

## *A rapid, low-dose, <sup>13</sup>C-urea tablet for the detection of Helicobacter pylori infection before and after treatment*

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### SUMMARY

**Background:** A new urea breath test (UBT) has been described which uses a tablet formulation of <sup>13</sup>C-urea with citric acid and allows breath sampling to be performed as early as 10 min after ingestion of the tablet.

**Aim:** To assess the diagnostic accuracy of tablet-based <sup>13</sup>C-UBTs (50 and 100 mg <sup>13</sup>C-urea) before and after *Helicobacter pylori* eradication treatment, compared with an endoscopy gold standard and a conventional <sup>13</sup>C-UBT (75 mg <sup>13</sup>C-urea).

**Methods:** Two hundred dyspeptic patients underwent endoscopy, followed by tablet-based <sup>13</sup>C-UBTs (50 and 100 mg <sup>13</sup>C-urea) and a conventional <sup>13</sup>C-UBT (75 mg <sup>13</sup>C-urea). *H. pylori*-infected patients were prescribed treatment and asked to return 4–6 weeks after the end of therapy for repeat endoscopy and <sup>13</sup>C-UBTs.

**Results:** One hundred and thirteen patients were infected with *H. pylori*. The sensitivity and specificity of the conventional <sup>13</sup>C-UBT were both 100%; the sensitivity and specificity of the 100-mg tablet-based <sup>13</sup>C-UBT were 100% and 98.85%, respectively. For the 50-mg tablet-based <sup>13</sup>C-UBT, cut-off values of the difference over baseline of between 1.65 and 3.15 provided a sensitivity and specificity of 100%. At follow-up, the sensitivity and specificity of the conventional and 100-mg tablet-based <sup>13</sup>C-UBTs were both 100%. For the 50-mg tablet-based <sup>13</sup>C-UBT, cut-off values of the difference over baseline of between 1.49 and 1.56 gave a sensitivity and specificity of 100%.

**Conclusions:** New 10-min <sup>13</sup>C-UBTs using tablet formulations of <sup>13</sup>C-urea with citric acid are reliable for the assessment of *H. pylori* status pre- and post-treatment.

### INTRODUCTION

The <sup>13</sup>C-urea breath test (<sup>13</sup>C-UBT) is a standard non-invasive method for the detection of *Helicobacter pylori* infection. It is accurate for both the initial diagnosis of *H. pylori* infection and for confirming the eradication of the organism after therapy. Since its first description, when 350 mg of <sup>13</sup>C-urea was used,<sup>1</sup> this test has been

extensively modified, including variations in the dose of labelled urea, sampling time, test meal and cut-off values. These modifications have been designed to reduce the duration of the test, improve its accuracy and reduce the amount of expensive substrate (<sup>13</sup>C) used in the test. Tests employing a dose of 75 mg of <sup>13</sup>C-urea have proved to be as accurate as those using larger amounts, and are less expensive. This dose has been increasingly adopted in research studies, as well as in most of the commercially available diagnostic kits.<sup>2</sup> Another area in which improvements in the conventional breath test are possible is in the duration of the

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test. Many breath test protocols require a baseline sample plus another sample obtained 30 min after ingestion of  $^{13}\text{C}$ -urea. Conventional liquid-based  $^{13}\text{C}$ -UBT protocols use a test meal to delay gastric emptying and to cause an even distribution of  $^{13}\text{C}$ -urea throughout the stomach. The longer the duration of the test, the more inconvenient it is for the patient, and therefore attempts have been made to decrease the duration of the test. Recently, a new type of  $^{13}\text{C}$ -UBT has been described using a tablet formulation of  $^{13}\text{C}$ -urea, which allows breath sampling to be performed as early as 10 min after ingestion of the tablet.<sup>3</sup> The tablet consists of a combination of  $^{13}\text{C}$ -urea and citric acid, formulated to disintegrate and dissolve rapidly in the stomach.

The aim of this study was to assess the diagnostic accuracy of tablet-based  $^{13}\text{C}$ -UBTs (50 and 100 mg of  $^{13}\text{C}$ -urea plus citric acid) in the initial diagnosis of *H. pylori* infection and in the confirmation of eradication, compared with a biopsy-based gold standard and a conventional  $^{13}\text{C}$ -UBT using 75 mg of  $^{13}\text{C}$ -urea.

## MATERIALS AND METHODS

### *Patients and endoscopy*

This was a prospective, blind, comparison study. A total of 200 consecutive dyspeptic patients (87 men and 113 women; mean age, 53 years; s.d., 15 years) were studied prospectively between December 2000 and August 2001. Dyspepsia was defined as pain or discomfort in the upper abdomen.<sup>4</sup> All patients had suffered from symptoms for at least 2 months. The patients had not been investigated or treated previously for *H. pylori* infection. None of the patients had taken antibiotics, bismuth preparations or antisecretory drugs ( $\text{H}_2$ -antagonists or proton pump inhibitors) during the 4 weeks before endoscopy. All endoscopies were performed by the same investigator (DV) using an Olympus GIF 100 video-endoscope. At endoscopy, six biopsy samples were obtained. Two biopsies were taken from the antrum and two from the corpus for histology. One sample from the antrum was obtained for culture (performed on selective blood agar), and one sample was obtained from the antrum for rapid urease test. After endoscopy, all patients underwent the following  $^{13}\text{C}$ -UBTs: (i) a 50-mg tablet-based  $^{13}\text{C}$ -UBT on the first day after endoscopy; (ii) a 100-mg tablet-based  $^{13}\text{C}$ -UBT on the third day after endoscopy; and (iii) a conventional 75-mg  $^{13}\text{C}$ -UBT on

the fifth day after endoscopy. According to the guidelines of the European *Helicobacter pylori* study group, infected patients were given 1-week triple therapy with omeprazole, 10 mg twice daily, amoxicillin, 1 g twice daily, and clarithromycin, 500 mg twice daily.<sup>5</sup> Endoscopy was repeated 4–6 weeks after the end of treatment, as were the  $^{13}\text{C}$ -UBTs, using the same schedule as before treatment. Patients were classified as infected with *H. pylori* at baseline if the rapid urease test and histology were positive and/or if the culture of gastric biopsy specimens was positive. All other patients were classified as negative. Patients were classified as having successful eradication at follow-up only if all three tests were negative. These criteria have been recommended by an expert panel for use in clinical trials of *H. pylori* eradication.<sup>6</sup>

### *Histology and bacterial culture*

Histological biopsies were stained with haematoxylin and eosin plus Giemsa stains, and gastritis was scored using the updated Sydney system.<sup>7</sup> The pathologist who performed the histological examinations (CR) was blind to the results of all the other tests carried out. Biopsies collected for bacterial culture were streaked on to Columbia agar enriched with 5% horse blood and containing vancomycin, trimethoprim, polymixin B and nalidixic acid to inhibit the growth of microbes other than *H. pylori*. The plates were incubated in a micro-aerobic environment at 37 °C for 7 days, and inspected daily from the third day. The isolates were identified by Gram stain and by oxidase, catalase and urease tests. The microbiologist who performed bacterial cultures (FP) was blind to the results of all the other tests carried out.

### *$^{13}\text{C}$ -UBT protocols*

*Conventional breath test.* Breath tests were carried out after an overnight fast. For the conventional  $^{13}\text{C}$ -UBT (HelicoKit 75 mg, Italchimici, Pomezia (Rome), Italy), citric acid (1.5 g) as test meal and 75 mg of  $^{13}\text{C}$ -urea as water solution were given to the patients after collection of a baseline sample, obtained by blowing through a disposable plastic straw into a 20-mL container; a further breath sample was collected 30 min later. The breath samples were analysed by a gas isotope ratio mass spectrometer (Finnigan, Bremen, Germany), and were considered to be positive if there was a greater than 5‰  $^{13}\text{C}$  difference over baseline (DOB), according to the manufacturer's recommendations.

**Tablet-based breath tests.** Breath tests were carried out after an overnight fast. For the 100-mg tablet-based <sup>13</sup>C-UBT, two tablets [each containing 50 mg of <sup>13</sup>C-urea with anhydrous citric acid (456 mg)] were used (Diabact AB, Uppsala, Sweden). For the 50-mg tablet-based <sup>13</sup>C-UBT, a single tablet (containing 50 mg of <sup>13</sup>C-urea with anhydrous citric acid (456 mg)) was used. The tablets were swallowed, together with 200 mL of tap water, after collection of a baseline sample, obtained by blowing through a disposable plastic straw into a 20-mL container. A further breath sample was collected 10 min later.

### Analysis

All the breath samples from the tablet-based <sup>13</sup>C-UBTs were analysed by a gas isotope ratio mass spectrometer (Finnigan, Bremen, Germany); the 100-mg tablet-based <sup>13</sup>C-UBTs were considered to be positive if there was a greater than 1.5‰ <sup>13</sup>CO<sub>2</sub> DOB, according to the manufacturer's recommendations. For the 50-mg tablet-based <sup>13</sup>C-UBT, there were no manufacturer's recommendations, and we obtained the best cut-off values in pre- and post-treatment samples using receiver operating characteristic curve (ROC) analysis. The conventional and tablet-based <sup>13</sup>C-UBTs pre- and post-treatment were performed by the same investigator (AT), who was unaware of the results of the histological and bacteriological examinations and rapid urease test.

### Statistics

The sensitivity, specificity and likelihood ratios for positive and negative tests were calculated using the methods recommended by Altman.<sup>8</sup> As they are dependent on the prevalence of infection, the positive and negative predictive values were not calculated, because they are not indicative of the values that might be observed in other clinical settings. The mean,

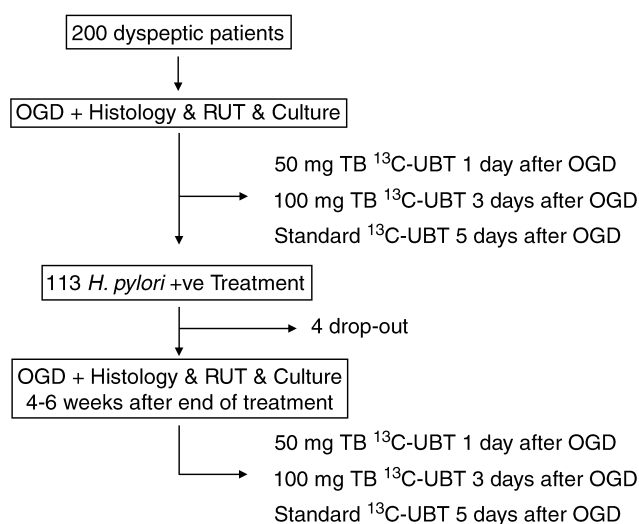


Figure 1. Study design. OGD, oesophagus gastroduodenal endoscopy; RUT, rapid urease test; TB <sup>13</sup>C-UBT, tablet-based <sup>13</sup>C-urea breath test.

standard deviation (s.d.) and non-parametric ROC analysis of the three different <sup>13</sup>C-UBTs performed were evaluated using Intercooled STATA 6 for Windows (College Station, TX, USA).

### Ethical committees

All patients gave written informed consent. The protocol was approved by the ethics committee of S. Orsola Hospital.

### RESULTS

One hundred and thirteen patients were infected with *H. pylori* according to the gold standard [prevalence rate of 56%; 95% confidence interval (CI), 49–63]. At endoscopy, the following observations were noted: gastric ulcers ( $n = 5$ ), duodenal ulcers ( $n = 15$ ), oesophagitis ( $n = 34$ ) and normal ( $n = 146$ ). Following

Table 1. Diagnostic accuracy of the <sup>13</sup>C-urea breath tests (<sup>13</sup>C-UBT) before treatment

	TP	FN	TN	FP	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	LR+ve (95% CI)	LR–ve (95% CI)
75-mg <sup>13</sup> C-UBT*	113	0	87	0	100 (96.7–100)	100 (95.7–100)	—	—
100-mg TB <sup>13</sup> C-UBT†	113	0	86	1	100 (96.7–100)	98.85 (93.77–99.80)	87 (16.05–492)	0 (0.00–0.03)

CI, confidence interval; FN, false negative; FP, false positive; LR+ve, likelihood ratio for a positive test; LR–ve, likelihood ratio for a negative test; TP, true positive; TN, true negative; TB, tablet-based.

\* Compared with endoscopy/biopsy gold standard.

† Compared with endoscopy/biopsy gold standard and 75-mg <sup>13</sup>C-UBT.

Table 2. Receiver operating characteristic curve (ROC) analysis of  $^{13}\text{C}$ -urea breath tests ( $^{13}\text{C}$ -UBT) before treatment\*

DOB	75-mg $^{13}\text{C}$ -UBT MR cut-off > 5 DOB			100-mg TB $^{13}\text{C}$ -UBT MR cut-off > 1.5 DOB			50-mg TB $^{13}\text{C}$ -UBT		
	≤ 3.10	3.11–6.84	≥ 6.85	≤ 1.51	1.52–2.99	≥ 3.00	≤ 1.64	1.65–3.15	≥ 3.16
Sensitivity (%)	100	100	< 100	100	100	< 100	100	100	< 100
Specificity (%)	< 100	100	100	< 100	100	100	< 100	100	100

DOB, difference over baseline; MR, manufacturer's recommendation; TB, tablet-based.

\* Compared with endoscopy/biopsy gold standard.

Table 3. Diagnostic accuracy of  $^{13}\text{C}$ -urea breath tests ( $^{13}\text{C}$ -UBT) after treatment

	TP	FN	TN	FP	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	LR+ve (95% CI)	LR-ve (95% CI)
75-mg $^{13}\text{C}$ -UBT*	23	0	86	0	100 (85.6–100)	100 (95.7–100)	—	—
100-mg TB $^{13}\text{C}$ -UBT†	23	0	86	0	100 (85.6–100)	100 (95.7–100)	—	—

CI, confidence interval; FN, false negative; FP, false positive; LR+ve, likelihood ratio for a positive test; LR-ve, likelihood ratio for a negative test; TP, true positive; TN, true negative; TB, tablet-based.

\* Compared with endoscopy/biopsy gold standard.

† Compared with endoscopy/biopsy gold standard and 75-mg  $^{13}\text{C}$ -UBT.

Table 4. Receiver operating characteristic curve (ROC) analysis of  $^{13}\text{C}$ -urea breath tests ( $^{13}\text{C}$ -UBT) after treatment\*

DOB	75-mg $^{13}\text{C}$ -UBT MR cut-off > 5 DOB			100-mg TB $^{13}\text{C}$ -UBT MR cut-off > 1.5 DOB			50-mg TB $^{13}\text{C}$ -UBT		
	≤ 4.88	4.89–7	≥ 7.01	≤ 1.44	1.45–7.36	≥ 7.37	≤ 1.48	1.49–1.56	≥ 1.57
Sensitivity (%)	100	100	< 100	100	100	< 100	100	100	< 100
Specificity (%)	< 100	100	100	< 100	100	100	< 100	100	100

DOB, difference over baseline; MR, manufacturer's recommendation; TB, tablet-based.

\* Compared with endoscopy/biopsy gold standard.

eradication therapy, 109 of the 113 *H. pylori*-positive patients were available for re-examination and 23 were still infected according to the gold standard (eradication rate, 78.9%; 95% CI, 70.3–85.5). At endoscopy, all the ulcers had healed and there were no pathological findings. Four patients refused to return for follow-up examination. Figure 1 shows the study design.

#### Performance of the conventional and tablet-based breath tests before eradication therapy

Table 1 shows the performance of the breath tests. Compared with the gold standard, the conventional  $^{13}\text{C}$ -UBT had a sensitivity of 100% (95% CI, 96.7–100) and a specificity of 100% (95% CI, 95.7–100). The

sensitivity of the 100-mg tablet-based  $^{13}\text{C}$ -UBT was 100% (95% CI, 95.7–100) and its specificity was 98.85% (95% CI, 93.77–99.80; Table 1).

ROC analysis (Table 2) demonstrated that a cut-off value for DOB of between 3.11 and 6.84 had a sensitivity and specificity of 100% for the conventional  $^{13}\text{C}$ -UBT. ROC analysis also established that the cut-off value from the manufacturer for the 100-mg tablet-based  $^{13}\text{C}$ -UBT yielded one false positive result (with DOB = 1.51) (Table 2). Selection of a cut-off value of between 1.52 and 2.99 would result in a sensitivity and specificity of 100%. For the 50-mg tablet-based  $^{13}\text{C}$ -UBT, ROC analysis determined that, in these patients, a cut-off value for DOB of between 1.65 and 3.15 would result in a sensitivity and specificity of 100% (Table 2).

*Performance of the conventional and tablet-based breath tests after eradication therapy*

The conventional breath test had a sensitivity and specificity of 100% in the post-treatment setting (95% CI, 85.6–100 and 95.7–100, respectively; Table 3). The sensitivity and specificity of the 100-mg tablet-based <sup>13</sup>C-UBT were also 100% (95% CI, 85.6–100 and 95.7–100, respectively; Table 3).

ROC analysis showed that the cut-off values for the conventional <sup>13</sup>C-UBT, as well as for the 100-mg tablet-based <sup>13</sup>C-UBT, were optimal (Table 4). For the 50-mg tablet-based <sup>13</sup>C-UBT, ROC analysis determined that, in these patients, a cut-off value for DOB of between 1.49 and 1.56 would result in a sensitivity and specificity of 100% (Table 4).

## DISCUSSION

Non-invasive testing for *H. pylori* is recommended for dyspeptic patients in primary care.<sup>5</sup> Serological tests have fallen out of favour because they have a high false positive rate,<sup>9</sup> and active methods of testing are currently recommended. Available active tests include the stool antigen test and UBT. Both tests have been shown to be accurate in the initial evaluation of *H. pylori* infection and in the confirmation of eradication after treatment.<sup>10</sup> The choice of a test in the clinical setting depends not only on the cost of the test, but also on the ease with which the test can be administered, read and interpreted. The conventional UBT is sensitive and specific, but takes 45 min to complete and is generally performed using 75 or 100 mg of <sup>13</sup>C-urea, an expensive substrate that increases the cost of the test.<sup>11</sup> The stool antigen test is a polyclonal antibody test that detects *H. pylori* antigens that are shed in the stool. It is as sensitive and specific as the UBT.<sup>12</sup> The disadvantage with this test is that the stool must be collected by the patient, which some find disagreeable.

Attempts to improve the UBT have focused on reducing the duration of the test and decreasing the amount of substrate used in order to reduce the cost of the test. The tablet used in this study is a proprietary preparation that is designed to disintegrate rapidly in the stomach, and includes citric acid which has been shown to improve the accuracy of the test.<sup>3</sup> There are advantages of supplying urea in a quick-dissolve capsule rather than as a conventional drink.<sup>13</sup> When administered as a drink, interference from urease-producing

bacteria in the oro-pharynx may cause false positive results in early breath tests.<sup>14, 15</sup> Therefore, most UBT protocols do not obtain the diagnostic breath sample until 20–30 min after administration. In contrast, when using a capsule or tablet, the problem of oropharyngeal bacteria is eliminated.

The results of this study demonstrate that a 10-min tablet-based <sup>13</sup>C-UBT, using 50- or 100-mg doses of <sup>13</sup>C-urea, is sensitive and specific for the detection of *H. pylori* infection before and after treatment. The short duration of the test is an advantage over current methods and the tablet formulation allows the test to be performed easily in a doctor's office. The manufacturer's recommendation for the cut-off of the 100-mg test, pre-treatment, yielded an excellent sensitivity (100%; 95% CI, 96.7–100) and a very high specificity (98.85%; 95% CI, 93.77–99.8). In the post-treatment setting, the performance was also excellent, with a sensitivity and specificity of 100%. Based on ROC analysis in our series, for the 50-mg tablet-based <sup>13</sup>C-UBT before treatment, a cut-off value for DOB of between 1.65 and 3.15 results in an excellent sensitivity (100%) and specificity (100%). In the post-treatment setting, a cut-off value for DOB of between 1.49 and 1.56 results in a sensitivity and specificity of 100%. Indeed, a value of DOB > 1.5 in our series would produce no false negative results (sensitivity of 100%; 95% CI, 96.7–100) and only one false positive result (specificity of 98.9%; 95% CI, 93.8–99.8) pre-treatment, with no false negative or false positive results after treatment. It should be noted that our estimates of accuracy are based on a cut-off value which is optimal for our series. These estimates might not necessarily be optimal for other series of patients, and the quoted values should be interpreted by taking into account the confidence intervals. Nevertheless, the population included in this study contained a reasonable spectrum of patients in whom the diagnostic tests would be applied in clinical practice. Furthermore, case-control design, work-up bias and expectation bias, which are well-recognized causes of over-estimations of the diagnostic performance, were avoided in this study.<sup>16, 17</sup>

As a commercial price for the 50-mg tablet has not yet been determined, a formal cost analysis for the 50-mg tablet-based <sup>13</sup>C-UBT is not possible. Several estimates are, however, possible. In the determination of *H. pylori* status before eradication therapy, the sensitivity and specificity of all the tests were 100%. The choice of test will therefore depend on the cost and convenience. The

tablet-based tests are more convenient because of the short duration of the test (10 min compared with 30 min). A substantial portion of the cost of the  $^{13}\text{C}$ -UBT is related to the substrate ( $^{13}\text{C}$ -urea), and a reduction in dose should result in a reduction in the cost of the test. In summary, tablet-based  $^{13}\text{C}$ -UBTs in our patients are sensitive and specific for the detection of *H. pylori*.

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