

EXPERT
REVIEWSEvaluation of Exalenz
Bioscience's BreathID for
Helicobacter pylori detection

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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 ¹³C-urea breath test relies upon the ability of the *H. pylori* urease to hydrolyze the orally administered ¹³C-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,

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 compared 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 $^{13}/^{14}\text{C}$ -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, $^{13}\text{CO}_2$ and $^{12}\text{CO}_2$ 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, $^{13}\text{CO}_2$ 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 $^{13}\text{C}/^{12}\text{C}$ 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 $^{13}\text{CO}_2$ 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 spectroscopy [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 spectroscopy 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 $^{13}\text{CO}_2$ – $^{12}\text{CO}_2$. 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.

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 $^{13}\text{CO}_2$ 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 $^{13}\text{CO}_2$ 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,

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.

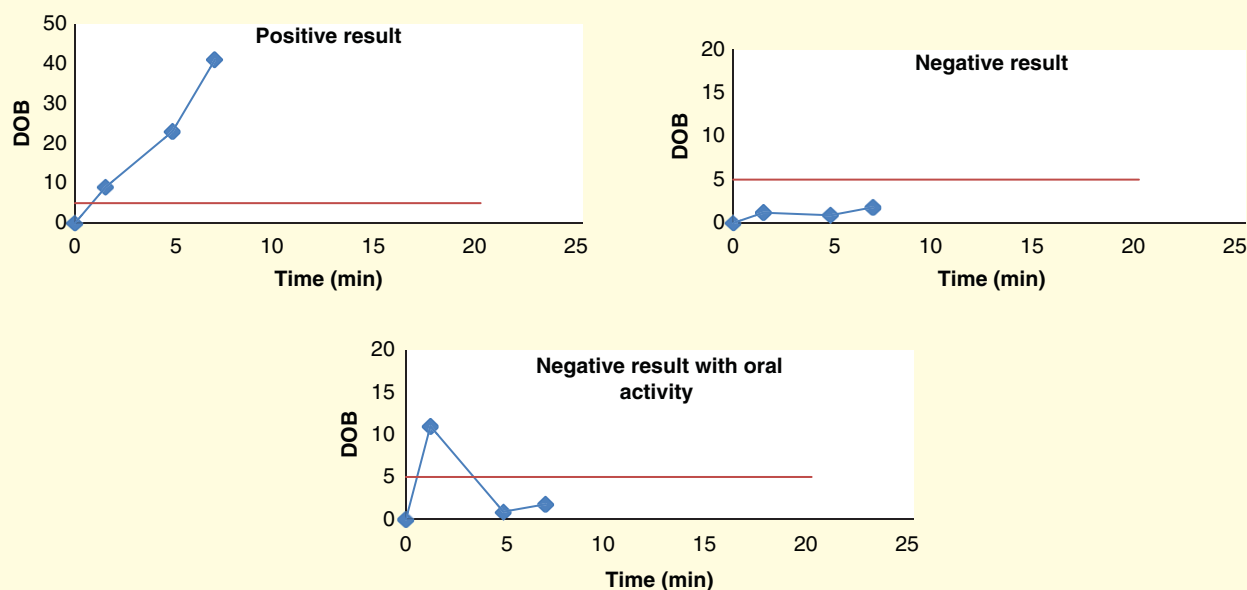


Figure 1. Sample breath test results with BreathID® *Helicobacter pylori* system.

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 $^{13}\text{CO}_2$ and NH_3 . The $^{13}\text{CO}_2$ 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:

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

where $^{13}\text{C}^{(\text{PDB})}/^{12}\text{C}^{(\text{PDB})}$ 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:

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

Excess $^{13}\text{CO}_2$ 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 $^{13}\text{C}/^{12}\text{C}$ 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

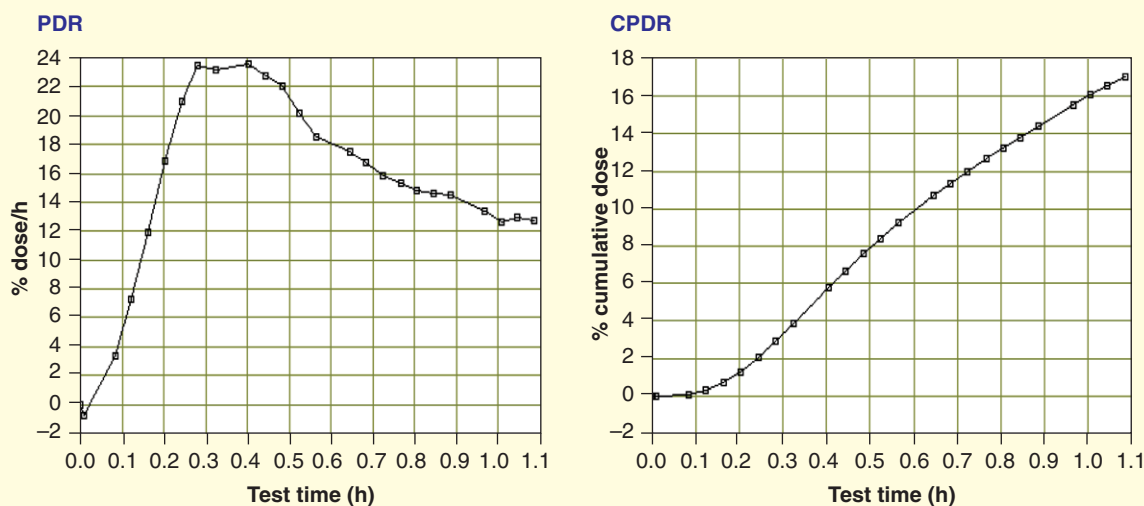


Figure 2. Percentage dose recovery and cumulative percentage dose recovery graphs displayed on BreathID® device in real time.

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 $^{13}\text{CO}_2$ and $^{12}\text{CO}_2$ 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 $^{13}\text{CO}_2$ and $^{12}\text{CO}_2$ gases. By using $^{13}\text{CO}_2$ and $^{12}\text{CO}_2$ charging lamps as two unique light sources, light absorption will be due only to the existence of $^{13}\text{CO}_2$ and $^{12}\text{CO}_2$ 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 $^{13}\text{CO}_2$ and $^{12}\text{CO}_2$ 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 $^{13}\text{CO}_2$ and $^{12}\text{CO}_2$ 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 $^{13}\text{CO}_2$ and $^{12}\text{CO}_2$ 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 $^{12}\text{CO}_2$, but a small portion of $^{13}\text{CO}_2$ is also exhaled in the breath. ^{13}C s natural abundance is approximately 1% in the environment and it is a stable isotope [35]. The baseline ratio between $^{13}\text{CO}_2$ and $^{12}\text{CO}_2$ is measured at the beginning of the test. After ingestion of a ^{13}C -labeled substrate, the ratio between the $^{13}\text{CO}_2$ and $^{12}\text{CO}_2$ 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 $^{13}\text{CO}_2/^{12}\text{CO}_2$ 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 $^{13}\text{CO}_2/^{12}\text{CO}_2$ 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.

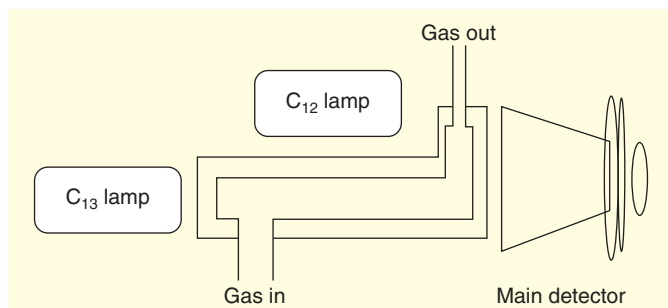


Figure 4. Schematic illustration of the principles of the BreathID® system.

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 discrete 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 discrete changes. The BreathID device continuously collects and analyzes the breath automatically as opposed to the IRMS. Therefore, the BreathID is less sensitive

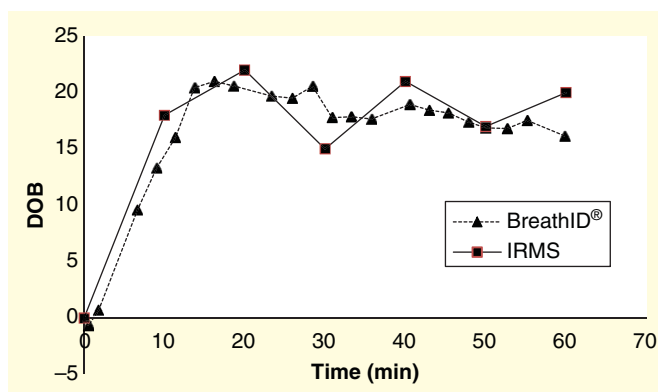


Figure 5. Noisy discrete breath collection versus BreathID® collection.

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 CO₂ 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 CO₂ 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 CO₂ 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 ¹⁴CO₂ up to 15 min after administration of non-capsulated ¹⁴C-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

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

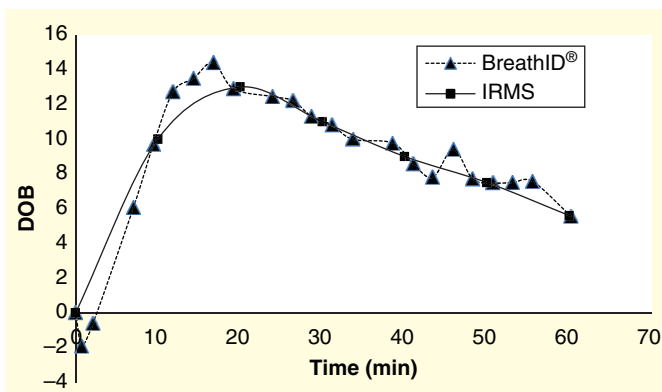


Figure 6. Examples of missed peaks; isotope ratio mass spectrometer versus BreathID®. (A) Normal emptying. (B) Delayed emptying.
DOB: Delta over baseline; IRMS: Isotope ratio mass spectrometer.

^{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].

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

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 \pm SD)		47.9 \pm 16.7	45.9 \pm 18.0	45.8 \pm 16.8	49.0 \pm 14.5
UBT results (DOB)	Baseline	31.7 \pm 31.6	27.5 \pm 19.6	28.7 \pm 23.7	23.8 \pm 18.3
	Day 14	33.8 \pm 29.5	24.8 \pm 21.4	27.1 \pm 28.1	19.1 \pm 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 metabolism 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 ($T_{1/2}$) 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 $^{13}\text{CO}_2$ in the exhaled breath, the end product of a sequence of events (e.g., ^{13}C -octanoic acid). Gastric scintigraphy with $^{99\text{m}}\text{Tc}$ 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 $^{13}\text{CO}_2$, which is eliminated by the lungs. This may limit the use of the test in patients with lung and liver disease, malabsorption or maldigestion. However, as in ^{13}C -urea with *H. pylori*, the quantity of $^{13}\text{CO}_2$ in the patient's exhaled breath is a function of the quantity of content leaving the stomach and reaching the intestine. By measuring the $^{13}\text{C}/^{12}\text{C}$ ratio in the expired air, clinicians can calculate the gastric emptying coefficient, the gastric $T_{1/2}$ 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

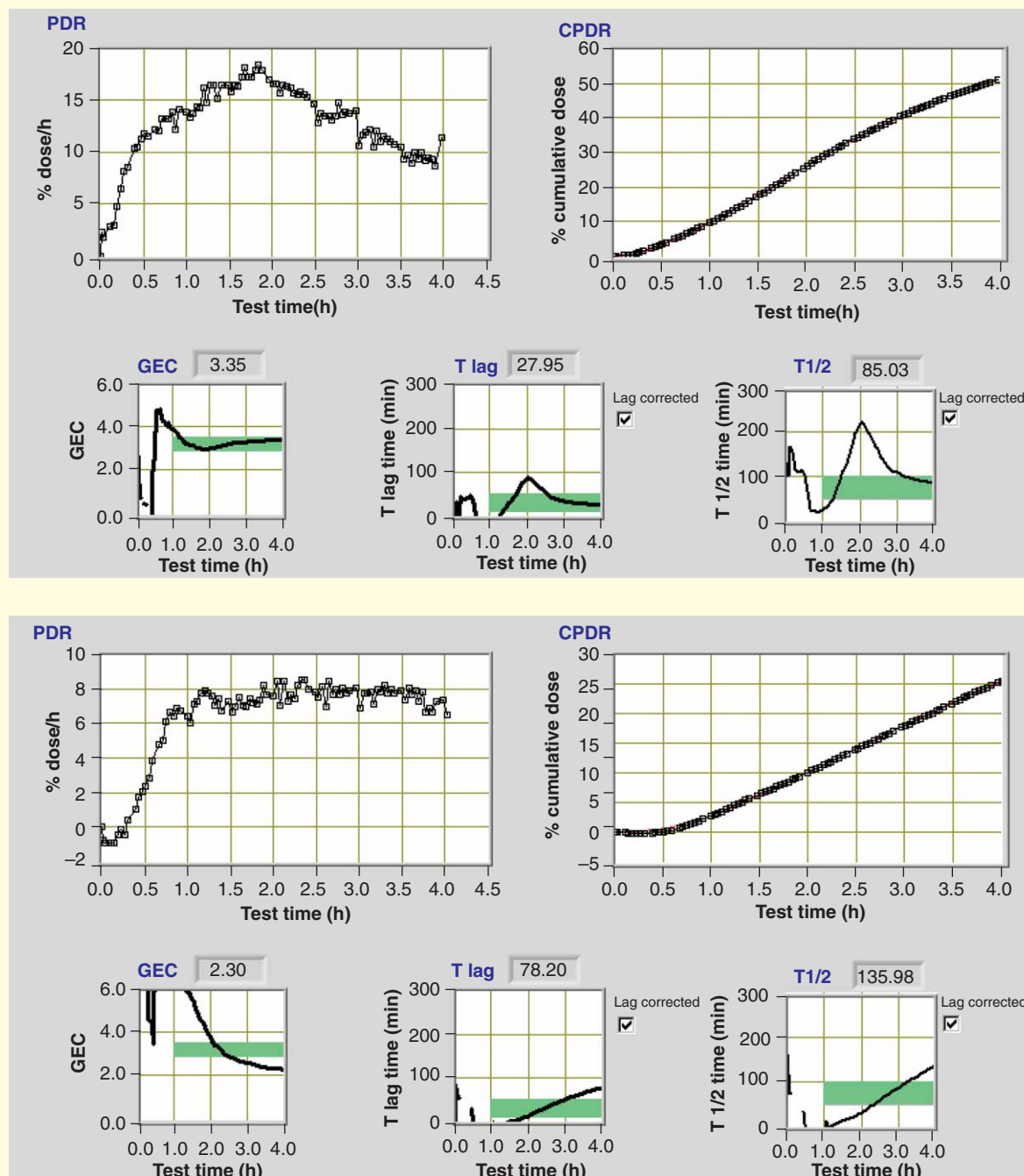


Figure 7. Percentage dose recovery, cumulative percentage dose recovery, gastric emptying coefficient, gastric $T_{1/2}$ and Tlag graphs displayed on BreathID® device in real time.

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

The BreathID automatically calculates the change in the $^{12}\text{CO}_2/^{13}\text{CO}_2$ 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 $T_{1/2}$ and Tlag according to the non-linear model described by Ghooos *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 $T_{1/2}$ 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

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 rationale for the use of breath test is that the ¹³C-labeled substrate given with the meal reaches the duodenum, where it is hydrolyzed by specific pancreatic enzyme to ¹³C-labeled metabolites. These are absorbed through the gut, metabolized in the liver while the ¹³CO₂ released during this process is absorbed in the bloodstream, reaches the lungs and is eliminated with expired air. Thus, the measurement of ¹³CO₂ 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 ¹³C-dipeptide breath test has the potential to become as easy, fast and practicable as the ¹³C-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 ¹³C-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 ¹³C-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 ¹³C-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 CO₂ 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.

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.

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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.

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