Evaluation of the Ez-HBT Helicobacter blood test to establish Helicobacter pylori eradication

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SUMMARY

Background: The urea blood test (Ez-HBT) has been shown to compare favourably with the urea breath test in the diagnosis of active *Helicobacter pylori* infection.

Aim: To examine the performance characteristics of the Ez-HBT Helicobacter blood test in establishing success or failure of therapy in *H. pylori*-infected adults using the ^{13}C urea breath test as the reference method.

Methods: ^{13}C urea breath test and Ez-HBT Helicobacter blood test were performed 4–6 weeks after completion of treatment in *H. pylori* positive subjects. Basal urea breath samples were collected; basal Ez-HBT Helicobacter blood test samples were not. Ez-HBT Helicobacter blood test results were reported as positive, negative, or indeterminate.

Results: Seventy patients generated 126 measurable sets of urea breath and blood tests. The *H. pylori* cure rate was 93%. The sensitivity, specificity, and accuracy of the Ez-HBT Helicobacter blood test were 100%, 97%, and 97%, respectively. Six of eight false positive and indeterminate Ez-HBT Helicobacter blood test results could be attributed to incomplete fasting or a ^{13}C enriched diet. After correcting for the non-fasting state, the positive predictive value of the Ez-HBT Helicobacter blood test improved from 56% to 86%.

Conclusion: The performance characteristics of the Ez-HBT Helicobacter blood test are comparable with that of ^{13}C-urea breath test in establishing *H. pylori* eradication after therapy. Errors related to incomplete fasting can be mitigated by collection of a basal blood sample.

INTRODUCTION

Currently recommended primary therapies for *Helicobacter pylori* yield eradication rates between 75% and 85%.[1] Because these therapies are far from perfect, testing to establish successful cure is recommended in a number of circumstances including uncomplicated and complicated peptic ulcer disease, low-grade gastric mucosa associated lymphoid tissue lymphomas, dyspeptic patients who remain symptomatic following *H. pylori* therapy, and in those who have not completed a full course of therapy.[2]

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Non-invasive tests such as the urea breath test (UBT)[3] and stool antigen tests[4–7] have been recommended as the preferred methods to establish *H. pylori* cure. Drawbacks of the UBT include the need for training in the collection, handling, transport, and storage of exhaled breath samples and the need for patient cooperation. The stool antigen test is easy to perform and can be used in children,[8, 9] however, variable sensitivity and specificity have been reported in the post-treatment setting.[6, 10, 11] Cross reactivity with antigens of other *Helicobacter* sp.[11], the effect of altered intestinal transit time, high fibre diets, and interactions with medications may potentially affect the results of this test.[12]

The Ez-HBT Helicobacter blood test (Ez-HBT), a test performed using serum ^{13}C-bicarbonate determination

in whole blood,[13] has been shown to compare favourably with the ^{13}C-UBT and endoscopic rapid urease testing in the diagnosis of active *H. pylori* infection with a specificity of 96% and a sensitivity of 89–92%.[14, 15] Since these published reports, the Food and Drug Administration (FDA) has approved the Ez-HBT with a modification to include an indeterminate category. In this study, we examined the performance characteristics of the FDA-approved Ez-HBT to accurately establish success or failure of therapy in *H. pylori*-infected adults using the ^{13}C-UBT as the reference method.

MATERIALS AND METHODS

Study patients

Adult subjects with treatment-naïve, active *H. pylori* infection determined by either histology, rapid urease testing, or ^{13}C-UBT, were eligible for this multi-centre out-patient study. Patients underwent *H. pylori* testing for either the evaluation of dyspeptic symptoms or upon finding peptic ulcer disease or gastritis on endoscopy. Exclusion criteria included use of bismuth or antibiotics within 30 days, proton pump inhibitor use within 14 days, renal or hepatic impairment, pathological hypersecretory syndromes, and inability to provide informed consent.

Enrolled patients were offered eradication therapy with two standard regimens: lansoprazole, tetracycline, pepto-bismol, and metronidazole or clarithromycin, amoxicillin, and lansoprazole. Patients underwent a ^{13}C-UBT and Ez-HBT 4–6 weeks after completion of therapy. Those with a negative ^{13}C-UBT underwent a second set of urea breath and blood tests 4–6 weeks later. Two successive negative ^{13}C-UBT results indicated successful treatment. Patients with a positive initial UBT were considered treatment failures and were treated with the alternative regimen after which they underwent two sets of urea breath and blood tests at 4 to 6-week intervals as described above. Patients with positive ^{13}C-UBT after retreatment were considered retreatment failures and were further treated at the discretion of their physicians.

Study procedures

After a 4-h fasting period, ^{13}C-UBT and Ez-HBT were performed according to the manufacturer's instructions by trained personnel at participating sites. Each study subject underwent baseline breath sample collection for the ^{13}C-UBT. According to current recommendations, basal blood samples were not collected for the Ez-HBT. Five minutes after drinking an 8 ounce can of Ensure (Abbot Laboratories, Abbot Park, IL, USA) to delay gastric emptying, study subjects ingested 125 mg of ^{13}C-urea (dissolved in 75 mL of sterile water). Thirty minutes after ingestion of the ^{13}C-urea, a 3-mL blood sample was obtained by standard venipuncture for the Ez-HBT and a breath sample was collected for the ^{13}C-UBT. ^{13}C-UBT and Ez-HBT samples were processed using gas isotope ratio mass spectrometry at a centralized location (Metabolic Solutions, Nashua, NH, USA).

For the UBT, breath $^{13}CO_2$ of delta ^{13}C over baseline >5 delta per mil indicated *H. pylori* infection. Ez-HBT results expressed as delta ^{13}C per mil (parts per thousand difference) relative to the international standard Pee Dee Belemnite (PDB) limestone were reported as positive (blood $^{13}CO_2$ level >−17.0 delta per mil), negative (blood $^{13}CO_2$ levels <−18 delta per mil), or indeterminate (blood $^{13}CO_2$ levels between −17 and −18 delta per mil).

Statistical analysis

We estimated, assuming an 80% eradication rate and that the UBT was 100% accurate in classifying cases, that at least 120 tests were required in the study to show that the urea blood test was greater than or equal to 90% accurate in detecting eradication, with an α risk of 0.05 and 80% power. Performance characteristics were calculated for the Ez-HBT using the ^{13}C-UBT as the gold standard in detecting *H. pylori* eradication. The sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of the Ez-HBT were determined. Only paired ^{13}C-UBT and Ez-HBT data were analyzed. Data sets with indeterminate Ez-HBT results were excluded from the initial analysis. This study was approved by the institutional review board at each participating site and all enrolled patients gave informed consent.

RESULTS

Seventy-four patients with active *H. pylori* infection (established by either histology, rapid urease testing or ^{13}C-UBT) were enrolled in the study. Seventy patients received *H. pylori* eradication therapy and underwent subsequent ^{13}C-UBT and Ez-HBT testing; data on these

Table 1. Characteristics of the 70 study subjects who received *Helicobacter pylori* therapy and underwent ^{13}C-urea breath test (^{13}C-UBT) and Ez-HBT testing

Age (years)	
Mean	62
Range	27–84
Gender *n* (%)	
Male	55 (79)
Female	15 (21)
Race *n* (%)	
Caucasian	57 (81)
African-American	13 (19)
Initial *H. pylori* diagnosis* *n* (%)	
One test	28 (40)
Two tests	42 (60)

* Histology, rapid urease testing, or ^{13}C-UBT.

70 patients is presented here. Active *H. pylori* infection was established by two diagnostic tests in 42 (60%) patients and by one diagnostic test in the remaining 28 (40%) patients (Table 1). Patient demographics are shown in Table 1. Success of eradication therapy was documented with the Ez-HBT using the ^{13}C-UBT for comparison as the gold standard. Five patients (7%) failed the first treatment regimen and three were retreated successfully giving an overall cure rate of 97% (Figure 1).

After *H. pylori* eradication therapy, 133 sets of urea breath and blood tests were generated (Figure 2). Fifty-eight subjects received two sets of paired urea breath and blood tests, 10 subjects received only pair of one urea breath and blood tests, one subject received three paired sets of tests, and one subject received four paired sets of tests. Four Ez-HBT tests had indeterminate results and there were three tests with no results (two UBTs had no breath gas, and one subject could not have blood drawn). These seven pairs of Ez-HBT and ^{13}C-UBT tests results were excluded, leaving 126 sets of measurable tests for analysis.

Using the ^{13}C-UBT as the diagnostic standard, there were five true positive (TP), 117 true negative (TN), four false positive (FP), and no false negative (FN) Ez-HBT results. The four indeterminate Ez-HBT results were excluded from the initial performance characteristic calculations. Results of the performance characteristics of the Ez-HBT are shown in Table 2.

The average basal breath $^{13}CO_2$ value in the four FP and the four indeterminate Ez-HBT patient groups were significantly higher than in the TN and TP groups ($P < 0.05$) (Table 3). Three of the four subjects with a FP Ez-HBT and three of the four indeterminate Ez-HBT test results had elevated basal breath $^{13}CO_2$, suggesting incomplete fasting or a ^{13}C-enriched diet. Ez-HBT performance characteristics were reanalyzed after correction for the non-fasting state by reassigning the six FP and indeterminate Ez-HBT test results from subjects with elevated basal breath $^{13}CO_2$, to the TN group. This resulted in improved performance characteristics of the Ez-HBT (Table 4); in particular, the positive predictive value increased from 56% to 86%.
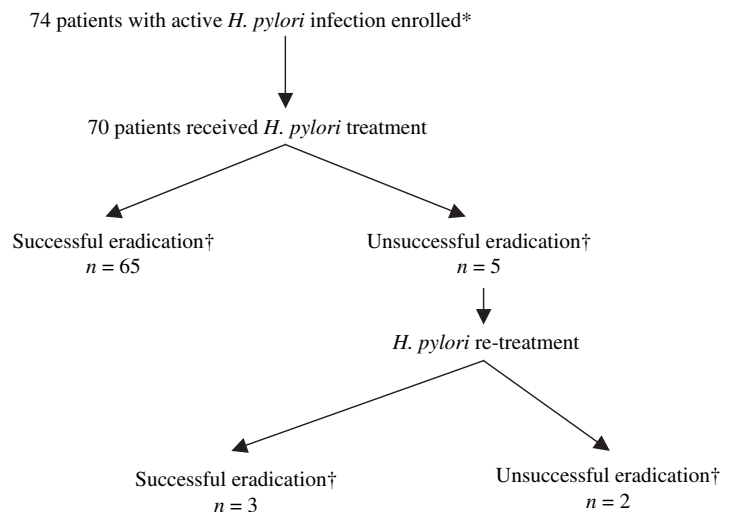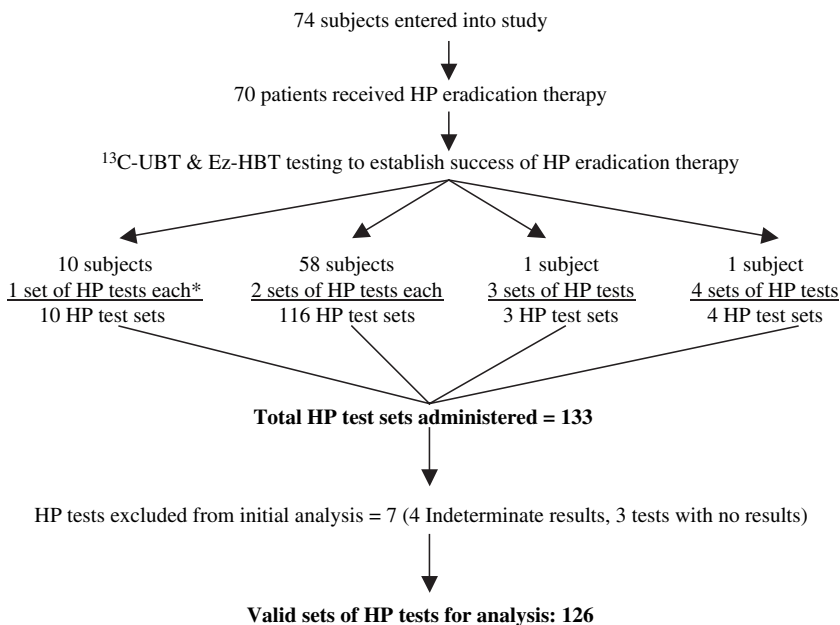
74 patients with active *H. pylori* infection enrolled*

↓

70 patients received *H. pylori* treatment

Successful eradication†
n = 65

Unsuccessful eradication†
n = 5

↓

H. pylori re-treatment

Successful eradication†
n = 3

Unsuccessful eradication†
n = 2

* Determined by histology, rapid urease testing, or ^{13}C-UBT

† Determined by ^{13}C-UBT testing

Figure 1. Schema of *Helicobacter pylori* eradication therapy.

74 subjects entered into study

↓

70 patients received HP eradication therapy

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^{13}C-UBT & Ez-HBT testing to establish success of HP eradication therapy

10 subjects	58 subjects	1 subject	1 subject
1 set of HP tests each*	2 sets of HP tests each	3 sets of HP tests	4 sets of HP tests
10 HP test sets	116 HP test sets	3 HP test sets	4 HP test sets

Total HP test sets administered = 133

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HP tests excluded from initial analysis = 7 (4 Indeterminate results, 3 tests with no results)

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Valid sets of HP tests for analysis: 126

* HP test set = ^{13}C-UBT and Ez-HBT

Figure 2. ^{13}C-urea breath test (^{13}C-UBT) and Ez-HBT testing to establish *Helicobacter pylori* eradication.

Table 2. Performance characteristics of the Ez-HBT compared with the ^{13}C-urea breath test as the reference

	n	%	95% CI
Sensitivity	5/5	100	100
Specificity	117/121	97	94–100
Positive predictive value	5/9	56	23–88
Negative predictive value	117/117	100	100
Accuracy	122/126	97	94–100

Table 3. Basal breath ^{13}C levels in the different Ez-HBT groups

Ez-HBT group	*n*	Mean	Median	s.d.
False positive + indeterminate	8	−20.09*	−19.84	±1.70
True negative	117	−22.77	−22.80	±1.15
True positive	5	−22.07	−21.76	±1.15

* Significantly different from true negative ($P = 0.003$) and true positive ($P = 0.031$) groups.

Table 4. Ez-HBT performance characteristics after correction for incomplete fasting

	n	%	95% CI
Sensitivity	6/6	100	100
Specificity	123/124	99	97–100
Positive predictive value	6/7	86	74–100
Negative predictive value	123/123	100	100
Accuracy	129/130	99	97–100

DISCUSSION

We have previously shown the utility of the Ez-HBT for the initial diagnosis of *H. pylori* infection.[14, 15] In this multi-centre study, we report similar sensitivity, specificity, negative predictive value and accuracy of the Ez-HBT compared with the ^{13}C-UBT in confirming the eradication of *H. pylori* infection after treatment. Advantages of the Ez-HBT are that it is easy to collect, store, and transport blood samples, and the test may be easier to use in uncooperative patients. However, like the ^{13}C-UBT, the Ez-HBT also requires the use of a mass spectrophotometer. Furthermore, like the ^{13}C-UBT and the stool antigen test, antibiotics and proton pump inhibitors may interfere with the accuracy of the Ez-HBT.[4]

The usefulness of the serum ^{13}C-bicarbonate test in confirming eradication of *H. pylori* after treatment was first demonstrated by Kim *et al.*[16] in a study of 20 patients. In a preliminary study, Cutler[17] demonstrated a specificity of 98% and a sensitivity of 86% in the post-treatment setting. These studies of the Ez-HBT were conducted before the FDA approval of the Ez-HBT and the introduction of the indeterminate result category, which has altered the interpretation of test results. The main limitation of our study is the small sample size, in particular the small number of patients with persistent *H. pylori* infection following therapy. Furthermore,

differences in the performance characteristics of the Ez-HBT related to gender and ethnicity could not be ascertained given the small number of female and African-American patients.

In this study, eight of 70 patients had FP or indeterminate Ez-HBT results. Achlorohydria, hypochlorhydria, gastric atrophy, and long term proton pump inhibitor therapy have been shown to decrease the accuracy of UBT results[18, 19] and the same could be expected of the Ez-HBT, although this remains to be demonstrated. In this study, however, patients with these conditions were excluded. False positive and FN UBT results have been attributed to the non-fasting state[20] or to ingestion of ^{13}C enriched foods (corn, cane, or maize), which have been shown to cause variations in the naturally occurring $^{13}CO_2/^{12}CO_2$ ratio.[21] In fact, restrictions limiting the ingestion of corn or cane sugar products 6 h before ^{13}C-UBT testing have been recommended to reduce FP UBT results.[21] Moayyedi *et al.*[22] do not recommend fasting prior to ^{13}C-UBT but suggest altering the cut off values for a positive test and avoiding foods containing corn. Six of eight patients with FP or indeterminate results in our study had elevated breath basal ^{13}C levels possibly indicating either incomplete fasting, a ^{13}C enriched diet, or exercise,[23] although these factors were not specifically assessed in this study.

Current ^{13}C-UBT procedures require breath sample collection at baseline and 30 min. Baseline abundance of $^{13}CO_2$ in breath samples reflects recent dietary intake and values in fasting subjects vary based on different dietary habits.[21] A basal breath $^{13}CO_2$ sample to establish the $^{13}CO_2/^{12}CO_2$ ratio is important to control for inter-subject dietary variability; high baseline values, which could lead to FP results, are attributable to the consumption of corn or cane products. The collection of basal and 30-min samples for the ^{13}C-UBT results in excellent performance characteristics of the test with <1% FP and 1.1% FN results.[24] Although others have reported that the elimination of basal samples may not significantly affect test results,[25–27] they stress the need for avoiding cane and corn products prior to testing.[25] In the pre-treatment setting where the *H. pylori* density is high, it is unlikely that dietary modifications will affect test results. However, in the post-treatment setting where the *H. pylori* density is much lower, it is important to minimize factors that may impair test accuracy.

In fact, initial descriptions of the serum ^{13}C-bicarbonate test compared basal with post-urea dose blood samples.[13, 24] The current FDA-approved Ez-HBT protocol, however, uses a single blood sample drawn 30 min after stable-labelled urea administration. Without the benefit of baseline breath samples, which were available to us because of the study design, we could not have determined that six of eight FP and indeterminate Ez-HBT tests were TN results. Although the performance characteristics of the Ez-HBT are acceptable in the post-treatment setting, we recommend the collection of a basal blood sample, which need only be tested in the event of an indeterminate or positive test result, will result in further improvement in test accuracy. In our study group, the use of basal samples might have prevented the unnecessary retreatment of 8.6% (6/70) of patients.

In conclusion, the Ez-HBT is a simple, non-invasive, accurate test with performance characteristics comparable with that of the ^{13}C-UBT in establishing *H. pylori* eradication after therapy. The majority of FP and indeterminate Ez-HBT test results are attributable to incomplete fasting or reflect dietary practices. We recommend further studies to investigate the utility of basal blood sample collection and the effect of non-fasting on the Ez-HBT results.

REFERENCES

1 Basset C, Holton J, Ricci C, *et al.* Review article: diagnosis and treatment of *Helicobacter*: a 2002 updated review. Aliment Pharmacol Ther 2003; 17(Suppl 2): 89–97.

2 Malfertheiner P, Megraud F, O'Morain C, *et al.* Current concepts in the management of *H. pylori* infection – The Masstricht 2-2000 Consensus Report. Aliment Pharmacol Ther 2002; 16: 167–80.

3 Peura DA. The report of the Digestive Health Initiative International Update Conference on *Helicobacter pylori*. Gastroenterology 1997; 113: S4–8.

4 Vakil N, Affi A, Robinson J, *et al.* Prospective blinded trial of a fecal antigen test for the detection of *Helicobacter pylori* infection. Am J Gastroenterol 2000; 95: 1699–701.

5 Vaira D, Vakil N. Blood, urine, stool, breath, money and *Helicobacter pylori*. Gut 2001; 48: 287–9.

6 Makristathis A, Pasching E, Schutze K, *et al.* Detection of *Helicobacter pylori* in stool specimens by PCR and antigen enzyme immunoassay. J Clin Microbiol 1998; 39: 2772–4.

7 Vaira D, Malfertheiner P, Megraud F, *et al.* Diagnosis of *Helicobacter pylori* infection with a new non-invasive antigen-based assay. Lancet 1999; 354: 30–33.

8 Oderda G, Rapa A, Marinello B, *et al.* Usefulness of *Helicobacter pylori* stool antigen test to monitor response to eradication treatment in children. Aliment Pharmacol Ther 2001; 15: 203–6.

9 Van Doorn OJ, Bosman DK, vna't Hoff BW, *et al. Helicobacter pylori* stool antigen tests: a reliable non-invasive test for the diagnosis of *Helicobacter pylori* infection in children. Eur J Gasatroenterol Hepatol 2001; 13: 1061–5.

10 Trevisani L, Sartori S, Galvani F, *et al.* Evaluation of a new enzyme immunoassay for detecting *Helicobacter pylori* in feces: a prospective pilot study. Am J Gastroenterol 1999; 94: 1830–3.

11 Forne M, Dominguez J, Fernandez-Banares F, *et al.* Accuracy of an enzyme immunoassay for the detection of *Helicobacter pylori* in stool specimens in the diagnosis of infection and post-treatment check-up. Am J Gastroenterol 2000; 95: 2200–5.

12 Demirturk L, Yazgan Y, Tarcin O, *et al.* Does *N*-acetyl cysteine affect the sensitivity and specificity of *Helicobacter pylori* stool antigen test? Helicobacter 2003; 8: 120–3.

13 Moulton-Barrett R, Triadafilopoulos G, Michener R, *et al.* Serum ^{13}C-bicarbonate in the assessment of gastric *Helicobacter pylori* urease activity. Am J Gastroenterol 1993; 88: 369–74.

14 Cutler AF, Toskes P. Comparison of [^{13}C]urea blood tests to [^{13}C]urea breath test for the diagnosis of *Helicobacter pylori*. Am J Gastroenterol 1999; 94: 959–61.

15 Chey WD, Murthy U, Toskes P, *et al.* The ^{13}C-urea breath test accurately detects active *Helicobacter pylori* infection: a United States, multicenter trial. Am J Gastroenterol 1999; 94: 1522–4.

16 Kim MJ, Michener R, Triadafilopoulos G. Serum ^{13}C-bicarbonate assay for the diagnosis of gastric *Helicobacter pylori* infection and response to treatment. Gastroenterology 1997; 113: 31–7.

17 Cutler AF. [^{13}C] urea blood test to confirm *H. pylori* cure. Gastroenterology 2000; 118: A698.

18 Perri F, Festa V, Clemente R, *et al.* Methodological problems and pitfalls of urea breath test. Ital J Gastroenterol Hepatol 1998; 30 (Suppl 3): S315–9.

19 Catherton J, Spiller RC. The urea breath test for *Helicobacter pylori*. Gut 1994; 35: 723–5.

20 Epple HJ, Kirstein FW, Bojarski C, *et al.* ^{13}C-urea breath test in *Helicobacter pylori* diagnosis and eradication. Correlation to histology, origin of 'false' results, and influence of food intake. Scand J Gastroenterol 1997; 32: 308–14.

21 Schoeller DA, Klein PD, Watkins JB, *et al.* ^{13}C abundances of nutrients and the effect of variations in ^{13}C isotopic abundances of test meals formulated for ^{13}CO$_2$ breath test. Am J Clin Nutr 1980; 33: 2375–85.

22 Moayyedi P, Braunholtz D, Heminbrough E, *et al.* Do patients need to fast for a ^{13}C-urea breath test? Eur J Gastroenterol Hepatol 1997; 9: 275–7.

23 Schoeller DA, Brown C, Nakamura K, *et al.* Influence of metabolic fuel on the ^{13}C/^{12}C ratio of breath CO$_2$. Biomed Mass Spectrom 1984; 11: 557–61.

24 Klein PD, Graham DY. Minimum analysis requirements for the detection of *Helicobacter pylori* infection by the ^{13}C-urea breath test. Am J Gastroenterol 1993; 88: 1865–9.

25 Gisbert JP, Benito LM, Lara S, *et al.* ^{13}C-urea breath test for the diagnosis of *Helicobacter pylori* infection: are basal samples necessary? Eur J Gastroenterol Hepatol 2000; 12: 1201–5.

26 Okawa K, Onda M, Matsukura N, *et al.* Detection of *H. pylori* infection by ^{13}C-urea breath test: with special references to the one point measurement without pretreated breath samples. Gastroenterology 1997; 112: A243.

27 Lotterer E, Ramaker J, Ludtke FE, *et al.* The simplified ^{13}C-urea breath test – one point analysis for detection of *Helicobacter pylori* infection. Z Gastroenterol 1991; 29: 590–4.