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Narrative review

Rapid diagnosis of parasitic diseases: current scenario and future needs

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ABSTRACT

Background: Parasitic diseases are one of the world's most devastating and prevalent infections, causing millions of morbidities and mortalities annually. In the past, many of these infections have been linked predominantly to tropical or subtropical areas. Nowadays, however, climatic and vector ecology changes, a significant increase in international travel, armed conflicts, and migration of humans and animals have influenced the transmission of some parasitic diseases from 'book pages' to reality in developed countries. It has also been noted that many patients who have never travelled to endemic areas suffer from blood-borne infections caused by protozoa. In the light of existing knowledge, this new trend can be explained by the fact that in the process of migration a large number of asymptomatic carriers become a part of the blood bank donor and transplant donor populations. Accurate and rapid diagnosis represents the crucial weapon in the fight against parasitic infections.
Aims: To review old and new approaches for rapid diagnosis of parasitic infections.
Sources: Data for this review were obtained through searches of PubMed using combinations of the following terms: parasitological diagnostics, microscopy, lateral flow assays, immunochromatographic assays, multiplex-PCR, and transplantation.
Content: In this review, we provide a brief account of the advantages and limitations of rapid methods for diagnosis of parasitic diseases and focus our attention on current and future research in this area. The approximate costs associated with the use of different techniques and their applicability in endemic and non-endemic areas are also discussed.
Implications: Microscopy remains the cornerstone of parasitological diagnostics, especially in the field and low-resource settings, and provides epidemiological assessment of parasite burden. However, increased use and availability of point-of-care tests and molecular assays in modern era allow more rapid and accurate diagnoses and increased sensitivity in the identification of parasitic infections. **S. Momčilović, Clin Microbiol Infect 2019;25:290**
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Introduction

The morphological identification of parasite life-cycle stages is the pillar on which the diagnosis of parasitic diseases has been supported for ages. Although it remains highly useful, especially in low-income communities and regions with a high parasite burden, the loss of the required 'skills' for achieving quick and accurate diagnoses using morphology/morphometrics is increasingly common with the continued loss of experienced diagnosticians, while detecting and identifying disease-causing pathogens during the prepatent period is impossible [1,2]. Even more challenging when using morphological data in isolation is the identification and matching of the life-cycle stages, with larvae typically bearing little resemblance to adults and having few, indistinct morphological

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features for diagnosis [3]. The advent of molecular biology and its natural progression for use in diagnostics has been a major step forward. PCR-based kits provide diagnosticians with a tremendous capacity to detect and identify simultaneously various parasitic organisms to the subspecies or strain level not only in clinical samples, but also in their natural vector [4–7]. Furthermore, these kits are independent of the patient's immunologic status or previous infection [8], which is of great significance in distinguishing active parasitic disease (e.g. *Trypanosoma brucei*, *Trypanosoma cruzi*, *Toxoplasma gondii*, *Wuchereria bancrofti*, and *Schistosoma* spp. infections) from past exposure especially in patients from endemic areas [9]. In patients with immune system dysfunction (e.g. patients with HIV/AIDS [10] or solid organ [11] and hematopoietic stem cell transplant recipients [12]) or malignant haematological disorders [13], serological testing, as opposed to molecular, may be unreliable because of the possible occurrence of false-negative results. Also, molecular assays provide differentiation between wild-type pathogens and mutants, which is especially useful for determination of drug resistance. Finally, nucleic acid-based detection systems can be carried out using chemically inactivated (fixed) samples or extracts, thereby reducing the risk of laboratory-associated infections [8]. Unfortunately, the unavailability of robust, reliable, and rapid molecular-based diagnostics for many parasitoses, and choosing the 'right' existing test(s) for others, represent new major challenges.

Microscopy

The use of the microscope to examine biological samples for parasite life-cycle stages is a relatively cheap method for the morphology-based diagnosis of disease and remains the reference standard in parasitology. Typically, highly experienced operators use light or fluorescent microscopes to examine wet mounts of sputum, urine, vaginal swabs, duodenal aspirates, sigmoidoscopic material, abscesses, and tissue biopsies [8,14]. Mounting specimens in saline or iodine are valuable for detecting trophozoites of *Entamoeba histolytica/dispar*, *Giardia duodenalis* [15], *Balantidium coli* [16], *Trichomonas vaginalis* [17], *Naegleria fowleri* [18], and *Acanthamoeba* [19], larvae (e.g. *Strongyloides* sp.), adults and eggs of helminths, and protozoan (oo)cysts (e.g. cysts of *Entamoeba histolytica*, *Giardia duodenalis* [8,20], and *Balantidium coli* [16], while oocysts of *Cryptosporidium* spp., *Cyclospora cayetanensis*, or *Isospora belli* can be visualized only in heavy infections [21]). The wet mount examination of blood is used to detect microfilariae of various nematode species and trypanosomes, while saline mounts of superficial skin snips are useful for the detection of *Onchocerca volvulus* [8].

Staining biological preparations with chemical or fluorescent dyes can improve the detection and visualization of parasite life-stages [22]. For example, acid-fast stains such as Ziehl-Neelsen and Kinyoun's stain are used to detect *Cryptosporidium*, *Isospora*, and *Cyclospora* spp. in stool preparations [23]. Likewise, Wright and Giemsa stains (Romanowsky stains) can provide a rapid method for the detection of species of *Plasmodium*, *Babesia*, *Trypanosoma*, and *Leishmania* and microfilariae in thick and thin blood films [24], *Leishmania* spp. in bone marrow aspirates and in aspirates or imprints of cutaneous or mucocutaneous lesions, trypanosomes in lymph node imprints, *Toxoplasma gondii* in imprints of brain, lung, or other tissues, and *Giardia duodenalis* and *Entamoeba histolytica* in imprints of gastrointestinal biopsy specimens [25].

Fluorescent stains can also be used in parasitological diagnostics. Auramine-rhodamine, an acid-fast stain, binds to mycolic acids found in protozoa such as *Cryptosporidium*. Although not as specific for acid-fast organisms compared with the Ziehl-Neelsen stain, it is more affordable and cheaper and therefore commonly used as a rapid screening tool. Acridine orange, an organic intercalating nucleic acid-selective fluorescent dye, can be employed to detect *Acanthamoeba* and *Leishmania* [24]. Calcofluor white, which selectively binds to cellulose and chitin in the cell walls of fungi, has also been used to detect *Acanthamoeba* and *Naegleria* [19,26,27].

The comparatively low cost of these methods has perpetuated their sustained use, especially in economically disadvantaged regions of the world, where the cost of molecular and immunological kits remains prohibitive (Table 1).

Disadvantages of conventional methods

The accurate identification of parasites underpins a holistic approach to disease control. However, the very basis of the microscopic method of diagnosis (i.e. the morphological and morphometric identification of a wide assortment of parasite life-stages from a variety of parasites in different biological samples) is in itself the major disadvantage of using this technique. Conventional microscopic analyses require a high level of parasitological knowledge [1,9], combined with an ability to objectively evaluate morphological variation(s) and find life-stages that are frequently just tens of micrometres in size [44]. These methods are labour- and time-intensive and characterized by low sensitivity and reproducibility, often requiring the examination of multiple independently collected specimens to reduce parasite-induced variability caused by differences in the day-to-day shedding of parasite life-cycle stages [45,46]. Moreover, conventional microscopic methods are unable to distinguish species within many parasite genera (e.g. *Cryptosporidium* [47], *Cyclospora* [48], and *Entamoeba* [49]) and therefore pathogenic from commensal parasite species (e.g. *Entamoeba histolytica* from *Entamoeba dispar* [49]). Finally, the highly invasive sampling for tissue parasites, which may induce medical complications, further undermines the utility of these methods [50,51]. The advantages and limitations of microscopy in the rapid diagnosis of specific parasitic infections are presented in Table 2.

Point-of-care testing

In low-income communities, clinical laboratories are constantly faced with financial and human resource constraints. Therefore, diagnosis of infectious diseases depends largely on the availability and accessibility of point-of-care tests (POCTs) [98]. To date, a large number of in-house developed and commercially available POCTs have been used for diagnosis of a wide range of bacterial, parasitic, viral, and fungal diseases [99,100]. The implementation of reliable POCTs into daily clinical practice offers a multitude of potential benefits such as cost-effectiveness, speed, user friendliness, equipment-free testing, treatment decision guiding, and improving patient satisfaction [101–103]. Early initiation of treatment guided by POCTs can dramatically improve clinical outcomes in potentially fatal infections (e.g. African trypanosomiasis, visceral leishmaniasis, and falciparum malaria) [98,104] and in patients with immunodeficiency disorders where a normally mild infection can be easily transformed into a massive, rapidly progressive threat to life [103,105]. However, POCTs are often used as part of a two-step screening which means that only POCT positive samples are sent to further parasitological diagnostics.

Lateral flow immunochromatographic assays (LFIA)

Many serious parasitoses, such as Chagas disease and malaria, are endemic in poor communities and the lack of equipment and trained personnel to carry out conventional parasitological examinations inhibits diagnosis and the timely initiation of treatment

Table 1
Comparative costs of microscopy, rapid diagnostic tests (RDTs), and molecular tests in diagnosis of parasitic infections

Parasitic infection	Test used for diagnosis	Baseline costs ($)	Total costs with technician's time ($)	Study area (year)	References
Malaria	Microscopy of blood smears	US$6.81	US$9.41	Nis, Serbia (2018)	Data from our laboratory [b]
		No data available	US$0.26	Tanzania (2007)	[28]
		US$0.2	No data available	Rawalpindi, Pakistan (2011)	[29]
		No data available	US$1.61–9.89	Afghanistan (2009)	[30]
	RDT CareStart™ malaria Pf (HRPII)/Pan (pLDH) (Access Bio, Inc., New Jersey, United States)	No data available	US$1.28–2.23	Afghanistan (2009)	
	RDT Rida®Quick Malaria (R-Biopharm, Darmstadt, Germany)	US$9.95	US$12.55	Nis, Serbia (2018)	Data from our laboratory [b]
	RDT Paracheck-Pf® (Orchid Biomedical Systems, Goa, India)	US$0.60	US$0.81	Tanzania (2007)	[28]
	RDT First Response®Combo Malaria Ag (pLDH/HRP2) card (Premier Medical Corporation Ltd., Mumbai, India)	US$0.87	US$1.00	Uganda (2011)	[31]
	RDT OptiMAL (Flow Inc, Portland, OR, USA)	US$2.75	No data available	Rawalpindi, Pakistan (2011)	[29]
	PlasmoNex™ multiplex PCR	US$9.00/ sample	No data available	Malaysia (unspecified)	[32]
	Multiplex qPCR [a]	US$14.80	No data available	Malaysia (unspecified)	
Visceral leishmaniasis	Microscopy of bone marrow aspirates	US$2.0	US$21.40	Morocco (2014)	[33]
		US$6.8	US$27.10	Brazil (2014)	[34]
	RDT IT-LEISH® (DiaMed Latino-America SA, Switzerland)	US$1.0	US$1.10	Morocco (2014)	[33]
	RDT Kalazar Detect™ (InBios International, Inc., Seattle, USA)	US$3.67	US$7.45	Brazil (2014)	[34]
	RDT Leishmania Dipstick Rapydtest® (Apacor LTD, Wokingham, UK)	US$13.71	US$16.31	Nis, Serbia (2018)	Data from our laboratory [b]
	PCR	US$19.35	US$32.72	Brazil (2014)	[34]
Intestinal protozoal infections	Microscopy - Ova and Parasite Exam	US$2.31	US$4.91	Nis, Serbia (2018)	Data from our laboratory [b]
		US$1.50	US$13.75	Florida, USA (2000)	[35]
		US$1.79	US$50	Washington, USA (2009–2011)	[36]
	RDT ImmunoCard STAT!® *Cryptosporidium/Giardia* (Meridian Bioscience Inc.)	~US$30	~US$38		
	RDT Remel™ Xpect™ *Giardia/Cryptosporidium*	~US$26	~US$38		
	RDT *Giardia/Cryptosporidium* Quik Chek™ (Techlab Inc., Blacksburg, Virginia, USA)	~US$34	~US$50		
	RDT Rida®Quick *Cryptosporidium*	US$3.67	US$6.27	Nis, Serbia (2018)	Data from our laboratory [b]
	FilmArray™ GI panel (BioFire Diagnostics, Salt Lake City, UT)	USD$155	Na data available	USA (unspecified)	[37]
	Luminex xTag® GI pathogen panel (GPP) (Luminex Corporation, Toronto, Canada)	USD$80–90	Na data available		
	NanoCHIP® Gastrointestinal Panel (GIP) (Savyon Diagnostics, Ashdod, IL)	<US$30/ sample	Na data available	Israel (2013–2014)	[38]
	BD MAX™ Enteric Parasite Panel (BD Diagnostics, Sparks, MD)	AUD$50/ sample	Na data available	Australia (unspecified)	[39]
	EasyScreen™ Enteric Parasite Detection Kit (Genetic Signatures, Sydney, Australia)	AUD$20/ sample	Na data available		
Schistosomiasis	Single Kato-Katz technique	US$1.09	US$6.89	Western Kenya (2010–2011)	[40]
		~US$0.20	US$ 1.73	Tanzania (2009)	[41]
	Duplicate Kato-Katz technique	~US$0.40	US$2.06	Tanzania (2009)	
	Triplicate Kato-Katz technique	US$2.79	US$17.54	Western Kenya (2010–2011)	[40]
	Formalin-ethyl acetate sedimentation technique	US$0.68	Na data available	Philippines (2010)	[42]

Table 1 (*continued*)

Parasitic infection	Test used for diagnosis	Baseline costs ($)	Total costs with technician's time ($)	Study area (year)	References
	The urine-CCA (Circulating Cathodic Antigen) cassette test (Rapid Medical Diagnostics, Pretoria, South Africa)	US$3.15	US$7.26	Western Kenya (2010–2011)	[40]
	qPCR	US$8.8/ sample	No data available	Philippines (2010)	[42]
		US$9.20/ sample	No data available	China (2013)	[43]

It is important to emphasize that the total cost of the tests varies depending on the level of education of the medical staff interpreting the test (technician, general practitioner, or the specialist in the field of parasitology), economic standard of the region, the cost of 1 hour of technologist time, and material costs. For example, material costs for microscopic examination are usually low, but this procedure often requires 1 hour of hands-on testing time for the interpretation of slides, which significantly increases the cost of testing especially in regions with higher hourly wages. On the other hand, RDTs are relatively cheap, require ~10–20 minutes of hands-on testing time, and the readout of results is usually done optically by a wide range of users. However, these tests are cost-effective only in settings in which parasitic infection rates are low or moderate, when the chosen test is highly specific to ensure appropriate treatment and if adherence to negative test results by healthcare providers is high. In comparison with microscopy and RDTs, molecular tests require expensive kits, highly trained staff (molecular biologists or specialists in the field of parasitology), and sophisticated equipment which can cost up to several hundred thousand dollars.

[a] Multiplex qPCR - Multiplex real-time PCR.

[b] Costs were collected in Serbian dinars (RSD) and converted to US dollars (US$) using an average of the last month of exchange rates, which ranged from RSD96.57 to RSD95.75 for US$1.

[106]. Commercial LFIA have recently been developed to alleviate these issues (see Table 3). LFIA are based on the simple principle: a liquid sample containing the biomarker of interest (e.g. antigens, antibodies, haptens, and even oligonucleotides) migrates via capillary force through various zones of a porous membrane, on which specific bio-recognition and detection components are immobilized [107,108]. As such, they overcome many of the disadvantages of conventional microscopy by providing simple, quick, cost-effective, and sensitive and accurate identification of parasites. They can also be used and interpreted by 'relatively' inexperienced personnel and performed directly on a variety of clinical specimens to detect antigens (ags) or antibodies (abs) against protozoa and helminths. For example, a recently developed immunochromatographic assay for diagnosis of visceral leishmaniasis can detect *Leishmania* ags from seven different species in the blood of patients, and divide these as past and active infections, monitor treatment success, and diagnose immunocompromised patients [109]. LFIA can also accurately differentiate multiple parasite genera/species simultaneously (i.e. the Rida®Quick Cryptosporidium/Giardia/Entamoeba Combi kit (R-BIOPHARM AG, Darmstadt, Germany) can diagnose infection by *Entamoeba histolytica*, *Giardia duodenalis*, and *Cryptosporidium* spp. and distinguish non-pathogenic species of *Entamoeba* from *Entamoeba histolytica*). Their simplicity of use and subsequent scalability means they apply to screening numerous samples collected during outbreak events as well as survey studies in low-income regions of the world [110]. Also, the ability to tie objective outcomes to other diagnostic tests (e.g. the fluorescent immunochromatographic assay for the diagnosis of *Acanthamoeba castellanii* in corneal scraping corresponds closely with quantitative real-time PCR for *Acanthamoeba* keratitis diagnosis [111]) increases their versatility. The characteristics of each test are shown in Table 3.

Problems with LFIA arise because of cross-reactivity when applied to whole blood, rather than sera samples, brought on by efforts to increase sensitivity, resulting in many assays losing their specificity. For example, VIRapid® HYDATIDOSIS (Vircell, Granada, Spain) test, used for the diagnosis of *Echinococcus*-specific abs, can give false-positive results when coinfections with *Taenia solium* occur [112]. The dipstick LFIA for the detection of *Shistosoma japonicum* abs can be positive when coinfections with intestinal *Clonorchis*, *Paragonimus*, and *Angiostrongylus cantanensis* occur [113,114]. Also, LFIA for the diagnosis of *Wuchereria bancrofti* infection has been shown to have higher specificity in patients without onchocerciasis, loiasis, and strongyloidiasis [115]. A consequence of this has been the need to apply many LFIA to sera, which necessitates the need for basic laboratory equipment for sera collection. The presence of cellular enzymes in whole blood can lead to nonspecific assay effects, especially in enzyme-based assays, while the presence of fibrinogen and paraproteins in unclotted plasma can affect the ability to accurately manoeuvre the sample during pipetting because of increased viscosity.

Multiplex molecular detection assays

The most significant progress in the diagnosis of infectious diseases has been made with the introduction of molecular tools. Currently, multiplexed molecular diagnostic tests are a hot research topic in the diagnosis of parasitic infections. These assays provide simultaneous detection of multiple pathogens, resistance genes, and can differentiate pathogenic from non-pathogenic species/strains/isolates in a single closed tube system [141,142]. Most tests are based on multiplex qPCR technology. The primers used in these assays are carefully designed to prevent unspecific hybridization with DNA from the host sample. In other words, each amplicon has a unique size, melting temperature, or probe binding sequence, which enables detection of different pathogens in the same sample [143]. Also, multiplexed methods include internal controls that identify the presence of PCR inhibitors and thus eliminate false-negative reports [144].

In syndromic parasitic diseases, similar clinical symptoms caused by the simultaneous presence of several pathogens can compound accurate differential diagnoses. Here, the application of molecular panels can greatly enhance diagnosis leading to optimized treatment, the prevention of secondary infection, and improved disease treatment and control. Molecular panels also represent invaluable tools for epidemiological surveillance programmes and research projects, and can quickly pinpoint the source of disease outbreaks, while also significantly improving use of hospital resources [145–154]. Despite the clear benefits provided by molecular panels, the assays are not infallible, and false-negative results may occur when the pathogen is present below the limit of detection, sequence polymorphism in target genes prevents the efficient binding of PCR primers, or because of operator error. Also, parasite nucleic acids may persist *in vivo* post-infection which can lead to the occurrence of false-positive results [155].

Gastrointestinal multiplex molecular panels

The microbiological aetiology of gastroenteritis may include bacterial, parasitic, or viral pathogens. Until recently, clinicians carried out a range of laboratory tests (e.g. stool culture on selective

Table 2
Major advantages and limitations of rapid microscopic examination in diagnosis of certain parasitic diseases

Parasitic infections	Microscopic techniques used for diagnosis	Advantages	Disadvantages	References
Malaria	• Giemsa- or acridine orange-stained blood preparations • Thin blood film • Thick blood film	• Simple and low cost technique which provides detection and identification of *Plasmodium* species in Giemsa-stained thick blood films (useful especially for screening), species confirmation in thin blood films, and assessment of parasite density • Provides essential information to the physician to guide the initial treatment decisions that need to be made acutely • Thick blood film represents the most sensitive preparation for the microscopic identification of *Plasmodium* parasites • Microscopic diagnosis at primary healthcare facilities in underdeveloped countries where malaria is endemic and remains one of the major causes of illness and death could reduce the prescription of antimalarial drugs, and improve the appropriate management of non-malarial fevers • High-level parasitaemia (>10% of RBCs infected) which includes only ring forms is suggestive of *Plasmodium falciparum* infection, even without observation of gametocytes	• The microscopy of thick blood film is only sensitive when the minimum *Plasmodium* parasite titre in blood sample is 50 000–500 000 parasites/mL • Low diagnostic efficacy of conventional microscopy can be expected in low-intensity or early infections which can be present among infected patients under chemoprophylaxis • Potential subjective physician/lab technician dependent results can influence misdiagnosis of malaria, particularly in infections caused by lethal *Plasmodium* species, which has significant negative impact on the clinical outcomes of patients • Staining and interpretation processes are labour-intensive, time-consuming, and require considerable expertise and trained healthcare workers, particularly for accurate identification of species at low parasitaemia or in mixed malarial infections • Not suitable for high-throughput use • Microscopic preparations should preferably be analysed within 2 to 3 hours of blood collection • If blood is collected in EDTA tubes, parasite morphology may be altered by delays in preparation of the slides • Quantitative buffy coat examination used widely for diagnosis of malaria poses difficulty in differentiating *Plasmodium* from *Babesia*	[8,44,52–56]
Babesia infections	• Giemsa- or Wright-stained blood smears • Thin blood film • Thick blood film	• Cheap, fast, and acceptable standard for diagnosis of active *Babesia* infections by visualization of parasites on Giemsa- or Wright-stained thin blood smears • The most frequently used technique for diagnosis of *Babesia microti* infection in the United States and *Babesia divergens* infection in Europe • In combination with an accurate patient's history and clinical presentation provides an appropriate diagnosis • Thin blood smear examination is used for speciation and determination of the degree of parasitaemia in high parasitaemia cases (the number of infected red blood cells/100 red blood cells) • Thick blood smear examination is helpful for determination of parasite density in low parasitaemia cases (number of parasites/200 white blood cells in combination with the WBC count in the patient)	• Subjective process which depends on the experience of the microscopist and the time spent examining the smear • Clinical manifestations of malaria and *Babesia* infection overlap thus leading to potential difficulties in distinguishing the ring forms of *Babesia* morphologically from those of *Plasmodium falciparum* on blood smears • The duration of detectable parasitaemia on blood smears varies from 3 weeks to 12 weeks, with the longest duration of smear positivity being 7 months for a splenectomized patients • The need to discriminate the subtleties of babesial morphology and possible low parasitaemias may result in inaccurate diagnoses which might necessitate further analysis • Diagnosis may be difficult or underappreciated in areas in which *Plasmodium* and *Babesia* parasites cocirculate or in travellers who have been exposed to both malarious and *Babesia*-endemic areas • *Plasmodium* and some *Babesia* species in the same hosts may appear to be morphologically similar • In cases with low parasitaemia, *Babesia* infections can be easily missed by evaluation of thin smear if 300 fields or multiple smears are not examined • *Babesia venatorum* organisms are morphologically indistinguishable from *Babesia divergens* • It is impossible to differentiate *Babesia microti* from *Babesia duncani* infections • *Babesia microti* and *Babesia duncani* are small organisms (diameter <3 μm) and may be missed by examination of thick blood smears	[54,55,57–60]
Chagas disease	• Fresh blood or buffy coat smears stained with Giemsa • Thin blood film • Thick blood film	• Highly specific method when performed by an experienced laboratory • Due to high level of parasitaemia in acute phase of Chagas disease (usually during a period of 6 to 8 weeks), microscopic examination of fresh blood or buffy coat	• Low sensitivity and labour-intensive method for routine application • The level of parasitaemia decreases within 90 days of infection, even without treatment, and is undetectable by microscopy in the chronic phase	[54,61–67]

Table 2 (*continued*)

Parasitic infections	Microscopic techniques used for diagnosis	Advantages	Disadvantages	References
		smears stained by Giemsa provides detection of circulating motile trypomastigotes • When *Trypanosoma cruzi*-infected organ donors are detected before or soon after the transplantation, microscopy and PCR of serial blood specimens are recommended to monitor possible infection in the recipient	• Only infants with very high parasitaemia born to *Trypanosoma cruzi*-seropositive mothers can be diagnosed using microscopic observation of blood samples at 1 month of age • Results of microscopic examinations of blood samples are highly dependent on the observer, the time of observation, and parasite motility • Because of morphological similarities, it can be difficult to distinguish *Trypanosoma cruzi* from human nonpathogenic *Trypanosoma rangeli* • Microscopy is less useful during the latent and chronic stages of infection when rates of parasitaemia are very low and the nonmotile (amastigote) intracellular form of *Trypanosoma cruzi* predominates • Specimens must be handled with care using standard precautions, including the use of gloves and other personal protective measures, because live trypanosomes are highly infectious	
African trypanosomiasis	• Fresh wet preparations of blood, buffy coat samples, aspirates of enlarged lymph nodes and cerebrospinal fluid • Giemsa-stained blood smears • Thin blood film • Thick blood film	• Provides visualization of live motile trypanosomes in fresh wet preparations of blood, buffy coat samples, or aspirates of enlarged lymph nodes during the acute phase of infection • The presence of mononuclear white blood cells and Mott cells (large plasma cells containing immunoglobulin) in cerebrospinal fluid is highly suggestive of African trypanosomosis in the appropriate clinical and epidemiological settings • Examination of stained blood in a thick blood film slightly improves sensitivity (detection is around 5000 trypanosomes/mL) of microscopy in *Trypanosoma brucei species* infections • The quantitative buffy coat technique allows concentration of parasites and their better discrimination from white blood cells	• Standard microscopy is estimated to miss 20% to 30% of cases • The entire slides should be examined because the parasites are often few in number and may be unexpectedly detected in blood submitted for malaria diagnosis • Detection limit of wet mount microscopy is 10 000 trypanosomes/mL (1 parasite/200 microscopic fields) for diagnosis of *Trypanosoma brucei* infections • Giemsa- or Field's-stained thin blood films have low sensitivity • Microscopy of blood is less useful during the later meningoencephalitic stages of infection when parasitaemia levels are low • Trypanosomes are rare and difficult to detect in cerebrospinal fluid • Specimens must be handled with care using standard precautions, including the use of gloves and other personal protective measures, because live trypanosomes are highly infectious	[54,57,68,69]
Leishmaniasis	**Mucocutaneous leishmaniasis** • Giemsa-stained preparations of scrapings, aspirates, and touch-impression smears of leading edge of ulcers **Visceral leishmaniasis** • Giemsa-stained preparations of bone marrow, lymph nodes, spleen and liver	• Microscopic examination of Giemsa- or haematoxylin-eosin-stained scrapings, aspirate samples, or biopsy samples of skin ulcers or mucosal lesions is the standard method for laboratory diagnosis of cutaneous and mucocutaneous leishmaniasis • Microscopic analysis of fixed aspirates and biopsies of bone marrow, lymph nodes, spleen, and liver stained with Giemsa or haematoxylin-eosin provides diagnosis of visceral leishmaniasis	• Requires well-trained and experienced microscopists because tissue amastigotes may be difficult to identify and may be confused with other organisms (e.g. *Histoplasma capsulatum*) • Depending on the skill of the observer, the sensitivity of the test varies from 42% to 70% for diagnosis of cutaneous leishmaniasis • Although splenic aspirate is a highly sensitive method (sensitivity over 95%) for diagnosis of visceral leishmaniasis, it is associated with risk for fatal haemorrhage in inexperienced hands and should be avoided in patients with a platelet count of less than 40 000 platelets/μL and a prothrombin time of more than 5 s over the control because of the risk of excessive blood loss • Compared with splenic aspirate, bone marrow aspirate is a more painful and less sensitive method (sensitivity 60% to 85%) for diagnosis of kala-azar • Tissue specimens are usually uneven in thickness; consequently, the amastigotes are unevenly distributed which may require long searches to demonstrate the parasites	[54,69–71]
Free-living amoebiasis	***Naegleria fowleri*** • Wet mount preparations of cerebrospinal fluid • Preparations of cerebrospinal fluid stained with Wright's, Giemsa, or haematoxylin and eosin stains	• Direct microscopic examination of wet mount preparations is the method of choice for detection of motile *Naegleria fowleri* trophozoites in cerebrospinal fluid • If *Naegleria fowleri* trophozoites are present in cerebrospinal fluid, definitive identification can be made by staining fixed preparations	• Wet-mount preparations of the cerebrospinal fluid should be examined immediately after collection under the microscope for the presence of actively moving trophozoites • Trophozoites from all pathogenic free-living amoeba species can be difficult to	[18,72,73]

(*continued on next page*)

Table 2 (*continued*)

Parasitic infections	Microscopic techniques used for diagnosis	Advantages	Disadvantages	References
	Acanthamoeba* spp.** • Wet mount preparations of corneal scrapings and cerebrospinal fluid • Giemsa-stained preparations of cerebrospinal fluid sediments • Acridine orange- and calcofluor white-stained preparations of biopsy samples • *In vivo* confocal microscopy ***Balamuthia mandrillaris • Preparations of brain biopsy specimens embedded in paraffin and stained with haematoxylin and eosin	with Wright's, Giemsa, or haematoxylin and eosin stain • Light microscopy is an efficient diagnostic modality to detect *Acanthamoeba* in wet-mount preparations of corneal scrapings (noninvasive method), in wet preparations of cerebrospinal fluid or Giemsa-stained preparations of cerebrospinal fluid sediments, in biopsy samples, and in keratoplasty specimens • Microscopic examination of preparations stained with acridine orange and calcofluor white can be used to observe *Acanthamoeba* cysts in tissues • *Acanthamoeba* trophozoites can be directly identified in corneal tissue without any invasive procedure using *in vivo* confocal microscopy which represents sensitive and specific diagnostic tool if applied by an experienced microscopist	differentiate from host inflammatory cells, especially in stained tissue sections • Because of the same nuclear structure, differentiation of *Acanthamoeba* trophozoites from *Balamuthia mandrillaris* trophozoites is not possible using light microscopy • Visualization of *Balamuthia mandrillaris* is generally not possible in cerebrospinal fluid preparations • In mixed fungal and *Acanthamoeba* infections, both pathogens will be stained using calcofluor white as both amoebic cysts and fungal cell walls are possible targets • The use of fluorescent dyes may lead to false-positive staining patterns of cell debris and therefore an experienced microscopist and a fluorescence microscope are necessary for an accurate diagnosis	
Trichomoniasis	• Wet mount preparations of urogenital secretions and urine sediment • Papanicolaou-stained endocervical preparations • Liquid-based Pap tests	• Microscopic examination of wet mount slides prepared from vaginal secretions, urine sediment, urethral or prostatic secretions provides identification of motile *Trichomonas vaginalis* trophozoites • Direct observation of the pear-shaped trichomonads with their characteristic jerky or tumbling motility is considered 100% specific for *Trichomonas vaginalis* • The wet mount examination represents the most cost-effective, simple, rapid and low-technology point-of-care test for diagnosis of *Trichomonas vaginalis* infection which provides immediate results • Liquid-based Pap tests appear to be more accurate for microscopic identification of *Trichomonas vaginalis* with 60–96% sensitivity and 98–100% specificity	• Requires experience of the observer • The method is characterized by poor sensitivity (from 44% to 68% according to one and from 38% to 82% according to another report) even with experienced microscopists and prompt examination of vaginal specimens • In inoculums which contain less than 10^4 organisms/mL, trichomonads cannot be observed by conventional microscopy • When not motile, a trichomonad can be difficult to differentiate from white blood cells or from the nucleus of a vaginal epithelial cell • Delay in transport and evaporation of moisture from the specimen reduces motility and, consequently, diagnostic sensitivity • Suboptimal specimen storage or transportation conditions, especially temperatures below 22°C, further reduce parasite motility and thus wet mount sensitivity • The conventional Pap smear technique is low sensitive and specific for diagnosis of *Trichomonas vaginalis* infection and requires confirmatory testing if positive results are obtained in asymptomatic women • Liquid-based Pap test results are typically available several days after specimen collection • Microscopic examination of male urethral specimens or urine sediment is unreliable for detection of *Trichomonas vaginalis* infection in men because of the lower organism burden	[17,57,74–76]
Intestinal protozoal infections	***Entamoeba histolytica*** • Wet mount preparations of fresh stool or concentrated fresh stool with/without iodine stain • Trichrome stained preparations of fresh or concentrated fresh stool ***Giardia lambila*** • Wet mount preparations of fresh or concentrated stool and concentrated duodenal contents • Trichrome or iron haematoxylin stained preparations of stool and duodenal contents after sedimentation/ concentration ***Cryptosporidium* spp.** • Modified acid-fast-stained preparations of stool	• The microscopic ova and parasite examination (O&P) represents cornerstone of diagnostic testing for the intestinal protozoa • Provides observation of *Giardia* cysts and trophozoites in fresh or permanently stained stool smears as well as duodenal concentrated wet mounts or permanently stained preparations • Permanently stained smears enable diagnosis of *Entamoeba histolytica* in nonendemic areas and easy identification of *Blastocystis hominis* • Modified acid-fast stains improves sensitivity of light microscopy in diagnosis of *Cryptosporidium* infections • *Cyclospora cayetanensis* and *Cystoisospora* oocysts intrinsically autofluoresce under UV light in a standard concentrated wet mount preparations • Detection of *Cystoisospora belli* oocysts from stool or duodenal samples is simplified by their distinctive size and shape	• Insensitive method • Examination should be performed by adequately trained technologists • Requires examination of multiple stool specimens (minimum of three stool samples collected over a period of 10 days) because of intermittent excretion of parasites • The presence of red blood cells in cytoplasm is not strictly specific for *Entamoeba histolytica* trophozoites • Diagnosis of *Entamoeba histolytica* infections by microscopy misses 40% of infections • *Entamoeba histolytica*, on one hand, cannot be distinguished morphologically from the nonpathogenic *Entamoeba dispar*, *Entamoeba coli*, *Entamoeba hartmanni*, *Entamoeba gingivalis, Endolimax nana*, and *Iodamoeba buetschlii*, and, on the other hand, *Entamoeba moshkovskii* and *Entamoeba bangladeshi* which may be pathogenic for humans • In diagnosis of *Entamoeba histolytica* infection, false-positive results may occur because of misidentification of nonmotile trophozoites as	[49,57,77–83]

Table 2 (*continued*)

Parasitic infections	Microscopic techniques used for diagnosis	Advantages	Disadvantages	References
	Cyclospora cayetanensis* and *Cystoisopora belli • Fluorescence microscopy of concentrated wet mount preparations of stool • Modified acid-fast or modified safranin stained preparations	• *Cystoisospora belli* oocysts are only easy to recognize in concentrated wet mounts from O&P exams	macrophages and polymorphonuclear cells (especially mature neutrophils) as cysts • For microscopic diagnosis of amoebiasis, the sample should be examined within 1 h of collection • *Giardia lambila* trophozoites and *Cystoisopora belli* oocysts are not always found in stool preparations • *Blastocystis* spp. may be confused with yeast, *Cyclospora* sp., or fat globules in wet mount preparations because of its polymorphic nature • Microscopic detection of *Cryptosporidium* and *Cyclospora cayetanensis* oocysts is difficult because of poor staining • Distinguishing *Dientamoeba fragilis* from nonpathogenic *Endolimax* nana or *Entamoeba hartmanni* can be difficult in cases when chromatin granules of this organism are covered with stain deposits, when the nuclear fragmentation is not obvious, or when the majority of trophozoites are mononucleated in the specimen • Visualization of trophozoites and cysts of *Dientamoeba fragilis* is difficult on wet mount preparations	
Intestinal nematode infections	• Wet mount preparations of fresh or concentrated stool • Kato-Katz method	• Rapid, easy-to-perform and low cost method which provides visualization of ova, larvae and adult forms of nematodes in stool samples • Microscopic examination enables detection of all infecting species presented in the same preparation ('true multiplex examination') • Concentration and flotation techniques can be used to increase diagnostic sensitivity of microscopy • The Kato-Katz, as the most commonly used microscopic technique, enables assessing the prevalence and intensity of infection in nematode control programmes • Larvae of *Ascaris lumbricoides* may be observed in sputum during pulmonary migration • In hyperinfection syndrome, microscopic examination of sputum or cerebrospinal fluid may visualize motile larvae of *Strongyloides stercoralis*	• The lack of sensitivity • Microscopic analysis of multiple samples with multiple slides per sample over several days is required for sensitive detection and quantification of nematode infections • In the Kato-Katz method, nematode eggs are visualized at different time intervals or 'clearing times' after the preparation of a slide which affects its specificity and sensitivity for the detection of hookworm infections • Microscopic-based techniques do not allow species-level identification of nematode eggs • Diagnosis of *Strongyloides stercoralis* is particularly challenging because of the excretion of small number of larvae in stool regardless of infection intensity • Flotation based techniques are labour-intensive, have poor reproducibility owing to operator error, and usually require centrifugation steps	[54,57,77,84]
Intestinal tapeworm infections	• Wet mount preparations of fresh or concentrated stool • Perianal scraping (Graham's test) • Haematoxylin and eosin staining of gravid proglottids obtained from stool samples	• Microscopic detection of eggs and proglottids in faeces provides diagnosis of taeniasis • Differentiation of the two human *Taenia* species is based on the number of uterine branches present in well-preserved gravid proglottids or on the absence or presence of hooks in the scolex of the tapeworm • Perianal scraping with adhesive tape (Graham's test) is highly sensitive for *Taenia saginata*, but not for *Taenia solium* • Pathohistological identification of gravid proglottids obtained from stool samples by simple haematoxylin and eosin staining is a simple, useful, cheap diagnostic technique which does not require any extra procedures or equipment and can be performed in any pathology or histology laboratory • Repeated microscopic examinations and concentration procedures will increase the probability of detecting light infections • Microscopy has very high genus specificity, especially with a trained operator, and thus remains one of the most commonly applied tools for the diagnosis of human taeniases in endemic countries • Most cases of diphyllobothriasis are correctly diagnosed at least at the genus level using microscopy	• Conventional coproscopical examination has a low sensitivity (even 60–70% of cases may be missed) because of the variable numbers of eggs excreted in stools and the small volume of examined samples • Although *Diphyllobothrium* eggs are easily distinguishable, eggs of *Taenia solium* and *Taenia saginata* are morphologically similar • Microscopic diagnostis of taeniasis is not possible during the first 3 months following infection (prior to development of adult tapeworms) • Morphological diagnosis of species on the basis of proglottid materials is technically difficult and impractical in cases when gravid proglottids are not available • Obtaining well-preserved and intact gravid proglottids or the scolex after treatment of the patient is often difficult because of the partial destruction of gravid proglottids or the recovery of only immature proglottids in the stool • *Diphyllobothrium* species can be misidentified with trematodes that may also possess operculate eggs of a similar size, or with segments of taenias that may have a similar shape • Embryonated eggs of *Diphyllobothrium* species are not present in fresh stool of definitive	**[85–93]**

(*continued on next page*)

Table 2 (*continued*)

Parasitic infections	Microscopic techniques used for diagnosis	Advantages	Disadvantages	References
		• Some *Diphyllobothrium* species can be differentiated morphologically on the basis of the shape and size of the embryonic hooks of the oncosphere	hosts, and embryonation requires several days in the water	
Liver, lung, and blood fluke infections	• Wet mount preparations of stool/bile/sputum/urine after the concentration by sedimentation or flotation techniques • Membrane filtration technique using Nucleopore filters for schistosomal infections • Kato-Katz method • Sedimentation or flotation techniques	• Microscopic techniques are the most widely used diagnostic approach for direct detection of trematode eggs in biological samples, such as faeces or bile aspirates for *Clonorchis sinensis*, *Fasciola* spp, and *Opisthorchis viverrini*, sputum for *Paragonimus* spp., or stool and urine for *Schistosoma* spp. • Provides determination of the infection intensity expressed by the number of parasite eggs per gram of faeces which allows quantification of treatment outcomes in terms of not only cure rate, but also egg reduction rate • Multiple stool sampling can enhance diagnostic accuracy (in case of schistosomal infections, one diagnostic study showed that the sensitivity increased from 85% for one stool to 100% for four stools, while another showed that the sensitivity increased from 70% for one smear to 92% for four smears) • Multivalent flotation method (FLOTAC) allows examination of considerably larger amounts of faeces compared with Kato-Katz or the McMaster techniques thus improving the sensitivity of microscopy • Formalin-based techniques for sedimentation and concentration may increase the diagnostic yield in patients with a light schistosomiasis, such as returned travellers • Kato-Katz thick smear stool examination, as rapid, simple, inexpensive and 100% specific technique, is widely used in field studies for diagnosis of high-intensity schistosomal infections • Intestinal schistosomiasis is diagnosed by visualization of a single egg in thick smears of stool specimens with or without suspension in saline • In heavy *Clonorchis sinensis* infections, adult flukes may be observed in faecal specimens • Microscopic visualization of *Fasciola* spp. eggs in bile is suggestive for *Fasciola hepatica* infection (*Fasciola buski* is limited to the small intestine)	• The sensitivity of microscopy, in particular for low-intensity infections, is frequently insufficient • Differential diagnosis of trematode species, such as *Fasciola hepatica* and *Fasciola gigantica*, or *Clonorchis sinensis*, *Opisthorchisvi verrini*, and various intestinal flukes (e.g. *Haplorchis taichui* and *Haplorchis pumilio*), using microscopic examination is difficult because of the similarity in egg size, shape, and colouration • Early stage (prepatent) infections cannot be diagnosed by microscopy considering that this test relies on the detection of parasite eggs (in the case of schistosomiasis, eggs can be visualized microscopically at least 2 months after the last known freshwater contact) • Due to intermittent egg shedding, even three specimens may be needed to make a diagnosis of schistosomiasis in some patients • Eggs of *Fasciola hepatica* are microscopically indistinguishable from *Fasciola buski* which is, because of the difference in treatment, a significant problem • The eggs of *Clonorchis sinensis* cannot be differentiated from the eggs of two intestinal flukes: *Heterophyes heterophyes* and *Metagonimus yokogawai*	[8,23,94,95]
Filariasis	• Wet preparations of blood or buffy coat samples • Thick and thin blood or buffy coat films stained with Giemsa • Wet preparations of skin snips • Concentration methods - Knott technique and Nuclepore polycarbonate filters	• Provides observation of live, motile microfilaria in fresh wet preparations of blood or buffy coat samples • Filariae may also be microscopically visualized in thick and thin blood or buffy coat films stained with Giemsa (*Wuchereria bancrofti*, *Brugia* spp, *Loa loa*, *Mansonella perstans*, and *Mansonella ozzardi*) or skin snips (*Onchocerca volvulus* and *Mansonella streptocerca*) • Fresh unstained wet preparations of skin snips after incubation in saline at 37ºC for 2–24 h provides laboratory diagnosis of onchocerciasis • Knott technique or the use of Nuclepore polycarbonate filters (Capitol Scientific, Inc., Austin, TX, USA) can increase the sensitivity of light microscopy	• The technique is tedious, has low sensitivity in low-density infections and infections where adult worms are present but produce no microfilariae, and is often impractical • The sensitivity of microfilariae detection depends on the volume of sampled blood and the time of blood collection • Requires highly skilled and dedicated professionals in slide preparation, microfilarial identification, and speciation • Night blood collection is necessary (between 22:00 h and 02:00 h) to match the circadian periodicity in microfilaria production of *Wuchereria bancrofti* and *Brugia malayi* • Preparation of microscope slides is labour-intensive • Microfilariae are frequently absent from the blood during the early and late stages of infection	[54,69,96,97]

media, morphological light, or electron microscopic examinations, biochemical and serological assays, single PCR analyses) with variable sensitivity, specificity, and turnaround times to perform diagnoses [156]. The practice has been complex, labour-intensive, time-consuming, expensive, and often requires the transport of clinical samples to reference laboratories. These factors affect patient management, infection control, and public health interventions. The limited palette of available laboratory tests mean that the underlying aetiological cause can often remain unidentified [46,157–159].

Compared with traditional diagnostic methods, newly developed multiplex molecular panels allow detection of multiple gastrointestinal pathogens from a single biological specimen (see Tables 4 and 5) [160–162]. Moreover, such assays can be quantitative, which is a significant contribution towards the diagnosis of parasitic infections where infection intensity is necessary to determine morbidity [163]. These methods provide significant improvements in the diagnosis and treatment of specific demographics within the human population. For example, FilmArray GI panel (BioFire Diagnostics, Salt Lake City, UT) and Luminex xTag GI pathogen panel (Luminex Corporation, Toronto, Canada), that have been cleared by the Food and Drug Administration (FDA) for the detection of GI pathogens, are capable of differentiating a broad range of potential causative agents of diarrhoea, including *Giardia* spp. and *Cryptosporidium* spp. [157,164]. Both groups are capable of developing into chronic infections in infants, the elderly and immuno-compromised or -suppressed patients, causing malabsorption, severe weight loss and/or failure to thrive, and can sometimes be fatal in the absence of treatment. As anti-cryptosporidial drugs and vaccines are not efficient/available, and treatment failures with nitroimidazoles have been reported in patients with *Giardia* infections because of drug resistance [165], the control of these diseases relies primarily on preventative measures and effective diagnosis.

Multiplex molecular panels have dramatic potential to increase the detection rate of the gastrointestinal pathogens in solid organ transplant (SOT) recipients [166]. Also, these panels can provide rapid differentiation between infectious and non-infectious diarrhoea in transplant patients, which is essential for timely initiation of appropriate treatment, prevention of unnecessary modifications of the immunosuppressive therapy, patient's and graft survival, and avoidance of invasive diagnostic procedures such as endoscopy [166–168]. Endoscopic examination carries significant risk of infection in these patients because of the possible presence of a number of microorganisms inside the endoscope or the growth of a biofilm. Also, this procedure may result in tissue damage or dissemination of infective foci during observation [169].

With the introduction of multiplex gastrointestinal assays in clinical practice, the detection rate of co-infections has increased [37,167]. This has important implications for assessing parasite-parasite interactions and the pathology of dual infections, not only on the human digestive tract but also the beneficial microbiome within this microenvironment [160]. It also has implications for proactive control. For example, immunization against human hookworm diseases can be affected if one species downregulates subsequent immune responses for another [170].

The major benefits of multiplex PCR testing include high sensitivity and accuracy, detection of difficult-to-identify parasites in stool samples, and the ability to differentiate closely related species, which aids understanding of sources of infection, treatment, and control [77,80,160,163]. Although some tests still require optimization, examples include:

(i) The detection of *Necator americanus, Ancylostoma* spp., *Ascaris* spp., *Trichuris trichiura, Entamoeba histolytica, Giardia duodenalis*, and *Strongyloides stercoralis* from minute quantities of starting material [77].
(ii) The identification of *Cryptosporidium* spp., *Cyclospora cayetanensis*, *Blastocystis hominis*, and *Dientamoeba fragilis* in stool samples, which would otherwise involve specific staining procedures of (oo)cysts [80,171,172].
(iii) The ability to distinguish *Ancylostoma duodenale*, which is linked to severe clinical symptoms, from *Necator americanus* [173]. This distinction, in turn, improves methods of control – *Necator americanus* is frequently detected only at sites of defaecation because the parasite cannot persist for long periods in the external environment compared with *Ancylostoma duodenale* [163]. In developing countries, these two nematode species continue to be recognized as a leading cause of iron deficiency anaemia and protein malnutrition [174]. On the other hand, the ability to identify species within the genus *Ancylostoma* [77] is of great importance given that *Ancylostoma ceylanicum* represents the second most common hookworm species infecting humans in Asia, while 6–23% of persons positive for hookworm eggs are infected with this species in Thailand, Laos, and Malaysia [174].
(iv) The ability to differentiate the non-pathogenic *Entamoeba dispar* from the human pathogen *Entamoeba histolytica*, a parasite that causes 100 000 deaths per year [78].
(v) The ability to distinguish *Clonorchis sinensis*, a trematode which has been reclassified as a Group 1 biological carcinogen, from *Opisthorchis* spp. and *Metagonimus yokogawai* [171].

Other benefits, especially in resource-rich areas, include:

(i) Significantly lower sample-to-answer turnaround time compared with conventional methods [156].
(ii) Dramatic decrease of hands-on-time [175].
(iii) Overall reduction of the total costs (costs of reagents, technicians' time, and equipment) compared with completing routine tests [37].
(iv) Reduction in the personnel involved in the workflow of infective gastroenteritis diagnosis [159].
(v) Improved treatment choices combined with earlier initiation without the need for additional confirmatory testing [159].
(vi) Prevention of unnecessary patient isolation, and a significant reduction in time when required [154].
(vii) Minimizing the occurrence of false-negatives [154].

Despite these improvements, drawbacks remain. One major drawback is the inability to differentiate viable pathogen agents, nonviable organisms, or free DNA/RNA. False-positive results may be obtained for extended periods following treatment because of the presence of cell free pathogen DNA/RNA in the patient. Multiplex gastrointestinal assays do not definitively differentiate between clinical disease, subclinical infection, asymptomatic carriage, or intermittent shedding [37,167]. Similarly, the methods are subject to the inconsistent distribution of parasitic elements (i.e. such as larvae, eggs and (oo)cysts) in biological samples, the day-to-day variation in excretion, as well as the nonhomogeneous and complex structure of biological (faecal) masses. In addition, errors can occur during sample collection and processing, although this is now less common with the introduction of systems that do not require post-PCR handling and integrate DNA extraction into the kits [77,162,176,177]. Finally, these methods require appropriately trained staff and well-equipped laboratories that can fulfill high standards. Therefore, these assays are not suitable for a point-of-care test, and the price of reagents and instruments remain great for resource-poor communities (Table 1) [77,157,177].

Plasmodium multiplex molecular panels

Rapid molecular multiplex diagnostic tests are an excellent alternative to conventional microscopy or immunological testing for malaria. Multiplex PCR assays offer many potential applications: large-scale screening of samples within a short period which is required in epidemiologic studies, the high diagnostic efficacy of malaria detection in states of low parasitemia levels, detection of malaria co-infection with other parasites [202–204], identification

Table 3
Performances of immunochromatographic tests for detection of parasites

Species	Type of sample	Detection of Ag/Ab	Time to obtain results	Sp/Sn of evaluated tests (%)	Tests used in the study	References
Plasmodium sp.	Whole blood	Ag (HRP2) *P. falciparum* Ag (PAN-pLDH) Ag *non-falciparum species* Ag of *P. vivax*	20 min	99.67/96.46 99.24/95.03 99.74/96.83 99.74/96.70	Wondfo® Rapid Diagnostic Kit (Pf-HRP2/PAN-pLDH), (Guangzhou Wondfo Biotech Co. Ltd, Guangdong, China)	[116]
Plasmodium falciparum and *Plasmodium vivax*	Whole blood	Ag (HRP2) *P. falciparum* Ag (pLDH) *P. vivax*	20 min	90.1/97.7 98.4/79.6	First Response® Combo Malaria Ag (pLDH/HRP2) card (Premier medical corporation Ltd, Daman, India)	[117]
		Ag (HRP2) *P. falciparum* Ag (pLDH) *P. vivax*	20 min	91.9/75.8 96.7/19.8	GENOMIX(Pf/Pv) Malaria Antigen Detection (Genomix Molecular Diagnostics Pvt. Ltd., Hyderabad, India)	
		Ag (HRP2) *P. falciparum* Ag (pLDH) *P. vivax*	15–30 min	84.1/89.4 98.6/45.7	FalciVax™ (Zephyr Biomedical, Verna, Goa, India)	
		Ag (HRP2) *P. falciparum* Ag (pLDH) *P. vivax*	15–30 min	85.8/88.9 97.7/49.5	Parascreen®,Pan/Pf Device (Zephyr Biomedicals, Verna, Goa, India)	
		Ag (HRP2) *P. falciparum* Ag (pan specific aldolaze) *P. vivax*	15–30 min	90.6/77.9 97.9/34.3	ParaHIT® Total Pan/Pf (Span Diagnostics Ltd., Surat, Gujarat, India)	
		Ag (HRP2) *P. falciparum* Ag (pLDH) *P. vivax*	15–30 min	85.4/86.8 98.1/49.5	SD BIOLINE Malaria Ag Pf/Pan (SD Bio Standard Diagnostics Pvt Ltd., Gurgaon, Haryana, India)	
		Ag (HRP2) *P. falciparum* Ag (pLDH) *P. vivax*	15–30 min	89.6/86.7 98.4/42.9	NecVIPARUM one step malaria Pf/Pv antigen detection (Nectar Life Science Ltd., Chandigarh, India)	
		Ag (HRP2) *P. falciparum* Ag (pLDH) *P. vivax*	20 min	92.8–98.5/ 87.5–95.9 87.5–95.9/ 92.8–98.5	CareStart™ Malaria HRP-2/pLDH (Pf/ pan) Combo (Access Bio, Inc., New Jersey, United States)	[118]
		Ag (HRP2) *P. falciparum* Ag (pan specific aldolaze) *P. vivax*	15–30 min	69.5–76.2/ 84.8–94.0 97.4–97.8/63.3 –68.4	Malascan™ Device (Pf/Pan) (Zephyr Biomedical, Verna, Goa, India)	[119]
Toxoplasma gondii	Serum	Ab (IgG and IgM)	20–30 min	96/97	Toxoplasma ICT IgG-IgM test (LDBIO Diagnostic, Lyon, France)	[120]
Leishmania spp.	Serum	Ag	15 min	98.7/95.8	Not registered, still in the research phase	[109]
		Ab (anti-rk39 IgG)		97.9/94.8	Kalazar Detect™ (InBios International, Inc., Washington, USA)	
		Ab (anti-rKE16)	15–30 min	98–99.2/ 36.8–92.8	CrystalKA (Span Diagnostics, Ltd., Surat, India)	[121]
			2–10 min	96.4–100/73.2 –100	Signal KA (Span Diagnostics, Ltd., India)	
		Ab (anti-rk39)	5 min	95.6–97.6/87.2 –98.8	DiaMed-IT LEISH® (Bio-Rad, Marnes-la-Coquette, France)	
		Ab (anti-rk39)	15 min	96.8/99.6	OnSite Leishmania Ab Rapid Test (CTK Biotech Inc, San Diego, United States)	
Trypanosoma cruzi	Whole blood Serum	Ab	25–35 min	98.8/87.3 100/95.7	WL Check Chagas test (Wiener Lab SAIC, Argentina)	[106,122]
	Peripheral blood Capillary blood Serum		10–15 min	93.6/92.1 95/86.4 70.7/84.9	Simple Chagas WB (Operon S.A., Cuarte de Huerva, Zaragoza, Spain)	[123] [122]
	Serum/plasma		10 min	91.6/100	Simple Stick Chagas (Operon S.A., Cuarte de Huerva, Zaragoza, Spain)	[123]
	Serum		20 min	96.8/99.5	PATH–Lemos Rapid Test (Laboratorio Lemos SRL, Buenos Aires Argentina)	[124]
			15 min	91/90.1	OnSite Chagas Ab Rapid test (CTK Biotech, San Diego, United States)	[122]
			15–25 min	79/76.6	Chagas Instantest (Silanes, Mexico)	
			10–15 min	94/92.9	Trypanosoma Detect™ Rapid Test (InBios International Ltd., Seattle, Washington)	
			10–15 min	93.2/92.9	Chagas Quick Test® (Cypress. Diagnostics, Belgium)	
			15 min	93.2–99.5/87.2 –95.3	Chagas STAT-PAK (Chembio Diagnostic Systems, Medford, NY)	[122,124]
			5–25 min	97/10.6	Immu-Sure Chagas (Millennium Biotech, United States)	[122]

Table 3 (*continued*)

Species	Type of sample	Detection of Ag/Ab	Time to obtain results	Sp/Sn of evaluated tests (%)	Tests used in the study	References
			15 min	94–100/ 90.7–99.3	SD Bioline Chagas Ab Rapid test (Standard Diagnostics, Seoul, Republic of Korea)	[122,125]
			120 min	94.7/94.2	SERODIA®-Chagas (Fujirebio Inc., Tokyo, Japan)	[122]
			60 min	94/97.2	ImmunoComb® II Chagas Ab Kit (Orgenics, Yavne, Israel)	
Trypanosoma brucei gambiense	Plasma Whole blood	Ab	15 min	96/98.5 98/97.5 99/93.9	HAT Sero-Strip (Coris BioConcept, Gembloux, Belgium) HAT Sero-K-SeT (Coris BioConcept, Gembloux, Belgium)	[126]
Entamoeba histolytica	Stool	Ag	10 min	96.1/62.5	Rida®Quick *Cryptosporidium* (N1202), *Giardia* (N1102) and *Entamoeba* (N1702) single tests; Rida®Quick *Cryptosporidium/Giardia* (N1122) combi test; Rida®Quick *Cryptosporidium/ Giardia/Entamoeba* combi test (R-Biopharm, Darmstadt, Germany)	[110]
Giardia duodenalis		Ag		>99/90–97		
Cryptosporidium spp.		Ag		93.6–94.9/ 70–72		
Cryptosporidium sp.				94/100	Cryptosporidium lateral flow (TechLab, Inc., Blacksburg, VA)	[127]
Acanthamoeba keratitis	Corneal scraping	Ag	300 min	No data available	Not registered, still in the research phase	[111]
Trichomonas vaginalis	Vaginal swab	Ag	10 min	99.37/97.98	OSOM® Trichomonas Rapid Test (Sekisui Diagnostics, Framingham, MA, USA)	[128]
Taenia solium	Serum	Ab (anti-ES33) Ab (anti-T24H)	25 min 15 min	96/94.5 98.9/93.9	Not registered, still in the research phase	[129]
Schistosoma japonica	Serum	Ab (anti-SEA IgG)	15 min	94.91–97.6/ 93.7–95.1	Not registered, still in the research phase	[113,114]
Schistosoma mansoni	Urine	Ag (circulating cathodic antigen - CCA)	20 min	74.2–78/ 85.4–89.1	Not registered, still in the research phase	[130,131]
Schistosoma haematobium	Urine	Ag (circulating cathodic antigen - CCA)	20 min	78.9/36.8	Not registered, still in the research phase	[131]
Echinococcus granulosus	Serum/Plasma	Ab (anti-5/B IgG)	30 min	87.5/96.8	VIRapid® HYDATIDOSIS (Vircell, Granada, Spain)	[112]
	Serum	Ab (abs to hydatid cyst fluid ags)	15 min	91.4/100	Not registered, still in the research phase	[132]
Echinococcus multilocularis	Serum	Ab (anti-Em 18)	20 min	95.4/94.2	Not registered, still in the research phase	[133]
Wuchereria bancrofti	Serum/Plasma	Ag (CAg)	20 min	95.3–96.4/ 91.6–92.6	Not registered, still in the research phase	[115]
Toxocara canis	Serum	Ab (anti-recTES-30) Ab (anti-rTES-26, rTES-30 and rTES-120)	20 min <15 min	90.1/85.7 No data available	iToxocara kit, still in the research phase Not registered, still in the research phase	[134] [135]
Angiostrongylus cantonensis	Serum	Ag (excretory-secretory Ags)	5–10 min	91.1/100	Not registered, still in the research phase	[136]
Capillaria philippinensis	Serum	Ab	15 min	96.6/100	Kapillariasis ICT kit, still in the research phase	[137]
Fasciola hepatica	Whole blood	Ab (IgG)	22 min	No data available	SeroFluke test, still in the research phase	[138]
	Serum		10 min	100/100		
Paragonimus skrjabini	Serum	Ab	10 min	94.12/94.44	Not registered, still in the research phase	[139]
Gnathostoma spp.	Serum	Ab (anti-rGslic18)	15 min	97.01/93.75	Not registered, still in the research phase	[140]

of malaria causing *Plasmodium* species [205,206], diagnosis of malaria in high-malaria-burdened regions where there are limited clinical personnel and experienced microscopists, diagnosis of malaria in patients with history of prior travel to endemic areas and refugees, and detection of *Plasmodium* molecular markers of drug resistance [205].

Many studies have been carried out with the aim of designing simpler, single-step multiplex PCR systems, which could be useful for diagnosis of malaria, a disease that requires prompt diagnosis and effective treatment (Table 6). Intentions are the development of tests with high sensitivity and specificity in identification and differentiation of all five human *Plasmodium* species as well as in detection of mixed-species infections that will be suitable for use in laboratories worldwide, especially in developing countries and endemic areas. On the other hand, the spread of resistant malaria parasites is a major challenge for disease control and elimination. Molecular markers of resistance, therefore, represent a useful tool to monitor the spread of anti-malarial resistance in the regions of the world where this infection is endemic [202–207].

Research priorities for the future

Continuous development and commercialization of various serological and molecular-based diagnostic tests have significantly contributed to better clinical outcomes in patients with parasitic diseases. However, there are still some challenges in this area. In the future, therefore, we will specifically need to:

Table 4
Performances of commercial molecular gastrointestinal multi-pathogen detection panels for detection of parasites

	Pathogens	Time to obtain results/hands-on time	Sn/Sp of evaluated tests (%)	Trade name	Manufacturer	Preextraction required	Principle	Detection methodology	References
Gastrointestinal detection panels	*Cryptosporidium* spp., *Cyclospora cayetanensis*, *Entamoeba histolytica*, *Giardia lamblia* + 11 bacteria +5 viruses + 2 toxins	1 h/2 min	94.5–100/ 97.1-100	FilmArray® GI	BioFire Diagnostics, Salt Lake City, UT, USA	No	Nested-multiplex PCR	Endpoint melting curve analysis	[157]
	Giardia lamblia, *Cryptosporidium* spp., *Entamoeba histolytica*, *Ascaris lumbricoides*, *Trichuris trichiura* + 9 bacteria +5 viruses	4 h/No data available	85/77 vs conventional assays 98/96 vs PCR-Luminex assays	TaqMan® Array Card	Life Technologies, Carlsbad, CA, USA	Yes	Multiplex tandem PCR	Fluorescence-labelled probe	[178]
	Giardia lamblia, *Cryptosporidium* spp., *Entamoeba histolytica* + 6 bacteria +3 viruses + 2 toxins	5–6 h/1 h	90–97/ 99.0–99.9	xTAG® GPP	Luminex Corporation, Austin, TX, USA	Yes	Multiplex PCR and hybridization	Fluorescence-labelled bead array	[164,179]
	Giardia lamblia, *Cryptosporidium* spp., *Entamoeba histolytica* + 7 bacteria +5 viruses	6 h/No data available	No data available	FTD® Gastroenteritis	Fast-track Diagnostics, Junglinster, Luxembourg	Yes	Multiplex qPCR based on TaqMan® technology	Multiple fluorophore detection	[180]
	Giardia lamblia, *Cryptosporidium parvum*, *Entamoeba histolytica* and *Dientamoeba fragilis*	1.5 h/No data available	No data available	RIDA®GENE Parasitic Stool Panel	R-Biopharm	Yes	Multiplex qPCR	Multiple fluorophore detection	[181]
	Giardia lamblia, *Cryptosporidium parvum*, *Entamoeba histolytica*, *Dientamoeba fragilis* + 2 bacterial pathogens + 6 viruses	No data available	No data available	Gastroenteritis Multiplex	Diagenode, Liege, Belgium	Yes	Multiplex qPCR/qPCR	Multiple fluorophore detection	[146]
	Giardia spp., *Giardia lamblia*, *Cryptosporidium* spp., *Cryptosporidium parvum*, *Entamoeba histolytica* + 6 bacterial pathogens + 5 viral pathogens	3 h/No data available	No data available	Faecal pathogens A	AusDiagnostics	Yes	Multiplex tandem PCR	Intercalating dye detection	[182]
	Giardia duodenalis, *Cryptosporidium* spp., *Entamoeba complex*, *Dientamoeba fragilis*, *Blastocystis* spp.,	3 h/10–20 min	92–100/100	EasyScreen™	Genetic Signatures, Sydney, Australia	Yes	Multiplex qPCR	Multiple fluorophore detection	[183,184]
	Giardia lamblia, *Entamoeba histolytica*, *Cryptosporidium* spp., *Dientamoeba fragilis* + 9 bacterial pathogens + 4 viral pathogens	5 h/No data available	60–100/No data available	GastroFinder™ Smart 17 Fast	PathoFinder, Netherlands	Yes	Multiplex qPCR	Endpoint melting curve analysis	[156]
	Giardia duodenalis, *Cryptosporidium hominis*, *Cryptosporidium parvum*, and *Entamoebahistolytica*	3.5 - 4.5 h/ 50 min for a batch of 24 samples	95.5–100/ 99.5–100	BD MAX™ Enteric Parasite Panel	Becton, Dickinson and Company, USA	No	Multiplex qPCR	Multiple fluorophore detection	[185,186]
	Giardia duodenalis, *Cryptosporidium* spp., *Entamoeba histolytica*, *Entamoeba dispar*, *Dientamoeba fragilis*, *Blastocystis hominis* + 3 bacterial pathogens	No data available	No data available	NanoCHIP® Gastrointestinal Panel	Savyon Diagnostics Ltd, Israel	Yes	Multiplex PCR and hybridization	Fluorescence-labelled probes – sandwich hybridization array	[38]
	Giardia lamblia and *Cryptosporidium* + 8 bacterial pathogens + 6 viral pathogens	3.5 h/No data available	No data available	OpenArray™ nanolitre real-time PCR platform	OpenArray, Life Technologies Corp., Carlsbad, CA	Yes	Multiple target nanolitre quantitative reverse transcriptase-qPCR	Multiple fluorophore detection	[187]

- Develop new, specific biomarkers: a) which will provide sero-diagnosis of trichinellosis during the early stage of disease (3–4 weeks between *Trichinella* infection and specific antibody positivity) [213], b) for *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense* infections considering the fact that these parasites express variable surface antigens which significantly limits diagnostic usefulness of serological examination [214], c) for *Toxoplasma gondii* infection suitable to divide

Table 5
Performances of in-house molecular gastrointestinal multi-pathogen detection panels for detection of parasites

	Pathogens	Sn/Sp of evaluated tests (%)	Principle	Detection methodology	References
Gastrointestinal detection panels	*Ascaris lumbricoides, Trichuris trichiura, Necator americanus*	87/83	Multiplex PCR	Gel electrophoresis	[188]
	Necator americanus, Ancylostoma spp. *(A. duodenale, A. ceylanicum), Ascaris* spp., *Trichuris trichiura* and equine herpes virus	No data available	Multiplex qPCR	Multiple fluorophore detection	[77]
	Necator americanus, Ancylostoma duodenale, Trichuris trichiura, Strongyloides stercoralis, and *Ascaris lumbricoides*	No data available	Multi-parallel qPCR	Multiple fluorophore detection	[189]
	Ascaris lumbricoides, Necator americanus, Ancylostoma duodenale, Giardia lamblia, Cryptosporidium spp., *Entamoeba histolytica, Trichuris trichiura* and *Strongyloides stercoralis*	No data available	Multi-parallel quantitative qPCR	Multiple fluorophore detection	[160,163]
	Entamoeba histolytica, Giardia lamblia, Cryptosporidium parvum, and Phocin Herpes Virus 1 (PhHV-1)	100/100	Multiplex qPCR	Multiple fluorophore detection	[190]
	Entamoeba histolytica, Entamoeba dispar, Giardia lamblia assemblages A and B, *Cryptosporidium parvum* types 1 and 2	No data available	Multiplex PCR and hybridization	Fluorescence-labelled probe/ microarray	[191]
	Entamoeba histolytica, Giardia intestinalis, Cryptosporidium spp.	88.33 (86–90)/98.33 (97–100)	Multiplex qPCR based on TaqMan® technology	Multiple fluorophore detection	[192]
	Entamoeba histolytica, Giardia lamblia and *Cryptosporidium parvum/Cryptosporidium hominis*, PhHV	No data available	Multiplex qPCR	Multiple fluorophore detection	[193]
	Ancylostoma duodenale, Necator americanus, Ascaris lumbricoides, Strongyloides stercoralis, Schistosoma, and PhHV-1	91.86 (83.3–98.2)/ 100	Multiplex qPCR	Multiple fluorophore detection	[194]
	Ancylostoma duodenale, Necator americanus, and *Oesophagostomum bifurcum*	95.57 (86.7–100) – 99.33 (98.5–100)/ 100	Multiplex qPCR	Multiple fluorophore detection	[195]
	Entamoeba histolytica, Cryptosporidium parvum sensu lato, Giardia lamblia and PhHV	No data available	Multiplex qPCR	Multiple fluorophore detection	[196]
	Cryptosporidium, Giardia lamblia and *Entamoeba histolytica* + 7 bacterial pathogens + 5 viral pathogens	86.2/≥ 95	Multiplex qPCR	Multiple fluorophore detection	[197]
	Microsporidia, *Cyclospora* and *Cryptosporidium* spp.	No data available	Multiplex nested PCR	Gel electrophoresis	[198]
	Cyclospora cayetanensis, Cystoisospora belli, Enterocytozoon bieneusi and *Encephalitozoon intestinalis*	87–100/88–100	Multiplex PCR	Fluorescence-labelled bead array	[172]
	Entamoeba histolytica, Cryptosporidium, Giardia lamblia; Ancylostoma duodenale, Necator americanus, Ascaris lumbricoides, and *Strongyloides stercoralis*	No data available	Multiplex qPCR	Multiple fluorophore detection	[199]
	Schistosoma mansoni, Schistosoma haematobium and PhHV-1	No data available/100	Multiplex qPCR	Multiple fluorophore detection	[200]
	Cryptosporidium spp., *Giardia intestinalis, Entamoeba histolytica, Ancylostoma duodenale, Ascaris lumbricoides, Necator americanus,* and *Strongyloides stercoralis*	83–100/88–100	Multiplex PCR-bead assay	Fluorescence-labelled bead array	[201]
	Cryptosporidium parvum, Giardia lamblia, Entamoeba histolytica, Blastocystis hominis, Dientamoeba fragilis, Clonorchis sinensis, Metagonimus yokogawai, and *Gymnophalloides seoi*	100/100	Multiplex qPCR	Multiple fluorophore detection	[171]

These are in-house molecular panels which need prior extraction, and there were no data available regarding time to obtain results or hands-on-time.

recent from past infections and replace a panel of tests (*Toxoplasma gondii* serologic profile (TSP)) which is still in use for this purpose [215], d) which will allow serological differentiation between *Toxocara canis* and *Toxocara cati* (or *Toxocara malaysiensis*) infections [216].

- Develop scalable tests, particularly for use in field situations (e.g. rapid test for: a) the diagnosis of *Babesia* infections which will allow screening of the large population of blood donors, monitoring the infectious status or parasite burden in the host, thus contributing to appropriate control strategies [217], b) the diagnosis of *Plasmodium knowlesi* infection suitable for use in remote rural areas [218]).
- Enhance the sensitivity and specificity of some current tests (e.g. a) rapid diagnostic strip tests for both *Schistosoma* sp. antigens and specific antibody detection [219], b) serological tests for diagnosis of cystic echinococcosis [220,221], c) and serological tests for diagnosis of cutaneous leishmaniasis [222]).
- Develop new, cost-effective, and technically simple molecular tests which will be suitable for widespread implementation in resource-poor settings, in which many of the parasitic diseases are endemic. Isothermal nucleic acid amplification tests (NAAT), such as loop-mediated isothermal amplification (LAMP) and nucleic acid sequence based amplification (NASBA), show promise for the future [57].
- Simplify procedures for sample handling and preparation. In addition, diagnostic instruments should be designed to lead partial or complete automation to reduce turnaround times [156,157].

Conclusions

Despite the substantial number of infected persons, most healthcare providers are not still familiar with some neglected

Table 6
Performances of molecular multi-pathogen detection panels for detection of *Plasmodium* spp. and *Plasmodium vivax* and *Plasmodium falciparum* molecular markers of drug resistance

	Pathogens	Time to obtain results/hands-on time	Sn/Sp of evaluated tests (%)	Trade Name	Manufacturer	Preextraction required	Principle	Detection methodology	References
Malaria detection panels	*Plasmodium falciparum, Plasmodium vivax, Plasmodium knowlesi, Plasmodium malariae* and *Plasmodium ovale*	3 h/No data available	62–100/100	PlasmoNex™	Reszon Diagnostics, Selangor, Malaysia	Yes	Multiplex PCR	Gel electrophoresis	[32,204,207]
	Plasmodium falciparum, Plasmodium vivax, Plasmodium knowlesi, Plasmodium malariae, and *Plasmodium ovale*	No data available	50/100	MSP-multiplex PCR	In-house	Yes	Multiplex PCR	Gel electrophoresis	[32]
	Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae, and *Plasmodium ovale*	3 h/No data available	100/100	Not registered	In-house	Yes	Multiplex qPCR based on TaqMan® technology	Multiple fluorophore detection	[52,208]
	Plasmodium falciparum and *Plasmodium vivax*	No data available	97.4–98.9/98.8–100	Not registered	In-house	Yes	Multiplex-nested reverse transcription PCR	Gel electrophoresis	[209]
	Plasmodium vivax dhfr, dhps and *mdr1* genes	No data available	No data available	Not registered	In-house	Yes	Multiplex nested PCR	Multiplex ligase detection reaction–fluorescent microsphere assay	[205]
	Plasmodium falciparum pfcrt, pfmdr1 and pfdhfr genes	No data available	No data available	Not registered	In-house	Yes	Multiplex PCR	Gel electrophoresis and restriction fragment length polymorphism analysis	[210]
	Plasmodium spp., *Plasmodium falciparum, Plasmodium vivax* and human RNaseP gene	No data available	No data available	Not registered	In-house	Yes	Multiplex quantitative qPCR	Multiple fluorophore detection	[211]
	Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae, Plasmodium ovale, and *Wuchereria bancrofti*	No data available	86/94	Not registered	In-house	Yes	Multiplex PCR	Multiplex ligase detection reaction–fluorescent microsphere assay	[202]
	Plasmodium falciparum, Plasmodium vivax, Plasmodium knowlesi, Plasmodium malariae, and *Plasmodium ovale*	No data available	No data available	Not registered	In-house	Yes	Semi-nested multiplex PCR	Gel electrophoresis	[212]
	Plasmodium falciparum, Plasmodium vivax, Plasmodium knowlesi, Plasmodium malariae and *Plasmodium ovale*	No data available	95.88 (89.5–100)/99.06 (98.4–100)	Not registered	In-house	Yes	Multiplex 5′ nuclease quantitative qPCR based on TaqMan® technology	Multiple fluorophore detection	[203]

parasitic diseases even in highly developed countries. Consequently, delays in accurate diagnosis of parasitic diseases severely affect the implementation of treatment and control strategies, which may have devastating consequences for the host and the prevention and spread of emerging pathogens. Therefore, clinicians should be adequately prepared through various education programmes to report parasitic diseases efficiently, while laboratory experts should be familiar with benefits and limitations of existing diagnostic procedures, especially those that are part of their everyday practice, to get the maximum from each test, avoid possible testing errors, and spend health resources rationally. Also, each parasitological laboratory must decide on an individual basis which tests are the most economical and the most appropriate to introduce into the routine work. In the future, based on the published results of molecular researchers with attempts and efforts to simplify parasitoses diagnosis, it can be expected that molecular assays will significantly improve parasitological diagnostics worldwide. Finally, a close relationship between laboratorians and clinicians remains a crucial issue in developing effective diagnostic strategies, and optimization and standardization of assays.

Transparency declaration

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