

# Faster Detection of *Helicobacter pylori* Infection by <sup>13</sup>C-Urea Breath Test. Comparing Short versus Standard Sampling Time

Emilio Molina-Molina<sup>1</sup>, Leonilde Bonfrate<sup>1</sup>, Michele Lorusso<sup>1</sup>, Harshitha Shanmugam<sup>1</sup>, Giuseppe Scaccianoce<sup>2</sup>, Theodore Rokkas<sup>3</sup>, Piero Portincasa<sup>1</sup>

1) Clinica Medica "A. Murri",

Department of Biomedical

Sciences and Human

Oncology, University of Bari

"Aldo Moro" Medical School,

Bari, Italy

2) Gastrointestinal Endoscopy,

"Perinei" Hospital, Altamura,

Bari, Italy

3) Department of

Gastroenterology, Henry

Durant Hospital Center,

Athens, Greece

## ABSTRACT

**Background & Aims:** <sup>13</sup>C-Urea Breath Test (UBT) is a non-invasive, highly accurate and recommended test to detect *Helicobacter pylori* (*H. pylori*) infection and to confirm post-therapy eradication. However, differences exist in terms of manufacturers, dose of labelled urea, addition of citric acid, solid vs. liquid formulation, and sampling times of breath samples. In this study, we compared the diagnostic accuracy of "short" (15 minutes) vs. "standard" (30 minutes) time for a single type of liquid UBT.

**Methods:** We compared the performance of a single UBT type (BREATHQUALITY, AB Analitica, Padua, Italy, 10 mL of 75 mg <sup>13</sup>C-Urea and 1.4 g citric acid) during a "short" vs. "standard" breath sampling time. Enrolled were 151 subjects requiring UBT as naïve (N=92) or post-eradication (N=59) checks.

**Results:** UBT at 15 and 30 minutes were highly comparable, showing optimal correlation in all subsets of patients (i.e. naïve vs. post eradication, negative vs. post eradication check). One discrepant result occurred at the borderline zone of the DOB 4%, but proved to be true positive at a later confirmation by a second UBT and stool antigen test.

**Conclusions:** By shortening the testing time of BREATHQUALITY to 15 minutes (-50%) comparable accuracy will be maintained and in addition, it will bring some benefits to patients' waiting lists, compliance, and hospital staff.

**Key words:** breath test – chronic gastritis – infrared analysis – peptic ulcer – stable isotope.

**Abbreviations:** DOB: delta over baseline; UBT: urea breath test.

**Address for correspondence:**

Prof. Piero Portincasa,

MD, PhD

Clinica Medica "Augusto

Murri"

Department of Biomedical

Sciences and Human

Oncology

University of Bari

Medical School

Piazza Giulio Cesare 11

70124 Bari - Italy

piero.portincasa@uniba.it

## INTRODUCTION

*Helicobacter pylori* (*H. pylori*) is a gram-negative spiral microaerophilic bacterium that colonizes gastric mucosa. The worldwide prevalence of *H. pylori* infections is approximately 50%, with a higher prevalence in low-income countries. *H. pylori* infection is associated with peptic ulcer disease, atopic disorders, gastric cancer and mucosa-associated lymphoid tissue (MALT) lymphoma [1]. Several guidelines recommend eradication of *H. pylori* infection in most of the diagnosed subjects [2, 3]. Current indications for diagnosis of *H. pylori* infection include non-invasive procedures

such as urea breath test (UBT), fecal *H. pylori* antigen (active infection) and serology (active or prior infections). Invasive tests for *H. pylori* infection include upper gastrointestinal endoscopy, coupled to rapid urease test or histology. In this context, choosing the right diagnostic test depends on several aspects, namely patients' choice, local availability of diagnostic tests, and clinical settings [4]. Urea breath test is the non-invasive, accurate and highly recommended test to detect *H. pylori* infection, employing the stable isotope <sup>13</sup>C. The urea breath test offers an important diagnostic role in younger subjects (generally aged less than 50) with upper uncomplicated gastrointestinal symptoms, who do not have "alarm" features (i.e., unintentional weight loss, unexplained iron deficiency anemia, persistent vomiting, progressive dysphagia or odynophagia, palpable mass or lymphadenopathy, and family history of upper gastrointestinal cancer). In these latter cases, upper gastrointestinal endoscopy or additional investigations are invariably performed [3, 4].

We incorporated the diagnosis of UBT in different settings looking at the impact of *H. pylori* infection in Italy, and outcome of different eradication therapies [5-8]. Patients

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undergo UBT according to standard protocols recommended by manufacturers, taking breath samples of exhaled air generally 30 minutes after ingestion of the  $^{13}\text{C}$ -urea-containing solution.

Because of the expanding role of UBT in the noninvasive diagnosis of *H. pylori* infection, there is a further and urgent need to rationalize the methodological aspects of the test. The time for breath sampling comprises a series of factors, which include time exposure of the gastric mucosa and *H. pylori* to the  $^{13}\text{C}$ -substrate, appearance of  $^{13}\text{CO}_2$  in breath, sample collection, and diagnostic outcome in terms of sensitivity and specificity. Additional factors also include the hospital stay of the patients, waiting lists and time devoted by doctors and nurses to the test, which generally requires at least 30 minutes between the substrate ingestion and the second breath sampling. In this study, therefore, we compared the diagnostic accuracy of “short” (15 minutes, T15) vs. “standard” (30 minutes, T30) time for a single type of liquid UBT currently adopted at our Hospital.

## METHODS

### Subjects

A total of 151 patients were enrolled (91 females, 60 males; age:  $49.3 \pm 1.3$  years) at the Academic Unit of Internal Medicine “Clinica Medica A. Murri”, in Bari, Italy. All subjects joining the study were outpatients from the public Hospital waiting list. Subjects were complaining of likely *H. pylori*-associated symptoms, including dyspepsia, gastroesophageal reflux (without alarm symptoms), a previous history of peptic ulcer, and use of chronic therapy with non-steroidal anti-inflammatory drugs (NSAIDs). Some patients had iron-deficiency-anaemia, vitamin B12 deficiency, and idiopathic thrombocytopenic purpura. A small subgroup of subjects was also referred by their own physicians because of a family history of *H. pylori* infection or personal concern about infection (risk of peptic ulcer, gastric cancer, atrophic gastritis), or with symptoms unrelated to *H. pylori* infection (urticarial, allergies, dermatological symptoms) [4]. All subjects underwent a visit including history, a general physical examination, UBT as well as the answer to a single question investigating the compliance of the short vs. standard UBT (see study protocol).

The study was totally noninvasive, and the protocol was approved by the local Ethics Committee of the University of Bari Medical School.

### Urea Breath Test

The UBT test is the standard diagnostic tool currently available from the central pharmacy of the Policlinico Hospital. The kit is registered as a medical device (BREATHQUALITY UBT, AB Analitica, Padua, Italy) at the Italian Ministry of Health (A.I.C. n. 034510014), and consists of 75 mg  $^{13}\text{C}$ -labelled urea in 10 mL of 1.4 g of citric acid solution. Subjects fasted for at least 8 hours and were free of medication which could influence the UBT results (i.e., of antibiotics in the prior month and no proton pump inhibitors in the previous two weeks) [4]. During the test, the patient swallowed the solution, added on-site to 200 mL tap water. UBT results depend on the gastric hydrolysis of urea by *H. pylori*. In case of infection,  $^{13}\text{CO}_2$

appears in breath, while ammonia is released into the stomach. Breath samples were analyzed by the same  $^{13}\text{C}$ -infrared analyzer (HeliFANplus®, FAN GmbH, Leipzig, Germany) kept at the Division of Internal Medicine. The differences between the values at T30 and baseline were expressed as delta over baseline (DOB,  $\delta\%$ ), with a normal cut-off up to 4%. As recommended by the manufacturer, the DOB at T30 was the reference value, and this was compared with the results obtained from a sample taken at T15.

### Study protocol

The protocol consisted of the following steps: 1) Full explanation of the purpose of the study and collection of the informed consent; 2) Collection of two baseline breath samples of expired air into glass vacutainers in the fasting subject at rest (8.00 AM). Smoking and physical exercise are forbidden for the whole duration of the test; 3) Ingestion of the 200 mL solution containing  $^{13}\text{C}$ -urea and citric acid; 4) Collection of duplicate breath samples at two time points: T15 and at T30; 5) Recording the answer to one question: “Would you prefer a 15 min rather than a 30 min UBT?”; 6) Calculation of  $^{13}\text{CO}_2$  concentrations in breath samples by infrared analyzer.

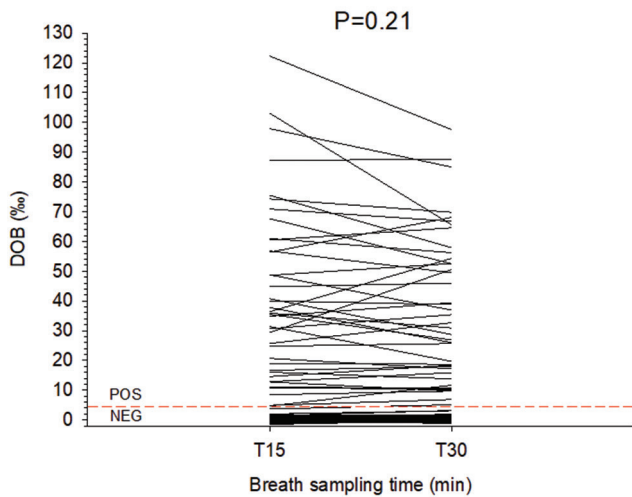
### Statistical analysis

Data were expressed as mean  $\pm$  SEM. Results of UBT were compared between T15 and T30 by a paired t-test and visual inspection of single results. The comparisons included the whole group and two subgroups, i.e. according to UBT results (positive or negative) and according to the patient’s status (naïve or post-eradication). Proportions were compared by contingency tables and Chi-Square or Fisher’s exact tests. Correlations were assessed by calculating Spearman’s test. Statistical analyses were performed with the NCSS statistical software (NCSS9 Statistical Software 2013. NCSS, LLC. Kaysville, Utah, USA, [www.ncss.com/software/ncss](http://www.ncss.com/software/ncss)) [9, 10]. The difference was considered statistically significant when the two-tailed probability (p-value) was less than 5%.

## RESULTS

Among the 151 enrolled subjects, females (n=91) tended to be younger than males (n=60) ( $48.2 \pm 1.6$  vs.  $50.9 \pm 2.1$  years respectively,  $p=0.30$ ). Physicians referred 75% of the patients; other specialists, including us, referred 20% of the patients, while 5% asked to undergo UBT because of their own decision and personal concern. Females were more represented than males (60% vs. 40%,  $p=0.0005$ ).

The results of the test comparing T15 and T30 appear in Fig. 1. With the given cut-off point set at DOB 4% at T30 (as recommended by the company), there were 41 UBT positive cases (27.2%) and 110 UBT negative patients (72.8%). Overall, mean DOBs were  $11.1 \pm 1.9\%$  (T15) and  $10.5 \pm 1.7\%$  (T30), with  $p=0.21$ . In addition, DOB was comparable in the case of a negative test ( $0.35 \pm 0.1\%$  at T15 vs.  $0.54 \pm 0.2\%$  at T30,  $p=0.06$ ) and positive test ( $40.8 \pm 4.6\%$  at T15 vs.  $38.1 \pm 3.9\%$  at T30,  $p=0.13$ ). The same occurred with respect to DOBs and patient’s status. For naïve patients there were 63 negative UBT (DOB  $0.22 \pm 0.1\%$  at T15 vs.  $0.35 \pm 0.1\%$  at T30,  $p=0.22$ ) and 29 positive UBT (DOB  $47.2 \pm 5.8\%$  at T15 vs.  $44.4 \pm 4.7\%$



**Fig. 1.** Comparison of exhaled breaths in subjects undergoing UBT after 15 (T15) and 30 minutes (T30). The dotted horizontal line is set at 4‰ DOB and indicates the normal cutoff limit. Differences between T15 and T30 are not significant. Legend: DOB: delta over baseline; NEG: negative; POS: positive.

at T30,  $p=0.19$ ). For post-eradication there were 47 negative UBT (DOB  $0.23 \pm 0.12$ ‰ at T15 vs.  $0.42 \pm 0.12$ ‰ at T30,  $p=0.16$ ) and 12 positive UBT (DOB  $23.2 \pm 4.0$ ‰ at T15 vs.  $21.3 \pm 3.9$ ‰ at T30,  $p=0.09$ ).

The overall correlations between DOB at T15 and T30 appear in Fig. 2. The highly significant correlation between the two time points was confirmed overall ( $n=151$ ,  $r=0.97$ ,  $p<0.001$ ) (Fig. 2A). The correlation also persisted in the two

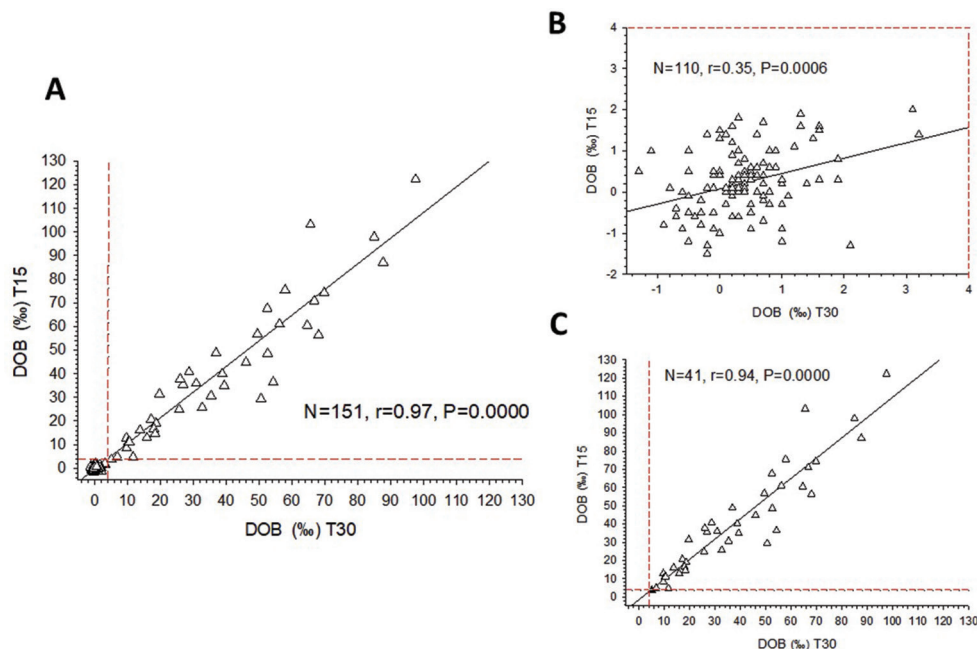
subgroups of patients with a negative UBT (Fig. 2B) and a positive UBT (Fig. 2C). Data dispersion, however, was much greater at lower DOB values, i.e. in the 110 UBT-negative cases ( $r=0.35$ ,  $p=0.0006$ ) (Fig. 2C).

The visual comparison of DOBs at T15 and T30 disclosed a single discrepant result at T15 close to the borderline cut-off of 4‰. In this unique patient, DOB was 3.7‰ (negative) at T15, and increased to 5.1‰ (positive) at T30 (see Fig. 1A, arrow). This patient underwent a second UBT one week apart, and the results were positive (DOB 6.1‰ and 5.7‰ at T15 and T30, respectively), and further confirmed by a positive stool antigen test. We arbitrarily considered this case a “false negative” result. Based on the first-round results from the 151 cases, therefore, the overall sensitivity and specificity at T15 was 97%, compared to the given 100% of sensitivity and specificity at T30. The answer to the question about preferring the short-15 min, rather than the standard 30 min UBT is summarized in Fig. 4. Over 90% of subjects were positive about the short version of UBT.

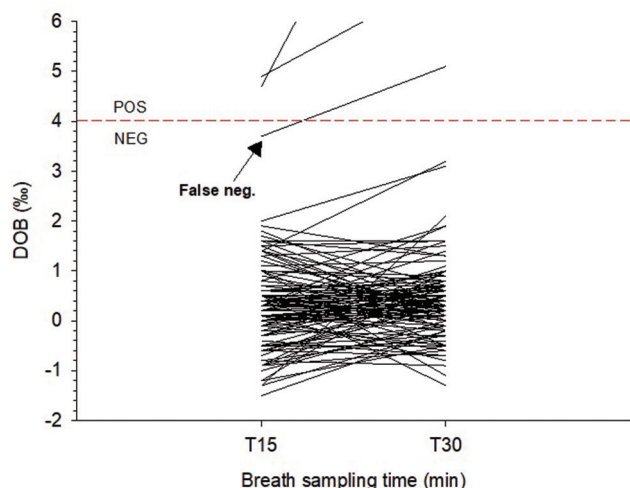
**DISCUSSION**

The UBT is one of the major non-invasive diagnostic tools for detection of *H. pylori* infection. The test is used worldwide, is totally non-invasive and can be used in children and pregnant women as well, with millions of subjects screened every year, both at a naïve level and after eradication therapies [4-7].

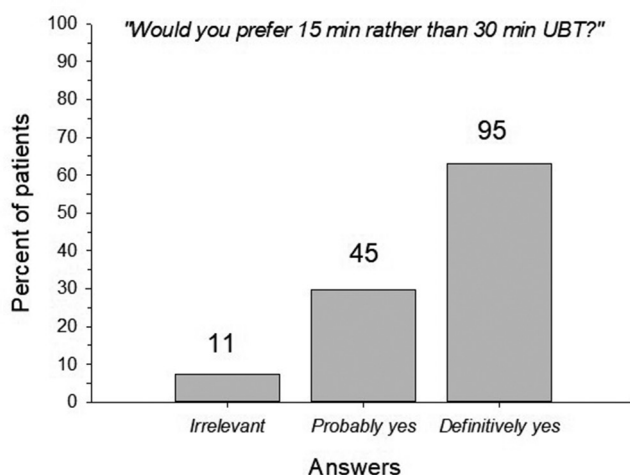
The results of the UBT provide important information which recapitulate further diagnostic and therapeutic decisions, in order to reduce the potential and deleterious



**Fig. 2.** A) Linear correlation between DOBs obtained at two time points (T15 and T30) in subjects undergoing UBT. A very strong correlation is disclosed by the  $r=0.97$ . B) Linear correlation between DOBs obtained at two time points (T15 and T30) in subjects negative for UBT. The dotted horizontal and vertical lines indicate the normal cutoff limit of 4‰ DOB. Data dispersion is visible and disclosed by the  $r=0.35$ . C) Linear correlation between DOBs obtained at two time points (T15 and T30) in subjects positive for UBT. The single black triangle at the intersection of the dotted lines represents the “false-negative” result at T15. A very strong correlation is disclosed by the  $r=0.94$ . Legend: DOB: delta over baseline; the dotted horizontal and vertical lines indicate the normal cutoff limit of 4‰ DOB. .



**Fig. 3.** Comparison of exhaled breaths at different times in subjects undergoing UBT. Compared to Fig. 1, the left Y-axis is set to a different scale (from -2 to 6 DOB) to emphasize the solitary “false negative” result around the normal cutoff limit of 4‰ DOB. Below the dotted horizontal line, only negative tests are shown. Legend: DOB: delta over baseline; NEG: negative; POS: positive.



**Fig. 4.** Judgement on UBT sampling times. Results are expressed as absolute cases over percentages (bars).

consequences of the chronically infected gastric mucosa in humans [3, 11, 12].

The sensitivity and specificity of UBT range from 88% to 95% and from 95% to 100%, respectively [13], with very uncommon false-positive results [14, 15]. In spite of such highly confident outcomes, differences still exist in terms of manufacturers, dose labelled urea (25, 50, 75 vs. 100 mg), addition of citric acid, solid vs. liquid formulation, and time required between each breath sampling (10, 15, 30 minutes) (Table I). In line with a previous study on 129 children and adolescents (age range: 2.1-19 years old), a dose of 25mg  $^{13}\text{C}$ -UBT was accurate for *H. pylori* diagnosis (prevalence: 41.1%), with an accuracy of 95.3% at T30 [16]. A need for evaluating different doses with bigger sample sizes across ages, populations and timing is therefore encouraged.

Thus, there is still room to rationalize further some methodological aspects of the test including testing time, i.e.,

time exposure of the gastric mucosa to the substrate without any significant diagnostic loss. In this study, therefore, we compared the sensitivity and specificity of “short” (T15) vs. “standard” (T30) time for a single type of liquid UBT provided by the local hospital pharmacy.

We show that this specific  $^{13}\text{C}$ -UBT (BREATHQUALITY) brings consistent and reproducible results in patients evaluated at a 3rd referral hospital for suspected *H. pylori* infection or for confirmation of post-therapy eradication. In this setting, the kit for UBT provided highly reproducible results when performed at 15 min, as compared with 30 min (as recommended by the manufacturer). The test performed well in naïve patients, irrespective of the following positive or negative results, and in patients checking for eradication after different antibiotic treatments [4].

The correlation of DOB between T15 and T30, however, was somewhat weaker at lower DOB values, i.e. when the results of the test were negative. Likely, the finding depends on the absence of ammonia-producing bacteria but larger low-level “physiological” fluctuations of  $^{13}\text{CO}_2$  in expired samples of healthy subjects.

Only in one case we found an apparent “false-negative” result at T15, as compared to T30, but it was very close to the borderline. This patient was enquired about potentially confounding factors, but all of them were later discarded. It is possible that the breath sample at T15 was insufficient, due to technical problems, especially at low DOB. That is why we performed a second UBT one week later, and found positive results at both T15 and T30, but still close to the borderline. In a real-life setting, this apparent discrepant result might have poor practical implications, since a crosscheck with a different test is advisable within the borderline range of  $4\pm 1\%$  DOB.

In terms of management, reducing the test time from 30 to 15 minutes would align BREATHQUALITY with several others (see Table I) and shorten the necessary time to assess the patient, while decreasing waiting lists and increasing patient enrollment and compliance. This latter aspect also emerged from the one-question survey we conducted at the end of the test. In addition, other indirect costs could be affected, such as those involving public and private transportation or absenteeism, as well as a decrease in the working time of hospital personnel.

## CONCLUSION

Shortening the testing time of BREATHQUALITY to 15 minutes (-50%) will keep comparable optimal accuracy and brings some benefits to patients’ waiting lists, compliance, and hospital staff.

**Conflict of interests:** None to declare.

**Authors’ contributions:** E.M.M. and M.L. designed the study protocol, analyzed the data and wrote the draft. L.B. and H.S. collected the data and helped in data analysis. G.S. and T.R. interpreted the results and participated in writing the paper. P.P. analyzed the data and reviewed the manuscript.

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**Table I.** Principal characteristics of kits for urea breath test.

Manufacturers	Sofar	Richen	Kibion	AB Analytica	BreathTek	Exalenz
Website	<a href="https://www.sofarfarm.it">https://www.sofarfarm.it</a>	<a href="https://www.richeneurope.eu">https://www.richeneurope.eu</a>	<a href="https://www.kibion.se/">https://www.kibion.se/</a>	<a href="https://www.abanalytica.com/it">https://www.abanalytica.com/it</a>	<a href="https://www.breathtek.com/">https://www.breathtek.com/</a>	<a href="http://int.exalenz.com/">http://int.exalenz.com/</a>
Nation	Italy	China	Sweden	Italy	USA	Israel
Name of test	Expirobacter ATC V04CX	Citredici UBTKit V04CX	Diabact UBT	BreathQuality UBT	BreathTek UBT	BreathID
<sup>13</sup> C-Urea	100 mg	50/75 mg	25/50 mg	75 mg	75 mg	75 mg
Citric acid	1.4 g	-	-	1.4 g	3.0 g	4.0 g
Formulation	Tablet	Powder	Tablet	Liquid	Powder	Tablet
Recommended Sampling	T0, T30	T0, T30	T0, T10	T0, T30	T0, T15	T0, T15

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