J Clin Gastroenterol 2003;37(2):139–141.

A Novel ^{13}C-Urea Breath Test Device for the Diagnosis of *Helicobacter pylori* Infection

Continuous Online Measurements Allow for Faster Test Results With High Accuracy

Eran Israeli, MD, Yaron Ilan, MD, Shimon Bar Meir, MD, Claudia Buenavida, MD, and Eran Goldin, MD

Abstract

Objective: The aim of this study is to determine the accuracy of a novel laptop sized ^{13}C-Urea breath test analyzer that continuously measures expired breath and to use its advantages to decrease testing time. **Methods:** One hundred and eighty-six subjects (mean age of 47.8 years) were tested simultaneously by the BreathID system (Oridion, Israel), and by the traditional IRMS. BreathID continuously measured the expired breath for a ratio of $^{13}CO_2$:$^{12}CO_2$. This value was expressed as delta over baseline (DOB) and displayed graphically on a screen in real time. **Results:** One hundred and one subjects were positive and 85 were negative for *H. pylori* by isotope ratio mass spectrometry (IRMS). The correlation for the BreathID system at 30 minutes was 100% for positive cases and 98% for negative cases. Analysis of the continuous curves generated by the BreathID for all patients permitted definition of different DOB thresholds for a positive or negative result at shorter time intervals. Thus, after 6 minutes a conclusive test result could be obtained for 64% of subjects, and after 10 minutes for 92% of subjects. **Conclusions:** The ^{13}C-Urea breath test utilizing the technology of molecular correlation spectrometry is an accurate method for determining infection by *H. pylori*. The advantage of continuous measurements can shorten testing time without compromising accuracy.

Key Words: Helicobacter pylori, ^{13}C-urea breath test, on-line breath test

Gastric infection by *Helicobacter pylori* is the main cause of chronic gastritis, promotes peptic ulcer disease, and is a risk factor for gastric malignancy. It has been recognized as a class I gastric carcinogen.[1] *H. pylori* eradication can be established reliably by histology, rapid urease testing, and urea breath test (UBT). Preliminary studies suggest that stool antigen test may also be a useful means.[2] UBT uses labeled urea (^{13}C or ^{14}C) that is metabolized by the presence of *H. pylori* to yield CO_2. The labeled gas is absorbed across the gastric mucosa and is subsequently measured in the patient's expired breath. The UBT has become the standard means of determining infection by *H. pylori* in several clinical settings such as post *H. pylori* eradication.[3,4] The ^{13}C-Urea breath test is a highly sensitive, non-invasive and safe method for detecting *H. pylori*.[5,6] Current ^{13}C-Urea breath test devices, which use isotope ratio mass spectrometry (IRMS), are expensive, cumbersome, and are usually restricted to large laboratories. Due to the remote analysis location, these methods were designed to minimize the number of samples, and results are not available on the spot.

We evaluated the use of a "laptop size" device for ^{13}C-analysis utilizing a novel technology of molecular correlation spectrometry[7] as compared with the traditional method of single point IRMS.

Submitted September 5, 2002. Accepted March 24, 2003

From the Department of Gastroenterology (E.I., Y.I., C.B.) Hadassah University Hospital, Jerusalem; and the Department of Gastroenterology (S.B.M.) Sheba University Hospital, Tel Hashomer[2], Israel.

Address correspondence and reprint requests to Dr. Eran Goldin, Hadassah University Hospital, Jerusalem Israel. E-mail: goldin@cc.huji.ac.il.

PATIENTS AND METHODS

Patient Population

One hundred and eighty-six subjects (80 women and 106 men, with a mean age of 47.8 years, range of 19 to 90 years) were tested for *H. pylori*. They comprised 6 groups: healthy volunteer subjects (n = 65); patients with a history of peptic disease and new onset dyspepsia (n = 42); new onset symptoms of dyspepsia without a history of peptic disease (n = 42), symptoms of gastroesophageal reflux (n = 10); post eradication of *H. pylori* (n = 17) and a group consisting of miscellaneous gastrointestinal symptoms (n = 10). Each subject was tested simultaneously by the standard ^{13}C-UBT IRMS method, and by The BreathID system as outlined below. Informed consent was obtained from participating subjects. Pregnant women, and children under the age of 18 years were not eligible. Subjects who had been taking antibiotics in the previous 6 weeks or Proton-pump inhibitors in the previous 7 days were excluded. This study was approved by the Ethical Committee of the Hadassah University Hospital, Jerusalem

^{13}C-UBT Utilizing the Method of Molecular Correlation Spectrometry

The BreathID system (Oridion, Israel) is composed of an analyzer designed to measure continuously the ratio of $^{13}CO_2$:$^{12}CO_2$ in exhaled air by an optical method. It also includes a laptop computer with a monitor that can be placed on the desktop. Results are recorded graphically on the monitor online and are available on the spot.

The system continuously sampled the subject's breath via a nasal cannula connected to the analyzer. First the baseline ratio of exhaled $^{13}CO_2$:$^{12}CO_2$ was measured. Then 75 mg of ^{13}C-urea, and

4 g of citric acid dissolved in 200 mL of water were given to the subjects.

$^{13}CO_2$:$^{12}CO_2$ ratios were expressed as delta over baseline ^{13}C (DOB) and displayed on the Breath ID screen in real time. The subjects were tested for 30 minutes for comparison to mass spectrometry. Eight samples were collected from each patient (including baseline and 30 minutes). Each sample was collected twice to assure concordance and the final result was averaged. Automatically filled tubes were used and sent for analysis by IRMS. The BreathID measured the same samples of expired gas simultaneously. The threshold for positive detection of *H. pylori* was 5 DOB for both methods as established previously for IRMS.[8,9]

Reference results were the traditional readings of the IRMS at 30 minutes. The operators of the BreathID or IRMS were unaware of the *H. pylori* status or the results of other tests.

Analysis of Results

The accuracy of the BreathID system was determined by comparing the 30 minutes DOB results of the IRMS (considered as gold-standard) to that of the BreathID system, thus determining the sensitivity and specificity. By analyzing the data curves generated for all of the subjects by the BreathID, criteria were defined that enabled reduction of the test time. These criteria included different DOB thresholds at earlier time intervals. Since these criteria were applicable by definition to 100% of subjects tested (with a positive or negative result), accuracy was not compromised.

RESULTS

One hundred and one subjects were positive and 85 were negative for *H. pylori* by IRMS at 30 minutes. These results were compared with the measurement of Breath ID at 30 minutes. The threshold of 5 DOB was used for both methods: for IRMS as well as for the breathID. The correlation between both methods was 100% for positive cases and 98% for negative cases. There were only 2 discordant cases, both of which were negative by IRMS and positive by BreathID. One subject was a healthy volunteer; the second was tested for *H. pylori* 6 weeks after eradication therapy. The readings by the IRMS were 4.7 DOB for the first patient and 4.9 DOB for the second patient, thus defining both patients as negative for *H. pylori*. The first patient had a reading of 5.1 DOB and the second patient a reading of 5.2 DOB by the breathID, thus defining these subjects as positive for *H. pylori* infection.

Since accuracy of the BreathID was determined to be comparable to IRMS at 30 minutes, the next step included analysis of the curves generated by the BreathID to reduce the test time.

Figure 1a is a representative curve of a subject that was positive for *H. pylori*. This curve shows a continuous rise of DOB ^{13}C values over time. The threshold of 5 DOB was reached already after 2 minutes. At 30 minutes the DOB reading was far above 5 DOB, thus defining this subject as *H. pylori* positive by the traditional IRMS criteria.

Analysis of the breath test curves obtained from all subjects permitted definition of different DOB thresholds at periods shorter than 30 minutes, such that 100% of subjects who exceeded these thresholds were also positive for *H. pylori* at 30 minutes. Thus, we calculated a threshold of 8 DOB at 6 minutes: all subjects that surpassed this threshold at 6 minutes were positive for *H. pylori* by the conventional 30-minute criterion of the IRMS. Subjects with a rising curve who did not reach this threshold were reevaluated at 8 minutes with a threshold of 7 DOB, and again at 10 minutes and at 18 minutes with a threshold of 6 DOB. Thereafter the traditional threshold of 5 DOB was used. It is noteworthy that the curves of all *H. pylori* positive subjects showed a similar trend of rising DOB values at the beginning of testing with later stabilization (each subject with an individual rate of elevation). This is opposed to the curves of *H. pylori* negative subjects that had fluctuating DOB values over time.

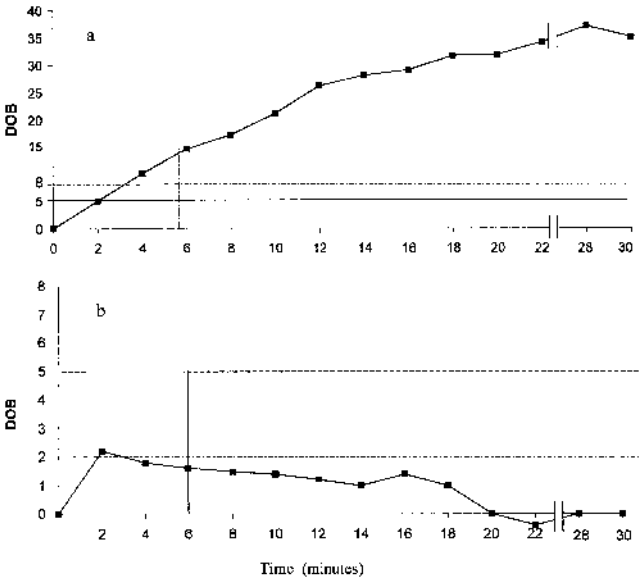

FIGURE 1. Delta over baseline (DOB) values of ^{13}C in breath samples. Representative curves of a single a: *H. pylori* positive subject; b: *H. pylori* negative subject. Values above 5‰ at 30 minutes are considered as representative of *H. pylori*- positivity.

Figure 1b demonstrates a representative curve of a subject negative for *H. pylori* by breath test. This curve shows fluctuating DOB values: after a preliminary elevation to 2 DOB the curve slowly declines to baseline values within 30 minutes. Similar analysis of all the curves of *H. pylori* negative subjects allowed definition of lower thresholds at shorter time periods to conclude a negative *H. pylori* test result earlier. A reading under 2 DOB was chosen for 6 minutes. Subjects not meeting this criterion were reevaluated at 8 minutes for a value under the threshold 3 DOB, and again at 10 minutes and 18 minutes for a value under 4 DOB, and thereafter for a value lower than the traditional threshold of 5 DOB.

Table 1 summarizes the threshold DOB ^{13}C values that were used for concluding a positive or negative test result at

TABLE 1. *Percent of subjects with a conclusive test result achieved at different time intervals from beginning of testing. At every time interval different threshold values were calculated based on analysis of all curves generated by the BreathID analyzer*

Time interval (min)	Threshold DOB for H pylori negative result	Threshold DOB for H pylori positive result	Percent of subjects with conclusive test results (positive & negative for H pylori infection)
6	<2	>8	64%
8	<3	>7	74%
10	<4	>6	92%
18	<4	>6	97%

earlier time intervals from the beginning of testing. For each time point the percentage of conclusive test results that were achieved are demonstrated. Since this analysis included the curves obtained from all subjects tested, the BreathID reached 100% sensitivity and specificity by definition, in reference to the test results at 30 minutes. Thus, after 6 minutes a conclusive test result can be obtained for 64% of subjects, and after 10 minutes for 92% of subjects.

DISCUSSION

Since the discovery of the role of *H. pylori* as a gastroduodenal pathogen, diagnosis of *H. pylori* infection has become a key step in the management of patients referred to the gastroenterologist. The ^{13}C-UBT has proven a reliable and safe means for this purpose. It is highly accurate but is expensive, as it requires cumbersome equipment and trained personnel for operation. This restricts its use to large laboratories. Mailing of breath samples to a central laboratory is often used. Therefore, results are often not available immediately, and a small number of samples per patient are examined. An ideal test for *H. pylori* would provide rapid results at low cost, preferably at the physician's office.

The present study confirms that ^{13}C-Urea breath test utilizing molecular correlation spectrometry is a reliable tool for detection of *H. pylori.* Advantages are: ease of use (sampling through a nasal cannula), low cost, and analysis of gas samples that does not require an experienced operator.

Continuous measurement of the subject's breath enables not only receiving repeat DOB results but also seeing the trend of the results over time. A conclusive positive test result could be ascertained within minutes by using a lower DOB threshold. This is based on the observation that all *H. pylori* positive subjects had a rising trend of DOB values over time, with later stabilization. In *H. pylori* negative subjects these time-specific thresholds were not reached, and the DOB readings over the same time intervals failed to demonstrate a pattern of rising values. Thus, we could reach a conclusive test result for over 90% of subjects within 10 minutes.

Breathing through a nasal cannula offers the possibility of using this method for testing small children.[10] Large breath gas volumes are not required, and cooperation on part of the subject is minimal. This on-line measurement of ^{13}C breath testing might also be of use for further applications such as gastric emptying studies or liver function tests.

REFERENCES

1. International Agency for Research on Cancer. World Health Organization. Infection with *Helicobacter pylori*. In: *Schistosomes, Liver Flukes, and Helicobacter pylori*. Lyon: IARC; 1994:177–202.
2. Vaira D, Malfertheiner P, Megraud F, et al. Noninvasive antigen-based assay for assessing *Helicobacter pylori* eradication: A European multicenter study. *Am J Gastroenterol*. 2000;95:925–929.
3. Rollan A, Giancaspero R, Arrese M, et al. Accuracy of invasive and noninvasive test to diagnose *Helicobacter pylori* infection after antibiotic treatment. *Am J Gastroenterol*. 1997;92:1268–1974.
4. Leodolter A, Dominguez-Munoz E, Arnim UV, et al. Validity of a modified ^{13}C-urea breath test for pre- and post-treatment diagnosis of *Helicobacter pylori* infection in the routine clinical setting. *Am J Gastroenterol*. 1999;94:2100–2104.
5. Logan RPH. Urea breath tests in the management of *Helicobacter pylori* infection. *Gut*. 1998;43(Suppl 1):S47–S50.
6. Savarino V, Vigneri S, Celle G. The ^{13}C urea breath test in the diagnosis of *Helicobacter pylori* infection. *Gut* 1999; (Suppl 1):118–122.
7. Ilan Y, Yatzkan Israelit Y, Bar Meir S, et al. A novel method & device for *Helicobacter pylori* detection: Continuos, fast, ^{13}C-urea breath test, abstract. *Gastroenterology*. 1998;114:GO642.
8. Braden B, Duan LP, Caspary WF, et al. More convenient ^{13}C-urea breath test modifications still meet the criteria for valid diagnosis of *Helicobacter pylori* infection. *Z Gastroenterol*. 1994;32:198–202.
9. Lotterer E, Ramakar J, Lüdke FE, et al. BauerFE. The simplified ^{13}C-urea breath test-one point analysis for detection of *Helicobacter pylori* infection. *Z Gastroenterol*. 1991;29:590–594.
10. Koletzko S, Haisch M, Seeboth I, et al. Isotope-selective non-dispersive infrared spectrometry for detection of *Helicobacter pylori* infection with ^{13}C-urea breath test. *Lancet*. 1995;345:961–962.

diagnostics

MDPI

Review

Current *Helicobacter pylori* Diagnostics

Dmitry S. Bordin [1,2,3,*], Irina N. Voynovan [1], Dmitrii N. Andreev [2] and Igor V. Maev [2]

1 A.S. Loginov Moscow Clinical Scientific Center, 111123 Moscow, Russia; i.voynovan@mknc.ru
2 A.I. Yevdokimov Moscow State University of Medicine and Dentistry, 127473 Moscow, Russia; dna-mit8@mail.ru (D.N.A.); igormaev@rambler.ru (I.V.M.)
3 Tver State Medical University, 170100 Tver, Russia
* Correspondence: d.bordin@mknc.ru

Abstract: The high prevalence of *Helicobacter pylori* and the variety of gastroduodenal diseases caused by this pathogen necessitate the use of only accurate methods both for the primary diagnosis and for monitoring the eradication effectiveness. There is a broad spectrum of diagnostic methods available for detecting *H. pylori*. All methods can be classified as invasive or non-invasive. The need for upper endoscopy, different clinical circumstances, sensitivity and specificity, and accessibility defines the method chosen. This article reviews the advantages and disadvantages of the current options and novel developments in diagnostic tests for *H. pylori* detection. The progress in endoscopic modalities has made it possible not only to diagnose precancerous lesions and early gastric cancer but also to predict *H. pylori* infection in real time. The contribution of novel endoscopic evaluation technologies in the diagnosis of *H. pylori* such as visual endoscopy using blue laser imaging (BLI), linked color imaging (LCI), and magnifying endoscopy is discussed. Recent studies have demonstrated the capability of artificial intelligence to predict *H. pylori* status based on endoscopic images. Non-invasive diagnostic tests such as the urea breathing test and stool antigen test are recommended for primary diagnosis of *H. pylori* infection. Serology can be used for initial screening and epidemiological studies. The histology showed its value in detecting *H. pylori* and provided more information about the degree of gastric mucosa inflammation and precancerous lesions. Molecular methods are mainly used in detecting antibiotic resistance of *H. pylori*. Cultures from gastric biopsies are the gold standard and recommended for antibiotic susceptibility tests.

Keywords: *Helicobacter pylori* (*H. pylori*); diagnosis; endoscopy; artificial intelligence; histology; molecular methods; serology; stool antigen test; urea breath test

Citation: Bordin, D.S.; Voynovan, I.N.; Andreev, D.N.; Maev, I.V. Current *Helicobacter pylori* Diagnostics. *Diagnostics* **2021**, *11*, 1458. https://doi.org/10.3390/diagnostics11081458

Academic Editor: Laurent Bélec

Received: 30 June 2021
Accepted: 9 August 2021
Published: 12 August 2021

1. Introduction

Helicobacter pylori (*H. pylori*) is one of the most common human pathogens and a leading etiological factor for various gastroduodenal diseases, including chronic gastritis, peptic ulcers, gastric adenocarcinoma, and MALT lymphoma [1,2]. According to the latest systematic review with meta-analysis, 44.3% (95% CI: 40.9–47.7) of the global population are infected with this microorganism [3]. Timely diagnosis and subsequent eradication of *H. pylori* in adults allows one to resolve inflammatory changes in the gastric mucosa and prevent the development of precancerous conditions (atrophic gastritis and intestinal metaplasia) [4–6].

There are several diagnostic methods for detecting *H. pylori* infections. All methods can be broadly classified as invasive or non-invasive (Table 1). Invasive methods require upper endoscopy and analysis of the gastric biopsy. Preference should be given to non-invasive diagnostic methods. If the patient requires upper endoscopy, a histological analysis, rapid urease testing, molecular methods, or culture can be performed to diagnose the *H. pylori* infection [7,8]. The main limitation of these methods is their invasiveness and the ability to analyze only a small part of the gastric mucosa. Table 1 shows the general characteristics of the diagnostic methods for *H. pylori*, their applications in clinical practices, as well as

the choice of diagnostic tests in different clinical conditions. Non-invasive tests include immunological methods (serology, stool antigen test), the 13 C-urea breath test (UBT), and molecular methods, i.e., a PCR study with determination of *H. pylori* DNA in feces (PCR from stool) [7].

Table 1. Overview of the diagnostic methods for *H. pylori*.

	Initial Diagnosis	Follow-up after Eradication	Requires Excluding PPI, Antibiotics, Bismuth Before Testing	Gastroduodenal Bleeding	Detection of Antibiotic Resistance	Sensitivity	Specificity
Invasive (require upper endoscopy)							
Histology	+	+	+	–	–	91–93%	100%
RUT	+	–	+	–	–	85–95%	95–100%
Culture	+	–	+	–	+	76–90%	100%
Molecular method (PCR)	+	+	+	+	+	95%	95%
Non-Invasive							
UBT	+	+	+	+	–	96–100%	93–100%
SAT	+	+	+	–	–	95.5%	97.6%
Serology	+	–	–	+	–	76–84%	79–90%
Stool PCR test	+	–	+	+	+	71%	96%

Any of the tests can be used for the primary diagnosis of *H. pylori*. The urease breath test is a "gold standard" in the diagnosis of *H. pylori* infection [7–10].

Modern non-invasive tests provide high reliability in *H. pylori* detection due to their high sensitivity and specificity. However, all of these methods have limitations. The choice of a particular test will depend on its sensitivity, specificity, and the clinical circumstances [11].

2. Invasive Methods for *H. pylori* Diagnostics

2.1. Endoscopic Imaging

Upper endoscopy is of particular importance in the diagnosis of *H. pylori* gastritis since *H. pylori* infection is strongly associated with gastric carcinogenesis. However, studies have shown that conventional image-enhanced endoscopy (IEE) with white light imaging (WLI) does not allow for the diagnosis of a wide range of inflammations of the gastric mucosa [12].

There is growing interest in improving the visualization of pathological changes in the gastric mucosa and in detecting *H. pylori* infections in real time during an upper endoscopy. *H. pylori* imaging in real time could reduce the cost of diagnosis and treatment.

Narrow band imaging is a new method of visual endoscopy based on the use of a laser light source and has opened up new possibilities for the diagnosis of not only precancerous changes in the gastric mucous but also of *H. pylori* infection.

A new IEE system that has two laser light sources offers four observation modes of white light imaging (WLI), blue laser imaging (BLI), BLI-bright, and linked color imaging (LCI). This is a new method of visual endoscopy developed in Tokyo called LASEREO (FUJIFILM Co., Tokyo, Japan) [13].

LCI and blue laser imaging (BLI) provide brighter endoscopic views and facilitate the diagnosis of inflammation and atrophy of the mucosal surfaces, allowing for the diagnosis of early gastric cancer. BLI improves the detection rate of early gastric cancer in comparison with that of white light imaging (93% vs. 50%, respectively, $p < 0.001$).

One study evaluated diffuse redness of the fundic mucosa, an endoscopic feature that could be correlated with *H. pylori* infection. The sensitivity and specificity for the diagnosis

of *H. pylori* using LCI was higher (85.8; 93.3 and 78.3%, respectively) compared to that of WLI (74.2; 81.7 and 66.7%, respectively) [12].

Magnifying endoscopy (ME) is another IEE tool that allows for the predicting of *H. pylori* by the microvascular architecture of the gastric mucosa. A meta-analysis was carried out to assess the diagnostic performance of ME to predict *H. pylori* infection. One endoscopic diagnosis criterion of *H. pylori* was "pit plus vascular pattern". The meta-analysis showed a high level of diagnostic accuracy of ME in predicting *H. pylori* infection [12]. ME accurately predicted *H. pylori* infection in both the white-light and chromoendoscopy modes [12]. However, ME requires specialized training in the interpretation of the images; therefore, is not widely used in everyday practice.

Artificial Intelligence

With the progress in computer technologies, artificial intelligence (AI) technologies have recently been applied in medicine to improve the quality of the diagnoses of diseases, to make an accurate diagnosis, and to predict disease progression and treatment planning [14]. Artificial intelligence, or neural networks known as "deep learning", is based on training computers on datasets containing a large number of images with their corresponding labels. The neural network then uses these learned functions to classify a given image [13].

Previous studies have demonstrated the capability of artificial intelligence in the prediction of *H. pylori* infection status for diagnosing gastritis. AI was efficiently created with IEE, BLI, and LCI. The studies evaluated the diagnostic accuracy of WLI and IEE for *H. pylori* gastritis, which found it to be 83.8% for *H. pylori* infection using WLI with the magnifying function. One pilot study showed that artificial intelligence based on BLI and LCI demonstrated an excellent ability to diagnose *H. pylori*. Sensitivity for BLI-bright and LCI was 96.7% and 10% superior to that using WLI [13].

A systematic review and meta-analysis were performed for assessing artificial intelligence in the forecasting of *H. pylori* infection, presenting diagnostic performance. The accuracy of the AI algorithm reached 82% for the discrimination between images of no infection and post-eradication images [15].

Artificial intelligence offers promising diagnostic performance using endoscopic imaging. It can help identify neoplastic or non-neoplastic lesions of the gastric mucosa and gastric cancer at an early stage and detect *H. pylori* in real time [15]. Soon, AI-assisted endoscopy will be feasible in clinical practice.

2.2. Histology

Histology is still one of the most commonly used diagnostic methods. This method allows for direct visualization of *H. pylori* and can be recommended for primary diagnosis if upper endoscopy is required. In addition to routine hematoxylin and eosin, various selective stains are used to detect *H. pylori* such as Warthin–Starry, Hp silver stain, Dieterle, Giemsa, Gimenez, acridine orange, McMullen, and immunostaining. Giemsa staining has become the most used method worldwide for the detection of *H. pylori* due to its low cost, ease of use, sensitivity, and reproducibility. It should be borne in mind that *H. pylori* can be detected only on sufficiently thin and well-stained sections [16].

It is recommended to take at least two biopsies to identify *H. pylori*; the best option is two biopsies from the antrum and one from the corpus. Biopsy from the corpus is especially valuable for yielding positive results if the patient has been taking PPI for a long time when *H. pylori* is translocated from the antrum to the corpus [16] and with a background of atrophic gastritis.

Moreover, in the area of intestinal metaplasia, *H. pylori* in most cases is not detected either with conventional or various selective stains. The disappearance of *H. pylori* correlates with the development of intestinal metaplasia and a decrease in gastric secretion [17]. The accuracy of the method can be affected by low bacterial density, for example, from taking

PPIs for a long time or an uneven distribution of *H. pylori* on the surface of the gastric mucosa [16].

The Maastricht V Consensus Report recommends patients to stop taking antibiotics and bismuth 4 weeks before the test and PPIs 2 weeks before testing [10].

The specificity of the histological method can reach 100%, and the sensitivity can reach 91–93% [18]. Some studies show that the sensitivity of these tests' ranges from 50% to 95% and depends on the quality, location, size, and frequency of the biopsy and the applied staining varieties [8]. Hematoxylin and eosin staining of biopsies has very poor sensitivity (66%) and suboptimal specificity (88%). The histological sensitivity decreases to 70% in patients with peptic ulcer bleeding; however, it remains a quite reliable test compared with the rapid urease test or culture, regardless of the presence of the bleeding [16].

Additional staining in gastric biopsies was investigated, such as using cresyl violet or immunohistochemistry for *H. pylori* detection [19–21]. Benoit A et al. [20] reported that it is not necessary to use this method to detect a *H. pylori* infection since conventional selective stains show good diagnostic accuracy. Immunohistochemistry can be used in cases of low bacterial density, atrophic gastritis with extensive intestinal metaplasia, and chronic active gastritis without *H. pylori* identification by standard staining. Immunohistochemistry is more specific; however, it is more expensive and not available in all laboratories.

A novel method using a γ-glutamyl transpeptidase (GGT) activatable fluorescent probe was proposed this year. The γ-glutamyl hydroxy methyl rhodamine green probe reacts with GGT and immediately produces fluorescence. The method allows for the quantification of the GGT activity of *H. pylori* on gastric biopsies within 15 min. However, the sensitivity is still limited (75–82%) [22].

Despite the high specificity and sensitivity, the histology has a higher cost and longer processing time, requires an upper endoscopy to obtain gastric biopsy samples, depends on the skills of the operator, and is not suitable for assessing the effectiveness of eradication since endoscopy is necessary.

The main advantage of histology is the ability to assess the condition of the gastric mucosa and diagnose precancerous lesions. The degree and stage of chronic gastritis, risk of carcinogenesis, and assessment according to the modern classification of chronic gastritis (OLGA—Operative Link for Gastritis Assessment and OLGIM) allow for the assessment of the prognosis of the disease [23]. The updated Sydney System recommends taking five biopsy specimens from different sites for the assessment of the degree and stage of *H. pylori* gastritis status. According to this system, two biopsies are taken from the antrum (from the lesser and greater curvature, both within 2–3 cm from the pylorus), two from the corpus (the lesser curvature about 4 cm proximal to the angulus; the middle portion of the greater curvature, approximately 8 cm from the cardia), and one from the incisura angularis [24].

Atrophic gastritis (AG) and intestinal metaplasia (IM) are considered precancerous lesions of the stomach. Studies have shown that with AG and IM, the sensitivity of histology for detecting *H. pylori* infection decreases to 30–55%, while the corpus lesser curvature side showed 80% sensitivity, and the corpus greater curvature side showed 95–100% sensitivity. Thus, the appropriate biopsy site for detecting *H. pylori* infection in AG and IM patients as well as in patients with gastric cancer is the corpus, especially the corpus greater curvature side [16].

2.3. The Rapid Urease Test

The rapid urease test (RUT) is based on detecting the activity of the *H. pylori* urease enzyme, which splits the urea test reagent to form ammonia. Ammonia increases pH, which is detected by the phenol red indicator [7,25,26].

The RUT is a low cost, rapid, and generally highly specific assay.

The Maastricht V Consensus Report allows for the use of RUT for primary diagnosis, and a positive test result allows for the prescription of eradication, but it does not recommend a rapid urease test to assess eradication after treatment due to its lack of sensitivity

and high false-negative rate [10]. Therefore, a negative rapid urease test should not be used to exclude *H. pylori*, which should also be taken into account in the initial diagnosis.

Commercially available RUT*s* (e.g., HpFast, GI-supply, Camp Hill, Pennsylvania; CLOTest, Delta West, Bentley, Western Australia; HpOne, GI Supply, Camp Hill, PA) have reported specificities from 95% to 100%, but their sensitivity is moderate (85% to 95%) [7,26]. However, the sensitivity of the test increases if we take biopsies from both the corpus and antrum [19].

RUT has limited sensitivity and can give false-negative results, for example, if less than 10^4 bacterial cells are present in the gastric biopsy or if a biopsy is taken from areas of atrophy and metaplasia of the gastric mucosa. It is necessary to exclude the use of antibiotics and bismuth for 4 weeks and PPIs for 2 weeks before the test [7,8].

In some instances, RUT may lead to false-positive test results due to the presence of other urease-producing bacteria such as *Staphylococcus capitis* subsp. ureolyticus, *Streptococcus salivarius*, and *Proteus mirabilis* in the stomach [8]. Bleeding peptic ulcers reduce the sensitivity of RUT by up to 70%.

False-negative test results are more common than false-positive test results, so a negative result cannot be used to exclude a diagnosis of *H. pylori*. Thus, a positive RUT result indicates the presence of *H. pylori* and makes it possible to prescribe treatment, but a negative result does not allow excluding *H. pylori*; therefore, it is recommended to confirm the diagnosis with an additional method [10].

2.4. Culture

The greatest information about *H. pylori* can be obtained in isolation cultivations of *H. pylori* from gastric biopsy specimens. The cultivations allows not only for the isolation of a pure culture of *H. pylori* and its identification, but also the study of the morphological, biochemical, and biological properties of the pathogen and the pathogenicity factors of *H. pylori*. The bacteriological method of research makes it possible to the determine antibiotic resistance in *H. pylori* and carry out dynamic monitoring of it [7].

Bacteriological examination is a very laborious method; it requires taking at least two biopsies from the stomach. It is necessary to strictly follow the rules of transporting biopsy material for culture in order to keep this microorganism in a viable state. It is advisable to sow the material on the day it arrives at the laboratory. The incubation of crops is carried out under microaerophilic conditions with an oxygen content of $\leq$5%. Later, the cultures are identified, and their morphological and tinctorial properties and sensitivity to antibiotics (e.g., amoxicillin, clarithromycin, and metronidazole) are determined [7].

The specificity of the method is 100% when performed under optimal conditions; the sensitivity is 76–90% [16], and according to other data it is 50–90% [17].

As with any diagnostic method, the bacteriological research method not only has advantages, but also has disadvantages, which often limit the widespread use of this method in clinical practice. Most importantly, the shortcomings include the need for special laboratory equipment and reagents, special nutrient media, and trained specialists. This is all associated with high material costs.

False-negative results arise from non-adherence or inaccurate adherence to the test method, such as poor sample quality, delayed transport, exposure to an aerobic environment, or an inexperienced microbiologist [7].

Patient factors such as low bacterial load; bleeding from the upper gastrointestinal tract; alcohol consumption; or taking PPIs, bismuth preparations, H_2RA, and antibiotics have an adverse effect on obtaining a culture of *H. pylori* [7].

PPIs, H_2RA, bismuth, and antibiotics should be stopped 4 weeks before the culture method. To avoid negative results due to the uneven distribution of *H. pylori* in the stomach and to increase the sensitivity and specificity of the method in the diagnosis of *H. pylori*, it is necessary to take several biopsies from the gastric mucosa: two from the antrum and two from the body of the stomach. Some authors believe that in order to increase the sensitivity

and specificity of the bacteriological method, taking biopsies for cultivation should be carried out 3 months after patients cease taking PPIs, antibiotics, and bismuth [27].

Although the culture is very laborious and requires special conditions for implementation, it is very valuable in clinical practice. The Maastricht V Consensus Report recommends culture and antibiotic-susceptibility testing in geographical areas where primary resistance to clarithromycin is higher than 20%. This method is recommended after failure of second-line treatments, when the further choice of antibiotics is determined by the sensitivity of *H. pylori* to them [10].

3. Non-Invasive Methods for *H. pylori* Diagnostics

3.1. Urea Breath Test

13C-UBT is a non-invasive method for the diagnosis of *H. pylori* based on a simple principle: patients ingest urea labeled with 13C or 14C, and *H. pylori* produces urease—an enzyme that splits urea into ammonia and 13C-labeled carbon dioxide; then, 13C carbon dioxide is absorbed into the bloodstream, enters the lungs, and is excreted with the exhaled air [7].

Urea is usually given to the patient with a citrus juice (lemon, orange) to delay gastric emptying and increase contact time with the mucosa.

Before taking the test solution, the exhaled air is collected in a sealed bag 30 min after the solution has been drunk. The collected air samples are analyzed on a mass spectrometer or by infrared spectroscopy, which is technically simpler and also cheaper than using a mass spectrometer. Infrared spectroscopy determines the 13C/12C isotopic ratio. The increase in labeled CO2 is expressed as delta over baseline (DOB). The DOB value is positively correlated with the *H. pylori* bacterial load.

Thus, from the appearance of 13C in the exhaled air, we can determine with high accuracy whether the patient is infected with *H. pylori*, and from the value of the 13C/12C ratio, we can estimate the degree of infection. The 13C urea breath test is similar to the 14C urea breath test except that 13C is a non-radioactive isotope.

The standard urea breath test uses 75 mg of 13C. The sensitivity of 13C-UBT is 96–100%; the specificity is 93–100% [7,28].

One study found that testing time could be shortened to 15 min (the BREATH QUALITY UBT) without affecting the accuracy of the method [29].

The conducted meta-analysis showed the high accuracy of the test in children of any age. In children >6 years, sensitivity and specificity were 96.6% and 97.7%, respectively; in children $\leq$6 years, they were 95% and 93.5%, respectively [30].

Recently, a new UBT technique has been proposed, which uses a 13C-urea tablet formulation. This technique allows for air sampling with high accuracy within 10 min after taking the pill. In addition, the tablet form has the advantage of preventing the formulation from interacting with the urease-producing bacteria in the oropharynx, which can cause false-positive results [8,31].

False-positive results are rare, but they can be observed after endoscopy with a biopsy immediately before the test in patients who underwent gastric resection and also those with a significant decrease in gastric secretion. False-positive tests most often cause hydrolysis of urea by bacteria in the mouth or bacteria containing urease in the stomach [31]. This is especially likely in the presence of achlorhydria or hypochlorhydria. A small number of false-negative results may be associated with a violation of the method of taking and storing samples of exhaled air or physical activity on the eve of and during the test. As with most other tests, a reliable UBT result can be obtained after a 2-week discontinuation of PPIs and no earlier than 4 weeks after stopping antibiotics and bismuth [7,10].

3.2. Stool Antigen Test

The stool antigen test (SAT) is based on the direct identification of the bacterium antigen in stools. There are two types of SATs used for *H. pylori* detection: enzyme immunoassay (EIA) and speedy in-office tests—immunochromatography assay (ICA)-

based methods, using either polyclonal antibodies or monoclonal antibodies. EIA provides more reliable results than does ICA. Monoclonal antibodies-based tests are more accurate than are polyclonal antibodies and give useful reports [32].

SAT is recommended both for the primary diagnosis of *H. pylori* infection and for the monitoring of therapy effectiveness. This test is noninvasive, quick, low cost, and easy to use. The test has a good sensitivity of 95.5% and a specificity of 97.6% (LIAISON® Meridian) [7,22].

The test requires a small amount of feces, and it is possible to collect a sample at home and send it to a laboratory at a suitable time. Stool samples can be frozen at −20 °C and stored for a long time. It is important to remember that the sensitivity of the test drops to 69% if the sample is kept at room temperature for 48–72 h. It is not recommended to perform the test during diarrhea or on watery stools [33].

SAT must be performed not earlier than four weeks after last intake of antibiotics and bismuth or two weeks after the last intake of PPI. To evaluate the eradication efficiency, the test must be used 30 or more days after the completion of eradication [33]. Uneven distribution of antigen in feces, destruction of antigen in constipation, ongoing bleeding of the gastrointestinal tract, and low bacterial load in the stomach are the reasons for false-negative results [33,34].

Stool monoclonal antigen is a convenient and effective test for the diagnosis of *H. pylori* in children [35].

3.3. Serology

The colonization of *H. pylori* induces a systemic immune response. Antibodies to *H. pylori* appear in the blood 3–4 weeks after infection. These antibodies can be determined by one of three methods: the enzyme-linked immunosorbent assay (ELISA) test, latex agglutination tests, and Western blotting. Of these, ELISA is the most commonly used method [36]. This method is based on the detection of specific circulating antibodies: IgG, IgA, and IgM. *H. pylori* is a chronic infection; therefore, only a validated IgG test should be used [10].

Serologic tests are widely available to diagnose *H. pylori*; they are non-invasive, rapid, do not require any special equipment, and can be used in screening populations.

However, serology may be positive due to the presence of an active infection at the time of the test, a previous infection, or because of non-specific cross-reacting antibodies [7,8].

Immunoglobulins (antibodies) against antigens appear due to the presence of active infection, previous infection, or because of non-specific cross-reacting antibodies [36]. Thus, a serological test can be used for primary diagnosis of *H. pylori* or another test confirmation. Quantitative antibodies levels do not decline significantly for a long time after successful eradication; therefore, serological testing should not be used for therapeutic follow-up. Furthermore, false-positive serologic tests are common in a population with a low prevalence (<40%) of *H. pylori* as the positive predictive value of serology depends on the prevalence of *H. pylori* infection in the considered area [33]. In such populations, it is not recommended to use serology, and in case of positivity of a serological test for *H. pylori*, it is necessary to confirm the test with a more reliable test, e.g., histological tests, culture of biopsy sample, the urea breath test, or the stool antigen test.

Serology is not affected by recent use of proton-pump inhibitors, antibiotics, or bismuth preparations, gastrointestinal bleeding, or atrophy of the gastric mucosa [10].

The specificity and sensitivity of serological testing varies. One meta-analysis showed that the sensitivity and specificity of the test were 85% and 79%, respectively. Another study demonstrated sensitivity ranging from 76% to 84% and specificities from 79% to 90% [33].

Several studies have shown that the levels of anti-*H. pylori* IgG were associated positively with the grade of histological gastritis, mucosal bacterial density, and levels of serum biomarkers for stomach function, including PGI, PGII, PGI/II ratio, and gastrin-17. Other studies found no associations; thus, the results are conflicting [37].

4. Molecular Invasive and Non-Invasive Methods for *H. pylori*

Molecular diagnostic methods are based on the amplification of nucleic acid using a conventional polymerase chain reaction (PCR) or PCR in real time (RT-PCR). Genetic material (DNA) of *H. pylori* can be detected in gastric biopsy, saliva, feces, or dental samples. PCR can be considered as either an invasive or non-invasive method for detecting *H. pylori* depending on the applied material. It demonstrates up to 95% sensitivity and 95% specificity [38]. Molecular methods are more expensive than other methods, and the laboratory must have appropriate equipment and experience. PCR allows for the detection of specific mutations leading to antibiotic resistance and bacterial virulence factors such as CagA and VacA.

There are a number of molecular assays commercially available for *H. pylori* and clarithromycin-resistance detection. Several studies have found different sensitivities and specificities of the method depending on the DNA extraction method and the PCR assay used. The *H. pylori* Taqman® real-time PCR assay in stool specimens shows a high sensitivity of 93.8%. The ClariRes assay shows a low sensitivity (ranging from 63% to 84%) for *H. pylori* detection in stool specimens when compared to those of the stool antigen test and *H. pylori* culture from gastric biopsy specimens [26].

One of the new approaches to diagnosing *H. pylori* is next-generation sequencing (NGS) by sequencing *H. pylori* DNA directly from formalin-fixed paraffin-embedded (FFPE) gastric biopsy specimens. NGS reveals mutations in genes that lead to resistance to antibiotics (clarithromycin, levofloxacin, and tetracycline) and their correlation with phenotypic drug resistance. Using NGS, mutations in the gyrA, 23S rRNA, and 16S rRNA genes were identified and analyzed [22]. The sensitivity of the method is 95%. The study showed the possibility of using NGS to detect multidrug resistance in culture-negative biopsies and on clinical specimens collected during the standard of care [39].

Studies show that clarithromycin resistance is based on point mutations at nucleotide positions A2146 and A2147 in the 23S rRNA gene [22,39]. The rRNA 16S gene is a much more sensitive method for detecting *H. pylori* in gastric biopsies compared to other methods [22].

Sequencing *H. pylori* DNA from gastric biopsy specimens is a laborious method. *H. pylori* must be cultured from multiple gastric biopsy specimens, then, multiple colonies must be picked from agar plates for DNA extraction in order not to miss the drug-resistant subpopulations; the strains should be sequenced with sufficient coverage to detect heteroresistance; usually, multiple susceptible and resistant strains of *H. pylori* are sequenced [26].

The detection of *H. pylori* DNA in stool samples is a very convenient, fast, sensitive, and accurate method. Stool RT-PCR analysis can detect *H. pylori* DNA sequences and antibiotic resistance point mutations. The conducted meta-analysis showed that most diagnostic candidate genes identified in stool samples were 23S rRNA, 16S rRNA, and glmM. Stool DNA PCR had a performance of 71% (95% CI: 68–73) sensitivity and 96% (95% CI: 94–97) specificity in the diagnosis of *H. pylori*. Analysis showed that the 23S rRNA gene has high sensitivity for the detection of *H. pylori* in clinical samples [40]. Three mutations (A2142G, A2143G, and A2142C) in a gene in 23S rRNA were associated with *H. pylori* resistance to clarithromycin, and these mutations have been associated with treatment failure [22].

Undoubtedly, stool DNA PCR has its advantages: it gives faster results, fewer bacteria are required in the sample for analysis, it does not need special processing supplies or transportation of the material, and the result can be obtained in a fairly short time (<4 h).

Despite the high specificity of the test, a number of studies have revealed a high percentage of false-positive results, especially when the test is carried out 4–6 weeks after successful eradication therapy. False-positive results in treated patients can be explained by persistence in the feces of coccoidal forms of *H. pylori*, which, over time, begin to decrease and completely disappear at 8–12 weeks [41].

In geographic regions with high clarithromycin resistance, stool RT-PCR testing with determination of clarithromycin resistance is a useful diagnostic option for young dys-

peptic patients who do not require endoscopy and should preferably be treated with clarithromycin-containing regimens [42].

5. Conclusions

The high prevalence and etiopathogenetic relationship of *H. pylori* with the most significant diseases of the stomach highlights the need to optimize the diagnosis of this infection, taking into account the sensitivity and specificity of the tests, as well as the conditions for their use. The infection must be detected before therapy is prescribed, and its success must be confirmed after treatment.

The developments of current diagnostic methods allow for a more accurate and reliable diagnosis of *H. pylori* infection. The choice of method will depend on the accessibility, their advantages and disadvantages, sensitivity and specificity, and different clinical circumstances of each patient.

Leading international experts dictate the rules for the diagnosis of *H. pylori* infection; however, the majority of mistakes are still made when assessing the effectiveness of eradication, namely, the use of inadequate methods or lack of control. According to the European Registry on *H. pylori* management (Hp-EuReg), confirmation of the eradication was performed in 94% of the cases [43].

Author Contributions: Conceptualization, D.S.B. and I.V.M.; investigation, I.N.V. and D.N.A.; writing—original draft preparation, I.N.V. and D.N.A.; writing—review and editing, D.S.B., D.N.A., and I.V.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Feldman, M.; Friedman, L.S.; Brandt, L.J. *Sleisenger and Fordtran's Gastrointestinal and Liver Disease E-Book: Pathophysiology, Diagnosis, Management*, 11th ed.; Elsevier: Amsterdam, The Netherlands, 2020.
2. Suzuki, H.; Warren, R.; Marshall, B. *Helicobacter Pylori*; Springer: Tokyo, Japan, 2016.
3. Zamani, M.; Ebrahimtabar, F.; Zamani, V.; Miller, W.H.; Alizadeh-Navaei, R.; Shokri-Shirvani, J.; Derakhshan, M.H. Systematic review with meta-analysis: The worldwide prevalence of Helicobacter pylori infection. *Aliment. Pharmacol. Ther.* **2018**, *47*, 868–876. [CrossRef]
4. Zhou, L.; Sung, J.J.; Lin, S.; Jin, Z.; Ding, S.; Huang, X.; Xia, Z.; Guo, H.; Liu, J.; Chao, W. A five-year follow-up study on the pathological changes of gastric mucosa after *H. pylori* eradication. *Chin. Med. J.* **2003**, *116*, 11–14. [PubMed]
5. Lee, Y.C.; Chen, T.H.; Chiu, H.M.; Shun, C.T.; Chiang, H.; Liu, T.Y.; Wu, M.S.; Lin, J.T. The benefit of mass eradication of *Helicobacter pylori* infection: A community-based study of gastric cancer prevention. *Gut* **2013**, *62*, 676–682. [CrossRef] [PubMed]
6. Okubo, M.; Tahara, T.; Shibata, T.; Nakamura, M.; Yoshioka, D.; Maeda, Y.; Yonemura, J.; Ishizuka, T.; Arisawa, T.; Hirata, I. Changes in gastric mucosal patterns seen by magnifying NBI during *H. pylori* eradication. *J. Gastroenterol.* **2011**, *46*, 175–182. [CrossRef]
7. Pichon, M.; Pichard, B.; Barrioz, T.; Plouzeau, C.; Croquet, V.; Fotsing, G.; Chéron, A.; Vuillemin, É.; Wangermez, M.; Haineaux, P.A.; et al. Diagnostic Accuracy of a Noninvasive Test for Detection of Helicobacter pylori and Resistance to Clarithromycin in Stool by the Amplidiag H. pylori+ClariR Real-Time PCR Assay. *J. Clin. Microbiol.* **2020**, *58*, e01787-19. [CrossRef]
8. Ricci, C.; Holton, J.; Vaira, D. Diagnosis of *Helicobacter pylori*: Invasive and non-invasive tests. *Best Pract. Res. Clin. Gastroenterol.* **2007**, *21*, 299–313. [CrossRef]
9. Gisbert, J.P.; De la Morena, F.; Abraira, V. Accuracy of monoclonal stool antigen test for the diagnosis of *H. pylori* infection: A systematic review and meta-analysis. *Am. J. Gastroenterol.* **2006**, *101*, 1921–1930. [CrossRef]
10. Malfertheiner, P.; Megraud, F.; O'Morain, C.A.; Gisbert, J.P.; Kuipers, E.J.; Axon, A.T.; Bazzoli, F.; Gasbarrini, A.; Atherton, J.; Graham, D.Y.; et al. European Helicobacter and Microbiota Study Group and Consensus panel. Management of *Helicobacter pylori* infection—The Maastricht V/Florence Consensus Report. *Gut* **2017**, *66*, 6–30. [CrossRef]
11. Patel, S.K.; Pratap, C.B.; Jain, A.K.; Gulati, A.K.; Nath, G. Diagnosis of *Helicobacter pylori*: What should be the gold standard? *World J. Gastroenterol.* **2014**, *20*, 12847–12859. [CrossRef] [PubMed]

12. Bessède, E.; Arantes, V.; Mégraud, F.; Coelho, L.G. Diagnosis of *Helicobacter pylori* infection. *Helicobacter* **2017**, *22* (Suppl. 1), 11221. [CrossRef]
13. Hirotaka, N.; Hiroshi, K.; Hiroshi, K.; Nobuhiro, S. Artificial intelligence diagnosis of *Helicobacter pylori* infection using blue laser imaging-bright and linked color imaging: A single-center prospective study. *Ann. Gastroenterol.* **2018**, *31*, 462–468. [CrossRef]
14. Zharikov, O.G.; Meshcherikov, I.V.; Litvin, A.A. Neuronet technologies in medicine. *Issues Organ. Informatiz. Healthc.* **2007**, *4*, 59–63. (In Russian).
15. Bang, C.S.; Lee, J.J.; Baik, G.H. Artificial Intelligence for the Prediction of Helicobacter Pylori Infection in Endoscopic Images: Systematic Review and Meta-Analysis of Diagnostic Test Accuracy. *J. Med. Internet Res.* **2020**, *22*, e21983. [CrossRef]
16. Lee, J.Y.; Kim, N. Diagnosis of *Helicobacter pylori* by invasive test: Histology. *Ann. Transl. Med.* **2015**, *3*, 10. [CrossRef]
17. Craanen, M.E.; Blok, P.; Dekker, W.; Ferwerda, J.; Tytgat, G.N. Subtypes of intestinal metaplasia and *Helicobacter pylori*. *Gut* **1992**, *33*, 597–600. [CrossRef]
18. Lopes, A.I.; Vale, F.F.; Oleastro, M. *Helicobacter pylori* infection—Recent developments in diagnosis. *World J. Gastroenterol.* **2014**, *20*, 9299–9313. [CrossRef] [PubMed]
19. Tonkic, A.; Vukovic, J.; Cindro, P.V.; Pisac, V.P.; Tonkic, M. Diagnosis of *Helicobacter pylori* infection. *Wien. Klin. Wochenschr.* **2018**, *130*, 530–534. [CrossRef]
20. Benoit, A.; Hoyeau, N.; Fléjou, J.F. Diagnosis of *Helicobacter pylori* infection on gastric biopsies: Standard stain, special stain or immunohistochemistry? *Ann. Pathol.* **2018**, *38*, 363–369. [CrossRef]
21. Makristathis, A.; Hirschl, A.M.; Mégraud, F.; Bessède, E. Review: Diagnosis of *Helicobacter pylori* infection. *Helicobacter* **2019**, *24* (Suppl. 1), e12641. [CrossRef]
22. Godbole, G.; Mégraud, F.; Bessède, E. Review: Diagnosis of *Helicobacter pylori* infection. *Helicobacter* **2020**, *25* (Suppl. 1), e12735. [CrossRef]
23. Rugge, M.; Fassan, M.; Pizzi, M.; Pennelli, G.; Nitti, D.; Farinati, F. Operative Link for Gastritis Assessment gastritis staging incorporates intestinal metaplasia subtyping. *Hum. Pathol.* **2011**, *42*, 1539–1544. [CrossRef]
24. Dixon, M.F.; Genta, R.M.; Yardley, J.H.; Correa, P. Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. *Am. J. Surg. Pathol.* **1996**, *20*, 1161–1181. [CrossRef]
25. Vaira, D.; Vakil, N.; Gatta, L.; Ricci, C.; Perna, F.; Saracino, I.; Fiorini, G.; Holton, J. Accuracy of a new ultrafast rapid urease test to diagnose *Helicobacter pylori* infection in 1000 consecutive dyspeptic patients. *Aliment. Pharmacol. Ther.* **2010**, *31*, 331–338. [CrossRef] [PubMed]
26. Pohl, D.; Keller, P.M.; Bordier, V.; Wagner, K. Review of current diagnostic methods and advances in *Helicobacter pylori* diagnostics in the era of next generation sequencing. *World J. Gastroenterol.* **2019**, *25*, 4629–4660. [CrossRef]
27. Leszczyńska, K.; Namiot, A.; Namiot, Z.; Leszczyńska, J.K.; Jakoniuk, P.; Chilewicz, M.; Namiot, D.B.; Kemona, A.; Milewski, R.; Bucki, R. Patient factors affecting culture of Helicobacter pylori isolated from gastric mucosal specimens. *Adv. Med. Sci.* **2010**, *55*, 161–166. [CrossRef]
28. Ferwana, M.; Abdulmajeed, I.; Alhajiahmed, A.; Madani, W.; Firwana, B.; Hasan, R.; Altayar, O.; Limburg, P.J.; Murad, M.H.; Knawy, B. Accuracy of urea breath test in Helicobacter pylori infection: Meta-analysis. *World J. Gastroenterol.* **2015**, *21*, 1305–1314. [CrossRef]
29. Mezmale, L.; Coelho, L.G.; Bordin, D.; Leja, M. Review: Epidemiology of *Helicobacter pylori*. *Helicobacter* **2020**, *25* (Suppl. 1), e12734. [CrossRef]
30. Leal, Y.A.; Flores, L.L.; Fuentes-Pananá, E.M.; Cedillo-Rivera, R.; Torres, J. 13C-urea breath test for the diagnosis of *Helicobacter pylori* infection in children: A systematic review and meta-analysis. *Helicobacter* **2011**, *16*, 327–337. [CrossRef] [PubMed]
31. Peng, N.J.; Lai, K.H.; Liu, R.S.; Lee, S.C.; Tsay, D.G.; Lo, C.C. Clinical significance of oral urease in diagnosis of *Helicobacter pylori* infection by [13C] urea breath test. *Dig. Dis. Sci.* **2001**, *46*, 1772–1778. [CrossRef] [PubMed]
32. Dalla Nora, M.; Hörner, R.; De Carli, D.M.; Rocha, M.P.; Araujo, A.F.; Fagundes, R.B. Is the immunocromatographic fecal antigen test effective for primary diagnosis of *Helicobacter pylori* infection in dyspeptic patients? *Arq. Gastroenterol.* **2016**, *53*, 224–227. [CrossRef] [PubMed]
33. Stefano, K.; Rosalia, A.; Chiara, B.; Federica, G.; Marco, M.; Gioacchino, L.; Fabiola, F.; Francesco, D.M.; Gian, L.D.A. Non-invasive tests for the diagnosis of *Helicobacter pylori*: State of the art. *Acta Biomed.* **2018**, *89* (Suppl. 8), 58–64. [CrossRef]
34. El-Shabrawi, M.; El-Aziz, N.A.; El-Adly, T.Z.; Hassanin, F.; Eskander, A.; Abou-Zekri, M.; Mansour, H.; Meshaal, S. Stool antigen detection versus ^{13}C-urea breath test for non-invasive diagnosis of pediatric *Helicobacter pylori* infection in a limited resource setting. *Arch. Med. Sci.* **2018**, *14*, 69–73. [CrossRef] [PubMed]
35. Zhou, X.; Su, J.; Xu, G.; Zhang, G. Accuracy of stool antigen test for the diagnosis of *Helicobacter pylori* infection in children: A meta-analysis. *Clin. Res. Hepatol. Gastroenterol.* **2014**, *38*, 629–638. [CrossRef] [PubMed]
36. Best, L.M.J.; Takwoingi, Y.; Siddique, S.; Selladurai, A.; Gandhi, A.; Low, B.; Yaghoobi, M.; Gurusamy, K.S. Non-invasive diagnostic tests for *Helicobacter pylori* infection. *Cochrane Database Syst. Rev.* **2018**, *3*. [CrossRef]
37. Tu, H.; Sun, L.; Dong, X.; Gong, Y.; Xu, Q.; Jing, J.; Yuan, Y. Serum anti-*Helicobacter pylori* immunoglobulin G titer correlates with grade of histological gastritis, mucosal bacterial density, and levels of serum biomarkers. *Scand. J. Gastroenterol.* **2014**, *49*, 259–266. [CrossRef]
38. Szymczak, A.; Ferenc, S.; Majewska, J.; Miernikiewicz, P.; Gnus, J.; Witkiewicz, W.; Dąbrowska, K. Application of 16S rRNA Gene Sequencing in *Helicobacter pylori* detection. *PeerJ* **2020**, *8*, e9099. [CrossRef]

39. Nezami, B.G.; Jani, M.; Alouani, D.; Rhoads, D.D.; Sadri, N. Helicobacter pylori mutations detected by Next-Generation Sequencing in Formalinfixed, paraffin-embedded gastric biopsy specimens are associated with treatment failure. *J. Clin. Microbiol.* **2019**, *57*, e01834-18. [CrossRef]
40. Khadangi, F.; Yassi, M.; Kerachian, M.A. Review: Diagnostic accuracy of PCR-based detection tests for Helicobacter Pylori in stool samples. *Helicobacter* **2017**, *22*, e12444. [CrossRef]
41. Trevisani, L.; Sartori, S.; Galvani, F.; Rossi, M.R.; Ruina, M.; Chiamenti, C.; Caselli, M. Evaluation of a new enzyme immunoassay for detecting *Helicobacter pylori* in feces: A prispective pilot study. *Am. J. Gastroenterol.* **1999**, *94*, 1830–1833. [CrossRef]
42. Giorgio, F.; Ierardi, E.; Sorrentino, C. *Helicobacter pylori* DNA isolation in the stool: An essential pre-requisite for bacterial noninvasive molecular analysis. *Scand. J. Gastroenterol.* **2016**, *51*, 1429–1432. [CrossRef]
43. Nyssen, O.P.; Vaira, D.; Tepes, B.; Kupcinskas, L.; Bordin, D.; Pérez-Aisa, Á.; Gasbarrini, A.; Castro-Fernández, M.; Bujanda, L.; Garre, A.; et al. Room for Improvement in the Treatment of *Helicobacter pylori* Infection: Lessons from the European Registry on *H. pylori* Management (Hp-EuReg). *J. Clin. Gastroenterol.* **2021**. [CrossRef] [PubMed]

EXPERT REVIEWS

Evaluation of Exalenz Bioscience's BreathID for *Helicobacter pylori* detection

Expert Rev. Mol. Diagn. Early online, 1–14 (2015)

Efrat Broide[1,2] and Haim Shirin*[1,2]

[1]*The Kamila Gonczarowski, Institute of Gastroenterology, Assaf Harofeh Medical Center, Zerifin, 70300 Israel*
[2]*Sackler School of Medicine, Tel-Aviv University, Tel-Aviv, Israel*
**Author for correspondence: haimsh@asaf.health.gov.il*

Carbon-labeled urea breath tests, which have high sensitivity and specificity, are the preferred method used in epidemiological studies, screening dyspeptic patients and assessing eradication or recurrence of *Helicobacter pylori* infection. The principle of the ^{13}C-urea breath test relies upon the ability of the *H. pylori* urease to hydrolyze the orally administered ^{13}C-urea. The BreathID® (Exalenz Bioscience Inc., Union, NJ, USA) provides a competitive solution for breath testing, including unique features such as automatic continuous breath collection and analysis. This is an unattended convenient test, with no human error as the correct part of the breath is collected and patients' assistance is not required. The test results are available in real time at the point of care and enable shortened breath testing procedures. Additionally, several studies showing expanded utility of the BreathID in pediatrics, after therapy and during proton pump inhibitors intake, further support the safety and performance of the BreathID in the diagnosis of *H. pylori*.

Keywords: BreathID® • gastric emptying • *Helicobacter pylori* • test substrate • urea breath test

Helicobacter pylori, the bacteria of the 20th century led to a dramatic change in our understanding the pathogenesis and the therapy of peptic ulcer. Moreover, it also clarified the association between chronic bacterial infection and gastric malignant diseases. The prevalence of *H. pylori* infection is decreasing in Western countries, but remains comparably high in developing regions [1]. *H. pylori* colonizes the human stomach during childhood and survives in the human stomach for the lifetime of the carrier. The exact mechanism whereby *H. pylori* is acquired is not well defined [2]. It has been hypothesized a human-to-human transmission, by oral-oral or fecal-oral contact or both. Human stomach is the only reservoir of the bacteria, which typically does not cause any clinical or endoscopic adverse effects. However, it is still a major cause for chronic gastritis, peptic ulcers and dyspepsia and increases risk of gastric mucosa-associated lymphoid tissue lymphoma and non-cardiac gastric adenocarcinoma. Atrophic gastritis, chronic use of anti-platelets agents or proton pump inhibitors (PPI) and family history of gastric cancer are also indications for testing and eradication of the bacteria [3].

Considering the broad spectrum of diagnostic methods, only highly accurate tests should be used in clinical practice. Currently, the sensitivity and specificity of an adequate test should exceed 90%. Diagnostic testing for *H. pylori* can be divided into invasive and non-invasive techniques, based upon the need for endoscopy which was the original gold standard for detection of *H. pylori* infection. Although the invasive, gastroscopic biopsy-based tests such as the rapid urease test (RUT), histological examination, culture and molecular methods (PCR) have been widely used to diagnose *H. pylori* infection, recently many investigators have attempted to categorize the endoscopic findings characteristic of a *H. pylori*-infected stomach [4,5].

The non-invasive methods include the serology, stool antigen test (SAT) and urea breath test (UBT) [6,7]. Each method has its advantages and disadvantages and each practitioner should choose the best diagnostic method according to the facilities available. Among the non-endoscopic procedures used in diagnosing *H. pylori*, serology remains the most accepted [8]. It is the only test, which is not affected by local changes in the stomach,

 10.1586/14737159.2015.982537 ISSN 1473-7159

Table 1. BreathID® versus other methods of assessment of *Helicobacter pylori*.

	Non-invasive	>98% sensitivity and specificity	Immediate results	Rapid test 10-min	Easy-to-do, simple training	Platform for multiple tests
BreathID®	✓	✓	✓	✓	✓	✓
Central lab breath test	✓	×	×	×	✓	×
Biopsy	×	✓	×	×	×	NA
Rapid urease test	×	×	×	×	×	NA
Serology	✓	×	×	×	✓	NA
Stool	✓	×	×	×	×	NA

NA: Not available.

widely available, inexpensive and has a high negative predictive value [9]. However, the tests are not accurate enough and therefore not recommended by the US [10], European [3] and Asia-Pacific Consensus Guidelines [11], as serology may not indicate active or current infection but only previous exposure to *H. pylori*. In addition, antibody titers may decrease up to 6 months after successful treatment, limiting the use of the test for post-eradication confirmation.

The SATs are relatively inexpensive non-invasive tests with high sensitivity and specificity. SATs using monoclonal antibodies are useful for primary diagnosis of active infection as well as for the assessment of eradication therapy [12]. SATs are also useful in the management of *H. pylori* infection in children and post-gastric surgery patients. However, test results may differ between kits and from one population to another with unacceptable low effectiveness in some kits [13].

Carbon-labeled UBTs, which have a high sensitivity and specificity, are commonly used as a non-invasive method in detecting an active *H. pylori* infection. UBTs are the preferred method used in epidemiological studies, screening dyspeptic patients and assessing eradication or recurrence of the infection. The UBT evaluates the presence of the bacteria in the whole gastric mucosa. This increases the sensitivity of the test compare with other diagnostic methods based on the analysis of focal samples obtained by gastric biopsy. Focal gastric sampling is susceptible to sampling error with higher rates of false negative results, probably due to the heterogeneous colonization of the *H. pylori* in the gastric mucosa. Table 1 summarizes the characteristics of the BreathID versus other methods of *H. pylori* detection. If *H. pylori* infection was detected and treated, a post-therapy follow-up breath test, no less than 1 month from completion of therapy, is the recommended method to confirm eradication after therapy [3].

Urea breath tests

Breath testing based on carbon-labeled substrates has been used for over 40 years, for diagnostic applications. The $^{13/14}$C-UBT, which has a high sensitivity and specificity, provides a 'gold standard' in detecting an active *H. pylori* infection [7]. All ^{13}C breath analyzers use a similar principle for analyzing breath by measuring different isotopes of carbon in CO_2. In all analyzers, $^{13}CO_2$ and $^{12}CO_2$ from the exhaled breath of the patient is collected and their ratio is calculated. The principle of the ^{13}C-UBT relies upon the ability of the urease, produced by *H. pylori* in the gastric mucosa, to hydrolyze the orally administered ^{13}C-urea. This enzyme breaks down the urea to ammonia and CO_2, which is absorbed into the bloodstream and then released from the lungs. The labeled carbon dioxide, $^{13}CO_2$ is detected in breath samples [14]. UBT detects much lower levels of *H. pylori* infection and by assessing the entire gastric mucosa, it avoids the risks of local gastric sampling error due to patchy distribution of the bacterium in the gastric mucosa. False-positive results are extremely rare, whereas false-negative results may occur in specific clinical settings. Several factors are associated with UBT results in the diagnosis of *H. pylori* including gastric emptying rate (GER) (may be delayed by a test meal), gastric pH (affected by test meal, H_2 blockers and PPIs), the dose of the labeled substrate (^{13}C-urea), bacterial urease activity (which is pH dependent), the sampling time or method and bacterial density (previous use of antibiotics or PPIs, gastrectomy), Antimicrobials, for example, should be avoided for 4 weeks prior to testing (UBT, SAT or endoscopy), as these agents also suppress infection and reduce test sensitivity [15].

^{13}C-labeled UBTs are safe in children and pregnant women and they are the preferred method used in epidemiological studies, screening patients for the presence of *H. pylori* and assessing eradication or recurrence of the infection [3].

The previous gold standard for performing UBTs for detection of *H. pylori*, used the mass spectrometry method for analysis. The capacity of this device to sequentially process hundreds of samples in an automated manner makes the system adequate for referral central laboratory performing high volume of analyses per day. These tests usually entail a two-point sampling with a 20- to 30-min gap. In this cumbersome method, the results of the test are not immediate and individual samples are collected and analyzed in a special laboratory equipped with an isotope ratio mass spectrometer (IRMS) device to determine

the $^{13}C/^{12}C$ ratio in each sample. Although relatively accurate, IRMS is not appropriate for a point of care (POC) environment or small-to-medium labs, requires patient cooperation, is subject to human error, entails high capital costs, specially trained personnel to operate the device and is relatively time consuming.

Several alternative methods for the detection of $^{13}CO_2$ have been described, including the use of laser or infrared spectroscopy. One of the most reliable tests for the diagnosis of *H. pylori* infection is ^{13}C-UBT non-dispersive, isotope-selective infrared spectroscope [16]. This device has been shown to be as accurate as IRMS but with the advantage of being faster, smaller and cheaper [17–19]. However, an important disadvantage of this equipment is that it can sequentially process only a few breath samples. Non-dispersive, isotope-selective infrared spectroscope also requires relatively large breath bags to be connected directly to the spectrometer for measurement, which greatly limits the possibility of storing and transporting breath samples to a measuring laboratory [7]. Another device, the laser-associated ratio analysis system, is based on laser spectroscopy that employs CO_2 lasers to excite a breath sample, producing an optogalvanic effect, which on analysis provides a measure of the ratio of $^{13}CO_2$–$^{12}CO_2$. Several studies using this equipment have confirmed encouraging results [20,21]. The laser-associated ratio analysis system has similar technical characteristics (the number of samples it can sequentially process, the volume of breath sample required and the cost of maintenance) as IRMS, but is limited in its market. Table 2 summarizes the characteristics of the BreathID versus other breath test methods of *H. pylori* detection.

Table 2. BreathID® compared with other breath tests.

Overall	BreathID®	Small NDIR	Large NDIR	IRMS
Continuous measurement and visual display	✓	×	×	×
Real-time results	✓	✓	×	×
Not sensitive to human errors	✓	×	×	×
Minimize test duration	~10 min	20 min	NA	NA
Unattended test	✓	×	×	×
Point of care	✓	✓	×	×
No special training needed for operation	✓	✓	×	×
Platform for multiple uses	✓	×	✓	✓
Device capital cost	✓	✓	✓✓	✓✓✓
No special training needed for interpreting results	✓	✓	×	×

IRMS: Isotope ratio mass spectrometer; NA: Not available; NDIR: Non-dispersive infrared.

One of the limitations of all the UBT is the lack of ability to assess antibiotic resistance detection to *H. pylori*. The economic benefits of tailoring first-line therapy are likely to depend on the local antibiotic resistance levels [22]. Considering the increasing failure rate of standard therapies, bacterial culture or molecular methods may have important implications as relevant alternatives for *H. pylori* diagnosis [23,24]. According to the recent Maastricht guidelines, this is not the first-line diagnostic recommendation. They suggest that culture and standard susceptibility testing should be considered in all regions before giving a second-line treatment after a first failure, if an endoscopy is carried out. After a second failure, it should be performed in all cases as already recommended at the previous Maastricht conference.

The test substrate

Evaluation of different ^{13}C-UBT protocols demonstrates that there is no consensus regarding the dosage of the ^{13}C-urea, the time and interval of breath sample collection or the test meal chosen to delay gastric emptying used in UBTs [19]. Each clinical center uses its own test protocol and this makes the comparison of results almost impossible. The test meal delays gastric emptying and enables better interaction between the bacteria and the ^{13}C-urea. These may decrease the doses of the ^{13}C-urea and increase the sensitivity of the test. Citric acid solution is currently one of the most widely used, and it has been stated that it may increase the maximum concentrations of $^{13}CO_2$ in comparison with other semi-liquid test meals previously used. Although Dominguez-Munoz *et al.* reported identical sensitivity and 100% specificity of ^{13}C-UBT for three different test meals (0.1 N citric acid solution, semi-liquid fatty meal and semi-liquid meal), the delta peak values of $^{13}CO_2$ were much higher when citric acid solution was used as the test drink [25]. Moreover, Graham *et al.*, using 1, 2 and 4 g citric acid, reported that the increase in urease activity is dose dependent [26]. Orange juice was originally proposed as test meal and is still utilized as alternative because of the unappealing taste of citric acid, which can reduce compliance. The sensitivity of the ^{13}C-UBT is lower with orange juice compared with 0.1M citric acid, probably because orange juice has a smaller content of citric acid (less significant decrease in gastric pH) and gastric emptying was significantly faster [27].

More than 90% of the bacterial urease, which generates ammonia to buffer the bacteria from the acid milieu, is located in the cytoplasm. Urease activity is low at neutral pH but as the external pH decreases between 6.5 and 5.5 there is a 10- to 20-fold increase in activity, which remains high through approximately pH 2.5 [28,29]. The transport of urea into the bacteria is regulated by Urel-dependent specific H^+-gated urea channels that are also pH dependent [30]. To minimize these pH-dependent effects, BreathID protocol uses a test drink which includes a 75 mg ^{13}C-labeled urea tablet, dissolved in 200 ml water with a high concentration (4.0 g) of citric acid,

doi: 10.1586/14737159.2015.982537

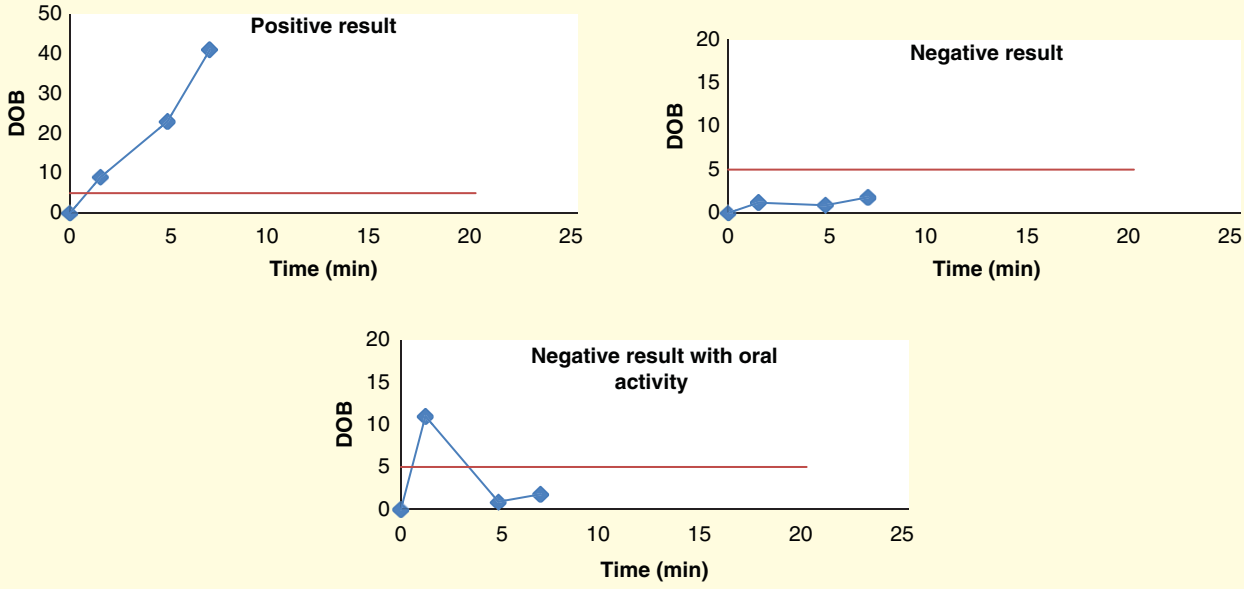

Figure 1. Sample breath test results with BreathID® *Helicobacter pylori* system.
Blue line: breath test result; red line: cutoff value.
DOB: Delta over baseline.

which delays gastric emptying and decreases gastric pH. However, recently Graham *et al.* hypothesized that these two factors *per se* appear unlikely to be the critical determinants in the increased access of urea to the urease enzyme *in vivo* [31].

BreathID breath test system

The ^{13}C-labeled substrate, in the case of *H. pylori*, is ^{13}C-urea, accompanied by citric acid powder. In the presence of urease associated with gastric *H. pylori*, ^{13}C-urea is decomposed into $^{13}CO_2$ and NH_3. The $^{13}CO_2$ is absorbed into the blood and exhaled. Delta is an expression of the change in the ^{13}C–^{12}C ratio and is defined as:

$$\delta\,(\text{delta}) = \frac{(^{13}C^{(n)}/^{12}C^{(n)}) - (^{13}C^{(PDB)}/\,^{12}C^{(PDB)})}{(^{13}C^{(PDB)}/\,^{12}C^{(PDB)})} \times 1000\ ‰ \quad (1)$$

where $^{13}C^{(PDB)}/^{12}C^{(PDB)}$ in this formula stands for the isotope ratio (1.1273%) of international reference material (Pee-Dee Belemnite standard) [32]. The formula shows carbon isotope ratio in CO_2 contained in exhaled breath. Delta over baseline (DOB) indicates the deviation of delta value from the standard delta value at a time point (i.e., before any substrate was ingested). It is defined as:

$$\text{DOB} = \frac{(^{13}C^{(n)}/^{12}C^{(n)}) - (^{13}C^{(0)}/^{12}C^{(0)})}{(^{13}C^{(PDB)}/\,^{12}C^{(PDB)})} \times 1000\ ‰ \quad (2)$$

Excess $^{13}CO_2$ in the breath compared with baseline translates into a positive breath test result if the final test results reach a value more than 5 DOB units, as can be seen in Figure 1.

The BreathID can also be used for other applications and received a CE mark for liver function, gastric emptying testing and other gastrointestinal-related applications. For these applications, a quantitative evaluation of the substrate metabolized is required and therefore, the BreathID device plots (not relevant in *H. pylori* mode) also the percentage dose recovery (PDR) and cumulative percentage dose recovery on the device's display and provides the PDR peak value as seen in Figure 2. PDR refers to the rate at which the ^{13}C substrate is metabolized. In the case of liver function testing, for example, the amount of ^{13}C-methacetin metabolized reflects hepatic metabolic activity. Its units are in %/h. PDR is similar to DOB in its expression of change in $^{13}C/^{12}C$ ratio, but includes a normalization factor based on specific test details such as weight, height, dose and substrate type and purity, thereby in essence normalizing the results independent of differences in external factors. Cumulative percentage dose recovery is the numeric integral of PDR, and indicates the total amount of substrate metabolized at any given accumulated time. It is given in units of percent.

It has been shown in several analytical and clinical studies in the *H. pylori* application as well as other breath test applications that the BreathID highly correlates to endoscopy pathology results, endoscopy-based RUT and IRMS measurements (considered the 'gold standard') [33,34]. Additionally, post-therapy testing was performed on a portion of the subjects. All results showed sensitivity and specificity 95% or more.

Principle of the BreathID technology

The BreathID System components include a test kit, containing a nasal cannula for collecting the breath output exhaled by the patient (Figure 3). The diagnostic drug substrate depends

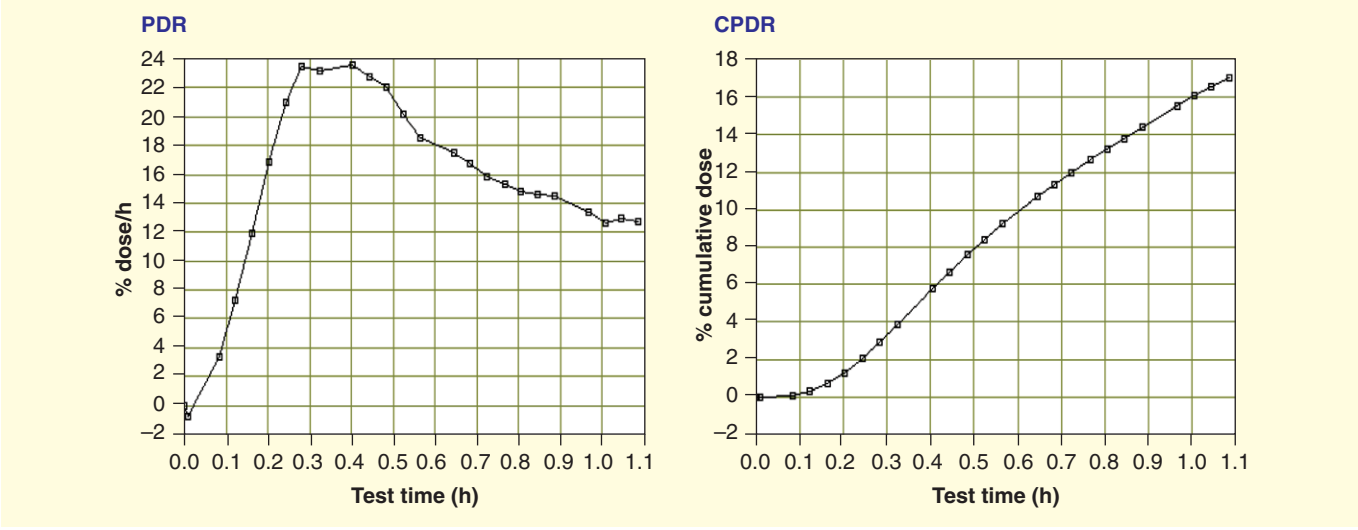

Figure 2. Percentage dose recovery and cumulative percentage dose recovery graphs displayed on BreathID® device in real time.

upon the application and is labeled with ^{13}C-urea for *H. pylori*. The BreathID device collects breath exhaled by the patient continuously for approximately 1 min into an internal bag, measures the average $^{13}CO_2$ and $^{12}CO_2$ concentrations of the accumulated breath present in the bag and computes their ratio and displays the results.

The BreathID uses a proprietary technology called Molecular Correlation Spectroscopy to measure ^{13}C and ^{12}C isotopes of CO_2 from the exhaled breath of patients. Molecular Correlation Spectroscopy is based on the optical absorption of specific radiation of $^{13}CO_2$ and $^{12}CO_2$ gases. By using $^{13}CO_2$ and $^{12}CO_2$ charging lamps as two unique light sources, light absorption will be due only to the existence of $^{13}CO_2$ and $^{12}CO_2$ in the gas mixture. Furthermore, by using this method the background radiation will be much reduced, leading to highly sensitive absorption curves. These allow the detection of a small variation in $^{13}CO_2$ and $^{12}CO_2$ concentrations. By modulating these different light sources with different frequencies, they can be measured at the same detector, called the main detector. In order to calculate the $^{13}CO_2$ and $^{12}CO_2$ gas concentrations, an absorption cell is fixed between the light source and the main detector (Figure 4). By measuring the light intensity with a given gas concentration in the absorption cells, specific absorption curves can be built. These absorption curves allow the $^{13}CO_2$ and $^{12}CO_2$ concentrations in the absorption cells to be calculated. The default test duration depends upon the application, 1 h in the case of liver function testing and 4 h for gastric emptying test.

Approximately 99% of the carbon dioxide exhaled comprise $^{12}CO_2$, but a small portion of $^{13}CO_2$ is also exhaled in the breath. ^{13}Cs natural abundance is approximately 1% in the environment and it is a stable isotope [35]. The baseline ratio between $^{13}CO_2$ and $^{12}CO_2$ is measured at the beginning of the test. After ingestion of a ^{13}C-labeled substrate, the ratio between the $^{13}CO_2$ and $^{12}CO_2$ is measured and compared with the baseline ratio. When the substrate containing the enriched levels of ^{13}C is metabolized, one of the by-products produced is carbon dioxide. The more metabolism that occurs, the larger the changes in $^{13}CO_2/^{12}CO_2$ ratio, leading to changes in the DOB. This in turn is translated into quantitative assessment of the targeted organ's ability to metabolize a given substrate. The measuring process is repeated continually throughout the test, enabling continual monitoring of the substrate metabolism. It has been shown that the BreathID device is a reliable device for measuring $^{13}CO_2/^{12}CO_2$ ratio, with regard to linearity over the entire relevant range of measurements and its results are reproducible in both healthy and non-healthy patients. Furthermore, it has been shown that the device remains stable over prolonged measurement durations.

Unique features of the BreathID system

The BreathID provides a competitive solution for breath testing compared with other ^{13}C breath analyzers and other methods of testing, including several unique features. The automatic breath collection and analysis makes the use convenient with no human errors. Instead of collection and analysis of discrete

Figure 3. Components of the BreathID® system.

doi: 10.1586/14737159.2015.982537

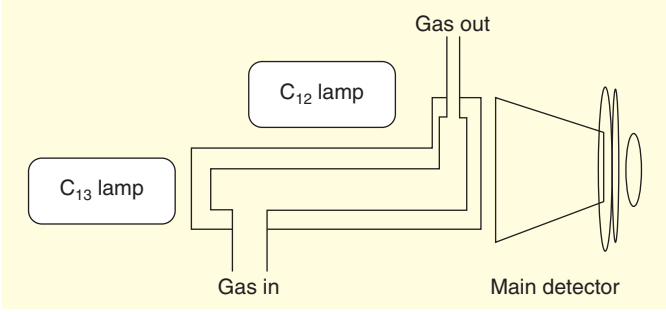

Figure 4. Schematic illustration of the principles of the BreathID® system.

samples, multiple samples are continually collected, providing additional information. Due to continual measurement, this simple and small device has excellent accuracy (>99% in comparison with gold standard in *H. pylori* detection in the US FDA study). Test results are available in real time for decision-making at the POC and enabling shortened breath testing procedures. Detailed explanations of these advantages are described below.

Automatic versus non-automatic breath testing

The automatic breath collection and analysis makes the test convenient unattended procedure that can be performed in POC environment and accurate, even compared with IRMS, with no human errors. The appropriate part of the breath sample is collected automatically (using a built-in 'capnograph').

Figure 5 illustrates the potential risk of sacrificed accuracy in non-automatic breath testing in a liver function breath test. This provides quantitative assessment of function at specific time points (compared with normal values). Noise in discreet points can lead to inaccurate readings at those specific time points. The BreathID collects breath over a period of time (~1 min) and analyzes the mixture, thereby enabling the device to be insensitive to discreet changes. The BreathID device continuously collects and analyzes the breath automatically as opposed to the IRMS. Therefore, the BreathID is less sensitive

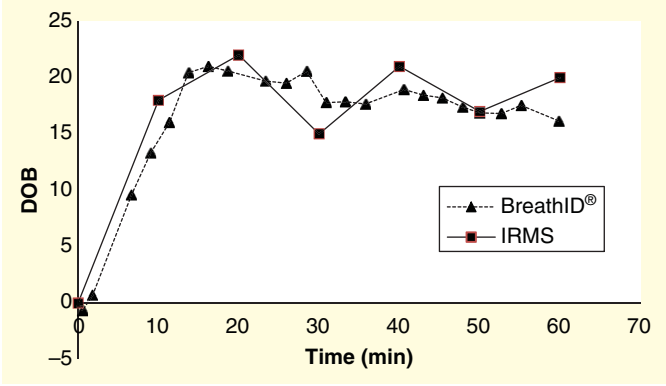

Figure 5. Noisy discrete breath collection versus BreathID® collection.
DOB: Delta over baseline; IRMS: Isotope ratio mass spectrometer.

to physiological fluctuations, enables to accurately detect the peak and does not require patient cooperation. In cases where the DOB is close to the threshold, physiological noise may affect the accuracy of the test. In that case, the fact that there are several points collected as opposed to a single point, the result will be more reliable. Furthermore, the device is less sensitive to the timing of the peak due to the multiple point collection. Lastly, the device automatically lengthens the test time when the results are close to the threshold.

Moreover, the patient is in a resting position during the test, which prevents rapid changes in physiology and CO_2 production. Lastly, patient's cooperation is not required. This provides an especially suitable test for adult, pediatric and intubated patients who may find it difficult to comply with breath collection requirements.

Continuous breath testing

One of the major advantages of continuous versus discrete breath testing is higher accuracy with approximately 2 min resolution that enables following of rapid physiological changes that may be missed with discrete sampling. Figure 6 demonstrates an example from a liver function utility test study with methacetin, of cases where the peak is missed by IRMS, even with the unusually high sampling rate of 10 min used in this study. This turned out to be a crucial factor in the liver function utility, where the peak has proven to be the most significant result parameter [36]. This additional information on physiological processes together with the online analysis enables the collection of useful clinical information and minimizing test duration. Continuous monitoring of the exhaled CO_2 is associated with lower sensitivity to physiological noise, since the trend can be analyzed, rather than single points (i.e., the general trend can be seen and parameters can be extracted, even in the case of a *noisy* response). This can enable dealing with the inaccuracies related to changes in the overall CO_2 production. In the case of UBT for the detection of *H. pylori*, several studies have shown that while performing the UBT, there is possibility of false-positive results due to the other urease-producing bacteria present in oropharynx. Usually, this DOB peak appears early during the test (1–3 min) and declines subsequently to baseline levels by 5–15 min (Figure 1) [37]. Pathak *et al.* showed that without mouth cleansing, oral micro flora excreted more $^{14}CO_2$ up to 15 min after administration of non-capsulated ^{14}C-urea. They proposed that two breath samples may be obtained either at 15 and 20 min without or at 10 and 15 min with mouth cleansing protocols. Continuous sampling of the breath samples identifies this oropharyngeal urease activity and terminates the test shortly after this peak, reducing the time taken to perform the test.

Real-time online analysis

BreathID provides immediate results with shorter test length than laboratory breath testing (i.e., the test can be stopped as soon as peak is detected which is unknown in off-line analysis) [38]. Results are not sensitive to changes in reference values

doi: 10.1586/14737159.2015.982537

in external laboratories. They are reproducible and available in real time for decision-making at the POC. Table 2 summarizes the characteristics of BreathID compared with other breath tests.

Specific clinical settings

Both invasive and non-invasive routine conventional methods for *H. pylori* detection have been increasingly focused on specific clinical settings and patient groups (concomitant use of PPIs or antibiotics, gastric atrophy and intestinal metaplasia, bleeding peptic ulcer, post-gastrectomy patients, children).

Concomitant use of PPIs

False-negative results may occur when using histological, RUT and UBT to detect *H. pylori* in biopsy specimens obtained during PPI use [39]. PPI-induced false-negative UBTs may be explained by a combination of marked gastric acid suppression and antimicrobial activity of these compounds against *H. pylori*. Consequently, all centers currently recommend cessation of PPIs 7–14 days before UBT [40]. This requirement means that symptomatic patients have to defer therapy for a significant period of time in order to be tested. Ideally, for both clinical and quality-of-life concerns, patients and physicians would prefer to start PPI treatment until the performance of the UBT. The BreathID results show that PPI-associated UBT masking can be kept to a minimum with judicious use of high-dose citric acid as a test meal and an appropriate PPI [41–43]. In our study, both pantoprazole and omeprazole had very low false-negative rates (2–4%), whereas lansoprazole and esomeprazole had unacceptably high false-negative rates ranging from 13 to 16% (Table 3, data have been taken from the citation). Concerning the use of anti-H_2 drugs, there is a general agreement that their effect on the UBT results is much less important compared with that observed for PPI, whereas the effect of antacids on false-negative results is negligible.

Partial gastrectomy

Partial gastrectomy and *H. pylori* infection are both considered as risk factors for gastric cancer. False-negative UBT results have been described in patients with gastric surgery, due to rapid gastric emptying of urea solution from the stomach and the small amount of the bacteria in the remnant stomach. Among the three commonly used tests (histology, RUT and UBT), histological examination performs the best, followed by the RUT, for the diagnosis of *H. pylori* infection after partial gastrectomy. Pooled sensitivity, specificity and diagnostic odds ratio (DOR) for the different methods were: UBT: 0.77 (95% CI: 0.72–0.82); 0.89 (95% CI: 0.85–0.93); and 27.86 (95% CI: 13.27–58.49). RUT: 0.79 (95% CI: 0.72–0.84); 0.94 (95% CI: 0.90–0.97) and 49.02 (95% CI: 24.24–99.14). Histology: 0.93 (95% CI: 0.88–0.97); 0.85 (95% CI: 0.73–0.93) and 97.28 (95% CI: 34.30–275.95) [44]. Kubota *et al.* reported that the use a specific protocol including ingestion of 100 mg ^{13}C-urea, use of mouthwash, horizontal position of the body to the left side increased the sensitivity of ^{13}C-UBT up to 95.7% [45]. Others improved the diagnostic accuracy of ^{13}C-UBT, over the standard UBT in patients with gastric resection, by multiple sampling [46]. Recently, Wardi *et al.* showed, when histology was considered as the gold standard method, a high negative predictive value by both BreathID and RUT, 0.92 and 0.95, respectively. The positive predictive value of the BreathID and the RUT was 0.64 and 0.35, respectively, with no difference for *H. pylori* positivity between patients with Billroth I or Billroth II operations [47].

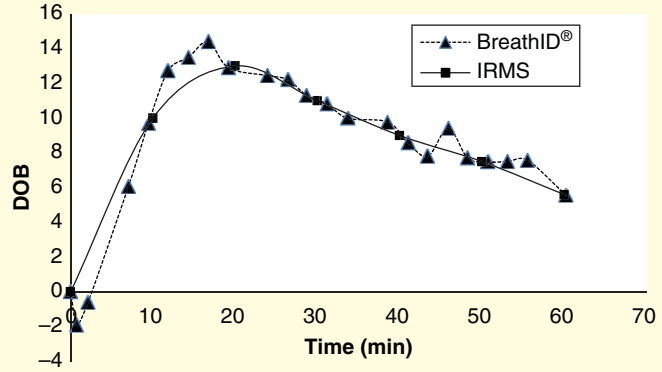

Figure 6. Examples of missed peaks; isotope ratio mass spectrometer versus BreathID®. (A) Normal emptying. **(B)** Delayed emptying.
DOB: Delta over baseline; IRMS: Isotope ratio mass spectrometer.

UBT in pediatric population

The ^{13}C-UBT has become the most convenient method for use in children because it is a non-invasive method and uses a stable and non-radioactive isotope. *H. pylori* infection is mainly acquired in childhood, and studies on the epidemiology of this infection depend on the availability of a non-invasive diagnostic test for use in children. UBT has shown variable accuracy in the pediatric population, especially in young children [48,49]. Most of the validation studies in children included only a few infants and toddlers. Only when the children were separated into subgroups by age it became apparent that the accuracy of most tests is lower in young children if the same cutoff values are used as established for older children or adults. In a recent meta-analysis including 31 articles and 135 studies, Leal *et al.* evaluated the diagnostic performance of the ^{13}C-UBT in children stratified in subgroups of <6 and ≥6 years of age. They also analyzed the effect of variables such as type of meal, cutoff value, tracer dose and delta time. The results showed good accuracy in all ages combined (sensitivity 95.9%, specificity 95.7%, likelihood ratio [LR]+ 17.4, LR– 0.06, DOR 424.9), with high accuracy in children >6 years (sensitivity 96.6%, specificity 97.7%, LR+ 42.6, LR– 0.04, DOR 1042.7). The ^{13}C-UBT test was less accurate in young children, but adjusting cutoff value, pretest meal and urea dose, this accuracy could be improved [50]. Indeed, recently Queiroz *et al.* evaluated a cohort of 414 infants (123 from Brazil and 291 from Peru) of ages 6–30 months living in impoverished

doi: 10.1586/14737159.2015.982537

Table 3. False-negative results at day 14 after proton pump inhibitors treatment. Comparison between omeprazole, pantoprazole, lansoprazole and esomeprazole.

Proton pump inhibitor		OME 20 mg	PAN 40 mg	LAN 30 mg	ESO 40 mg
Patients (N)		48	45	42	44
Male/Female		20/28	24/21	24/18	21/23
Age (years ± SD)		47.9 ± 16.7	45.9 ± 18.0	45.8 ± 16.8	49.0 ± 14.5
UBT results (DOB)	Baseline	31.7 ± 31.6	27.5 ± 19.6	28.7 ± 23.7	23.8 ± 18.3
	Day 14	33.8 ± 29.5	24.8 ± 21.4	27.1 ± 28.1	19.1 ± 17.5
False negative	Day 14	2/48 (4.1%)[†]	1/45 (2.2%)[‡]	7/42 (16.6%)	6/44 (13.6%)
True negative	Day 14	0/48	1/45	0/42	3/44

[†]OME versus LAN p = 0.05.
[‡]PAN versus LAN p = 0.02; PAN versus ESO p = 0.05.
DOB: Delta over baseline; LAN: Lansoprazole; OME: Omeprazole; PAN: Pantoprazole.
Adapted from [43].

regions of two developing countries in South America. They showed excellent agreement between the results of the ^{13}C-UBT and the SAT for infants and toddlers indicating that UBT is a reliable method for the diagnosis of *H. pylori* infection in very young children [51]. Similar results were reported by Pacheco *et al.* [52].

BreathID was prospectively evaluated in 72 consecutive children and adolescents aged 5–18 years who were referred for gastroscopy or for ^{13}C-UBT. Results were obtained within 10 min in 96% of patients. The test was rapid and had 100% concordance with conventional diagnostic methods [53]. Similar results were reported by Hino *et al.* showing that the BreathID was very effective in diagnosing and confirming eradication of *H. pylori* infection in children (100% sensitivity and 96.9% specificity [97.5% positive predictive value and 100% negative predictive value]) [54]. Although there are no sufficient data regarding the accuracy of the BreathID in young children, the automatic, rapid and continuous sampling method with no need of active cooperation makes the BreathID an optimal breath test for the use in this population.

Additional potential applications of BreathID

The concept of using non-invasive ^{13}C-labeled substrates in conjunction with a breath analyzer as a diagnostic tool or as an aid in management of patients with different gastrointestinal disorders has been gaining more attention due to the lack of reliable, easy-to-use function tests for gastric emptying, liver, pancreas and other gastro intestinal organs. ^{13}C-labeled substrates are chosen to target a specific metabolization process of the targeted organ. These breath tests, once validated, can potentially, in many situations, accurately replace other expensive, unpleasant and/or invasive procedures such as endoscopy, biopsy, stool tests, scintigraphy and others. Non-invasive breath tests may be repeated at high frequencies, allowing monitoring of the organ functionality in patients with chronic/acute conditions, in determining effectiveness of therapy and in optimizing therapy dose.

Assessment of GER

GER serves as a marker of various functional gastrointestinal disorders [55]. It is assessed by calculating the percentage of food retained or eliminated by the stomach after a standard solid meal at defined intervals of time. The gastric half-emptying time (T½) is the most practical and common clinical parameter. However, gastric retention above 10% after 4 h seems to be a better marker for the diagnosis of delayed gastric emptying [56]. Gastric scintigraphy measures the change in radioactivity within the stomach, which is directly proportional to its emptying rate, whereas breath test measures the concentration of $^{13}CO_2$ in the exhaled breath, the end product of a sequence of events (e.g., ^{13}C-octanoic acid). Gastric scintigraphy with 99mTc exposes patients and staff to low, but measurable doses of radiation. The test is not always readily available because it requires specialized and expensive equipment, trained personnel and licensure for the medical use of radioactive materials.

Ghoos *et al.* [57] were the first to show the benefits of the ^{13}C-enriched octanoic acid-based breath test for measuring GER. ^{13}C-octanoic acid is absorbed in the small intestine; from there, it is transported to the liver, producing $^{13}CO_2$, which is eliminated by the lungs. This may limit the use of the test in patients with lung and liver disease, malabsorbtion or maldigestion. However, as in ^{13}C-urea with *H. pylori*, the quantity of $^{13}CO_2$ in the patient's exhaled breath is a function of the quantity of content leaving the stomach and reaching the intestine. By measuring the $^{13}C/^{12}C$ ratio in the expired air, clinicians can calculate the gastric emptying coefficient, the gastric T½ and the lag phase (Tlag) [57]. The long duration of the test and the need for multiple sampling (up to 18 test tubes per patient at 15–30 min intervals) renders the test cumbersome to both patients and by laboratory staff. Several studies using octanoic acid-based breath test have provided reproducible results that were correlated with gastric scintigraphy, with a reported sensitivity of 67–95% and specificity ranging from 78 to 94% [57,58]. Still, the lack of standardization and normative values have raised

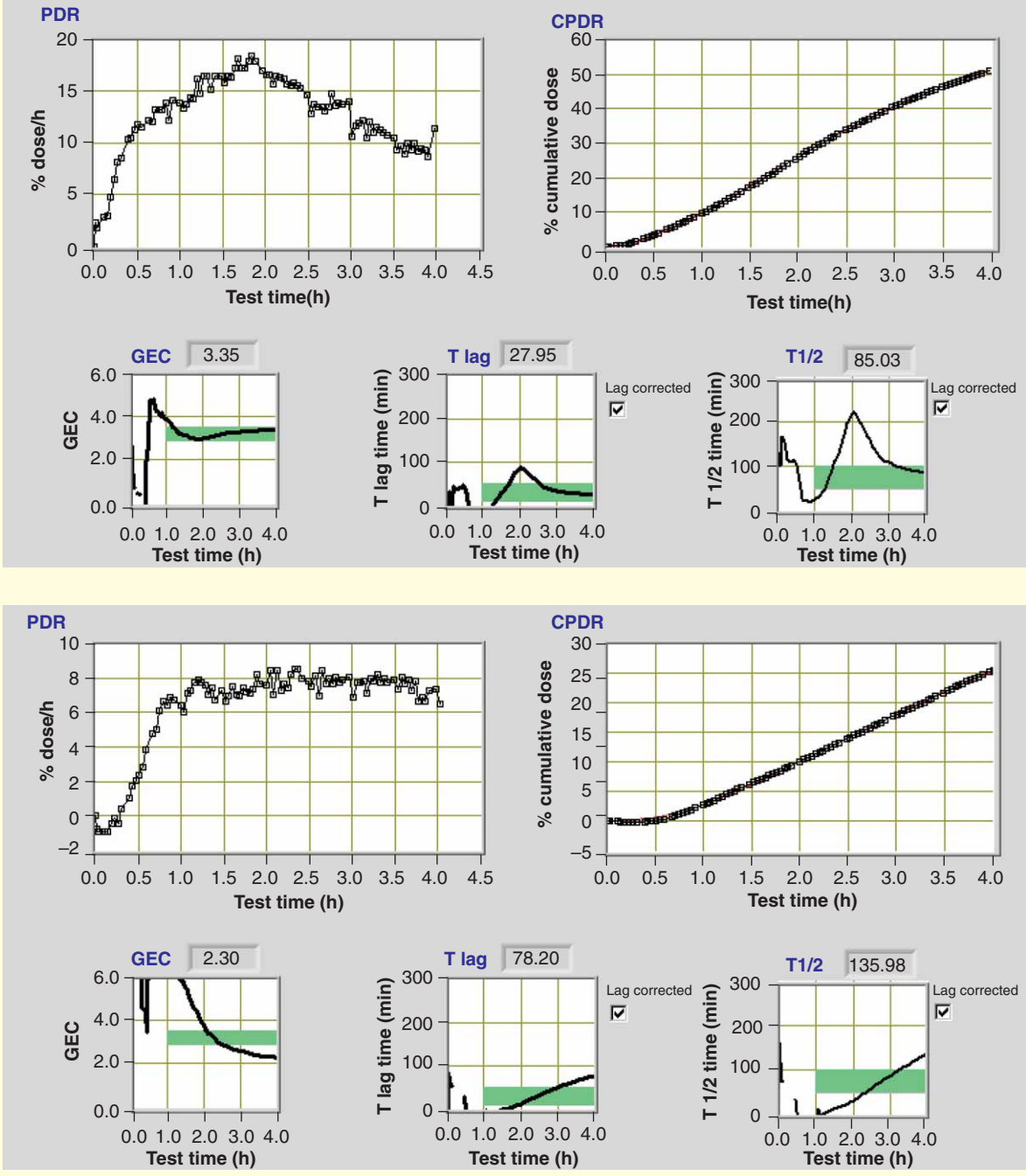

Figure 7. Percentage dose recovery, cumulative percentage dose recovery, gastric emptying coefficient, gastric T½ and Tlag graphs displayed on BreathID® device in real time.

concerns about the clinical application of the test and its routine use [59].

The BreathID automatically calculates the change in the $^{12}CO_2/^{13}CO_2$ ratio at various points after ingestion of ^{13}C-labeled octanoic acid compared with baseline (Figure 7).

The system calculates the gastric emptying coefficient, gastric T½ and Tlag according to the non-linear model described by Ghoos *et al.* [57]. In a recent prospective study conducted by our group, simultaneous GER measurements in a small group of dyspeptic patients using both the BreathID and gastric scintigraphy provided comparable qualitative results (normal/abnormal results) [60]. In this study, we recorded both gastric T½ and retention during gastric scintigraphy; however, assessment of retention by BreathID was not feasible. In a future study, there is a need to validate a method that will accurately calculate gastric retention by BreathID. Table 4 summarizes the

doi: 10.1586/14737159.2015.982537

Table 4. BreathID® versus other methods of assessment in gastric emptying.

	BreathID®	Scintigraphy	Mass spectrometer/ infrared
Radioactive	No	Yes	No
Gastric emptying rate patterns	Yes	No (unless continuous measurement is used)	Partial
Point-of-care	Yes	No	Partial
Results comprehensive	Yes	No ($T_{1/2}$ only)	Partial
Nurse/tech involvement	Low	High	High
Immediate results	Yes	No	No
Patient's active cooperation	Low	High	High
Operator errors	No	Yes (and variability)	Yes

characteristics of the BreathID test in the assessment of gastric emptying.

Assessment of pancreatic disorders

There is a need for a reliable and practical tool for evaluation of pancreatic function. The rational for the use of breath test is that the ^{13}C-labeled substrate given with the meal reaches the duodenum, where it is hydrolyzed by specific pancreatic enzyme to ^{13}C-labeled metabolites. These are absorbed through the gut, metabolized in the liver while the $^{13}CO_2$ released during this process is absorbed in the bloodstream, reaches the lungs and is eliminated with expired air. Thus, the measurement of $^{13}CO_2$ in the expired air is an indirect measure of pancreatic digestion. Braden [61] reviewed the different methods of testing for pancreatic function and observed that mixed triglycerides (MTG) breath test is the most studied reliable method of breath testing for this purpose. However, the ^{13}C-dipeptide breath test has the potential to become as easy, fast and practicable as the ^{13}C-UBT for *H. pylori* detection. While currently available clinical and laboratory parameters are either not sensitive enough or cumbersome, these preliminary data are promising. The breath tests can provide a novel alternative for management of patients with chronic (and acute) pancreatic disorders. Dominguez has shown that a ^{13}C-MTG breath test is an accurate method to evaluate the effect of enzyme therapy on fat digestion. This method is simpler than the standard fecal fat test to assess therapy in patients with pancreatic exocrine insufficiency. It can be used to tailor the optimal therapy in normalizing fat absorption and improving the nutrition in these patients [62]. However, still the ^{13}C-mixed triglyceride breath test could only diagnose pancreatic insufficiency that typically occurs in advanced stages of pancreatic disease, which limits the use of the test [63].

A BreathID preliminary trial has been carried out to evaluate exocrine pancreatic function and to differentiate between patients with and without normal exocrine pancreatic function, and the correlation between the breath test to standard function tests. Preliminary results seem promising (unpublished data). The BreathID, in contrast to other techniques that would require collection of many samples during 6 h when MTG is used, can minimize test length.

Clinical use of the BreathID in patients with acute & chronic liver disorders

Currently available blood-and-imaging tests or even liver histology do not provide accurate measures of hepatic metabolic function. The dream of every hepatologist is to develop a non-invasive surrogate liver function marker/test just like the glomerular filtration rate of the nephrologist or the ejection fraction of the cardiologist. It is based on the principle that a measurable metabolite of an ingested substrate is expelled by the respiratory system. The ideal substrate would be metabolized solely by the liver and therefore selectively reflect liver metabolic function. The principle assumption is that an accurate measurement of one metabolic pathway can reflect the status of other hepatic metabolic pathways [64,65]. This aim has been stalled by the complexity of the numerous metabolic pathways of the liver and its diverse functions.

Clinically used probes of ^{13}C-labeled substrates for liver assessment include: aminopyrine, caffeine, diazepam, phenacetin and erythromycin [66,67]. The safety displayed by methacetin in non-clinical studies and the high hepatic clearance by *O*-demethylation and subsequent exhalation of CO_2 led to its early use in exploratory clinical studies dating back to the late 1970s [68]. Methacetin is considered a preferred substrate because of its rapid metabolism in normal subjects, the apparent minimal effect of smoking and anticonvulsants and the lack of toxicity at over the 10-fold doses range tested. Other substrates can be used to assess mitochondrial/beat oxidation, which may be important in the context of specific etiologies. Examples of such substrates include methionine and sodium octanoate.

Recently, multiple trials conducted using the BreathID system, including populations with chronic viral liver disease (hepatitis C virus, hepatitis B virus), subjects with normal alanine aminotransferase, non-alcoholic fatty liver disease/non-alcoholic steatohepatitis, acute liver failure, bariatric surgery, hepatic venous gradient pressure, subjects that underwent chemoembolization, pediatric use and animal testing (showing ability to monitor functional liver mass) [69–74]. These studies show applications of the BreathID test in a wide variety of etiologies, where there is an unmet need for a simple routine monitoring test for those with chronic liver disease and fatty liver disease, thereby enabling early non-invasive prediction of decompensation. The BreathID provides a novel measure, which may be complementary to the currently used diagnostic liver function tests.

doi: 10.1586/14737159.2015.982537

Summary

The BreathID with its continuous breath test characteristic, provides several advantages over IRMS breath testing, including: higher accuracy (does not depend on operators, assured collection of 'end tidal' exhaled waveform), immediate results and convenience as an 'unattended test' that can be performed in any environment. Furthermore, the continuous testing allows shorter testing duration due to a propriety algorithm that allows test shortening if result is conclusive. An observational study involving approximately 13,000 subjects, indicated that completion of the BreathID test required 10–13 min on average. Only eight subjects (0.1%) from the total population had inconclusive results and needed further time to reach a conclusive result. Additionally, several studies showing expanded utility in pediatric, after therapy, during PPI intake, further support the safety and performance of the BreathID in the diagnosis of *H. pylori*.

Expert commentary

Data from recent studies show that the prevalence of *H. pylori* infection is still high in most countries worldwide [75]. There are continuous attempts to improve the existing serologic antibody tests that are still widely used regardless of the clear guidelines that these serum tests are not accurate [76]. Because serology is prone to inaccuracy, the choice that most of the experts are clearly recommending is non-invasive 'active' diagnostic tests, namely SAT or UBT. Active *H. pylori* testing is outlined as preferred by the American College of Gastroenterology, the American Gastroenterological Association, the European and Japanese societies in their patient test and treat approach to dyspepsia [3,10,77]. Additional support to this concept came in those days when Cigna was the first large national payer in the USA to decide that it will no longer reimburse serology testing as of 15 August 2014. This provides a great opportunity to further convert serology testing into active *H. pylori* testing, with either the UBT or the SAT, for initial diagnosis or to confirm eradication.

Comparison between SAT and UBT reveals advantages and disadvantages to each of them [12]. The cost of UBT is still relatively high (because of the price of ^{13}C-urea), while SATs are less expensive. In addition, patients are required to fast before UBT testing, but not before a SAT. False-negative results are noted in patients who have been taking PPIs in both UBT and SAT but some monoclonal antibody-based SATs, that are currently available, are not affected by PPIs [78]. Although both tests are useful for the diagnosis of *H. pylori* infection in children, the specificity of the UBT may be less than 90% in very young children. Therefore, monoclonal antibody-based SATs seem to be more effective in this population. In the setting of a mass survey, compared with serology, both tests may have high levels of false-negative results, mainly in patients with severe atrophic gastritis and intestinal metaplasia. Finally, a potential problem with the SATs appears to be patient reluctance about stool handling and this could prove a significant obstacle to patient compliance and the acceptability of the test in everyday clinical practice [79].

In our experience, patients prefer to avoid stool testing so that we anticipate that the UBT will be the dominant diagnostic test for *H. pylori* in patients not requiring endoscopy. The simplicity and the accuracy of the UBT will enable to replace the serum-based tests. The BreathID can optimize the management flow, as the patient will receive an answer immediately and the physician will be able to provide appropriate treatment in the same visit. Furthermore, the UBT is also a simple solution to provide post-eradication confirmation or lead physician to other treatment options to confirm eradication.

Five-year view

Although the guidelines recommend to refrain from serology, the majority of testing for *H. pylori* is still being done by serology for the acute diagnosis and follow-up of treatment (according to MediCare: 66% in 2012). It is expected that this number will gradually decrease, once the guidelines are adopted. Based on the current guidelines [9], the use of breath testing is expected to increase in the near future, as these guidelines recommend the use of the UBT both for the diagnosis and follow-up of eradication treatment. In addition, the current recommendation to use the 'test and treat' pathway for patients who have dyspepsia, without alarming symptoms, is also expected to increase the number of breath testing [80–82]. As the percentage of patients being successfully treated is decreasing (due to resistance to antibiotics) [83,84], using a reliable non-invasive test to assess *H. pylori* density and the activity and degree of gastritis became significantly important. High pre-treatment UBT results have been demonstrated to be an independent predictor of eradication therapy [85–89]. Further evaluation of this issue may potentially lead to more effectively targeted therapies and more individualized treatments, targeting the specific needs of a given patient.

It is likely that competitive pricing and ease of use of the real-time methodology will initially determine whether physicians and their practices will transition to this methodology. However, the likelihood will be increased by the development of other ^{13}C real-time breath methods for other indications, such as liver function testing, pancreatic function and gastric emptying estimations. As these are rolled out over the next few years, we predict that the real-time device will be marketed successfully as serving multiple purposes for gastroenterology practices and this will accelerate the move from the conventional ^{13}C-urea to the real-time ^{13}C-urea platform.

Financial & competing interests disclosure

H Shirin has received grants and stock options from Exalenz. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

doi: 10.1586/14737159.2015.982537

Key issues

- The prevalence of *Helicobacter pylori* infection is decreasing in Western countries, but remains comparatively high in developing regions.
- The discovery of *H. pylori* led to a dramatic change in our understanding the pathogenesis of peptic ulcer and gastric malignant diseases.
- *H. pylori* is a major contributory factor in the development of human gastric cancer and has been classified as a group 1 carcinogen by WHO.
- Carbon-labeled urea breath tests, which have a high sensitivity and specificity, are the preferred non-invasive method used in epidemiological studies, screening dyspeptic patients and assessing eradication or recurrence of *H. pylori* infection.
- The use of urea breath tests, allowing identification of bacterial density and grading of the gastritis may potentially lead to more individualized effective therapies and increase the eradication rates.
- Technological advancements made over the past decade have not yet led to new diagnostic methods of clinically proven benefit in the diagnosis of *H. pylori* infection.

References

1. Go MF. Review article: natural history and epidemiology of Helicobacter pylori infection. Aliment Pharmacol Ther 2002; 16(Suppl 1):3-15
2. Brown LM. Helicobacter pylori: epidemiology and routes of transmission. Epidemiol Rev 2000;22(2):283-97
3. Malfertheiner P, Megraud F, O'Morain CA, et al. Management of Helicobacter pylori infection–the Maastricht IV/Florence Consensus Report. Gut 2012;61(5):646-64
4. Cho JH, Chang YW, Jang JY, et al. Close observation of gastric mucosal pattern by standard endoscopy can predict Helicobacter pylori infection status. J Gastroenterol Hepatol 2013;28(2):279-84
5. Kato T, Yagi N, Kamada T, et al. Diagnosis of Helicobacter pylori infection in gastric mucosa by endoscopic features: a multicenter prospective study. Dig Endosc 2013;25(5):508-18
6. Di Rienzo TA, D'Angelo G, Ojetti V, et al. 13C-Urea breath test for the diagnosis of Helicobacter pylori infection. Eur Rev Med Pharmacol Sci 2013;17(Suppl 2):51-8
7. Logan RP. Urea breath tests in the management of Helicobacter pylori infection. Gut 1998;43(Suppl 1):S47-50
8. Megraud F. The most important diagnostic modalities for Helicobacter pylori, now and in the future. Eur J Gastroenterol Hepatol 2012;9(Suppl 1):S13-15.discussion S15
9. Burucoa C, Delchier JC, Courillon-Mallet A, et al. Comparative evaluation of 29 commercial Helicobacter pylori serological kits. Helicobacter 2013; 18(3):169-79
10. Chey WD, Wong BC. American College of Gastroenterology guideline on the management of Helicobacter pylori infection. Am J Gastroenterol 2007;102(8): 1808-25
11. Fock KM, Katelaris P, Sugano K, et al. Second Asia-Pacific Consensus Guidelines for Helicobacter pylori infection. J Gastroenterol Hepatol 2009;24(10): 1587-600
12. Shimoyama T. Stool antigen tests for the management of Helicobacter pylori infection. World J Gastroenterol 2013; 19(45):8188-91
13. Nardone G, Rocco A, Fiorillo M, et al. Gastroduodenal lesions and Helicobacter pylori infection in dyspeptic patients with and without chronic renal failure. Helicobacter 2005;10(1):53-8
14. Graham DY, Klein PD, Evans DJ Jr, et al. Campylobacter pylori detected noninvasively by the 13C-urea breath test. Lancet 1987; 1(8543):1174-7
15. McColl KE. Clinical practice. Helicobacter pylori infection. N Engl J Med 2010;362: 1597-604
16. Calvet X, Sanchez-Delgado J, Montserrat A, et al. Accuracy of diagnostic tests for Helicobacter pylori: a reappraisal. Clin Infect Dis 2009;48(10):1385-91
17. Gisbert JP, Gomollon F, Dominguez-Munoz JE, et al. Comparison between two 13C-urea breath tests for the diagnosis of Helicobacter pylori infection: isotope ratio mass spectrometer versus infrared spectrometer. Gastroenterol Hepatol 2003;26(3):141-6
18. Savarino V, Mela GS, Zentilin P, et al. Comparison of isotope ratio mass spectrometry and nondispersive isotope-selective infrared spectroscopy for 13C-urea breath test. Am J Gastroenterol 1999;94(5):1203-8
19. Gisbert JP, Pajares JM. Review article: 13C-urea breath test in the diagnosis of Helicobacter pylori infection - a critical review. Aliment Pharmacol Ther 2004; 20(10):1001-17
20. Savarino V, Landi F, Dulbecco P, et al. Isotope ratio mass spectrometry (IRMS) versus laser-assisted ratio analyzer (LARA): a comparative study using two doses of. Dig Dis Sci 2000;45(11):2168-74
21. Braden B, Gelbmann C, Dietrich CF, et al. Qualitative and quantitative clinical evaluation of the laser-assisted ratio analyser for detection of Helicobacter pylori infection by (13)C-urea breath tests. Eur J Gastroenterol Hepatol 2001;13(7):807-10
22. Cosme A, Montes M, Martos M, et al. Usefulness of antimicrobial susceptibility in the eradication of Helicobacter pylori. Clin Microbiol Infect 2013;19:379-83
23. Garza-González E, Perez-Perez GI, Maldonado-Garza HJ, et al. A review of Helicobacter pylori diagnosis, treatment, and methods to detect eradication. World J Gastroenterol 2014;20(6):1438-49
24. Smith SM, O'Morain C, McNamara D. H. pylori-associated disease. Antimicrobial susceptibility testing for Helicobacter pylori in times of increasing antibiotic resistance. World J Gastroenterol 2014;20(29):9912-21
25. Dominguez-Munoz JE, Leodolter A, Sauerbruch T, et al. A citric acid solution is an optimal test drink in the 13C-urea breath test for the diagnosis of Helicobacter pylori infection. Gut 1997;40(4):459-62
26. Graham DY, Runke D, Anderson SY, et al. Citric acid as the test meal for the 13C-urea breath test. Am J Gastroenterol 1999;94(5): 1214-17
27. Leodolter A, Dominguez-Munoz JE, Von Arnim U, et al. Citric acid or orange juice for the 13C-urea breath test: the impact of pH and gastric emptying.

doi: 10.1586/14737159.2015.982537

Aliment Pharmacol Ther 1999;13(8):1057-62

28. Rektorschek M, Weeks D, Sachs G, et al. Influence of pH on metabolism and urease activity of Helicobacter pylori. Gastroenterology 1998;115(3):628-41
29. Scott DR, Weeks D, Hong C, et al. The role of internal urease in acid resistance of Helicobacter pylori. Gastroenterology 1998;114(1):58-70
30. Weeks DL, Eskandari S, Scott DR, et al. A H+-gated urea channel: the link between Helicobacter pylori urease and gastric colonization. Science 2000;287(5452):482-5
31. Shiotani A, Saeed A, Yamaoka Y, et al. Citric acid-enhanced Helicobacter pylori urease activity in vivo is unrelated to gastric emptying. Aliment Pharmacol Ther 2001;15(11):1763-7
32. Schoeller DA, Klein PD, MacLean WC Jr, et al. Fecal 13C analysis for the detection and quantitation of intestinal malabsorption. Limits of detection and application to disorders of intestinal cholylglycine metabolism. J Lab Clin Med 1981;97(3):440-8
33. Shirin H, Kenet G, Shevah O, et al. Evaluation of a novel continuous real time (13)C urea breath analyser for Helicobacter pylori. Aliment Pharmacol Ther 2001;15(3):389-94
34. Israeli E, Ilan Y, Meir SB, et al. A novel 13C-urea breath test device for the diagnosis of Helicobacter pylori infection: continuous online measurements allow for faster test results with high accuracy. J Clin Gastroenterol 2003;37(2):139-41
35. Jonderko K, Kasicka-Jonderko A, Kamińska M, et al. A systematic study of a neutral meal suitable for subjects undergoing 13CO2 breath tests. Med Sci Monit 2008;14(10):CR543-6
36. Braun M, Zuckerman E, Ben-Ari Z, et al. The innovative real time BreathID® test system diagnoses and predicts the extent of hepatic injury in patients with nonalcoholic fatty liver disease. EASL; VIenna: 2006
37. Pathak CM, Avti PK, Bunger D, et al. Kinetics of 14carbon dioxide excretion from 14C-urea by oral commensal flora. J Gastroenterol Hepatol 2008;23(10):1603-7
38. Schmilovitz-Weiss H, Sehayek-Shabat V, Eliakim R, et al. Applicability of a short/rapid 13C-urea breath test for Helicobacter pylori: retrospective multicenter chart review study. BMC Gastroenterol 2012;12:8
39. Gatta L, Vakil N, Ricci C, et al. Effect of proton pump inhibitors and antacid therapy on 13C urea breath tests and stool test for Helicobacter pylori infection. Am J Gastroenterol 2004;99(5):823-9
40. Savarino V, Bisso G, Pivari M, et al. Effect of gastric acid suppression on 13C-urea breath test: comparison of ranitidine with omeprazole. Aliment Pharmacol Ther 2000;14(3):291-7
41. Shirin H, Frenkel D, Shevah O, et al. Effect of proton pump inhibitors on the continuous real time (13)C-urea breath test. Am J Gastroenterol 2003;98(1):46-50
42. Chey WD, Chathadi KV, Montague J, et al. Intragastric acidification reduces the occurrence of false-negative urea breath test results in patients taking a proton pump inhibitor. Am J Gastroenterol 2001;96(4):1028-32
43. Levine A, Shevah O, Shabat-Sehayek V, et al. Masking of 13C urea breath test by proton pump inhibitors is dependent on type of medication: comparison between omeprazole, pantoprazole, lansoprazole and esomeprazole. Aliment Pharmacol Ther 2004;20(1):117-22
44. Tian XY, Zhu H, Zhao J, et al. Diagnostic performance of urea breath test, rapid urea test, and histology for Helicobacter pylori infection in patients with partial gastrectomy: a meta-analysis. J Clin Gastroenterol 2012;46(4):285-92
45. Kubota K, Hiki N, Shimizu N, et al. Utility of [13C] urea breath test for Helicobacter pylori detection in partial gastrectomy patients. Dig Dis Sci 2003;48(11):2135-8
46. Lombardo L, Masoero G, Della Monica P, et al. Multiple sampling 13C-urea breath test: improvement of diagnosis in postgastrectomy patients. Minerva Gastroenterol Dietol 2003;49(3):181-6
47. Wardi J, Shalev T, Shevah O, et al. A rapid continuous-real-time 13C-urea breath test for the detection of Helicobacter pylori in patients after partial gastrectomy. J Clin Gastroenterol 2012;46(4):293-6
48. Kindermann A, Demmelmair H, Koletzko B, et al. Influence of age on 13C-urea breath test results in children. J Pediatr Gastroenterol Nutr 2000;30(1):85-91
49. Machado RS, Patricio FR, Kawakami E. 13C-urea breath test to diagnose Helicobacter pylori infection in children aged up to 6 years. Helicobacter 2004;9(1):39-45
50. Leal YA, Flores LL, Fuentes-Panana EM, et al. 13C-urea breath test for the diagnosis of Helicobacter pylori infection in children: a systematic review and meta-analysis. Helicobacter 2011;16(4):327-37
51. Queiroz DM, Saito M, Rocha GA, et al. Helicobacter pylori infection in infants and toddlers in South America: concordance between [13C]urea breath test and monoclonal H. pylori stool antigen test. J Clin Microbiol 2013;51(11):3735-40
52. Pacheco SL, Ogata SK, Machado RS, et al. Diagnosis of Helicobacter pylori infection by means of reduced-dose (13)C-urea breath test and early sampling of exhaled breath. J Pediatr Gastroenterol Nutr 2013;57(5):607-11
53. Levine A, Shevah O, Miloh T, et al. Validation of a novel real time 13C urea breath test for rapid evaluation of Helicobacter pylori in children and adolescents. J Pediatr 2004;145(1):112-14
54. Hino B, Eliakim R, Levine A, et al. Comparison of invasive and non-invasive tests diagnosis and monitoring of Helicobacter pylori infection in children. J Pediatr Gastroenterol Nutr 2004;39(5):519-23
55. Parkman HP, Hasler WL, Fisher RS. American Gastroenterological Association technical review on the diagnosis and treatment of gastroparesis. Gastroenterology 2004;127(5):1592-622
56. Abell TL, Camilleri M, Donohoe K, et al. Consensus recommendations for gastric emptying scintigraphy: a joint report of the American Neurogastroenterology and Motility Society and the Society of Nuclear Medicine. Am J Gastroenterol 2008;103(3):753-63
57. Ghoos YF, Maes BD, Geypens BJ, et al. Measurement of gastric emptying rate of solids by means of a carbon-labeled octanoic acid breath test. Gastroenterology 1993;104(6):1640-7
58. Bromer MQ, Kantor SB, Wagner DA, et al. Simultaneous measurement of gastric emptying with a simple muffin meal using [13C]octanoate breath test and scintigraphy in normal subjects and patients with dyspeptic symptoms. Dig Dis Sci 2002;47(7):1657-63
59. Choi MG, Camilleri M, Burton DD, et al. [13C]octanoic acid breath test for gastric emptying of solids: accuracy, reproducibility, and comparison with scintigraphy. Gastroenterology 1997;112(4):1155-62
60. Dickman R, Zilper T, Steinmetz A, et al. Comparison of continuous breath test and gastric scintigraphy for the measurement of gastric emptying rate in healthy and

doi: 10.1586/14737159.2015.982537

dyspeptic individuals. Eur J Gastroenterol Hepatol 2013;25(3):291-5

61. Braden B. (13)C breath tests for the assessment of exocrine pancreatic function. Pancreas 2010;39(7):955-9
62. Dominguez-Munoz JE. Pancreatic exocrine insufficiency: diagnosis and treatment. J Gastroenterol Hepatol 2011;26(Suppl 2): 12-16
63. Laterza L, Scaldaferri F, Bruno G, et al. Pancreatic function assessment. Eur Rev Med Pharmacol Sci 2013;17(Suppl 2):65-71
64. Ilan Y. Review article: the assessment of liver function using breath tests. Aliment Pharmacol Ther 2007;26(10):1293-302
65. Lalazar G, Adar T, Ilan Y. Point-of-care continuous (13)C-methacetin breath test improves decision making in acute liver disease: results of a pilot clinical trial. World J Gastroenterol 2009;15(8):966-72
66. Nista EC. 13C-Breath tests in the study of microsomal liver function. Eur Rev Med Pharmacol Sci 2004;8:33-46
67. Klein PD. Clinical applications of 13CO2 measurements. Fed Proc 1982; 41(10):2698-701
68. Schneider JF, Schoeller DA, Schreider BD, et al. Use of 13C-phenacetin and 13C-methacetin for the detection of alterations in hepatic drug metabolism. In: Klein ER, Klein PD, editors. Proceedings of the 3rd International Conference on Stable Isotopes, May 1978. Academic Press; New York: 1979. p. 507-16
69. Lalazar G, Pappo O, Hershcovici T, et al. A continuous 13C methacetin breath test for noninvasive assessment of intrahepatic inflammation and fibrosis in patients with chronic HCV infection and normal ALT. J Viral Hepat 2008;15(10):716-28
70. Goetze O, Selzner N, Fruehauf H, et al. 13C-methacetin breath test as a quantitative liver function test in patients with chronic hepatitis C infection: continuous automatic molecular correlation spectroscopy compared to isotopic ratio mass spectrometry. Aliment Pharmacol Ther 2007;26(2):305-11
71. Mawatari H, Inamori M, Fujita K, et al. The continuous real-time 13C-octanoate breath test for patients with nonalcoholic steatohepatitis using the BreathID system. Hepatogastroenterology 2009;56(94-95): 1436-8
72. Mizrahi M, Lalazar G, Adar T, et al. Assessment of insulin resistance by a 13C glucose breath test: a new tool for early diagnosis and follow-up of high-risk patients. Nutr J 2010;9:25
73. Shteyer E, Lalazar G, Hemed N, et al. Continuous 13C-methacetin breath test differentiates biliary atresia from other causes of neonatal cholestasis. J Pediatr Gastroenterol Nutr 2013;56(1):60-5
74. Shirin H, Aeed H, Shalev T, et al. Utility of a 13C-methacetin breath test in evaluating hepatic injury in rats. J Gastroenterol Hepatol 2008;23(11): 1762-8
75. Eusebi LH, Zagari RM, Bazzoli F. Epidemiology of Helicobacter pylori infection. Helicobacter 2014;19(Suppl 1): 1-5
76. Megraud F, Bessede E, Lehours P. Diagnosis of Helicobacter pylori infection. Helicobacter 2014;19(Suppl 1):6-10
77. Asaka M, Kato M, Takahashi S, et al. Guidelines for the management of Helicobacter pylori infection in Japan: 2009 revised edition. Helicobacter 2010;15: 1-20
78. Kodama M, Murakami K, Okimoto T, et al. Influence of proton pump inhibitor treatment on Helicobacter pylori stool antigen test. World J Gastroenterol 2012;18:44-8
79. Cullen KP, Broderick BM, Jayaram J, et al. Evaluation of the Helicobacter pylori stool antigen (HpSA) test in routine clinical practice–is it patient-friendly? Ir Med J 2002;95(10):305-6
80. Talley NJ, Vakil N. Guidelines for the management of dyspepsia. Am J Gastroenterol 2005;100(10):2324-37
81. Gisbert JP, Calvet X. Helicobacter pylori "Test-and-Treat" strategy for management of dyspepsia: a comprehensive review. Clin Trans Gastroenterol 2013;4:e32
82. McNulty C, Teare L, Owen R, et al. Test and treat for dyspepsia—but which test? BMJ 2005;330(7483):105-6
83. Liou JM, Chen CC, Chen MJ, et al. Sequential versus triple therapy for the first-line treatment of Helicobacter pylori: a multicentre, open-label, randomised trial. Lancet 2013;381(9862):205-13
84. Gatta L, Vakil N, Vaira D, et al. Global eradication rates for Helicobacter pylori infection: systematic review and meta-analysis of sequential therapy. BMJ 2013;347:f4587
85. Sheu BS, Lee SC, Yang HB, et al. Quantitative result of 13C urea breath test at 15 minutes may correlate with the bacterial density of H. pylori in the stomach. Hepatogastroenterology 1999; 46(27):2057-62
86. Onal IK, Gokcan H, Benzer E, et al. What is the impact of Helicobacter pylori density on the success of eradication therapy: a clinico-histopathological study. Clin Res Hepatol Gastroenterol 2013;37(6):642-6
87. Lai YC, Yang JC, Huang SH. Pre-treatment urea breath test results predict the efficacy of Helicobacter pylori eradication therapy in patients with active duodenal ulcers. World J Gastroenterol 2004;10(7):991-4
88. Moshkowitz M, Konikoff FM, Peled Y, et al. High Helicobacter pylori numbers are associated with low eradication rate after triple therapy. Gut 1995;36(6):845-7
89. Graham DY, Klein PD. Accurate diagnosis of Helicobacter pylori. 13C-urea breath test. Gastroenterol Clin North Am 2000;29(4): 885-93x

doi: 10.1586/14737159.2015.982537

ORIGINAL ARTICLE

AG-2019-63
dx.doi.org/10.1590/S0004-2803.201900000-38

Prospective study for validation of a single protocol for the 13C-urea breath test using two different devices in the diagnosis of *H. pylori* infection

Luiz Gonzaga Vaz **COELHO**, Osmar Reni **TRINDADE**, Laiane Alves **LEÃO**, Henrique Gomes **RIBEIRO**, Izabella Silva **FREITAS** and Maria Clara Freitas **COELHO**

Received 8/4/2019
Accepted 4/6/2019

ABSTRACT – **Background** – ^{13}C-urea breath test (UBT) is the gold-standard, noninvasive method for *H. pylori* diagnosis. However, there is no uniform standardization of the test. This situation can be unpractical for laboratories running with two or more devices. **Objective** – To perform a prospective comparison validation study of UBT employing one validated protocol for two different devices: BreathID Hp Lab System® (Exalenz Bioscience Ltd, Israel), here called device A and IRIS-Doc2® (Wagner Analysen-Technik, Germany, now Mayoly Spindler Group, France), here called device B, in the diagnosis of *H. pylori* infection. **Methods** – A total of 518 consecutive patients (365 females, 153 males, mean age 53 years) referred for UBT were included. All patients received device A protocol as follow: after at least one hour fasting, patients filled two bags prior to the test, then ingested an aqueous solution containing 75 mg of ^{13}C-urea with a 4.0 g citric acid powder and filled another two bags 15 min after ingesting the test solution. One pair of breath sample bags (before and after ingestion) was analyzed by the two different devices. A delta over baseline (DOB) ≥5‰ indicated *H. pylori* infection. Statistics: Wilcoxon test, kappa coefficient with 95% CI, Wilson's method. **Results** – Considering the device A protocol as the gold standard, its comparison with device B showed a sensitivity of 99.3% (95% CI: 96.3–99.9) and a specificity of 98.9% (95% CI: 97.3–99.6). Kappa coefficient was 0.976 (95% IC: 0.956–0.997). **Conclusion** – Correlation between the two devices was excellent and supports a uniform standardization of UBT.

HEADINGS – *Helicobacter* infections, diagnosis. Breath tests. Urea, analysis. Carbon isotopes, analysis.

INTRODUCTION

Helicobacter pylori is recognized as the main etiologic agent of peptic ulcers, with a pathogenic role equally well established in gastric adenocarcinoma and MALT (mucosa-associated lymphoid tissue) lymphoma[1,2]. First identified in 1983 by Marshall and Warren[3] from gastric tissue fragments, since then, different diagnostic methods have been developed for their detection, including invasive and non-invasive tests. Invasively, the bacterial presence can be identified through gastroscopy by the collection of gastric fragments for histology, culture, urease test or molecular tests. Non-invasive tests consist of serological tests, stool antigen detection, and the ^{13}C urea breath test (UBT)[4].

UBT is the gold-standard noninvasive method for *H. pylori* diagnosis, with a sensitivity and specificity greater than 95%[5-7]. Due to its high accuracy, low cost and easy performance, it is considered the first option in the control of *H. pylori* infection treatment or recurrence and it is a fundamental diagnostic tool in the "test and treat" strategy; it is also an excellent option in epidemiological studies[8-10]. Such tests are based on the property of *H. pylori* in producing high amounts of urease enzyme. The principle of the test is based on the ability of *H. pylori* (if present in the gastric environment) to break down orally absorbed ^{13}C-labelled urea. $^{13}CO_2$ diffuses into the blood and is excreted via the lungs and can be easily measured in the expired air using mass or non-dispersive, isotope-selective infrared spectroscopy[11]. Thus, the analysis of samples of expired air collected before (control) and after substrate ingestion will indicate the change in the $^{13}CO_2/^{12}CO_2$ ratio caused by the metabolic activity induced by the administration of the labelled urea.

Since its original description in 1987 by Graham et al.[12], UBT has undergone several modifications involving the need or not of fasting before the test[13], dose of ^{13}C-urea employed[11], concomitant administration of ^{13}C-urea in any citrus meal[14], ideal sample collection time for exhaled air after substrate ingestion, optimal cut-off point and performance of the device used to perform UBT[13,15]. Thus, there is no single worldwide standardization for testing to date, although numerous individual validation studies confirm the high accuracy of the method for the diagnosis of active *H. pylori* in adults and children over 6 years of age[4-10,16-18].

Considering the current moment, where there are several devices in the world market, including hospitals and clinics working with more than one device from different manufacturers in the daily routine, the search for a single UBT preparation and reading protocol should be investigated to standardize processes and improve efficiency.

Declared conflict of interest of all authors: none
Disclosure of funding: no funding received
Universidade Federal de Minas Gerais, Instituto Alfa de Gastroenterologia do Hospital das Clínicas, Belo Horizonte, MG, Brasil.
Corresponding author: Luiz Gonzaga Vaz Coelho. E-mail: lcoelho22@gmail.com

The aim of this study is to perform a prospective comparison validation study of ^{13}C-urea breath test for the diagnosis of *H. pylori* infection employing one validated protocol for two different devices.

METHODS

The study was performed at the Breath Tests Laboratory of the Alfa Institute of Gastroenterology at Clinics Hospital of Federal University of Minas Gerais, Belo Horizonte, MG, Brazil.

Patients

We recruited individuals consecutively referred for UBT for the purpose of the initial diagnosis or control of *H. pylori* infection treatment from November 2017 to September 2018. Informed consent was obtained from all patients to participate in the study after being duly informed about the purpose of the study. The study inclusion criterion was the non-use of proton pump inhibitors or antibiotics in the last 14 or 30 days, respectively, prior to UBT.

^{13}C-urea Breath Test (UBT)

All study participants underwent UBT, which was processed and analysed simultaneously by two different devices. Our laboratory has two devices: the BreathID HP Lab System® (Exalenz Bioscience Ltd, Israel), here called device A, and IRIS-Doc 2® (Wagner Analysen-Technik, Bremen, Germany, now Mayoly Spindler Group, France), here called device B. Although these two devices have independent protocols previously validated and recommended by the manufacturers[17,19], we chose a single protocol as recommended by the manufacturer of device A, cleared by FDA in November 2016, due its particularities and practicity[19].

The protocol employed can be summarized as follows: after at least 1h fasting, exhaled air samples were initially collected from the participants in two small collection bags (120 mL), which corresponded to time zero (sample-1, control). Next, the patients ingested, within 2 min, an aqueous solution (200 mL) containing 75 mg of ^{13}C-urea and 4.0 g of citric acid powder, with added edulcorant. A second exhaled air collection was performed 15min after the ingestion of the substrate in two new small collection bags, which corresponded to sample-2. Each pair of collected material (sample-1 and sample-2) was analysed and processed by one of the two infrared analyser devices of the study. According to the manufacturer's instructions, patients were considered positive for *H. pylori* when they had a delta over baseline (DOB) equal to or greater than 5‰[19]. This parameter indicates the change in the $^{13}CO_2/^{12}CO_2$ ratio in metabolic activity induced by the administration of the labelled urea.

For statistical analysis, the data were expressed as percentages, means (standard deviation), median and minimum and maximum values. Continuous variables were compared using t-test or the Mann-Whitney test (non-parametric data) and Wilcoxon test for paired samples. The coefficient of concordance (kappa) of the tests between the two devices was calculated. Considering device A, whose protocol was used in the study as the gold standard, the sensitivity, specificity and 95% confidence interval were calculated for the results obtained with device B. Wilson's method was used to calculate the confidence intervals[20]. All statistical analyses were performed using the MINITAB statistical package (Minitab Inc., State College, PA, USA) version 16 and Excel (Office 10).

RESULTS

Five hundred eighteen patients were consecutively included in the study: 365 (70.5%) patients were women and 153 were men (29.5%), with a mean age of 53 years (10-89 years) and a standard deviation (SD) of 15.3 years. Among the 518 patients, 161 had never received anti-*H. pylori* treatment and 357 patients underwent the test to evaluate the anti-*H. pylori* treatment result.

TABLE 1 shows the observed values in DOB‰ in the UBT obtained using the single protocol (device A) in the two different devices.

FIGURE 1 shows the absolute values of DOB‰ observed in all 518 study participants. Considering the cut-off point of DOB ≥5‰ for the presence of *H. pylori,* only 5/518 participants (FIGURE 2) presented discordant results between the two devices, a positive participant on device A and a negative one on device B, while four subjects were negative on device A and positive on device B. Four in five participants with discordant results underwent the test for post-

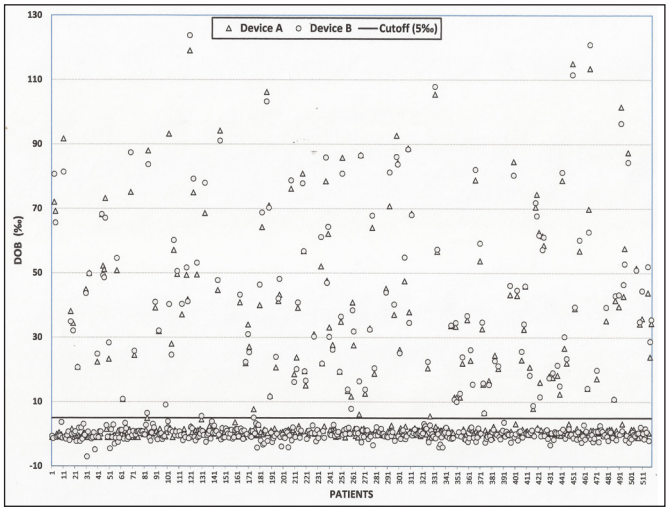

FIGURE 1. Absolute values of DOB‰ observed in device A and device B for all 518 study participants. DOB: delta over baseline.

TABLE 1. UBT results employing two different devices with a unique protocol (n=518).

DOB ‰	Device A			Device B		
	All	Negative	Positive	All	Negative	Positive
Mean value (SD)	13.1 (24.7)	0.4 (1.0)	44.8 (26.9)	12.9 (24.9)	-0.1 (1.5)	44.6 (26.7)
Median value	0.9	0.4	39.4	0.7	-0.1	40.4
Min. value	-1.0	-1.0	5.6	-7.0	-7.0	5.1
Max. value	119.1	4.9	119.1	123.8	3.9	123.8

UBT: ^{13}C-urea breath test; DOB: delta over baseline; SD: standard deviation.

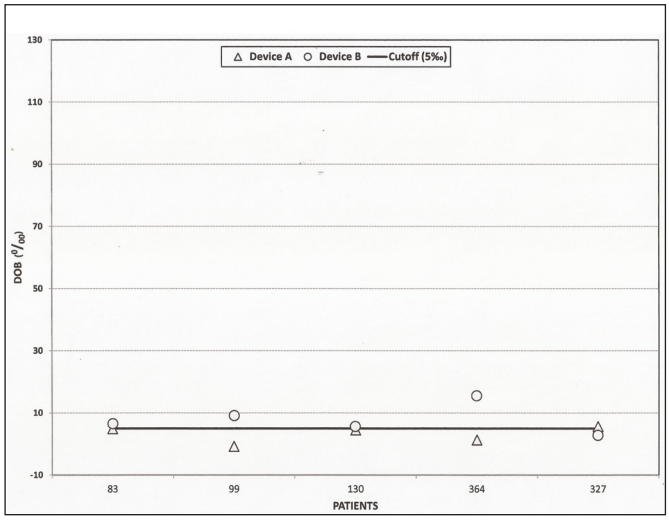

FIGURE 2. Discordant absolute values of DOB ‰ between device A and device B in 518 cases. DOB: delta over baseline.

treatment infection control. The kappa coefficient of concordance between the two devices was 0.976 (95% CI: 0.956–0.997). Considering the device A protocol as the gold standard, its comparison with device B showed a sensitivity of 99.3% (95% CI: 96.3–99.9) and a specificity of 98.9% (95% CI: 97.3–99.6).

TABLE 2 shows the absolute values of DOB‰ observed in patients with confirmed *H. pylori* in both devices (n=147); significantly higher values are observed in both devices for the female population compared with the male population.

TABLE 2. DOB‰ absolute values in *H. pylori*-infected patients (n=147), regarding sex.

Variables	Device A		Device B	
	Male	Female	Male	Female
Mean (SD)	36.4 (21.9)	47.5 (27.8)*	35.8 (21.2)	47.4 (27.8)**
Median	33.5	41.4	30.3	43.1
Min	6.1	5.6	5.6	5.1
Max	87.4	119.1	84.4	123.8

DOB‰: delta over baseline; *P=0.036; **P=0.027.

DISCUSSION

Our study shows that the UBT employing the same protocol for two different devices from two different manufacturers presented very similar results. This allows their standardization in daily practice. Only 5/518 (0.97%) participants (four of them performed for treatment control) showed discordant results, and the DOB values were close to the cut-off point in three of them. Characteristically, in the UBT, DOB‰ values in individuals infected or not infected by *H. pylori* are situated far from the established cut-off point, and our study shows very similar absolute values, both positive and negative, between the two devices analysed. Typically, borderline cut-off UBT observed values (grey or indeterminate zone) should be interpreted with caution, and it is suggested that the test should be repeated or the diagnosis should be confirmed by another method. The prevalence of UBT results in the grey area has been estimated at 1% to 2%, similar to the one observed here[21,22].

In its initial description, UBT was performed using isotope ratio mass spectrometry technology, which has a high cost and complexity regarding use and maintenance[12]. More recently, UBT has been performed using non-dispersive, isotope-selective infrared spectroscopes, which has a lower cost, smaller size, easy maintenance and operation, and which results are available a few minutes after the procedure; this allows UBT to be carried out in doctors' offices or small-to-medium sized laboratories. Its excellent performance has made this methodology attractive to the industry and physicians, since there are numerous devices being commercialized in the international market[11,23,24]. Despite the existence of small variations in the methods employed by each device, regarding fasting time before UBT, ideal test meal, best ^{13}C-urea dose, optimal breath sampling after ingestion of the substrate and best cut-off point to discriminate infected from non-infected individuals, several local validation studies have shown high diagnostic accuracy to detect *H. pylori* infection[6,8]. However, the absence of definitive standardization of the test makes it impractical to manage different devices at the same place and to compare the results from different studies.

In our study, although the protocols for the two devices were previously validated, we chose to use the protocol suggested by device A for its practicity, especially in relation to fasting time (1h instead of 8h), use of citric acid instead of orange juice and 15 min instead of 30 min for the optimal breath sampling after ingestion of the substrate. The fasting time of only one hour makes it more convenient to perform the test at different times, with several studies demonstrating that the differences between DOB‰ values fasting or not fasting are minimal or non-existent[25-28]. The incorporation of citric acid as a test meal instead of orange juice administered together with ^{13}C-urea is currently well established[14,29]. Its administration delays gastric emptying, allowing the labelled substrate to distribute throughout the stomach and maximizes the reaction with the preformed bacterial urease. Additionally, it increases the hydrolysis of urea, both by increasing the availability of intracytoplasmic urea and increasing the activity of intragastric urease, providing higher $^{13}CO_2$ recovery values[14,29]. Its effect is dose dependent, and the 4.0 g dose used here seems to be palatable and efficient to maximally enhance urease activity[29,30]. Recently, a new test meal using a high-dose mixture of citric, malic and tartaric acid has been proposed to be used as a test meal in patients taking proton pump inhibitors with good accuracy, although 7.2% of the patients complained of dyspeptic symptoms with the test meal[31]. The dose of ^{13}C-urea administered is between 50 mg and 100 mg, and 75 mg is the dose most commonly used currently[11]. Studies have shown that doses above 125 mg are unnecessary[32], and a dose of 25 mg is inefficient to provide good accuracy[33]. The interval of 15min instead of 30 min as optimal breath sampling after ingestion of the substrate has been considered sufficient to avoid interference of the pharyngeal flora with the possible presence of urease-producing bacteria and sufficient to allow hydrolysis ^{13}C-urea by contact with the preformed urease by *H. pylori*. The use of citric acid, might also contribute to obtain greater accuracy of UBT with the collection performed 15 min after substrate ingestion by increasing the saliva production and reducing possible interaction with the urease that might be present in the oral cavity[11,27,34].

Our study also demonstrated that mean UBT values, in both devices, were significantly higher in females (device A: 47.5‰, SD:27.8, P=0.036 and device B:47.4‰, SD:27.8, P=0.027) than

in males (device A:36.4‰, SD:1.9, and device B:35.8‰, SD:21.2). These results demonstrate that, for the first time, using two distinct devices simultaneously corroborate findings already described in studies using only one device. This demonstrates that UBT has absolute values significantly higher in adult females than in adult males, with a significant increase varying from 4.5‰ to 11‰ in the median UBT, and similar to our results from 11.1‰ in device A and 11.6‰ in device B[35-39]. The reasons for these findings are still largely unknown. Variables such as higher bacterial density in women, hormonal changes, body surface area and sex differences in intragastric pH are being investigated, but further investigations are clearly needed[38,39].

In conclusion, our study showed that UBT performed by two different devices employing a single protocol presents excellent agreement between them. This harmonization, while improving and simplifying the operational procedures, represents an important contribution in the search for a single standardization for UBT.

Orcid

Luiz Gonzaga Vaz Coelho. Orcid: 0000-0002-8721-7696.
Osmar Reni Trindade. Orcid: 0000-0003-1686-7365.
Laiane Alves Leão. Orcid: 0000-0001-6547-3798.
Henrique Gomes Ribeiro. Orcid: 0000-0001-8187-6378.
Izabella Silva Freitas. Orcid: 0000-0002-5500-8881.
Maria Clara Freitas Coelho. Orcid: 0000-0001-8028-6114.

ACKNOWLEDGEMENTS

The authors thank to Maurílio M. Fernandes and Maria de Lourdes M. Fernandes for technical and statistical assistance.

Authors' contribution

Coelho LG and Trindade OR contributed to the conception and design of the study; Leão LA and Trindade OR contributed to collection of the samples; Ribeiro HG and Freitas IS interpreted and analysed the data and Coelho LG and Coelho MC wrote the paper.

Coelho LGV, Trindade OR, Leão LA, Ribeiro HG, Freitas IS, Coelho MCF. Estudo prospectivo para validação de protocolo único para o teste respiratório com ureia marcada com carbono-13 empregando dois dispositivos diferentes, no diagnóstico da infecção por *H. pylori*. Arq Gastroenterol. 2019;56(2):197-201.

RESUMO – **Contexto** – O teste respiratório com ureia-marcada com carbono-13 (TR-13C) é o método padrão-ouro para o diagnóstico não invasivo da infecção por *H. pylori.* Apesar disto, não existe uma uniformização de protocolos para a sua realização, trazendo dificuldades operacionais para laboratórios ou clínicas que operam com equipamentos de fabricantes diferentes. **Objetivo** – Estudo prospectivo e comparativo para validação do TR-13C para o diagnóstico de infecção por *H. pylori*, com emprego de protocolo único para dois equipamentos diferentes, a saber: BreathID Hp Lab System® (Exalenz Bioscience Ltd, Israel), aqui denominado equipamento A e IRIS-Doc2® (Wagner Analysen-Technik, Alemanha, agora Mayoly Spindler Group, França), aqui denominado equipamento B. **Métodos** – Um total de 518 pacientes (365 mulheres e 153 homens, idade média de 53 anos) consecutivamente encaminhados para a realização do TR-13C foram incluídos no estudo. Todos os participantes realizaram TR-13C, que foi processado e analisado simultaneamente pelos dois equipamentos. Embora os dois equipamentos possuam protocolos independentes previamente validados, foi optado, por sua maior praticidade, pela utilização de um único protocolo, conforme recomendado pelo fabricante do equipamento A, e assim resumido: após jejum mínimo de 1h, foram amostras de ar expirado coletadas em dois pequenos sacos coletores (120 mL), correspondendo ao tempo-zero (amostra-1, controle). Em seguida, os pacientes ingeriram, em até 2 min, uma solução aquosa (200 mL) contendo 75 mg de 13C-ureia e 4,0 gramas de ácido cítrico em pó, adicionado com edulcorante. Uma segunda coleta de ar expirado era realizada 15 min após a ingestão do substrato em dois novos pequenos sacos coletores, correspondendo à amostra-2. Foram considerados positivos para a presença da infecção por *H. pylori* quando apresentavam um delta over baseline (DOB) igual ou maior que 5‰. Análise estatística foi realizada com o teste de Wilcoxon, coeficiente kappa com IC 95% e método de Wilson. **Resultados** – Considerando o protocolo do equipamento A como o padrão-ouro, sua comparação com o equipamento B mostrou sensibilidade de 99,3% (IC 95%: 96,3–99,9) e especificidade de 98,9% (IC 95%: 97,3–99,6). O coeficiente kappa observado foi de 0,976 (IC 95%: 0,956–0,997). **Conclusão** – A correlação entre os dois equipamentos foi excelente e contribui para uma uniformização de protocolos para TR-13C.

DESCRITORES – Infecções por *Helicobacter*, diagnóstico. Testes respiratórios. Ureia, análise. Isótopos de carbono, análise.

REFERENCES

1. Suerbaum S, Michetti P. Helicobacter pylori infection. N Engl J Med. 2002; 10;347:1175-86.
2. Fock KM, Graham DY, Malfertheiner P. Helicobacter pylori research: historical insights and future directions. Nat Rev Gastroenterol Hepatol. 2013;10: 495-500.
3. Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic-ulceration. Lancet. 1984;1:1311-5.
4. Megraud F, Lehours P. Helicobacter pylori detection and antimicrobial susceptibility testing. Clin Microbiol Rev. 2007;20:280-322.
5. Ling D. Carbon-13 urea breath test for Helicobacter pylori infection in patients with uninvestigated ulcer-like dyspepsia: an evidence-based analysis. Ont Health Technol Assessment Series [Internet]. 2013;13:1-30. Available from: https://www.hqontario.ca/Portals/0/Documents/evidence/reports/full-report-urea-breath-test-131003-en.pdf. Accessed March 3, 2019.
6. Ferwana M, Abdulmajeed I, Alhajiahmed A, Madani W, Firwana B, Hasan R, et al. Accuracy of urea breath test in Helicobacter pylori infection: meta-analysis. World J Gastroenterol. 2015;21:1305-14.
7. Best LMJ, Takwoingi Y, Siddique S, Selladurai A, Gandhi A, Low B, et al. Non-invasive diagnostic tests for Helicobacter pylori infection. Cochrane Database of Systematic Reviews 2018, Issue 3. Art. No.: CD012080. DOI: 0.1002/14651858.CD012080.pub2.
8. Graham DY, Klein PD. Accurate diagnosis of Helicobacter pylori. 13C-urea breath test. Gastroenterol Clin North Am. 2000;885-93.
9. Malfertheiner P, Megraud F, O'Morain CA, Gisbert JP, Kuipers EJ, Axon AT, et al. Management of Helicobacter pylori infection-the Maastricht V/Florence Consensus Report. Gut. 2017;66:6-30.
10. Coelho LGV, Marinho JR, Genta R, Ribeiro LT, Passos MDCF, Zaterka S, et al. 4th Brazilian Consensus Conference on Helicobacter pylori infection. Arq Gastroenterol. 2018;55:97-121.
11. Gisbert JP, Pajares JM. 13C-urea breath test in the diagnosis of Helicobacter pylori infection – a critical review. Aliment Pharmacol Ther. 2004;20:1001-7.
12. Graham DY, Klein PD, Evans DJ Jr, Evans DG, Alpert LC, Opekun AR, Boutton TW. Campylobacter pylori detected noninvasively by the 13C-urea breath test. Lancet. 1987;1:1174-7.

13. Mana F, Franken PR, Ham HR, Reynaert H, Urbain D. 13C urea breath test with nondispersive isotope-selective infrared spectrometry: reproducibility and importance of the fasting status. Helicobacter. 2000;5:104-8.
14. Dominguez-Mufioz JE, Leodolter A, Sauerbruch T, Malfertheiner P. A citric acid solution is an optimal test drink in the 13C-urea breath test for the diagnosis of Helicobacter pylori infection. Gut.1997;40:459-62
15. Parente F, Bianchi Porro G. The (13)C-urea breath test for non-invasive diagnosis of Helicobacter pylori infection: which procedure and which measuring equipment? Eur J Gastroenterol Hepatol. 2001;13:803-6.
16. Perri F. Diagnosis of Helicobacter pylori infection: which is the best test? The urea breath test. Dig Liver Dis. 2000;32 (Suppl 3):S196-8
17. Coelho LG, Reber M, Passos MC, et al. Application of isotope-selective non-dispersive infrared spectrometry for the evaluation of the 13C-urea breath test: comparison with three concordant methods. Braz J Med Biol Res. 1999;32: 1493-7.
18. Kawakami E, Machado RS, Reber M, Patrício FR. 13C-urea breath test with infrared spectroscopy for diagnosing Helicobacter pylori infection in children and adolescents. J Pediatr Gastroenterol Nutr. 2002;35:39-43.
19. Shirin H, Gonzalez JO, Hazan S, Gottlieb G, Friedenberg K, Gatof D, et al. The validity of breath collection bags using the novel BreathID® Hp Lab System: a multicenter clinical study in 257 subjects. Ther Adv Gastrointest Endosc. 2019;12:2631774519843401.
20. Altman DG, Bryant TN, Gardner MJ (eds). Statistics with confidence. Bristol: British Medical Journal; 2000.
21. Gisbert JP, Olivares D, Jiménez I, Pajares JM. Long-term follow-up of 13C-urea breath test results after Helicobacter pylori eradication: frequency and significance of borderline Δ13CO2 values. Aliment Pharmacol Ther. 2006;23:275-80.
22. Chen TS, Chang FY, Chen PC, Huang TW, Ou JT, Tsai MH, et al. Simplified 13C-urea breath test with a new infrared spectrometer for diagnosis of Helicobacter pylori infection. J Gastroenterol Hepatol. 2003;18:1237-43.
23. Opekun AR, Gotschall AB, Abdalla N, Agent C, Torres E, Sutton FM, et al. Improved infrared spectrophotometer for point-of-care patient 13C-urea breath testing in the primary care setting. Clin Biochem. 2005;38:731-4.
24. Gisbert JP, Gomollon F, Dominguez-Munoz JE, et al. Comparison between two 13C-urea breath tests for the diagnosis of Helicobacter pylori infection: isotope ratio mass spectrometer versus infrared spectrometer. Gastroenterol Hepatol. 2003;26:141-6.
25. Graham DY, Malaty HM, Cole RA, Martin RF, Klein PD. Simplified 13C-urea breath test for detection of Helicobacter pylori infection. Am J Gastroenterol. 2001;96:1741-5.
26. Ng FH, Lai KC, Wong BC, Wong WM, Wong SY, Chow KC, et al. [13C]-urea breath test without prior fasting and without test meal is accurate for the detection of Helicobacter pylori infection in Chinese. J Gastroenterol Hepatol. 2002;17:834-8.
27. Wang WM, Lee SC, Wu DC, Chen LT, Liu CS, Peng CF, et al. Simplified 13C-urea breath test for the diagnosis of Helicobacter pylori infection–The availability of without fasting and without test meal. Kaohsiung J Med Sci. 2000;16:607-13.
28. Moayyedi P, Braunholtz D, Heminbrough E, Clough M, Tompkins DS, Mapstone NP, et al. Do patients need to fast for a 13C-urea breath test? Eur J Gastroenterol Hepatol. 1997;9:275-7.
29. Graham DY, Miftahussurur M. Helicobacter pylori urease for diagnosis of Helicobacter pylori infection: A mini review. J Adv Res. 2018;13:51-7.
30. Graham DY, Runke D, Anderson SY, Malaty HM, Klein PD. Citric acid as the test meal for the 13C-urea breath test. Am J Gastroenterol. 1999;94:1214-7.
31. Tepes B, Malfertheiner P, Labenz J, Aygen S. Modified Helicobacter test using a new test meal and a 13C-urea breath test in Helicobacter pylori positive and negative dyspepsia patients on proton pump inhibitors. Prospective Study. World J Gastroenterol. 2017;23:5954-61.
32. Klein PD, Malaty HM, Martin RF, Graham KS, Genta RM, Graham DY. Noninvasive detection of Helicobacter pylori infection in clinical practice: the 13C urea breath test. Am J Gastroenterol. 1996;91:690-4.
33. Coelho LGV, Silva Jr AE, Coelho MCF, Penna FGC, Ferreira ROA, Santa-Cecilia EV. Does low dose 13C-urea breath test maintain a satisfactory accuracy in diagnosing Helicobacter pylori infection? Arq Gastroenterol. 2011;48:104-8.
34. Eggers RH, Kukp A, Tegeler R, Ludtke FE, Lepsien G, Meyer B. A methodological analysis of the 13C-urea breath tests for detection of Helicobacter pylori infections: high sensitivity and specificity within 30 minutes using 75 mg of 13C-urea. Eur J Gastroenterol Hepatol. 1990;2:437-44.
35. Shmuely H, Yahav J, Samra Z, Chodick G, Ofek I. Elevated 13C urea breath test values females infected with Helicobacter pylori. Dig Dis Sci. 2007;52:402-4.
36. Zevit N, Niv Y, Shirin H, Shamir R. Age and gender differences in urea breath test results. Eur J Clin Invest. 2011;41:767-72.
37. Marinho FP, Trindade OR, Ferreira KN, Amaral LA, Coelho LGV. 13C-urea breath test values: a large database study focusing at gender differences. Helicobacter. 2012;17(Suppl. 1):92 [abstract].
38. Moshkowitz M, Horowitz N, Beit-Or A, Halpern Z, Santo E. Gender-associated differences in urea breath test for Helicobacter pylori infection referrals and results among dyspeptic patients. World J Gastrointest Pathophysiol. 2012;3:80-4.
39. Eisdorfer I, Shalev V, Goren S, Chodick G, Muhsen K. Sex differences in urea breath test results for the diagnosis of Helicobacter pylori infection: a large cross-sectional study. Biology of Sex Differences. 2018;9:1.

The validity of breath collection bags method in detecting *Helicobacter pylori* using the novel *BreathID®Hp Lab System*: a multicenter clinical study in 257 subjects

Ther Adv Gastrointestinal Endoscopy

2019, Vol. 12: 1–10

DOI: 10.1177/2631774519843401

Vered Richter, Jeff O. Gonzalez, Sabine Hazan, Gary Gottlieb, Keith Friedenberg, David Gatof, Ravi Ganeshappa, Jorge-Shmuel Delgado, Dov Abramowitz, Robert Hardi, Allan Coates, Mahmudul Haq, Nilesh Mehta, Blake A. Jones, Steven F. Moss and Haim Shirin

Abstract

Background and Aim: The *BreathID®Hp* urea breath test provides several advantages over other ^{13}C breath analyzers for the detection of *Helicobacter pylori*. We evaluated the sensitivity and specificity of a new *BreathID®Hp Lab System* (Exalenz Bioscience Ltd, Israel), a ^{13}C-urea breath test system using breath sampling bags that facilitates multiple testing in a multicenter international clinical study.

Methods: A total of 257 subjects with evaluable results for urea breath test, rapid urease test, and histology were enrolled into two study groups: 189 naïve subjects were included in the pre-therapy group, and 68 subjects comprised the post-eradication therapy group. Analytical studies were conducted to evaluate the stability, reproducibility, and repeatability of the ^{13}C-urea breath test results using a delta over baseline cut-off value of 5.

Results: Among the pre-therapy subjects evaluated with the composite results from the rapid urease test and histology/immunohistochemistry, 176 results matched those of the urea breath test, resulting in an overall agreement of 98.3% with a sensitivity of 100% and specificity of 97.9%. In the post-eradication therapy cohort, the overall agreement between the urea breath test and the biopsy diagnosis was 98.5%; the sensitivity of the urea breath test in this cohort was 92.3% and the specificity was 100%. There was uniformly high overall reproducibility (99.48%) of the test results over different batches of breath sample bags, when analyzed on different days and under different storage conditions, showing stability of the breath samples in the breath collection bags

Conclusion: The *BreathID®Hp Lab System* is a highly accurate and dependable method for the diagnosis of *H. pylori* infection.

Keywords: diagnostic tests, *Helicobacter pylori*, lab mode, safety, sensitivity, specificity, urea breath test

Received: 20 October 2018; revised manuscript accepted: 14 March 2019.

Correspondence to:
Vered Richter
Institute of Gastroenterology, Liver Diseases and Nutrition, Assaf Harofeh Medical Center, Tel-Aviv University, Zerifin 70300, Israel.
richtervd@gmail.com

Vered Richter
Dov Abramowitz
Haim Shirin
Institute of Gastroenterology, Liver Diseases and Nutrition, Assaf Harofeh Medical Center, Tel-Aviv University, Zerifin, Israel

Jeff O. Gonzalez
Palmetto Research, LLC, Hialeah, FL, USA

Sabine Hazan
Ventura Clinical Trials, Ventura, CA, USA

Gary Gottlieb
Del Sol Research Management, LLC, Tucson, AZ, USA

Keith Friedenberg
Great Lakes Medical Research, Mentor, OH, USA

David Gatof
Innovative Clinical Research, Lafayette, CO, USA

Ravi Ganeshappa
Digestive Disease Center of South Texas, P.L.L.C, San Antonio, TX, USA

Jorge-Shmuel Delgado
Barzilai Medical Center, Ben-Gurion University of the Negev, Ashkelon, Israel

Robert Hardi
Chevy Chase Clinical Research, Chevy Chase, MD, USA

Allan Coates
West Michigan Clinical Research Center, Wyoming, MI, USA

Mahmudul Haq
Hope Clinical Research, Kissimmee, FL, USA

Nilesh Mehta
Digestive Disease Care PC, New Hyde Park, NY, USA

Introduction

Biopsies taken via esophagogastroduodenoscopy (EGD) and the carbon-labeled urea breath test (UBT) are considered the 'gold standard' methods for the diagnosis of active *Helicobacter pylori* infection.[1] The Maastricht V Consensus Report recommended ^{13}C-UBT as the best approach for the diagnosis of *H. pylori* infection, due to its high sensitivity, specificity, and excellent performance, especially in patients in whom endoscopy is not indicated.[2]

The ^{13}C-UBT is a noninvasive test for detecting the presence of *H. pylori* infection via changes in

Blake A. Jones
Innovative Clinical Research, Rapid City, SD, USA

Steven F. Moss
Brown University, Providence, RI, USA

the ratio of $^{13}CO_2/^{12}CO_2$ in exhaled breath.[3] In the presence of *H. pylori*, ingested ^{13}C-urea is metabolized to $^{13}CO_2$. The resulting $^{13}CO_2/^{12}CO_2$ ratio is compared with baseline values obtained before ingestion of the labeled urea. UBTs have high accuracy and reproducibility because they are functional tests that essentially sample the entire stomach. These tests are not prone to the same level of sampling error as biopsy-based tests, and false-positive results are uncommon.[4] The sensitivity and specificity of the breath test range from 90% to 100% and, in most cases, it is above 95%.[5–7]

UBTs are the preferred method for epidemiological studies, screening dyspeptic patients, and assessing eradication or recurrence of the infection after treatment.[3,8–10] A major disadvantage of the ^{13}C-UBT is the inconvenience related to the $^{13}CO_2$ analysis. In most medical centers, there is a need for collecting, storing, and transporting the samples to a central laboratory that is equipped with an isotope ratio mass spectrometer. This makes UBTs inconvenient to both the patient and physician.

A Food and Drug Administration (FDA)-cleared continuous UBT (using a nasal cannula) using the *BreathID®Hp* (Exalenz Bioscience Ltd, Modiin, Israel) provides several unique advantageous features. Instead of collecting and analyzing discrete breath samples, breath samples are continually evaluated, providing excellent accuracy (>99%) and enabling shortened breath testing procedures. Moreover, test results are available in real time for decision-making at the point of care.[11,12] A user-friendly interface for operation and point-of-care testing is another advantage.

Although the *BreathID®Hp Lab System* has the advantage of real-time point-of-care analyses, using this system has its disadvantages: patients need to be present at the site where this system is located. In addition, only one patient's breath can be measured at a time. This can limit the number of tests that can be done in a short time period. Using bags to collect breath samples allows accumulation of up to 10 sets of breath collection bags for up to 2 weeks. The *BreathID®Hp Lab System* performs automated analyses sequentially. This system may be located in a central laboratory or the system can be installed on-site. Its user-friendly interface, compact design, maintenance-free use, compatibility to lab information management system (LIMS), low cost compared to mass spectrometer, and automated operation make it an ideal method for *H.pylori* testing. Testing of each set of bags takes approximately 5 min. This study was aimed at validating the breath sampling bag test method in comparison to a composite reference standard of *H. pylori* status, comprising a combination of a histological examination for *H. pylori* and a rapid urease test (RUT).

Methods

Subjects

We determined the diagnostic accuracy of the *BreathID®Hp Lab System* in two prospectively enrolled sets of patients: initial diagnosis and post-eradication follow-up. This clinical study was conducted at 13 clinical sites in the USA and in Israel. Study participants were men and women 18 years of age or older who had a clinical indication for *H. pylori* testing for either initial diagnosis or post-eradication therapy verification (provided that their initial positive *H. pylori* diagnosis was confirmed through endoscopic biopsy). The study was approved by each clinical site's Institutional Review Board or an Independent Ethics Committee (in the USA only) and was registered at clinicaltrials.gov. Each subject provided informed consent prior to participating in the study.

Subjects were included in the initial diagnosis cohort if they had not received *H. pylori* treatments in the preceding 18 months and had not been tested for *H. pylori* within the 6 months prior to enrollment. Subjects were included in the post-eradication therapy cohort if they had biopsy documentation of *H. pylori* infection prior to eradication therapy and had documentation of receiving *H. pylori* eradication therapy within 6 months and completion of therapy at least 6 weeks prior to the UBT.

Evaluation of H. pylori *status*

Each subject was evaluated for *H. pylori* status by three diagnostic methods: histology, RUT, and UBT. For histopathology and RUT, each subject underwent an EGD according to the standard clinical practice at each site. If the UBT was planned to be performed on the same day as the endoscopy, the subjects performed the UBT before the EGD. The American College of Gastroenterology guidelines[1] recommend that a

Figure 1. The *BreathID® Lab System*.

minimum of three biopsies be obtained (one from the angularis, one from the greater curvature of the corpus, and one from the greater curvature of the antrum) in order to maximize the diagnostic yield.[13] In this study, all biopsies were taken in duplicate (for histology and RUT) resulting in a minimum of six biopsies: two from the angularis, two from the greater curvature of the corpus, and two from the greater curvature of the antrum. Biopsies from each of the three sites within the stomach were analyzed by histology and RUT.

Histopathology: at least three biopsy specimens were fixed with formalin, sectioned at a thickness of 4–5 μm and then stained with hematoxylin and eosin and by an immunohistochemistry (IHC) assay (Novacastra lyophilized polyclonal, product code NCL-HPp, purchased from Leica). The stain was performed on a Ventana ULTRA slide staining system. All slides were examined and analyzed by an experienced pathologist at a central laboratory.

RUT: at least three biopsy specimens, (similar to the ones taken by for histology) were analyzed on-site for urease activity after 1 h, with an FDA-cleared RUT (Pronto Dry®, Warsaw, Poland) according to the manufacturer's instructions. The principle of the RUT test is as follows: if *H. pylori* is present in the gastric biopsy, it secretes the urease which results in breakdown of urea, which in turn causes the pH to increase, and the color of the pH indicator changes.

The UBT was performed within 1 week before or after the EGD. After fasting for at least 1 h, each participant filled two bags prior to the UBT test (baseline bags). Next, participants ingested a test solution containing the ^{13}C-urea test solution (IDkitHp™ Two, Exalenz Bioscience Ltd) and filled two bags between 15 and 20 min after ingestion (test bags). The substrate of the drink contains 75 mg of ^{13}C-urea and citric powder which are dissolved in a cup of tap water. Antibiotics and bismuth preparations were avoided by all participants for 4 weeks prior to the breath test for the pre-therapy cohort and for 6 weeks for the post-therapy cohort. Proton pump inhibitors or H_2 blockers were avoided by all participants for 2 weeks prior to the breath test for both cohorts. Sample analysis using the *BreathID®Hp Lab System* (Figure 1) was performed either on-site or at a remote location.

The *BreathID®Hp Lab System* collects CO_2 from exhaled breath and analyzes its different isotopes in real time based on specific optical-radiation emission and absorption by $^{13}CO_2$ and $^{12}CO_2$. The system calculates the change in the $^{13}CO_2/^{12}CO_2$ ratio (R) from exhaled breath before and after ingestion of ^{13}C-labeled urea and produces a delta over baseline (DOB) value. DOB is defined as $[(^{13}CO_2(n)/^{12}CO_2[n]—^{13}CO_2(0)/^{12}CO_2(0))*1000]/[^{13}CO_2(PDB)/^{12}CO_2(PDB)]$, where PDB is the standard $^{13}C/^{12}C$ isotope ratio (=1.1273%), (0) is the baseline measurement and (*n*) is the measurement of interest. Normally, *R* remains constant in the expired breath of an individual patient. However, it can be changed via an external source of ^{13}C. *H. pylori* bacteria decompose ^{13}C-urea to ammonia and $^{13}CO_2$. Administering ^{13}C-enriched urea to a patient infected with *H.pylori* will cause an increase in the $^{13}CO_2/^{12}CO_2$ ratio in the patient's breath. The system uses the Exalenz MCS™ technology, with $^{13}CO_2$ and $^{12}CO_2$ discharge lamps as light sources. The light absorption will correlate directly to the presence of $^{13}CO_2$ and $^{12}CO_2$ in the gas samples. This approach results in highly sensitive and specific absorption curves which can detect minute (less than 1/1000) variations in $^{13}CO_2/^{12}CO_2$ ratios.

A DOB ⩾ 5 indicates an *H. pylori* infection which was determined by preliminary studies (described in the Lab System's publicly available package insert). The *BreathID®Hp Lab System* contains an autosampler unit and has an application to control the process that can measure up to 10 sets of bags consecutively and automatically within approximately 25 min. The bags are produced by Exalenz Bioscience Ltd. They are made of a printed flexible laminate LDPE 80 having a one-way valve and their volume (capacity) is 240 ml.

Based on the test results, the subject's status was classified as *H. pylori* positive, *H. pylori* negative, or non-evaluable. These results were compared to the classification results obtained by histopathology, RUT, and both combined (composite) according to FDA classification guidelines.[14] RUT was considered positive if any of the samples showed a positive result. If all samples were negative, the patient was classified as RUT negative. All biopsy samples were assessed together to provide a conclusive histology outcome. A subject was considered histologically positive when at least one of the samples showed positive IHC. Only if all three biopsy samples were IHC negative, the patient was classified as histologically negative. Finally, to determine if a patient was positive or negative when combining the RUT and histology results, FDA guidelines were used.[14] If a patient was in the initial diagnosis cohort, only if there were concordant results between RUT and histology was the patient classified as positive or negative. Patients with discordant results were considered non-evaluable. For a patient in the post-eradication cohort, any positive outcome (RUT, histology or both) would render the subject's classification as positive. Only if both RUT and histology were found to be negative would the post-eradication cohort patient be classified as negative.

The investigators remained blinded to the UBT results and the central pathology readings throughout the whole study to ensure that the patients would only be treated based on current clinical practice, without introducing bias from the UBT results or the central pathology laboratory assessments and to avoid any potential enrollment bias. Patient management decisions were made according to standard medical practice based on local testing results.

Stability of breath samples over time

To assess the stability of the breath samples in the breath sample bags, each pair of breath sample bags (before and after ingestion), obtained from the initial diagnosis cohort, was analyzed at a different time point up to 14 days after collection (on two different reading days). The stability was evaluated by the fact that the $^{13}CO_2/^{12}CO_2$ ratio did not change between the different bag's sampling. The first evaluable set of bags was used for the primary analysis. The second set of bags was collected and measured in order to assess the stability of the breath samples over time.

Reproducibility and repeatability results

Analytical studies were conducted to evaluate the reproducibility and precision (repeatability) of the results of the ^{13}C-UBT for measurements by different technicians and using different *BreathID®Hp Lab System*, or when testing was done on different days and at different sites, and on samples that were stored up to 14 days at different temperature and humidity conditions. Three different gas isotope pairs were used with DOB values of 3.3, 6.4, and 15.5, as determined via a bench study. Reproducibility was assessed by two operators who were asked to operate each of three *BreathID®Hp Lab System* at three different sites for 5 days in order to measure the DOB values for samples from each of the three batches. Standard deviation (*SD*) was calculated. The package insert states that the *SD* is less than the natural variability of the DOB measurement, which is defined in the device specification as 1 DOB for results under 5 DOB and 20% for results over 5 DOB.

Statistical analysis

All statistical analyses were performed after the study was completed, and the database was locked. Statistical programming and analyses were performed using SAS® version 9.4. The results are presented in two-way contingency tables. The exact binomial distribution was used to calculate the lower and upper limits of the 95% confidence intervals (CI) for the performance statistics.

Results

A total of 189 subjects (78 women and 111 men, mean age 48.4 ± 14.9 years) were included in the initial diagnosis cohort. The post-eradication therapy cohort included 68 subjects (43 women and 25 men, mean age 49.7 ± 15.3 years). The characteristics of the study participants are summarized in Table 1. In both groups, the most

Table 1. Subject baseline characteristics.

			Initial diagnosis cohort	Post eradication therapy cohort
			Per protocol set	**Per protocol set**
Age (years)				
		N	189	68
		Mean (*SD*)	48.4 (14.85)	49.7 (15.33)
		Median [range]	48.3 [20.2; 82.8]	50.0 [18.5; 82.3]
Gender				
	Male	% (*n*/*N*)	41.3% (78/189)	36.8% (25/68)
	Female	% (*n*/*N*)	58.7% (111/189)	63.2% (43/68)
Ethnic origin				
	Caucasian	% (*n*/*N*)	52.9% (100/189)	23.5% (16/68)
	African-American	% (*n*/*N*)	5.3% (10/189)	5.9% (4/68)
	Asian-Pacific	% (*n*/*N*)	2.1% (4/189)	5.9% (4/68)
	Hispanic	% (*n*/*N*)	38.6% (73/189)	64.7% (44/68)
	Other	% (*n*/*N*)	1.1% (2/189)	–
BMI (kg/m^2)				
		Mean (*SD*)	28.9 (6.12)	30.6 (7.44)
		Median [range]	28.1 [16.3; 50.3]	29.0 [19.7; 61.3]

BMI: body mass index; *SD*: standard deviation.

common indications for EGD were heartburn and abdominal pain. The most common endoscopic finding was antral erythema.

Comparison of ^{13}C-UBT results to endoscopy biopsy results

Initial diagnosis cohort. Among the initial diagnosis subjects evaluated with the composite results from the two endoscopy biopsy based methods (RUT and histological exam), 176 results matched those of the first evaluable UBT resulting in an overall agreement between the breath test and the reference biopsy result of 98.3% (95% CI: 95.2%, 99.7%) and the kappa (95% CI) was calculated to be 0.95; 37 results were positive, and 139 results were negative, showing a sensitivity of 100% (95% CI: 90.6%, 100.00%) and specificity of 97.9% (95% CI: 94.0%, 99.3%). Three subjects had false-positive results. However, 2 of the 3 false positives had a DOB result that was close to the predefined clinical cut-off value of 5 DOB. RUT and histology results alone were similar to those of the composite results (Table 2).

Comparing the breath test to RUT only showed a sensitivity of 88.1% (95% CI: 75.0%, 94.8%) and a specificity of 95.2% (95% CI: 90.5%, 97.7%), and the kappa (95% CI) was 0.82 (Table 3). These results are slightly lower than the other sensitivity and specificity results presented in this study. This is mainly due to the addition of the subjects that were classified as non-evaluable per the composite reference standard when the RUT and histology results were discordant. In the majority of those cases, the breath test agreed with the histology results and not with the RUT result. RUT results can sometimes be ambiguous due to the need to determine a clear change in color by visual inspection. Comparing the breath test to the histology classification showed a sensitivity of 97.6% (95% CI: 87.7%, 99.6%) and a specificity of 98.0% (95% CI: 94.2%, 99.3%), and kappa (95% CI) was 0.94 (Table 3).

Table 2. Comparative results of UBT, histology, RUT and composite test results.

UBT	Composite		RUT		Histology (IHC)	
	HP (+)	HP (–)	HP (+)	HP (–)	HP (+)	HP (–)
Initial diagnosis						
HP (+)	37	3	37	7	41	3
HP (–)	0	139	5	140	1	144
Post-eradication therapy						
HP (+)	12	0	11	1	12	0
HP (–)	1	55	0	56	1	55

HP: *H. pylori*; IHC: immunohistochemistry; RUT: rapid urease test; UBT: urea breath test.

Table 3. Diagnostic performance of the tests.

	Composite	RUT	Histology (IHC)
Initial diagnosis			
Sensitivity (%)	100 (90.6–100.0)	88.1 (75.0–94.8)	97.6 (87.7–99.6)
Specificity (%)	97.9 (94.0–99.3)	95.2 (90.5–97.7)	98.0 (94.2–99.3)
Kappa value	0.95	0.82	0.94
Post-eradication therapy			
Sensitivity (%)	92.3 (66.7–98.6)	100 (74.1–100.0)	92.3 (66.7–98.6)
Specificity (%)	100 (93.5–100.0)	98.3 (90.7–99.7)	100 (93.5–100.0)
Kappa value	0.95	0.95	0.95

IHC: immunohistochemistry; RUT: rapid urease test.

Post-eradication therapy cohort. There were 68 evaluable histology, RUT and composite reference assessments with corresponding breath test results. In 67 subjects, the first evaluable breath test results matched those of the composite reference standard biopsy results: 55 biopsy results were negative and 13 results were positive. In one subject, the first evaluable breath test results did not match the composite reference standard biopsy result as classified for post-eradication. This subject classified as a false negative had a breath test result that was close to the predefined clinical cut-off value of 5 DOB, but they were classified as positive per the composite reference standard based on a positive histology assessment despite the fact that all three RUT samples produced a negative result.

The overall agreement between the UBT diagnosis and the biopsy diagnosis in the post-eradication therapy cohort was 98.5% (95% CI: 92.1%, 100.0%) and the kappa (95% CI) was 0.95. The sensitivity of the breath test in this cohort was 92.3% (95% CI: 66.7%, 98.6%) and its specificity was 100.0% (95% CI: 93.5%, 100.0%; Table 3).

Comparing the breath test to RUT only showed a sensitivity of 100.0% (95% CI: 74.1%, 100.0%) and a specificity of 98.3% (95% CI: 90.7%, 99.7%), and kappa (95% CI) 0.95. Comparing the breath test to histology demonstrated a sensitivity of 92.3% (95% CI: 66.7%, 98.6%) and a specificity of 100.0% (95% CI: 93.5%, 100.0%), and kappa (95% CI) 0.95; Table 3).

Table 4. Results of the reproducibility analytical study.

Expected DOB	Parameter	*SD* value	95% CI	CV (%)
DOB: 3.3‰				
	Reproducibility	0.53	[0.46–0.63]	14.8
	Between days precision	0.54	[0.46–0.60]	14.9
	Between devices precision	0.54	[0.45–0.59]	14.9
	Between operators precision	0.53	[0.44–0.58]	14.8
DOB: 6.4‰				
	Reproducibility	0.60	[0.52–0.71]	9.7
	Between days precision	0.62	[0.54–0.68]	10.0
	Between devices precision	0.60	[0.51–0.65]	9.7
	Between operators precision	0.60	[0.51–0.70]	9.7
DOB: 15.5‰				
	Reproducibility	0.65	[0.57–0.77]	4.3
	Between days precision	0.65	[0.56–0.72]	4.3
	Between devices precision	0.66	[0.56–0.73]	4.4
	Between operators precision	0.65	[0.55–0.76]	4.3

DOB: delta over baseline; CI: confidence interval; CV: coefficient of variance; *SD*: standard deviation.

Safety. Overall, there were a total of four adverse events in the initial diagnosis cohort and one adverse event in the post-eradication therapy cohort: one subject had a cyst found on the epiglottis, one had a gastric ulcer, two patients had nausea for approximately 2 min after drinking the breath test mixture and one felt lightheaded due to fasting for the EGD procedure. None of them were serious or severe, and none were related to the study device. Hence, the test procedure itself was found to be very safe and well-tolerated by all subjects.

Stability of breath samples over time. The stability of the breath samples over a period of 14 days was evaluated on samples from 191 subjects from the pre-therapy cohort who had two breath test bags per subject analyzed on two separate occasions. This analysis also included subjects who were not evaluable based on the biopsy results. Of 45 samples that were positive on the first measurement, 44 of the samples in the same bags remained positive on the second measurement [percent-positive agreement: 97.8% (95% CI: 88.43, 99.61)]. Out of 146 samples negative on the first measurement, all 146 remained negative on the second measurement [percent negative agreement: 100.0% (95% CI: 97.44, 100.0)].

Reproducibility and repeatability results. The results demonstrated that the *SD* and overall reproducibility of the results of the ^{13}C-UBT were stable over different batches for both the operator, the devices, and between days. The reproducibility *SD* was 0.65 or less for all batches, and the between days, devices and operators *SD* was 0.66 or less in all cases; this is less than the natural variability of the DOB measurement (Table 4). Repeatability was assessed by measuring the DOB values for samples from each of the three batches twice a day for 12 days. The results demonstrated that the *SD* and overall repeatability were stable over different batches and different days. The repeatability *SD* was 0.64 or less and the overall between-days *SD* was 0.72 or less; this is less than the natural variability of the DOB measurement (Table 5).

Table 5. Results of the precision analytical study.

Expected DOB	Parameter	*SD* value	95% CI	CV (%)
DOB: 3.3‰				
	Repeatability	0.56	[0.44–0.78]	16.9
	Between days precision	0.63	[0.52–0.80]	17.4
DOB: 6.4‰				
	Repeatability	0.59	[0.46–0.82]	9.2
	Between days precision	0.68	[0.56–0.87]	10.6
DOB: 15.5‰				
	Repeatability	0.64	[0.50–0.89]	4.3
	Between days precision	0.72	[0.60–0.92]	4.8

DOB: delta over baseline; CI: confidence interval; CV: coefficient of variance; *SD*: standard deviation.

Table 6. Results of the bag storage analytical study per storage condition.

Expected DOB	Storage condition	Parameter	*SD* value	95% CI	CV (%)
DOB: 3.3‰					
	15°C	Overall repeatability	0.57	[0.45–0.78]	15.0
		Between days precision	0.57	[0.45–0.68]	15.0
	35°C + 70% humidity	Overall repeatability	0.60	[0.48–0.82]	16.9
		Between days precision	0.60	[0.47–0.72]	16.9

DOB: delta over baseline; CI: confidence interval; CV: Coefficient of Variance; *SD*: standard deviation.

Breath sample bags were stored at two different storage conditions representing the two extreme temperatures of the recommended storage range (15°C and 35°C) and at the high limit for the recommended relative humidity (70%). The DOB values for samples from each storage condition were measured on the *BreathID®Hp Lab System* seven times during 14 consecutive days for each storage condition, specifically on days 2, 4, 8, 9, 10, 11, and 14. The results demonstrated that the *SD* and overall repeatability were stable over different batches, days, and storage conditions. The overall repeatability *SD* and the between days precision *SD* were 0.60 or less; this is less than the natural variability of the DOB measurement (Table 6).

Discussion

Active *H. pylori* testing is the preferred modality according to guidelines by the American College of Gastroenterology, the American Gastroenterological Association, and the European and Japanese societies in the test-and-treat approach to dyspepsia.[1,2,15] Additional support for this concept came when Cigna became the first large national payer in the United States to decide that it will no longer reimburse serology testing as of 15 August 2014. This provided a great opportunity to further convert serology testing into active *H. pylori* testing via either the UBT or stool antigen test for initial diagnosis or to confirm eradication.

The *BreathID®Hp* was launched in the United States in the second half of 2010, offering a cannula-based test kit that features continuous breath sampling and an expected 10 min total test time. It also offers a bag-based test kit for those practices that prefer this method. The cannula kit is simple for patients and staff and provides real-time results in 10–15 min. This improved convenience has

enabled physicians to bring *H. pylori* breath testing in-house. An additional advantage of the device is that it is a relatively small and portable instrument that may be located at large-volume patients clinics such as a hospital outpatient gastroenterology clinic, preventing the requirement for the transportation of the bags for breath testing. However, as we discussed earlier, the *BreathID®Hp* may also have some negative aspects such as limitation of its use to only a single patient at a time and the inability to evaluate mailed or transported samples. The *BreathID®Hp* and the cannula-based method of testing are differentiated from the *BreathID®Hp Lab System*, the subject of this article, which is a bag-based breath collection method and offer notable advantages. These include significantly larger sample size (approximately 50–75 times more breath samples for the same time period), shortening the testing time by approximately 50% and maximizing accuracy.

The aim of this article is to evaluate the *BreathID®Hp Lab System*, when breath is collected into bags that are then either tested on-site or delivered to a central laboratory, instead of using the original continuous collection system. The results show a high diagnostic accuracy for both pre- and post-eradication setting. Accuracy reaches 100% sensitivity and 97.9% specificity for initial diagnosis of *H.pylori* than composite reference standard. UBT had a better sensitivity than biopsy urease test (approximately 90%).[2] These could be attributed to sampling error associated with endoscopic biopsy, due to patchy distribution of *H. pylori*, a very low number of *H. pylori* in the tissue sample or sampling of gastric atrophy or intestinal metaplasia that associated with decreasing *H. pylori* colonization.[16] Another reason for false-negative results is the recent use of proton pump inhibitors, bismuth, or antibiotics. Nonetheless, all of the tests for active infection including RUT, histology, UBT, and culture may become false negative during the use of these drugs. In addition, the possible effect of the storage of the bags was evaluated, and it was found that storage for up to 14 days and under different conditions does not significantly affect breath test results.

The *BreathID®Hp Lab System* has several advantages compared to previous *BreathID®Hp* device: First, the *BreathID®Hp Lab System* can perform sequential diagnosis on 10 pairs of breath collection bags within approximately 25 min via a fully automated process, thereby minimizing potential human error, as opposed to the previous *BreathID®Hp Lab System* that measures only one subject at a time. Second, it allows performing the breath test in locations that do not have the device itself (due to cost or other reason), and the test cannot be performed on-site. Third, this test takes 15 min with high accuracy than composite reference standard (97%). Finally, this is a reliable system, user-friendly with touch-screen operation, maintenance free, compact, and the system has availability to be connected to a laboratory Lab Information Management Software (LIMS) system.

The optimal ^{13}C-UBT conditions for diagnosing *H. pylori* infection are still being perfected. The optimal diagnostic cut-off point discriminating between positive and negative ^{13}C-UBT results is still a controversial issue. Therefore, the results for ^{13}C-UBT often affect the diagnostic accuracy when the results are very close to the cutoff as at the onset of the infection or when the level lies in a so-called gray zone.[3] The *BreathID®Hp Lab System* shares with its predicate device the same underlying technology, test substrate, and diagnostic capabilities. Both the subject and predicate systems use molecular correlation spectroscopy (MCS) technology and measure the ratio of $^{13}CO_2/^{12}CO_2$ in exhaled breath prior to and after administration of the test substrate (^{13}C-Urea). MCS technology measures the light absorbance of the sample by infrared spectrometry; this correlates to the CO_2 concentrations of the different carbon isotpoes in the breath sample. The output results from both systems are the DOB, and a positive/negative determination is based on the same assay cutoff ($\geqslant$5 DOB). Indeed, this study has shown that the cutoff of 5 DOB for the *BreathID®Hp Lab System* is precise and accurate when compared to the gold standard (EGD biopsy results). However, when assessing gastric biopsies, it should be noted that RUT is considered to be less accurate than IHC assessments.[16] Indeed, comparison of the UBT results in our study, to RUT and histology, respectively, revealed higher agreement with IHC, a finding consistent with a recent publication.[17]

There are several limitations to this study. First, the breath test was not compared to culture which is one of the recommended reference standards, as the efforts needed to insure proper conditions for culture growing were very difficult logistically to arrange in a clinical study and prone to human error. The FDA accepts a reference composite score using RUT and histology alone. Furthermore, the RUT test, whose results are determined by change in color of the substrate, can be interpreted differently by different users in borderline cases

(pink is positive and yellow is negative), contributing to human error.

In conclusion, the *BreathID®Hp Lab System* (Exalenz Bioscience Ltd) has been demonstrated to be as safe and effective as its predicate device, that is, the FDA-cleared Exalenz Bioscience Ltd *BreathID®Hp Lab System*. It is substantially equivalent to *BreathID®Hp* without raising new safety or efficacy issues. Based on this study, the *BreathID®Hp Lab System* also received marketing clearance from FDA for *H. pylori* detection in November 2016.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was funded by Exalenz Bioscience Ltd.

Conflict of interest statement

The authors declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: H.S. has received grants and stock options from Exalenz Bioscience Ltd. None of other authors report conflicts.

Trial Registry

NCT02528721, for protocol titled:#DM2-HP-0715 (https://clinicaltrials.gov/ct2/results?cond=&term=NCT02528721&cntry=&state=&city=&dist=)

ORCID iD

Vered Richter https://orcid.org/0000-0001-6003-9220

References

1. Chey WD and Wong BC. American College of Gastroenterology guideline on the management of *Helicobacter pylori* infection. *Am J Gastroenterol* 2007; 102: 1808–1825.
2. Malfertheiner P, Megraud F, O'Morain CA, *et al.* Management of *Helicobacter pylori* infection-the Maastricht V/Florence Consensus Report. *Gut* 2017; 66: 6–30.
3. Gisbert JP and Pajares JM. ^{13}C-urea breath test in the diagnosis of *Helicobacter pylori* infection—a critical review. *Aliment Pharmacol Ther* 2004; 20: 1001–1017.
4. Howden CW and Hunt RH. Guidelines for the management of *Helicobacter pylori* infection. *Am J Gastroenterol* 1998; 93: 2330–2338.
5. Chey WD. Accurate diagnosis of *Helicobacter pylori*. ^{14}C-urea breath test. *Gastroenterol Clin North Am* 2000; 29: 895–902.
6. Graham DY and Klein PD. Accurate diagnosis of *Helicobacter pylori*. ^{13}C-urea breath test. *Gastroenterol Clin North Am* 2000; 29: 885–893.
7. Perri F. Diagnosis of *Helicobacter pylori* infection: which is the best test? The urea breath test. *Dig Liver Dis* 2000; 32: S196–S198.
8. Nocon M, Kuhlmann A, Leodolter A, *et al.* Efficacy and cost-effectiveness of the ^{13}C-urea breath test as the primary diagnostic investigation for the detection of *Helicobacter pylori* infection compared to invasive and non-invasive diagnostic tests. *GMS Health Technol Assess* 2009; 5: Doc14.
9. Gisbert JP and Calvet X. *Helicobacter Pylori* 'test-and-treat' strategy for management of dyspepsia: a comprehensive review. *Clin Transl Gastroenterol* 2013; 4: e32.
10. Leodolter A, Dominguez-Munoz JE, von Arnim U, *et al.* Validity of a modified ^{13}C-urea breath test for pre- and posttreatment diagnosis of *Helicobacter pylori* infection in the routine clinical setting. *Am J Gastroenterol* 1999; 94: 2100–2104.
11. Schmilovitz-Weiss H, Sehayek-Shabat V, *et al.* Applicability of a short/rapid ^{13}C-urea breath test for *Helicobacter pylori*: retrospective multicenter chart review study. *BMC Gastroenterol* 2012; 12: 8.
12. Broide E and Shirin H. Evaluation of Exalenz Bioscience's BreathID for *Helicobacter pylori* detection. *Expert Rev Mol Diagn* 2015; 15: 299–312.
13. el-Zimaity HM. Accurate diagnosis of *Helicobacter pylori* with biopsy. *Gastroenterol Clin North Am* 2000; 29: 863–869.
14. Staff FDA. Characteristics of in vitro diagnostic devices for the detection of *Helicobacter pylori*, 2010, www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm425025.htm
15. Asaka M, Kato M, Takahashi S, *et al.* Guidelines for the management of *Helicobacter pylori* infection in Japan: 2009 revised edition. *Helicobacter* 2010; 15: 1–20.
16. Miftahussurur M and Yamaoka Y. Diagnostic methods of *Helicobacter pylori* infection for epidemiological studies: critical importance of indirect test validation. *Biomed Res Int* 2016; 2016: 4819423.
17. Kocsmar E, Szirtes I, Kramer Z, *et al.* Sensitivity of *Helicobacter pylori* detection by Giemsa staining is poor in comparison with immunohistochemistry and fluorescent in situ hybridization and strongly depends on inflammatory activity. *Helicobacter* 2017; 22: 12387.