

Simplified single sample ¹³C Carbon urea breath test for *Helicobacter pylori*: comparison with histology, culture, and ELISA serology

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Abstract

There is no ideal method for detecting *Helicobacter pylori*. The 'standard' ¹³C carbon urea breath test (¹³C-UBT), which involves collecting eight to 15 breath samples and subsequent costly analysis, was modified by pooling 2 l samples of expired breath taken at five minute intervals for 40 minutes into a collecting bag, from which a single 20 ml aliquot was taken and analysed by mass spectrometry. This test was evaluated on 50 patients after routine upper gastrointestinal endoscopy, and results were compared with those from the standard ¹³C-UBT, bacteriology, ELISA serology, and histology - the latter being taken as the gold standard. *H pylori* were seen in 34 of 50 (68%) patients (in three it was detected in biopsy specimens from the corpus alone). The modified ¹³C-UBT was positive (pooled excretion $\delta^{13}\text{CO}_2 > 5$ per mil) in 31 patients and negative in 19 (three false negative results), specificity was 100% (standard ¹³C-UBT 94%) and sensitivity 92% (standard ¹³C-UBT 93%). The modified ¹³C-UBT had a coefficient of variation within subjects of 3.7%. For the ELISA serology and culture the specificities were both 100%, but the sensitivities were 82% and 68% respectively. The ¹³C-UBT results correlated with the grade of histological gastritis. The modified ¹³C-UBT is simpler, cheaper, more reproducible, and provides an easy non-invasive method for the detection of *H pylori*.

subject (helpful for the serial monitoring of treatment), and makes it ideal for epidemiological studies, particularly as it can be used in children and women of child bearing age and because no radiation is involved, a licence is not required.

Since the initial report of the ¹³C-UBT,⁸ however, the technique has not been further refined; little is known of its reproducibility and as multiple samples are required to produce an excretion curve, interpretation of results may be difficult and the test costly. Therefore, the test has so far remained essentially a research tool.

We describe a modified protocol for the ¹³C-UBT and results of reproducibility studies, which together should make this test for *H pylori* easier to use in routine clinical practice.

Patients and methods

Fifty patients attending for routine upper gastrointestinal endoscopy were invited to enter the study, which had Parkside District Health Authority ethical committee approval. The patients gave written informed consent. Height, weight, and respiratory function (measured by peak flow, forced vital capacity, and forced expiratory volume in one second) were recorded. Exclusion criteria were recent (within two months) use of bismuth containing compounds, antibiotics known to be active in vivo against *H pylori*, or previous resective gastric surgery.

Endoscopy

Endoscopy (Olympus GIF1T20) was performed under intravenous sedation (diazepam or midazolam), and endoscopic findings were recorded. Four biopsy specimens were taken from the antrum and two from the body of the stomach. Biopsy forceps (Olympus FB13K) were sterilised by autoclaving and endoscopes were disinfected between patients, as recommended,¹³ using an automatic washing machine (KeyMed EW20). At endoscopy, 10 ml of blood were taken, and the serum was separated and stored at -70°C.

Histology

Two biopsy specimens from the antrum and two from the gastric body were processed routinely, embedded in paraffin wax, and stained with haematoxylin and eosin and by the Gimenez technique.¹⁴ Every specimen was examined by the same experienced histopathologist (MW) without knowledge of the results of the other

Helicobacter pylori infection of the human gastric mucosa is now recognised as the cause of type B (non-autoimmune) gastritis¹ and an important factor in duodenal ulceration.² The diagnosis is usually established by histological examination or culture of gastric biopsy specimens,^{3,4} although non-invasive serological methods of detection have been described.^{4,5} *H pylori* produces a powerful urease and this provides the basis for its detection in endoscopic biopsy specimens by the CLO test (and other similar colorimetric methods),^{6,7} and non-invasively by the labelled carbon urea breath test.^{8,12} In the latter, labelled carbon dioxide released by hydrolysis of labelled urea by the urease is collected serially from exhaled breath and the labelled fraction of carbon dioxide is measured.

Although mass spectrometry is needed for the ¹³C-urea breath test (¹³C-UBT), this technique has advantages over tests using radioactive ¹⁴C-urea.⁹⁻¹¹ Thus, ¹³C-urea is a non-radioactive isotope that allows repeated tests in the same

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tests. Gastritis was classified according to Whitehead¹⁵ as chronic, chronic atrophic, active atrophic, or acute, and severity was graded 1 (mild), 2 (moderate), or 3 (severe). The number of *H pylori* was assessed and scored as 0 (none), 1+ (few), 2+ (moderate), 3+ (many), or 4+ (teeming). The histological results were taken as the reference gold standard.

MICROBIOLOGY

Two antral biopsy specimens were placed in brain heart infusion (BHI) broth and promptly cultured. Mucus was scraped from the specimens and plated onto selective media and non-selective blood agar for routine culture. Plates were incubated at 37°C microaerobically (Campybak, BBL, Cockeysville, USA) for up to six days. *H pylori* was identified by typical colony appearance and positive oxidase and urease tests. The growth of colonies was graded as 0 (none), 1+ (few), 2+ (moderate), or 3+ (many).

SEROLOGY

Serology was performed by ELISA using the acid glycine extract (flagellated) antigen as described previously,¹⁶ but using sera at a 1:200 dilution. The threshold for seropositivity was taken as 10 µg specific IgG/ml.

¹³C-UBT

The ¹³C-UBT was performed three hours after the endoscopy in all patients recruited to the study, and was repeated either 24 hours, one week, or more than one week later in the 21 patients able to attend for a repeat test. In six patients, the ¹³C-UBT was done on three consecutive days.

METHODS

Patients exhaled into a 2 l collecting bag. Using a needle and syringe, a 20 ml baseline sample was aspirated from the bag and stored in a 20 ml vacutainer (Beckton-Dickinson). A fatty test meal (one sachet of Complian, 50 ml of semi-skimmed milk, and Calogen 100 ml) to delay gastric emptying of the isotope, was taken 12 minutes before ¹³C-urea (125 mg dissolved in 30 ml of sterile water), which was drunk at time zero. Distribution within the stomach was aided by turning the patient onto each side and head down, each for two minutes.

STANDARD COLLECTION OF SAMPLES FOR THE

¹³C-UBT

At 10, 20, 30, 40, and 60 minutes, patients filled a 2 l collecting bag with expired breath from which duplicate 20 ml aliquots were taken into 20 ml vacutainers.

SIMPLIFIED COLLECTION OF SAMPLES FOR THE

¹³C-UBT

Concurrently, and at five minute intervals from 10 to 40 minutes, patients filled a second 2 l collecting bag, the entire contents of which were

then expelled into a large reservoir bag. At the end of the test two 20 ml duplicate aliquots were aspirated from the reservoir bag and stored in two 20 ml vacutainers.

The ratio of ¹³CO₂ to ¹²CO₂ in the expired air samples was measured by mass spectrometry, with cryogenic extraction of the CO₂, using a dual inlet ratio mass spectrometer (VG SIRA II, BSIA, Brentford, Middlesex, UK). Results were expressed as parts per thousand (per mil) excess δ¹³CO₂ (by subtraction of the baseline pre-¹³C-urea breath sample), and were compared with the reference gold standard. For both the modified and standard ¹³C-UBT, a positive test was defined as any breath sample with a value more than 3 SD above the mean value of the ¹³C-UBT for those patients without *H pylori* as determined by the gold standard.

ANALYSIS OF RESULTS

All procedures were done by the same operators, without knowledge of the patients' *H pylori* status by other methods. The results of histology were taken as the gold standard and compared with the results of culture, serology, modified, and standard ¹³C-UBT. The variables recorded were subjected to statistical correlation and multivariate analysis. Reproducibility was determined by calculating the coefficient of variation within subjects using an analysis of variance.

Results

ENDOSCOPY

Fifty patients (26 men), median age 50.5 years (range 20–85) were recruited into the study. Indications for endoscopy were most commonly abdominal pain (34%), peptic ulcer follow up (23%), and dyspepsia (16%). The most frequent findings were macroscopic gastritis (33%), normal appearances (18%), or duodenal ulcer (12%).

¹³C-UBT

Thirty one patients with *H pylori* present on antral or gastric body histology had positive standard and modified ¹³C-UBTs (pooled excretion excess δ¹³CO₂>4.5 per mil). Fourteen patients with no *H pylori* seen on histology had negative standard and pooled breath tests (mean (SD) pooled excretion excess δ¹³CO₂=1.35 per mil (1.01), Fig 1). Three patients with positive histology had negative modified and standard ¹³C-UBT. However, in two of these three, a positive breath test was recorded on repeat testing within 24 hours and also in the third a week later. There were two false positive results with the standard ¹³C-UBT, each with the 10 minute breath sample, but none with the modified ¹³C-UBT.

Twenty one patients underwent serial ¹³C-UBTs, 12 within 24 hours of the first, and 11 more than six days after the first test. Six other patients had three breath tests on three consecutive days. The reproducibility of the standard ¹³C-UBT at 24 hours and at six days or longer (Table) was not as good as that of the modified

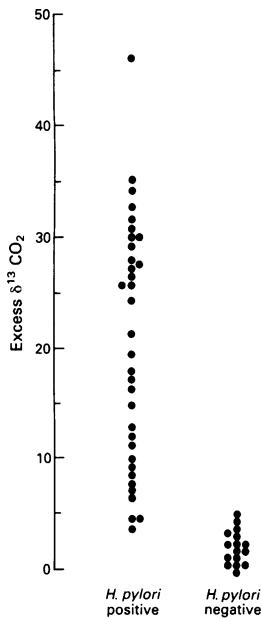


Figure 1: The excess $\delta^{13}\text{CO}_2$ per mil production as determined by the modified ^{13}C urea breath test (^{13}C -UBT) using a pooled breath sample for patients with and without *H. pylori* (determined by the result of two or more biopsy specimen based methods).

Reproducibility of the ^{13}C carbon urea breath test

Time (min)	Excess $\delta^{13}\text{CO}_2$ per mil	
	0 and 24 Hours	0 and >6 Days
10: mean (SD)	12.9 (2.9)	15.2 (9.7)
c of v (%)	22	64
20: mean (SD)	14.5 (3.7)	18.4 (7.8)
c of v (%)	26	43
30: mean (SD)	14.5 (3.3)	18.4 (7.3)
c of v (%)	23	40
40: mean (SD)	15.0 (3.3)	18.7 (7.6)
c of v (%)	22	41
60: mean (SD)	13.6 (2.0)	18.0 (4.2)
c of v (%)	15	24
Pooled sample: mean (SD)*	14.1 (0.5)	
c of v (%)	3.7	

*For all pooled samples.

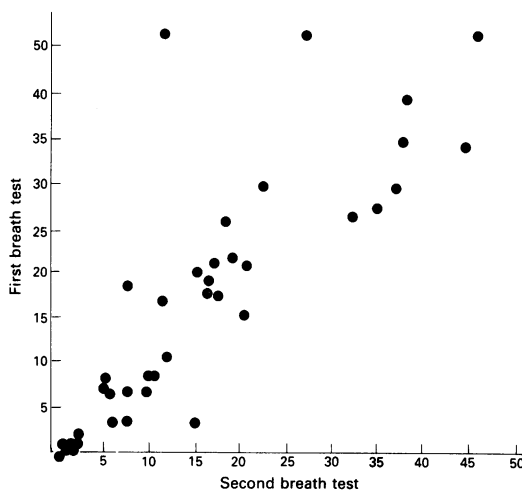


Figure 2: A scattergram showing the reproducibility of the modified ^{13}C urea breath test (^{13}C -UBT).

^{13}C -UBT, which had an overall excellent reproducibility (coefficient of variation = 3.7%, Fig 2).

There was a correlation between the histological grade of gastritis and the results of the modified breath test (Fig 3), but there was no correlation between the modified breath test result and the extent of colonisation of *H. pylori* as measured by either histology or culture. None of the correlations between height, weight, sex, forced vital capacity, or peak flow were statistically significant.

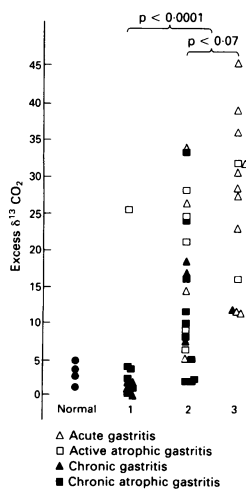


Figure 3: Relation between the excess $\delta^{13}\text{CO}_2$ per mil production and the histological type and grade of gastritis.¹⁵

HISTOLOGY

H. pylori was present in antral (n=31) or corpus (n=24) biopsy specimens in 34 of 50 patients (68%). In three patients, *H. pylori* was found in the corpus biopsy specimens alone. None of these three patients had histological appearances suggesting antral bile reflux gastritis¹⁷ or intestinal metaplasia.

Antral and body gastritis was recorded in all the patients with *H. pylori*. In the 16 patients with no *H. pylori*, four had normal histology and 12 had grade 1 or 2 chronic or chronic atrophic antral gastritis. None of these patients had histological evidence of intestinal metaplasia or bile reflux gastritis on antral biopsy specimen.¹⁷

MICROBIOLOGY

Culture of antral biopsy specimens was positive in 23 of the 34 patients with *H. pylori* seen on histology (specificity 100%), but failed to

identify 11 patients with *H. pylori* (sensitivity 68%). Two of the patients with false negative culture also had negative antral histology (but positive body biopsy specimens), while a further three patients had only scanty numbers of antral *H. pylori* seen on histology. There was no correlation between the extent of growth seen on culture with the result of the modified ^{13}C -UBT or with the number of organisms seen by light microscopy.

SEROLOGY

Serology correctly identified 28 patients with histological evidence of *H. pylori* (specificity 100%), but failed to identify six patients with positive antral histology (sensitivity 82%). Two of the six false negative serology results were also negative on culture.

Discussion

A major advantage of ^{13}C -UBT over the other methods of detection of *H. pylori* is the potential for frequent, multiple, non-invasive repeat assessments of *H. pylori* status. It is therefore important to develop, modify, and standardise this test into a cheap, easy, reproducible, non-invasive method for the detection of *H. pylori*. Since the first description of the ^{13}C -UBT, there have been only brief descriptions of alternative methods, mainly using different test meals or doses of isotope. This is the first study to compare the standard histological, bacteriological, and serological methods available for the detection of *H. pylori* with the standard ^{13}C -UBT (Graham),⁸ and with the new modified ^{13}C -UBT.

The standard ^{13}C -UBT used in this study has been shown to have a sensitivity and specificity similar to previous data.^{8,12} The new modified ^{13}C -UBT was as good as the standard method, but is much easier and cheaper to perform. In addition, the reproducibility of the new modified ^{13}C -UBT was greater than the standard method. Both methods had a sensitivity and specificity equivalent to antral histology and ELISA serology.

The lower dose of isotope used in this study, using half the amount of ^{13}C -urea used in the original method,⁸ has not affected the sensitivity or specificity of the test, and indeed even lower doses have been proposed.¹⁸ The use of a fatty meal theoretically minimises the influence of gastric emptying on the ^{13}C -UBT results, and in those patients who were *H. pylori* negative there was no late rise in $\delta^{13}\text{CO}_2$ excretion as a result of small bowel bacterial activity – a potential problem when using carbohydrate based meals. Previous studies have all expressed the results of the ^{13}C -UBT in slightly different ways, but as we have shown that body mass has no effect on the results, it is reasonable, as previously suggested,¹⁹ to express the results as excess $\delta^{13}\text{CO}_2$ excretion. This will also allow direct comparisons to be made between different centres using the ^{13}C -UBT without the need to standardise the mass spectrometers.

Every method for the detection of *H. pylori* has its own inherent advantages and disadvantages. Using antral histology as the sole gold standard,

there would have been three apparently false positive breath tests. However, an accurate assessment of the patients' *H pylori* status was made by additional histological examination of biopsy specimens taken from the body (two of these patients were also negative on culture). *H pylori* is most often found in the gastric antrum. However, any method of detection confined to antral biopsy specimen alone (especially in the presence of duodenogastric bile reflux, intestinal metaplasia, or low levels of colonisation by *H pylori*) is liable to sampling error, which may be decreased by taking multiple biopsy specimens from the antrum and elsewhere in the stomach. The modified ¹³C-UBT, however, provides an easier and alternative method for the detection of *H pylori* without the risk of sampling error.

Bacterial culture gave the lowest sensitivity of all the methods used, and while this also may be partly due to sampling error, it may also reflect the inherent technical difficulties associated with culture. Unlike Rauws's experience with the ¹⁴C-UBT we were unable to correlate the extent of *H pylori* growth in culture with the result of the breath test. This, as with Graham's report, may have been because of the size of our study. The modified ¹³C-UBT result, however, did correlate with the grade and type of gastritis. This may be a result of the residual histological response resolving more slowly after *H pylori* has migrated to colonise other areas of the stomach.

For any longitudinal study involving serial assessments it is important to know the extent to which results from the ¹³C-UBT may vary spontaneously with time. Results in the 21 patients with serial ¹³C-UBTs show that there is very little day to day variation, but over longer periods the variability increases. It is not yet known if these minor differences are a result of fluctuations in the bacterial load of *H pylori* or changes in urease activity.

The results of this study show that a simple modification of the ¹³C-UBT provides an accurate, non-invasive, sensitive means for the detection of *H pylori*, with results which are more easily interpretable and cheaper than the original method.

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