

Evaluation of a novel continuous real time ^{13}C urea breath analyser for *Helicobacter pylori*

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SUMMARY

Aim: To evaluate the sensitivity and specificity of a new ^{13}C urea breath test, Oridion BreathID, for the diagnosis of *Helicobacter pylori*.

Methods: A total of 97 consecutive symptomatic patients referred for upper endoscopy were included in the 'pre-therapy' part of the study. After endoscopy the patients were analysed for *H. pylori* by Oridion BreathID. BreathID continuously sampled the subject's breath for 20 min, and displayed the results on the BreathID screen in real time. Results of the BreathID were compared with the 'gold standard' (rapid urease test and histology). We also prospectively tested the validity of BreathID in comparison to isotope ratio mass

spectrometry, in 40 patients referred to monitor the efficacy of *H. pylori* eradication treatment.

Results: Complete agreement was observed between the 'gold standard' and the Breath ID test in 96.9% (94 out of 97) of the patients. The sensitivity and specificity of BreathID were 97.8% and 96.1%, respectively. The correlation between BreathID and isotope ratio mass spectrometry breath test was 100%.

Conclusions: The Oridion BreathID has comparable sensitivity and specificity to the claims of the currently available urea breath tests. Furthermore, BreathID has the potential advantages of ease of use with minimal medical staff requirement, and real time rapid results (20 min maximum) which may make the BreathID preferable to other urea breath test assays.

INTRODUCTION

Helicobacter pylori, the common aetiological agent of gastritis and peptic ulceration, may infect the gastric mucosa of more than half of the world's population.¹ Although the prevalence of *H. pylori* infection has declined in recent years in developed countries, those who are infected remain at risk of peptic ulceration, gastric cancer, or gastric lymphoma.^{2, 3} Before urea breath test, the diagnosis of *H. pylori* infection was usually established invasively by histology, culture and

rapid urease test, or non-invasively by serology. As the pathogenic role of *H. pylori* in peptic ulcer disease and other gastrointestinal conditions is well-established, there is an increasing demand for rapid non-invasive, office-based tests for the identification of *H. pylori*. One approach to the management of patients with dyspeptic symptoms is to empirically eradicate *H. pylori* before referring patients for endoscopy.⁴ Although serology is quick, simple, widely available and inexpensive, the detection of *H. pylori* by whole-blood or serum-based serologic tests may not reflect current active infection.^{5–7}

In contrast to serologic methods, the currently available urea breath tests detect active infection, with high sensitivity and specificity, and therefore they may be considered the preferred method for epidemiological

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studies, to screen dyspeptic patients and to assess eradication or recurrence of the infection.⁸ The major disadvantage of the ^{13}C urea breath test is the cost of the $^{13}\text{CO}_2$ analysis and in many medical centres there is still a need for collecting, storing, and transporting the samples to an isotope ratio mass spectrometer or gas chromatography laboratory. This makes the urea breath test inconvenient to both patient and physician.

In the present study, we tested the accuracy of a novel rapid continuous real time urea breath test, BreathID, using molecular correlation spectrometry. We prospectively evaluated the sensitivity and specificity of BreathID to detect the presence of *H. pylori* and the ability of this device to monitor the efficacy of bacterial eradication.

MATERIALS AND METHODS

Pre-therapy (diagnostic) evaluation

A total of 115 consecutive patients, over 18 years of age, referred for upper endoscopy because of upper gastrointestinal symptoms were included in the pre-treatment part of the study. All patients underwent diagnostic gastroscopy, including gastric biopsies of the antrum and corpus. The presence of *H. pylori* in the biopsy specimens was detected on histological examination (H&E and Giemsa) and direct detection of urease activity by the rapid urease test (CUTest, Temmler Pharma, Germany). Following recovery from endoscopy, the patients were also analysed for *H. pylori* by Oridion BreathID. The results by Oridion BreathID were compared to the results from the invasive test methods. Patients meeting any of the following exclusion criteria were not eligible to participate in the study: treatment for the eradication of *H. pylori* during the previous 4 weeks; administration of antibiotics and/or bismuth preparations within 4 weeks before the date of entry to the study; administration of proton pump inhibitors within 1 week before the date of entry to the study; pregnant or breast-feeding women. Gastrointestinal conditions were classified on the basis of symptoms (dyspepsia, epigastric pain, etc.) and endoscopic findings (duodenal ulcer, gastric ulcer, etc.). The technicians were blinded to the results of the other tests assessing *H. pylori* status. Informed consent was obtained from each patient before enrolment in the study.

Upper gastrointestinal endoscopy was performed under sedation with midazolam (5–10 mg, intravenously), and the endoscopic findings were recorded. Four gastric biopsies were taken from each patient, two from the antrum, 1–3 cm from the pyloric channel, and two from the mid corpus.

The first tissue sample from the antrum and one of the corpus were immediately tested by rapid urease test in the same tube. CUTest was monitored for colour change up to 24 h at room temperature.

'Gold standard'

The reference gold standard was considered the detection of *H. pylori* both in the rapid urease test and by Giemsa stain of biopsy specimens. If there was disagreement between the rapid urease test and histology, the patient was excluded from the study.

Oridion breath test system

Molecular correlation spectrometry is based on the optical absorption of specific radiation of $^{13}\text{CO}_2$ and $^{12}\text{CO}_2$ gases. The Oridion breath system is based on two unique light sources, a $^{13}\text{CO}_2$ and a $^{12}\text{CO}_2$ charging lamp. By using a $^{13}\text{CO}_2$ and a $^{12}\text{CO}_2$ charging lamp as 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 a $^{13}\text{CO}_2$ and a $^{12}\text{CO}_2$ charging lamp as light sources, 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 $^{12}\text{CO}_2$ concentration and $^{13}\text{CO}_2/^{12}\text{CO}_2$ ratio measurement. By modulating these different light sources with different frequencies, they can be measured at the same detector, called the main detector (Figure 1). In order to calculate the $^{13}\text{CO}_2$ and $^{12}\text{CO}_2$ gas concentrations, an absorption cell is fixed between

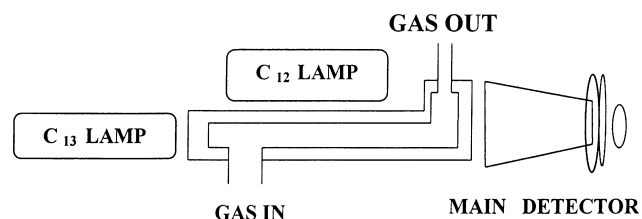


Figure 1. Schematic illustration of the principles of the BreathID breath system.

the light source and the main detector. 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, by measuring the intensities.

BreathID ^{13}C urea breath test

The BreathID is capable of collecting breath samples continuously from a patient who is connected to the instrument through a nasal canula based circuit. The instrument analyses breath samples before and after ^{13}C enriched urea is administered to the patient and generates multiple points on a graph with $^{13}\text{CO}_2/^{12}\text{CO}_2$ from the base line. The BreathID system comprises of the following components: 75 mg ^{13}C -urea (tablet), 4.5 g Citrica (citric acid based power), IDCircuit-sampling device, and the BreathID device. The ^{13}C labelled urea and citric acid are used as a test drink. The IDCircuit, a nasal breath sampling device, continuously transports the breath sample from the patient to the BreathID. Based on molecular correlation spectrometry, the BreathID continuously measures $^{13}\text{CO}_2$ and $^{12}\text{CO}_2$ concentrations from the patient's breath and establishes the $^{13}\text{CO}_2/^{12}\text{CO}_2$ ratio which is displayed vs. time on the screen. The results are also printed on a thermal printer and saved on the hard disk (Figure 2).

The determination of positive or negative results is based on a device algorithm. If, after 5 min, more than two points are above 6 delta over baseline (DOB), the patient is considered positive. If, after the same time, more than two points are below 3 DOB, the patient is considered negative. In our study the test automatically ended after 20 min. If there were no conclusive results after 20 min the nominal 5 DOB threshold was considered to distinguish positive from negative patients. The DOB was measured and displayed on the BreathID screen in real time with a threshold of 5 DOB. In many instances, the test time can be further reduced when a conclusive positive or negative case of *H. pylori* has been identified.

Evaluation of post therapy efficacy

In this part of the study we prospectively tested the validity of BreathID in comparison to isotope ratio mass spectrometry (Analytical Precision, AP 2003, UK). Forty patients testing positive for infection with

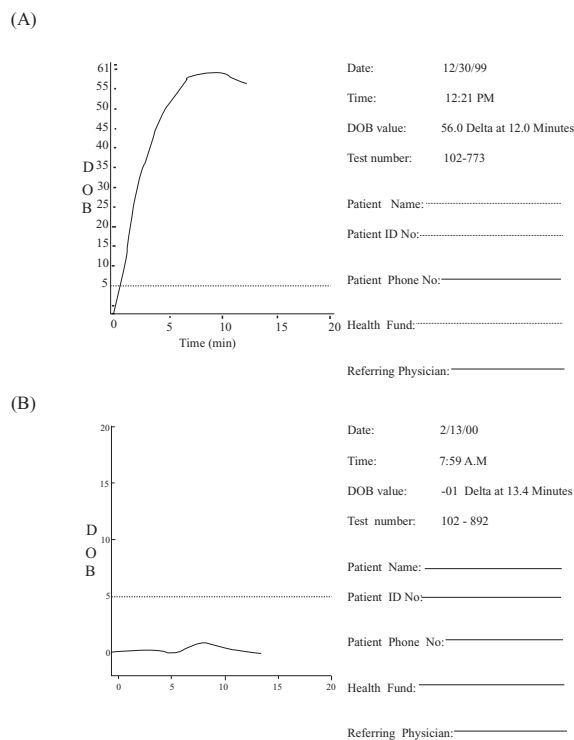


Figure 2. Positive (A) and negative (B) *Helicobacter pylori* tests as detected on real time and presented on the BreathID screen. The threshold is defined as 5 DOB and marked by the dash line.

H. pylori who agreed to monitoring of the efficacy of the treatment, were included in a follow-up evaluation 4–6 weeks after the end of treatment for *H. pylori* eradication. The eradication triple therapy included a proton pump inhibitor, clarithromycin 250 mg b.d. and amoxicillin 1 g b.d. or metronidazole 500 mg b.d. for 7 days. In the follow-up examination, breath samples at base line and at 30 min were collected for the isotope ratio analysis by isotope ratio mass spectrometry, whilst during this period, in parallel, the post-therapy eradication efficiency was assessed with BreathID.

RESULTS

A total of 115 consecutive symptomatic patients who underwent upper endoscopy in our gastrointestinal unit were studied in the pre-therapy part of the study. A full set of test data was available for 97 patients (41 men and 56 women) who were classified either as positive or negative by the gold standard. The mean patients' age was 58.6 years (range 18–85 years). A summary of the demographic characteristics and endoscopic findings of the patients is presented in Table 1. In four patients the

Table 1. Characteristics of 97 symptomatic patients undergoing upper gastrointestinal tract endoscopy. Some patients underwent more than one endoscopic diagnosis

Age (years \pm s.d.)	58.6 \pm 16.2
Sex [n (%)]	
Male	41 (42)
Female	56 (57)
Oesophagitis	10 (10)
Hiatal hernia	17 (17)
Gastritis	46 (47)
Gastric ulcer	12 (12)
Duodenitis	20 (20)
Duodenal ulcer	15 (15)
Normal	16 (16)
Other	10 (10)

CUTest was missing and 14 patients were considered as not evaluable because of a discrepancy between the CUTest and histology. The *H. pylori* status of those patients by the various diagnostic modalities is summarized in Table 2.

The gold standard identified the presence of *H. pylori* in 46 patients (47.4%). Chronic antral inflammation was found in all of the *H. pylori*-positive patients, with a similar prevalence to the gold standard. Complete agreement was observed between the gold standard test and the BreathID in 96.9% (94 out of 97) of patients (45 positive and 49 negative). Disagreement was found in three patients. Two were negative by the gold standard and positive by BreathID and one was found to be *H. pylori*-positive by the gold standard, and negative by the urea breath test. Table 3 shows the

sensitivity, specificity, and positive and negative predictive values of BreathID when compared with the gold standard. The sensitivity of BreathID was 97.8% and the specificity 96.1%.

Forty patients who initially had positive tests for *H. pylori* were studied again after 4–6 weeks, to monitor the efficacy of the treatment. They were included in a follow-up post therapy monitoring of *H. pylori* eradication. The correlation between BreathID and isotope ratio mass spectrometry breath tests in those patients was 100% and the eradication success rate was 82.5%.

DISCUSSION

Since first described by Graham *et al.*, the reported sensitivities and specificities of both ^{14}C - and ^{13}C -labelled urea breath tests are in the 90–100% range.^{9–13} The results of the present study show that the new Oridion BreathID urea breath test for the detection of *H. pylori* has comparable sensitivity and specificity to the currently available urea breath tests. Our results are compatible with previous data showing that both the sensitivity and specificity of BreathID are comparable to mass spectrometry (Micromass, UK), when measured at 18 min.¹⁴

In the current study the prevalence of *H. pylori*, as determined by a gold standard, by positive urease test and the identification of *H. pylori* in Giemsa stain, was 47.4% (46 out of 97). Our results also showed a clear association between the presence of *H. pylori* and chronic gastritis (data not shown) in agreement with

Histology*	<i>H. pylori</i> colonization	CUTest	Oridion BreathID	Patient
Ac.CG	Mild	Negative	Negative	1
Ac. CG, AG, IM	Mild	Negative	Negative	2
Ac. CG, IM	Mild	Negative	Negative	3
Ac. CG, AG, IM	Mild	Negative	Negative	4
Normal	Mild	Negative	Negative	5
Ac. CG, IM	Mild	Negative	Negative	6
Ac. CG	Mild	Negative	Negative	7
Ac. CG, AG, IM	Marked	Negative	Negative	8
Ac. CG, IM	Moderate	Negative	Positive	9
Ac. CG	Moderate	Negative	Positive	10
Ac. CG, AG, IM	Moderate	Negative	Positive	11
Ac. CG	Marked	Negative	Positive	12
Ac. CG	Marked	Negative	Positive	13
Ac. CG	Absent	Positive	Positive	14

* Ac.CG, active chronic gastritis AG, atrophic gastritis; IM, intestinal metaplasia.

Table 2. The presence of *H. pylori* by various diagnostic modalities in 14 patients that were excluded because of discrepancy between histology and CUTest

Table 3. Performance characteristics of the new urea breath test, BreathID, in symptomatic patients

Test	BreathID
Sensitivity	97.8
Specificity	96.1
PPV	95.7
NPV	98.0

PPV, positive predictive value; NPV, negative predictive value.

earlier reports.^{15, 16} The Oridion BreathID evaluated in our study had a sensitivity and negative predictive value of 97.8% and 98%, respectively. False-positive BreathID results were obtained in two patients. In the elderly, biopsy-based tests may be less sensitive than in younger patients.¹⁷ This may be due to sampling errors or the patchy distribution of *H. pylori* in the stomach—perhaps connected with an age-related atrophy or intestinal metaplasia of the gastric mucosa.^{18, 19} In our definition of the gold standard, failure of the biopsy methods to detect the organism may decrease the urea breath test true-positives and increase the false-positives.

The reported sensitivity and specificity of IgG serology is highly variable, ranging from 30% to 100%.^{20–24} Moreover, antibody titres against *H. pylori* may be high up to several months after successful eradication, thus limiting the use of serology in the efficient evaluation of *H. pylori* after treatment.²⁵ There is also doubt about the accuracy of serological tests in different ethnic populations.²⁶ However, serology still remains the most extensively used non-invasive test because the ¹³C-urea breath test is relatively expensive, and time consuming—in many medical centres it is necessary to collect, store, and transport the breath test samples to a central isotope ratio mass spectrometer or gas chromatography laboratory. Several novel technologies for the measurement of ¹³CO₂ were developed recently in order to reduce the high cost of the isotope ratio mass spectrometry, which is still not widely available. Indeed, the non-dispersive isotope-selective infrared spectrometry, and laser assisted ratio analyser tests are being considered as alternatives to isotope ratio mass spectrometry.^{27–29} These two systems require the patient's breath to be collected into vacutainer tubes and aluminized bags. These usually show two time points, and are collected manually. These protocols may cause problems for geriatric and

paediatric patients who do not have the lung capacity to fill the containers. Furthermore, samples may be confused during measurement when performed manually or stacked during automatization. Another limitation to these methods is that the results of non-real time measurements may be inconclusive if they are close to the threshold. The continuous monitoring of ¹³CO₂ by BreathID appears to have great potential in solving many disadvantages of the currently available urea breath tests, by giving real time, accurate and quick results at a reasonable cost. It should be emphasized that this new system may also have some negative aspects. For example, the BreathID cannot be used for gastroenterological centres requiring a large quantity of automated analysis. It also does not have yet the facility for the evaluation of mailed or transported samples, thus patients need to be present in the site where this system is allocated.

With regard to the cost of Oridion BreathID compared to the other commercially used urea breath test devices, such as laser assisted ratio analyser or isotope ratio mass spectrometry, which are in use at all the major test centres in Europe and in USA, it should be emphasized that the device is not yet approved either in the US or in Europe. The manufacturers expect registration in the US soon. The final price of the tests will be established after obtaining the Health Authorities' approvals and will be adjusted so that the test cost will be completely covered by the reimbursement which is given for these tests by the medical insurance organizations/companies.

Based on our data, we conclude that the Oridion BreathID urea breath test for the detection of *H. pylori* has comparable sensitivity and specificity to the currently available urea breath tests. Furthermore, BreathID has the advantages of ease of use, with minimal medical staff requirements, real time rapid results (20 min maximum), which may be further reduced when a conclusive positive or negative case of *H. pylori* has been identified before this time, and a reasonable cost. All these factors may make the BreathID preferable to other urea breath test assays.

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