

The ^{13}C urea breath test in the diagnosis of *Helicobacter pylori* infection

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Summary

The urea breath test (UBT) is one of the most important non-invasive methods for detecting *Helicobacter pylori* infection. The test exploits the hydrolysis of orally administered urea by the enzyme urease, which *H pylori* produces in large quantities. Urea is hydrolysed to ammonia and carbon dioxide, which diffuses into the blood and is excreted by the lungs. Isotopically labelled CO_2 can be detected in breath using various methods.

Labelling urea with ^{13}C is becoming increasingly popular because this non-radioactive isotope is innocuous and can be safely used in children and women of childbearing age. Breath samples can also be sent by post or courier to remote analysis centres. The test is easy to perform and can be repeated as often as required in the same patient. A meal must be given to increase the contact time between the tracer and the *H pylori* urease inside the stomach. The test has been simplified to the point that two breath samples collected before and 30 minutes after the ingestion of urea in a liquid form suffice to provide reliable diagnostic information. The cost of producing ^{13}C -urea is high, but it may be possible to reduce the dosage further by administering it in capsule form.

An isotope ratio mass spectrometer (IRMS) is generally used to measure ^{13}C enrichment in breath samples, but this machine is expensive. In order to reduce this cost, new and cheaper equipment based on non-dispersive, isotope selective, infrared spectroscopy (NDIRS) and laser assisted ratio analysis (LARA) have recently been developed. These are valid alternatives to IRMS although they cannot process the same large number of breath samples simultaneously.

These promising advances will certainly promote the wider use of the ^{13}C -UBT, which is especially useful for epidemiological studies in children and adults, for screening patients before endoscopy, and for assessing the efficacy of eradication regimens.

Introduction

Many diagnostic methods have been developed over the past 15 years to detect *Helicobacter pylori* infection—some invasive (rapid urease test, histology, culture, and polymerase chain reaction) because they cannot be performed without endoscopy, and others non-invasive (serology, urea breath test (UBT) and, more recently, *H pylori* antigen determination on faeces). Of the latter, the UBT is being increasingly used both in pretreatment and post-treatment phases.

Since it was first described by Graham *et al*,¹ the test has been modified extensively to

simplify and optimise it, including changes to the dose of urea used, sample timing, test meal, and cut off values to distinguish infected from uninfected subjects. Despite these modifications, the accuracy of the test has remained high and this is the best confirmation of its robustness.

The test exploits the large amount of urease produced by *H pylori*, as this enzyme hydrolyses the orally administered, labelled urea into ammonia and labelled CO_2 , which is absorbed through the mucus layer of the stomach and then transported to the lungs via the bloodstream for excretion. Isotope enrichment can be measured by various methods in breath samples collected at appropriate times.

Urea can be labelled with two different carbon isotopes: ^{14}C and ^{13}C . The main difference between them is that the former is radioactive, whereas the latter is stable. The advantages of using ^{14}C -urea are that it is cheap, so rapid that administering ^{14}C -urea in a gelatin capsule allows an accurate response to be obtained from a single 10 minute breath sample,^{2,3} and does not require any test meal. However, although the dose of ^{14}C has become progressively smaller and the test can now be performed with $1\ \mu\text{Ci}$,³ which is equal to the natural background radiation received in one day,^{3,4} the main problems are still the availability of a nuclear medicine department or centres licensed for storage and disposal of radioactive substrates, shipping difficulties, and the copious amounts of labelled tracer needed to perform large scale epidemiological studies. The use of this unstable tracer can be recommended when the number of UBTs per annum in a given gastroenterological centre is less than 2500 and ^{14}C facilities are available on site.⁵

In contrast, ^{13}C is a non-radioactive isotope that can be used safely for repeated testing, which is frequently required in clinical practice, and for detecting *H pylori* infection in children and women of childbearing age. Furthermore, ^{13}C -urea has been the most widely used substrate in methodological studies performed to validate this kind of diagnostic test. Another relevant advantage of using the stable isotope is that breath samples can be sent by post or courier to remote analysis centres, thus promoting the distribution of the test, which can even be performed at home if the patients are adequately selected and instructed.⁶ The major drawbacks of ^{13}C -urea are the higher cost compared with

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Abbreviations used in this paper: UBT, urea breath test; IRMS, isotope ratio mass spectrometer; NDIRS, non-dispersive, isotope selective, infrared spectroscopy; LARA, laser assisted ratio analysis; PPI, proton pump inhibitor; ROC, receiver operator characteristic.

Table 1 Comparison of the main characteristics of three different types of equipment for measuring the $^{13}\text{CO}_2$ to $^{12}\text{CO}_2$ ratio in breath samples

Characteristics of the instruments	Mass spectrometer (IRMS)	Infrared spectrometer (NDIRS)	Laser assisted ratio analyser (LARA)
Weight	90 kg	12 kg	350 kg
Reference gas	Necessary	Enclosed	Necessary
Carrier gas	Helium supply	None	None
Analysis time	120 seconds	90 seconds	60 seconds
Automation	For 220 samples	For 16 samples	For 60 samples
Breath sample	10 ml glass tube	1200 breath bag	12 ml plastic tube
Sample mailing	Favourable	Not practicable	Favourable
Multitasking software	Commonly available	Unnecessary	Planned
Technical expertise	Easy to operate	Easy to operate	Easy to operate
After sales support	Often required	Low maintenance	Low maintenance
Reported reliability	90–100%	90–98%	Limited experience
Approximate price	75 000 Euros	40 000 Euros	60 000 Euros

^{14}C -urea, and the need for expensive mass spectrometry, which is the most preferable device for measuring ^{13}C enrichment in breath samples of subjects infected with *H pylori*.

This review focuses on the most recent advances in the machines used to measure the ^{13}C isotope and on the most important aspects regarding the main UBT variables.

Measuring equipment

^{13}C is measured as the $^{13}\text{CO}_2$: $^{12}\text{CO}_2$ isotope ratio and is expressed as delta over baseline (DOB) per mil (‰) with respect to the international reference standard represented by the Pee Dee Belemnite limestone. The difference in isotope masses (45:44) is detected with extreme accuracy by the sector magnet contained in the mass spectrometer (IRMS). This equipment also requires a gas chromatograph because CO_2 has to be carefully purified from the whole breath prior to introduction into the mass spectrometer. The precision of measurements made by conventional IRMS can be as high as 0.01‰⁷ and this allows very low isotopic enrichments to be detected. As ^{13}C is measured as the ratio of $^{13}\text{CO}_2$ to $^{12}\text{CO}_2$, there is no need for a large volume of expired air and a 10 ml sample is enough to obtain reliable diagnostic information.⁵ Furthermore, the examination results do not depend on the body mass, so it is not necessary to take this variable into account.⁸

If a mass spectrometer is the most preferable machine for detecting ^{13}C excess in breath samples, its high price has limited the spread of the test and has prompted the biomedical industry to develop new instruments that are capable of measuring the stable isotope at a lower cost. Over the past few years, analytical devices besides IRMS have been developed to perform $^{13}\text{CO}_2$ and $^{12}\text{CO}_2$ analysis, such as infrared spectrometry^{9–10} and laser optogalvanic spectroscopy.¹¹ Several comparative studies^{12–15} have shown the reliability of these new, cheaper machines in measuring ^{13}C enrichment, thus allowing them to be considered as valid alternatives to IRMS.

Table 1 presents the main characteristics of IRMS, non-dispersive, isotope selective, infrared spectroscopy (NDIRS), and laser assisted ratio analyser (LARA) equipment. The advantage of IRMS is that it processes the highest number of breath samples and works in an automated manner. However, it is the most expensive and requires the longest analysis

time. NDIRS does not require helium as the carrier gas and has the lowest weight and price, but it can analyse only a small number of samples and so does not need multitasking software. The LARA system has the quickest analysis time; the other characteristics are average compared with the other two machines, especially with regard to the number of samples it can process and the cost. Integrated bar code readers and the use of multitasking programs have made running IRMS instruments much easier; however, they do require more maintenance than NDIRS and LARA systems. IRMS and LARA are more suitable for gastroenterological centres requiring large quantity, automated analysis, whereas NDIRS is more suitable for small laboratories in which the daily number of assays is not high. In this light, a small, new, cheap, infrared device for examination of only two breath samples has been developed to be used exclusively in the doctor's office.¹⁶ In the endless attempt to render the test cheaper and to increase the number of laboratories where it can be performed, other investigators have shown that a gas chromatograph coupled with a mass selective detector, which is available in many analytical and biomedical settings, can also be reliably used for ^{13}C -UBT.^{17–18}

Concomitant medication

The ^{13}C -UBT is a simple and innocuous assay which requires only few precautions in order to obtain accurate results. Besides having to avoid several dietary constituents with a natural abundance of ^{13}C ,¹⁹ such as maize, cane, and cornflour, there is still some debate concerning both the type of antisecretory drugs that may influence the test and the optimal timing of breath testing after their discontinuation in order to exclude false negative results. This is a relevant point because many patients requesting UBT are dyspeptic and thus reluctant to discontinue their antisecretory drugs. Although there is general consensus^{20–25} regarding the adverse effect of proton pump inhibitors (PPIs) on the UBT (false negative results range from 17 to 61%), the timing of their cessation prior to testing is not so clear. In many clinical trials aimed at assessing the efficacy of eradication regimens, for instance, it is generally reported that patients must not have taken PPIs for at least one month before the UBT, but it has been shown that five to seven days is sufficient to reverse their adverse effect.^{21–23}

Laine *et al.*,²⁴ however, suggest that patients should remain off PPIs for 14 days in order to be sure of avoiding false negative results. Although H₂ blockers are regarded as antisecretory drugs with no effect on UBT,²⁶ some recent investigations have shown that even standard and high dose ranitidine can cause up to 20% of false negative breath tests.^{22–25} These studies have shown that the reversal of this negative effect occurs within five to seven days of drug cessation and therefore withdrawal for one week suffices to rule out any adverse influence the drugs may have on the UBT.

Antibiotics, particularly those with anti-helicobacter action, and bismuth preparations should be withdrawn at least one month prior to UBT, as is usually suggested.

Amount and formulation of ¹³C-urea

The urea dose has been progressively reduced from the 350 mg (5 mg/kg) dose initially used in the study by Graham *et al.*,¹ thus decreasing the cost of the substrate needed to perform the test. At present three different dosages are utilised. A dose of 125 mg has been validated in the United States by Klein *et al.*,²⁷ who showed that a sensitivity and specificity as high as 100% can be reached with this dosage. Logan *et al.* used 100 mg in their proposal of a standard diagnostic protocol²⁸ and this dosage has received the greatest amount of attention in Europe. Finally, 75 mg (approximately 1 mg/kg) has been shown to be as reliable as the higher doses²⁹ and is used satisfactorily in an increasing number of research studies.^{13 15 17 30–34} This low dosage is also included in many commercial kits for ¹³C-UBT,⁴ as it limits the cost of the test. However, it may be possible to reduce the dosage of ¹³C-urea further by administering the tracer in capsules. It has been shown that a dose of either 38 mg³⁵ or 45 mg,³⁶ given in rapidly dissolving gelatin capsules, maintains the high sensitivity and specificity of the test and also reduces its duration to 15–20 minutes. In fact, this peculiar formulation bypasses the problem of contact of the substrate with the urease producing bacteria present in the oropharynx, thus avoiding false positive results if breath samples are taken too soon. Obviously, there is no need to delay gastric emptying by an appropriate meal, which is fundamental in tests performed with the liquid form of ¹³C-urea.

Test meal

One of the most important advantages of the UBT is that it samples the whole stomach and is not prone to sampling error as are biopsy based tests, which can be influenced by the patchy distribution of *H pylori* infection within the gastric mucosa. This global test can be improved if prolonged contact of the substrate with the urease enzyme is promoted. The use of test meals capable of increasing the residence time of ¹³C-urea in the stomach is recommended in most protocols. The usefulness of turning the patient instead of leaving him/her seated, in order to favour the contact between labelled substrate and urease activity, has recently been disproved.³⁷

Test meals containing fat are usually chosen due to the well known ability of this nutrient to

delay gastric emptying. Recently, citric acid, which acts by lowering duodenal pH, which in turn reduces antral motility and relaxes the gastric fundus,³⁸ has been shown to be an optimal test meal.³⁴ In this study citric acid, which was compared with two other frequently used semiliquid meals, Meritene and Ensure/Calogen, provided the highest and earliest δ peaks; however, data from uninfected patients were not shown in the published paper and the extent of the excess $\delta^{13}\text{CO}_2$ excreted because of the citric acid was only reported as the ratio above the baseline excess $\delta^{13}\text{CO}_2$ values, making it difficult to judge.⁴ One advantage of using citric acid is that the likelihood of contamination by urease produced by bacterial flora in the mouth is reduced greatly.²⁹

Is pretest fasting necessary?

Many protocols recommend that patients fast for at least four hours before testing in order to avoid any interference from food. Some recent studies, however, have addressed the question of whether fasting is really necessary in patients undergoing UBT. Perri and colleagues³¹ and Moayyedi and colleagues³⁹ found that there is no statistical difference between tests performed in fasting and non-fasting conditions and therefore do not recommend fasting before UBT. On the contrary, Epple and colleagues³³ and Savarino and colleagues¹³ observed that feeding causes a significant decrease in the 30 minute δ values compared with the fasting ones and an increase in false negative results. They suggest that fasting before testing should be mandatory. These contradictory findings probably result from the different meals used in the various studies, but at present there are no clear guidelines in this area.

Time of breath collection

The test has been progressively simplified since the first report by Graham *et al.*,¹ who took breath samples every 10 minutes for three hours. At present, there is universal consensus on the use of a two point examination—that is, a basal sample collected before and another sample collected 30 minutes after ingestion of the tracer, using whatever dosage of ¹³C-urea is chosen.^{5 20 29–34 40} As already mentioned, the use of citric acid as test meal has been shown to be better than other semiliquid fatty meals in the maximal recovery of ¹³CO₂ at this sampling time.³⁴

Reducing the number of breath samples to only one by omitting the baseline collection in order to decrease cost and duration of the test further has also been proposed.^{30 40} Lastly, shortening the sampling time to 20 minutes and omitting the meal has been shown to maintain the excellent sensitivity and specificity of the test⁴¹; however, this study was performed using 125 mg of ¹³C-urea, which is the usual dose in the USA but not in Europe.

Cut off values

Discrimination between infected and uninfected subjects is based on a strict cut off value which has been validated in many studies. The most widely used value is 5%, which has been

proposed by Logan and colleagues²⁸ in their European standard protocol. Later, by using receiver operator characteristic (ROC) curves, Johnston and colleagues⁴² showed that this cut off value can be lowered to 3.5‰ without compromising the sensitivity and specificity of the test. Cluster analysis was used by Mion *et al.*,⁴³ who also suggested adopting 3.0‰ as the optimal cut off point with the need, however, for a grey zone in which the results of UBT are inconclusive; there was no reference to a gold standard in their study. However, there is no universal agreement on the use of a lower than usual cut off value. Zagari and colleagues⁴⁴ also used ROC analysis on a large sample of patients and were not able to decrease the cut off level below the value of 4.5‰ with either 75 mg or 100 mg doses of ¹³C-urea. It must be stressed, however, that the 5‰ value remains a strong index for distinguishing infected subjects from uninfected ones and therefore this cut off value is still widely used.

Diagnostic accuracy and clinical applications

The ¹³C-UBT can be used in many clinical settings because of its non-invasiveness, simplicity, and safety. Furthermore, the sensitivity and specificity in untreated subjects are very high and range from 90 to 98% and from 92 to 100%, respectively.^{5 13 28 39 40 45 46} Accuracy in the assessment of eradicating efficacy is more controversial because some investigators⁴⁷⁻⁵⁰ found excellent reliability even in the post-treatment phase, whereas others⁵¹ did not confirm these good results. Accordingly, it has been recommended recently that two UBTs, performed at separate post-treatment time points (for example, two months apart), can equate to two different tests⁵² when assessing the success of anti-helicobacter treatments. Obviously, this applies to research trials on eradication treatments rather than to routine clinical practice.

It must be stressed, however, that the UBT has sometimes proved to be poorly diagnostic, because its accuracy has frequently been assessed against gastric biopsies as gold standard⁴ and it is well known that this latter test is susceptible to sampling error. As confirmation of this fact, Epple and colleagues³³ have shown that several apparently false positive UBT results were in fact correct findings, as clearly shown by the analysis of additional multiple biopsy specimens taken in the same patients who underwent a second gastroscopy. Furthermore, in a recent multicentre trial performed in Belgium, Glupzcynski and colleagues⁵³ showed that the UBT was the most reliable test for assessing the efficacy of eradication treatment, whereas the sensitivity of histology was low because of the frequently insufficient size and/or number of biopsy samples.

Considering the above mentioned points, the UBT is the test of choice for screening patients before endoscopy, in epidemiological studies on the prevalence of *H pylori* infection in various populations, and in the non-invasive evaluation of the efficacy of eradication regimens.

The other non-invasive test, serology, has relevant limits in assessing the success of anti-helicobacter regimens due to the considerable amount of time (at least six months) needed to show a consistent decrease in serum antibody levels. Lastly, ¹³C-UBT is the ideal test for children, using a lower dose of urea (50 mg); provided that a mask is used, breath samples can be collected from those under three years of age.⁴

Conclusions

Currently, the UBT is considered to be one of the most important and reliable non-invasive methods for the diagnosis of *H pylori* infection. The examination is simple, innocuous when ¹³C is used, easy to repeat, highly accurate, and requires a low number of precautions in order to obtain reliable results. It is particularly suitable for epidemiological studies in children and adults, in all clinical conditions where endoscopy is not strictly necessary, and to check the success of eradication regimens. The new and cheaper measuring equipment, the possibility of using a lower urea dose in capsules, thus omitting the meal, as well as shorter than usual sampling times, will certainly promote an increasingly broader application of this test.

- Graham DY, Klein PD, Evans DJ Jr, *et al.* Campylobacter pylori detected noninvasively by ¹³C-urea breath test. *Lancet* 1987;i:1174-7.
- Hamlet AK, Eriandsson KIM, Olbe L, *et al.* A simple, rapid, and highly specific reliable capsule-based ¹³C urea breath test for diagnosis of Helicobacter pylori infection. *Scand J Gastroenterol* 1995;30:1058-63.
- Peura DA, Pambianco DJ, Dye KR, *et al.* Microdose ¹³C-urea breath test offers diagnosis of Helicobacter pylori in 10 minutes. *Am J Gastroenterol* 1996;91:233-8.
- Logan RPH. Urea breath tests in the management of Helicobacter pylori infection. *Gut* 1998;43(suppl 1):S47-50.
- Goddard AF, Logan RPH. Review article: urea breath tests for detecting Helicobacter pylori. *Aliment Pharmacol Ther* 1997;11:641-9.
- Thijs WJ, Thijs JC, Kleibeuker JH, *et al.* Evaluation of clinical and home performance of the ¹³C-urea breath test for the detection of Helicobacter pylori. *Eur J Gastroenterol Hepatol* 1995;7:603-7.
- Guilluy R, Billion-Rey F, Pachiandi C, *et al.* On-line purification and carbon-13 isotopic analysis of carbon dioxide in breath: evaluation of on-line gas chromatography-isotope ratio mass spectrometry. *Analytica Chimica Acta* 1992;259:193-202.
- Logan RPH, Polson RJ, Misiewicz JJ, *et al.* Simplified single sample ¹³carbon urea test for Helicobacter pylori: comparison with histology, culture, and ELISA serology. *Gut* 1991;32:1461-4.
- Koletzko S, Haisch M, Seeboth I, *et al.* Isotope-selective non-dispersive infrared spectrometry for detection of Helicobacter pylori infection with ¹³C-urea breath test. *Lancet* 1995;345:961-2.
- Braden B, Schäfer F, Caspary WF, Lembecke B. Nondispersive isotope-selective infrared spectroscopy: a new analytical method for ¹³C-urea breath tests. *Scand J Gastroenterol* 1996;31:442-5.
- Murnick DE, Peer BJ. Laser-based analysis of carbon isotope ratios. *Science* 1994;263:945-7.
- Braden B, Haisch M, Duan LP, *et al.* Clinically feasible stable isotope technique at a reasonable price: analysis of ¹³CO₂/¹²CO₂-abundance in breath samples with a new isotope selective nondispersive infrared spectrometer. *Z Gastroenterol* 1994;32:675-8.
- Savarino V, Mela GS, Zentilin P, *et al.* Comparison of isotope ratio mass spectrometry and non-dispersive isotope-selective infrared spectroscopy for ¹³C-urea breath test. *Am J Gastroenterol* (in press).
- Van der Hulst RWM. Laser assisted ratio analyser-¹³C-urea breath testing, a novel non-invasive system for the diagnosis of *H. pylori* infection: a prospective diagnostic multicenter study [abstract]. *Gut* 1997;41(suppl 1):A72-3.
- Savarino V, Vaira D, Dulbecco P, *et al.* Comparison of laser assisted ratio analyser (LARA) and isotope ratio mass spectrometry (IRMS) for ¹³C-urea breath test (UBT) [abstract]. *Gut* 1998;43(suppl 2):A50.
- Ohara S, Kato M, Asaka M, Toyota T. The UbiT-100 ¹³CO₂ infrared analyser: comparison between infrared spectrometric analysis and mass spectrometric analysis. *Helicobacter* 1998;3:49-53.

- 17 Kasho VN, Cheng S, Jensen DM, *et al.* Feasibility of analysing [¹³C] urea breath tests for *Helicobacter pylori* by a gas chromatography-mass spectrometry in the selected ion monitoring mode. *Aliment Pharmacol Ther* 1996;10:985-95.
- 18 Lee HS, Gwee KA, Teng LY, *et al.* Validation of [¹³C]urea breath test for *Helicobacter pylori* using a simple gas chromatograph-mass selective detector. *Eur J Gastroenterol Hepatol* 1998;10:569-72.
- 19 Schoeller DA, Klein PD, Watkins JB, *et al.* ¹³C abundances of nutrients and the effect of variations in ¹³C isotopic abundances of test meals formulated for ¹³CO₂ breath tests. *Am J Clin Nutr* 1980;33:2375-85.
- 20 Atherton JC, Spiller RC. The urea breath test for *Helicobacter pylori*. *Gut* 1994;35:723-5.
- 21 Chey WD, Spybrook M, Carpenter S, *et al.* Prolonged effect of omeprazole on the ¹⁴C-urea breath test. *Am J Gastroenterol* 1996;91:89-92.
- 22 Chey WD, Woods M, Scheiman JM, *et al.* Lansoprazole and ranitidine affect the accuracy of the ¹⁴C-urea breath test by a pH-dependent mechanism. *Am J Gastroenterol* 1997;92:446-50.
- 23 Katelaris PH, Connor SJ, Seow F, *et al.* The effect of short-term omeprazole use on the accuracy of ¹³C-urea breath test in *Helicobacter pylori* infected patients [abstract]. *Gut* 1998;43(suppl 2):A53.
- 24 Laine L, Estrada R, Trujillo M, *et al.* Effect of proton pump inhibitor therapy on diagnostic testing for *Helicobacter pylori*. *Ann Intern Med* 1998;129:547-50.
- 25 Savarino V, Bisso G, Pivari M, *et al.* Effect of omeprazole and ranitidine on the accuracy of ¹³C-urea breath test (UBT) [abstract]. *Gut* 1998;43(suppl 2):A51.
- 26 Cutler AF, Elnaggar M, Brooks E, *et al.* Effect of standard and high dose ranitidine on [¹³C] urea breath test results. *Am J Gastroenterol* 1998;93:1297-9.
- 27 Klein PD, Malaty HM, Martin RF, *et al.* Noninvasive detection of *Helicobacter pylori* infection in clinical practice: the ¹³C urea breath test. *Am J Gastroenterol* 1996;91:690.
- 28 Logan RPH, Dill S, Bauer FE, *et al.* The European ¹³C-urea breath test for the detection of *Helicobacter pylori*. *Eur J Gastroenterol Hepatol* 1991;3:915-21.
- 29 Eggers RH, Kulp A, Tegeler R, *et al.* A methodological analysis of the ¹³C-urea breath test for detection of *Helicobacter pylori* infections: high sensitivity and specificity within 30 min using 75 mg of ¹³C-urea. *Eur J Gastroenterol Hepatol* 1990;2:437-44.
- 30 Lotterer E, Ramaker J, Lüdtkke FE, *et al.* The simplified ¹³C-urea breath test—one point analysis for detection of *Helicobacter pylori* infection. *Z Gastroenterol* 1991;29:590-4.
- 31 Perri F, Maes B, Geypens B, *et al.* The influence of isolated doses of drugs, feeding and colonic bacterial ureolysis on urea breath test results. *Aliment Pharmacol Ther* 1995;9:705-9.
- 32 Ellenrieder V, Glasbrenner B, Stoffels C, *et al.* Qualitative and semi-quantitative value of a modified ¹³C-urea breath test for identification of *Helicobacter pylori* infection. *Eur J Gastroenterol Hepatol* 1997;9:1085-9.
- 33 Epple HJ, Kirstein FW, Bojaeski C, *et al.* ¹³C-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.
- 34 Dominguez-Muñoz JE, Leodolter A, Sauerbruch T, *et al.* A citric acid solution is an optimal test drink in the ¹³C-urea breath test for the diagnosis of *Helicobacter pylori* infection. *Gut* 1997;40:459-62.
- 35 Bielanski W, Konturek SJ. New approach to ¹³C-urea breath test: capsule-based modification with low-dose of ¹³C-urea in the diagnosis of *Helicobacter pylori* infection. *J Physiol Pharmacol* 1996;47:545-53.
- 36 Canena J, Santos AM, Reis J, *et al.* ¹³C urea breath test (UBT) for *Helicobacter pylori* diagnosis. Is it possible to use a new low dose procedure? *Am J Gastroenterol* 1997;92:1610.
- 37 Johnston BJ, Gruer N, Johnson P. Effect of subject position on the performance of the ¹³C-urea breath test for the detection of *Helicobacter pylori* [abstract]. *Gut* 1998;43(suppl 2):A57.
- 38 Dominguez-Muñoz JE, Manes G, Leodolter A, *et al.* Effect of intraduodenal application of acid and lipids on gastric tone in healthy subjects [abstract]. *Gut* 1996;39(suppl 3):A110.
- 39 Moayyedi P, Braunholtz D, Heminbrough E, *et al.* Do patients need to fast for a ¹³C-urea breath test? *Eur J Gastroenterol Hepatol* 1997;9:275-7.
- 40 Klein PD, Graham DY. Minimum analysis requirements for the detection of *Helicobacter pylori* infection by the ¹³C-urea breath test. *Am J Gastroenterol* 1993;88:1865-9.
- 41 Malaty HM, El-Zimaity HMT, Genta RM, *et al.* Twenty-minute fasting version of the US ¹³C-urea breath test for the diagnosis of *H. pylori* infection. *Helicobacter* 1996;1:165-7.
- 42 Johnston BJ, Levi S, Johnson PG. Cut-off point for ¹³C-urea breath test [abstract]. *Gut* 1996;39(suppl 2):A122.
- 43 Mion F, Rosner G, Rousseau M, *et al.* ¹³C-urea breath test for *Helicobacter pylori*: cut off point determination by cluster analysis. *Clin Sci* 1997;93:3-6.
- 44 Zagari RM, Pozzato P, Bazzoli F, *et al.* A comparative trial to optimize the protocol of the ¹³C urea breath test for the diagnosis of *Helicobacter pylori* infection. *Digestion* 1998;59(suppl 3):461.
- 45 Braden B, Duan LP, Caspary WF, *et al.* More convenient ¹³C-urea breath test modifications still meet the criteria for valid diagnosis of *Helicobacter pylori* infection. *Z Gastroenterol* 1994;32:198-202.
- 46 Cutler AF, Havstad S, Ma CK, *et al.* Accuracy of invasive and noninvasive tests to diagnose *Helicobacter pylori* infection. *Gastroenterology* 1995;109:136-41.
- 47 Logan RPH, Gummatt PA, Misiewicz JJ, *et al.* One week eradication regimen for *Helicobacter pylori*. *Lancet* 1991;338:1249-52.
- 48 Bazzoli F, Zagari RM, Fossi S, *et al.* The ¹³C-urea breath test for early assessment of *Helicobacter pylori* infection [abstract]. *Gastroenterology* 1995;108:A57.
- 49 Slepman N, Cox S, Olson C. A comparison of culture, histology and ¹³C-urea breath test for detection of *H. pylori* [abstract]. *Gastroenterology* 1995;108:A221.
- 50 Rollán A, Giancaspero R, Arrese M, *et al.* Accuracy of invasive and non-invasive tests to diagnose *Helicobacter pylori* infection after antibiotic treatment. *Am J Gastroenterol* 1997;92:1268-74.
- 51 Van de Wouw BAM, De Boer WA, Hermsen HWEM, *et al.* Usefulness of the ¹⁴C urea breath test as a semi-quantitative monitoring instrument after therapy for *Helicobacter pylori* infection. *Scand J Gastroenterol* 1997;32:112-17.
- 52 Working Party of the European *Helicobacter pylori* Study Group. Guidelines for clinical trials in *Helicobacter pylori* infection. *Gut* 1997;41(suppl 2):S1-23.
- 53 Glupczynski Y, Ectors N, Burette A, *et al.* Methodological lessons learned from a multicenter trial of *Helicobacter pylori* infection using centralised laboratory facilities [abstract]. *Gut* 1997;41(suppl 1):A71.