

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

## Continuous Online Measurements Allow for Faster Test Results With High Accuracy

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### Abstract

**Objective:** The aim of this study is to determine the accuracy of a novel laptop sized  $^{13}\text{C}$ -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  $^{13}\text{CO}_2$ : $^{12}\text{CO}_2$ . 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  $^{13}\text{C}$ -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*,  $^{13}\text{C}$ -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.<sup>1</sup> *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.<sup>2</sup> UBT uses labeled urea ( $^{13}\text{C}$  or  $^{14}\text{C}$ ) that is metabolized by the presence of *H. pylori* to yield  $\text{CO}_2$ . 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.<sup>3,4</sup> The  $^{13}\text{C}$ -Urea breath test is a highly sensitive, non-invasive and safe method for detecting *H. pylori*.<sup>5,6</sup> Current  $^{13}\text{C}$ -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  $^{13}\text{C}$ -analysis utilizing a novel technology of molecular correlation spectrometry<sup>7</sup> as compared with the traditional method of single point IRMS.

### 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  $^{13}\text{C}$ -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

#### $^{13}\text{C}$ -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  $^{13}\text{CO}_2$ : $^{12}\text{CO}_2$  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  $^{13}\text{CO}_2$ : $^{12}\text{CO}_2$  was measured. Then 75 mg of  $^{13}\text{C}$ -urea, and

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

$^{13}\text{CO}_2$ : $^{12}\text{CO}_2$  ratios were expressed as delta over baseline  $^{13}\text{C}$  (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.<sup>8,9</sup>

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.

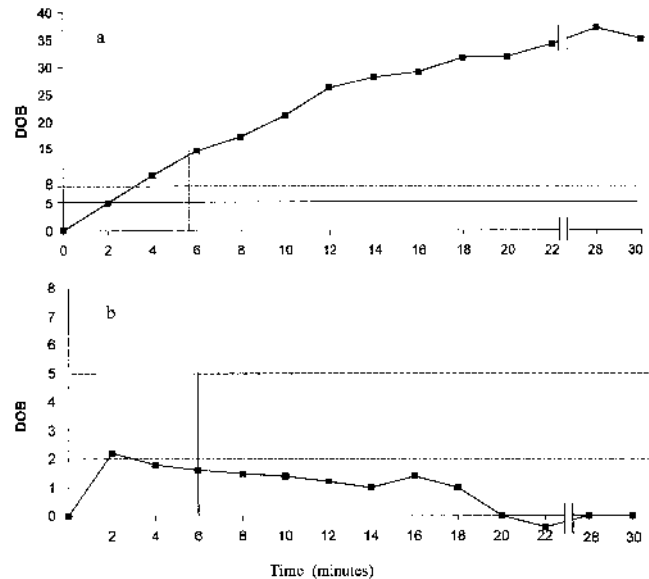
## RESULTS

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  $^{13}\text{C}$  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.*



**FIGURE 1.** Delta over baseline (DOB) values of  $^{13}\text{C}$  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.

*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 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  $^{13}\text{C}$  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		Percent of subjects with conclusive test results (positive & negative for H pylori infection)
	H pylori negative result	H pylori positive result	
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 gastro-duodenal pathogen, diagnosis of *H. pylori* infection has become a key step in the management of patients referred to the gastroenterologist. The <sup>13</sup>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 <sup>13</sup>C-Urea breath test utilizing molecular correlation spectrometry is a reliable tool for detection of *H. pylori*. Advantages are: ease of use (sam-

pling 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.<sup>10</sup> Large breath gas volumes are not required, and cooperation on part of the subject is minimal. This on-line measurement of <sup>13</sup>C breath testing might also be of use for further applications such as gastric emptying studies or liver function tests.

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