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 detection 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., 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 $^{13}$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, 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 µCi, which is equal to the natural background radiation received in one day, 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 $^{13}$C facilities are available on site.

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. The major drawbacks of $^{13}$C-urea are the higher cost compared with

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.
Urea breath test in the diagnosis of *H pylori* infection

Advances in the machines used to measure the 

sensitivity of the test to reverse their adverse effects. 

Concerning the main UBT variables.

**Measuring equipment**

\[ ^{13}C \] is measured as the ratio of \(^{13}CO_2\) to \(^{12}CO_2\) isotope enrichment which is not affected by the body mass, so it is not necessary to take this variable into account.

A small, new, cheap, infrared device for measuring \(^{13}C\)-enrichment in breath samples, its high price has limited the spread of this equipment in analytical and biomedical settings, can also be used for small laboratories in which the daily number of assays is not high. In this light, a small, new, cheap, infrared device for measurement of only two breath samples has been developed to be used exclusively in the doctor’s office. 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.

**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 \(^{13}C\), 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 their cessation 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 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.

### Table 1 Comparison of the main characteristics of three different types of equipment for measuring the \(^{13}CO_2\) to \(^{12}CO_2\) ratio in breath samples

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

\(^{13}C\)-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.

\[^{14}\text{C}\text{-}\text{UBT}\].

The following table summarises the main characteristics of the different types of equipment:

- Mass spectrometers (IRMS): high accuracy, but expensive and require a gas chromatograph coupled with a mass spectrometer. These machines require helium as the carrier gas and have the highest 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 measurement of only two breath samples has been developed to be used exclusively in the doctor’s office. 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.

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 \(^{13}C\), 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 their cessation 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 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.
Although H$_2$ blockers are regarded as antisecretory drugs with no effect on UBT, some recent investigations have shown that even standard and high dose ranitidine can cause up to 20% of false negative breath tests. 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 $^{13}$C-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., 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., 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 protocol 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 doses and is used satisfactorily in an increasing number of research studies. This low dosage is also included in many commercial kits for $^{13}$C-UBT, as it limits the cost of the test. However, it may be possible to reduce the dosage of $^{13}$C-urea further by administering the tracer in capsules. It has been shown that a dose of either 38 mg or 45 mg, 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 $^{13}$C-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$_2$ 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 $^{13}$C-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.

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

**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 colleagues 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, Eppe and colleagues and Savarino and colleagues observed that feeding causes a significant decrease in the 30 minute $\delta$ 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., 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 $^{13}$C-urea is chosen. 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 $^{13}$CO$_2$ at this sampling time.

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. Lastly, shortening the sampling time to 20 minutes and omitting the meal has been shown to maintain the excellent sensitivity and specificity of the test; however, this study was performed using 125 mg of $^{13}$C-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
Urea breath test in the diagnosis of H pylori infection

I21

100%, respectively.51 32 83 94 04 54 6 Accuracy in specificity in untreated subjects are very high.

tings because of its non-invasiveness, simplicity, and safety. Furthermore, the sensitivity and,
ty, and show a consistent decrease in serum antibody levels. Lastly, 13C-UBT is the ideal test for chil-

diagnosis of H pylori infection

Diagnostic accuracy and clinical applications

The 13C-UBT can be used in many clinical set-
tings because of its non-invasiveness, simplic-
ty, 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 26 39 40 41 46 Accuracy in

the assessment of eradicating efficacy is more controversial because some investigators57–50

found excellent reliability even in the post-
treatment phase, whereas others51 did not con-

firm 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

standard5 and it is well known that this latter

test is susceptible to sampling error. As confir-
mation of this fact, Epple and colleagues33 have

shown that several apparently false positive

patients and were not able to decrease the cut

t 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 sub-

decision rules.

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 endos-

copy 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.

1 2 3


16 Ohara S, Kató M, Asaka M, Toyota T. The UBT-100 CO2 infrared analyser: comparison between infrared spectrometric analysis and mass spectrometric analysis. Helico-


