Jennifer L.-E36, W12
Stephenson, Peter R.-C9
Stewart, James-B23
Stockwell, David C.-B46
Stolorow, Mark D.-F12
Stonebarger, Deborah R.-B113, K61
Sulner, Andrew-F29, W20
Sutton, Nichole-B183
Swartz, Alicia-B126
Swiatkowski, Sean A.-W1
Swofford, Henry J.-E71
Symes, Steven A.-H42

T

Tabencki, Michelle U.-A6
Tanaka, Kyle C.-G27
Taniguchi, Rodrigo N.-H58
Tarantino, Francesca-H5
Taylor, Adrian M.-K24
Taylor, Melissa K.-E61, E72
Templeton, Jennifer E.-B178
Thomas, Greg-W9
Thomas, Richard M.-A10
Thompson, Robert M.-B115
Thompson, William C.-W20
Tipple, Brett J.-A122
Tiscione, Nicholas B.-F49
Tise, Meredith L.-A76, E6
Tong, Mingsi-B115, B117
Tontarski, Karolyn L.-B177
Torbet, Tyler-B26
Trapella, Patrizia-F1, I10
Trejos, Tatiana-J18
Tremaine, Ladd-W1
Trignano, Claudia-K29
Troy, Meghan-B136
Truesdell, Janamarie-A78
Tseng, Zian-H134
Tuazon, Jennilee-I22



Presenting Author Index- 2015

Tuerkheimer, Deborah-W20
Turner, Edgar W.-G32
Tytell, Peter V.-J15, W3, W13

U

Ubelaker, Douglas H.-S1
Ueda, Momoko-E62
Urbanová, Petra-C6, H82
Urfer, Sarah-K50
Usui, Kiyotaka-K15
Uvaydov, Yuriy-B192

V

Vaira, Michele-F23
Valente, Alexander-B175
Valentine, Julie L.-E54
Van Deest, Traci L.-A41
van der Meer, Dieudonné J.-H117
van der Sluijs, Carlijn - K34
Vandell, Victor-K22
Vanderpuye, Oluseyi A.-K40
Vanin, Stefano-H35
Vastrick, Thomas W.-J3, J25
Vergnault, Marion-A55
Vieira, Duarte N.-B6, I17
Vijaychander, Sharada-E10
Vinueza, Nelson R.-B36
Vo, Eleanor B.-I37
Vogelsberg, Caitlin C.M.-A99
Vollner, Jennifer M.-A101
Volpini, Laura-K45
Vorder Bruegge, Richard-C2, W14
Vosacek, Carly S.-F45
Vosk, Ted W.-F24
Vuille, Joelle V.-F51

W

Walch, Mark A.-B111
Walta, David T.-A66
Walter, Richard D.-W18
Waltke, Heather E.-E46
Wang, Hanqing-H78
Wang, Ling-B24

Wang, Young-E59
Ward, Parris-E43
Ward-Trupp, Jennifer A.-J9
Ware, Charlotte W.-C7
Warner, Monica M.-A123
Warnica, Josie-B165
Warren, Daniel J.-W10
Warren, Michael W.-A112
Warren, Wendy S.-H124
Watson-Horzelski, Erin J.-H34
Watt, Brittany M.-K1, K62
Wax, Paul-W15
Waxse, Rebecca A.-F44
Wayman, James L.-D54
Wedel, Vicki-A105
Weedn, Victor W.-B40, C16, S2
Weems, Richard A.-G49
Wegner, Kristin E.-K14
Weiss, Kurt D.-D49
Welch, Todd W.-W19
Welner, Michael-F54, K43, L1
Wescott, Daniel J.-A120, W23
West, Rachel-B27
White, Joseph L.-C8
White, Michael-B51
Wiersema, Jason M.-A67
Wietbrock, Matthew C.-E17
Williams, Charmaine L.-B74
Williams, John A.-A118
Williams, Ken-S2
Williams, Kona-H22
Williams, Shannan-E61, F39
Williams, T.L.-E63
Wilson, Mark R.-W4
Wilson-Taylor, Rebecca J.-A51
Winborn, Jessica-B65
Winburn, Allysha P.-A111
Winecker, Ruth E.-S2
Winkler, Darcie L.-C11
Winters, Kelsey R.-B163
Wolf, Barbara C.-W6



Presenting Author Index- 2015

Wong Vui Foo, Dickens-K12
Wood, Robert E.-G39, G54
Word, Charlotte J.-F25, W24
Wright, Diana M.-B155
Wright, Franklin D.-G10
Wright, Ronald K.-H41
Wulff, Paula H.-W12

X

Xu, Baiyang-H70

Y

Yagmur, Fatih-I20
Yarbrough, Allyson D.-D57
Yeakel, Jillian K.-K7
Yocca, Stephanie A.-B63
Young, John L.-I31

Z

Zaferes, Andrea-E28, E66, W21
Zaidi, Arslan-B105
Zapico, Sara C.-A87, B5
Zein, Mustapha-B32
Zeliff, David J.-E15
Zhang, Xiang-K26
Zheng, Xiaoyu A.-B116
Zirpoli, Patrick-W18
Zmich, Cynthia R.-E29
Zubel, Brian C.-F55