Clinical practice – breath tests

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The underlying principle of the two non-invasive radio-labelled urea breath tests is similar. Both are positive when the patient's stomach is colonised by *Helicobacter pylori* because the organism's urease enzyme splits the orally administered urea isotope to labelled CO₂ which is then detected in the expired breath. The tests thus reflect active infection and are ideally suited to monitoring the success or failure of different eradication therapies as well as studying rates of acquisition and re-infection/late recrudescence post treatment. [¹³C]-urea should always be used in children since it is the stable non-radioactive isotope but the [¹⁴C]-urea breath test is suitable for most adults, since the dose of radioactivity is minimal.

The [¹³C]-urea breath test (UBT-13) was originally described by David Graham and colleagues from Texas in 1987. In writing this chapter, I have relied heavily on British experience. Recent reviews provide a detailed account of these tests.

Urease content of *H. pylori*

*H. pylori* is the urease-producing bacteria par excellence. The extraordinarily active *H. pylori* urease, first described by Tytgat's group from Holland, is the basis of the observation that patients infected with the organism tend to have higher levels of gastric ammonia and lower levels of gastric urea than uninfected individuals. The fact that the *H. pylori* urease splits urea into ammonia and carbon dioxide (via bicarbonate), and hence causes a detectable change in pH, is the basis of all the so-called rapid urease tests performed upon endoscopically obtained gastric biopsy material.

It occurred to us and others that if bacterial urease activity could be detected using a relatively crude chemical test on unprocessed gastric biopsy material, how much simpler, more accurate and elegant, it would be to measure *H. pylori* urease activity using some form of non-invasive isotopically labelled urea breath test.
Measurement of $^{14}$CO$_2$

Most District General hospitals will have a liquid scintillation counter to enable the clinician to measure $^{14}$CO$_2$ excretion in the patient's breath. To collect the breath, samples at time 0 and at various time intervals after oral administration of the $[^{14}$C]-urea substrate, the patient exhales through a tube of anhydrous calcium chloride into a vial containing 2 mmol of the CO$_2$ trapping agent hyamine hydroxide in 2 ml of ethanol with phenolphthalein as indicator. When 2 mmol of exhaled CO$_2$ has been trapped by the hyamine hydroxide, the vial is decolourised. Then, 10 ml of liquid scintillant is added and the $^{14}$C activity measured by liquid scintillation counting. A 1% standard is also counted and the activity expressed as a percentage of the administered dose per millimole of expired CO$_2$. This is multiplied by body weight (kg) to allow for endogenous CO$_2$ production.

It may be necessary for the patient to exhale into the hyamine hydroxide for 1–3 min to achieve decolourisation of the solution (and, by inference, the trapping of 2 mmol of CO$_2$). Marshall's group$^{18}$ employ only 1 mmol of hyamine in their trapping fluid, which obviously halves the time the patient is required to blow into the solution, since only 1 mmol of CO$_2$ is trapped.

We now use 0.1 MBq of $[^{14}$C]-urea for our UBT-14 test having initially used 4 times this amount$^2$. Using the data of Stubbs and Marshall$^{20}$, the effective dose a patient might receive from a single UBT-14 would vary between 4 micro Sieverts for a $H.~pylori$ negative man to 8 micro Sieverts for a $H.~pylori$ positive woman, which is (at maximum) less than 1/500 of that a patient receives when a barium meal is performed$^{10}$.

Measurement of $^{13}$CO$_2$

As described in much greater detail elsewhere$^{10,14}$, the natural abundance of $^{14}$CO$_2$ is extremely low, while that of $^{13}$CO$_2$ is relatively quite high. $^{13}$C is a natural non-radioactive isotope and can be measured relative to $^{12}$C by gas isotope ratio mass spectroscopy. Because $^{13}$C is expressed as a ratio of $^{12}$C, the volume of expired CO$_2$ for any given sample is not critical, and the analysis can be performed on less than 0.1 ml of exhaled CO$_2$. The $^{13}$C/$^{12}$C ratio is usually expressed as parts per thousand (per ml) relative to an international primary standard known as PDP calcium carbonate$^{10,14}$.

Certain plant materials, such as corn and sugar cane, are enriched with $^{13}$C. Thus, during the day, the natural abundance of $^{13}$CO$_2$ normally fluctuates slightly in response to dietary consumption of corn, sugar cane, etc. Following ingestion of $[^{13}$C]-urea, patients infected by $H.$
pylori nearly always have a $^{13}\text{C}/^{12}\text{C}$ ratio in their breath which greatly exceeds that of PDP calcium carbonate, thus giving a positive result.

One of the great advantages of the UBT-14 and UBT-13 tests over an endoscopically obtained, biopsy-based test other than the obvious ones of being considerably cheaper and non-invasive is that it is possible to define a cut-off between a positive and a negative result. One way to determine this arbitrary cut-off for both tests is to take the UBT result (be it UBT-14 or UBT-13) of a large number of patients whose ‘gold standard’ gastric biopsy results are unequivocally \textit{H. pylori} negative on culture, histology and the rapid urease test and calculate a normal range plus or minus 3 standard deviations from the mean and call this the upper limit of normal. Previously, in a study described by Logan \textit{et al}\textsuperscript{13}, the cut-off in adults was said to be 4.9 per ml. It has now been realised that, in many children and some adults, the true cut-off is about 3.5 per ml.

As with the UBT-14 test, the amount of $^{13}\text{C}$-urea used to conduct the test has been falling. When Graham and colleagues from Texas first described the test in 1987\textsuperscript{1}, they opted to use 200 mg of $^{13}\text{C}$-urea. Now the ‘Standard European Method’ for the UBT-13 test employs just 100 mg of $^{13}\text{C}$-urea without loss of sensitivity or specificity\textsuperscript{13}. Our own group conducted a comparison of the UBT-14 test with the UBT-13 test using just 75 mg of the latter and found an excellent correlation between the two methods\textsuperscript{21}.

\textbf{Test meals versus citric acid drink and timing of breath samples for the UBT-13 and UBT-14 tests}

Up until very recently, most groups have agreed that giving some form of suitable test meal before administering either $^{14}\text{C}$-urea or $^{13}\text{C}$-urea was important because it delayed gastric emptying and prolonged the time that the bacterial urease is exposed to the radio-labelled urea\textsuperscript{8,10,14}. Good results had been obtained in preliminary studies also using a citric acid drink\textsuperscript{23,24} instead of a standard test meal and so Malfertheiner and colleagues decided to study this more systematically\textsuperscript{22}.

Eighty patients (mean age 40 years) presenting for routine OGD with dyspeptic symptoms were studied\textsuperscript{33}. All had gastric biopsies taken from both antral and body regions of the stomach and \textit{H. pylori} status checked by: (i) rapid urease test; (ii) histology; and (iii) bacterial culture. Patients were classified as being \textit{H. pylori} positive if either bacterial culture or both rapid urease and histology were positive. The UBT-13 test was conducted in all patients after an overnight fast on 3 consecutive days. On each study day, a different test meal was given in a randomised order: (i) 200 ml 0.1 N citric acid solution, with the addition of 25 mg
Fig. 1 Curves obtained with the three \(^{13}\text{C}\)-UBT procedures in the same patient population \((n = 48)\). On each \(^{13}\text{C}\)-UBT, the same \(^{13}\text{C}\)-urea dose \((75 \text{ mg})\) but different test meal was given. Results expressed as means (SEM). Reproduced from Dominguez-Munoz et al. with the permission of the publishers of Gut.

saccharin as sweetener; (ii) 250 ml of a standard semiliquid meal (Meritene, Wander Pharma 237 kcal; 5 g fat, 20 g protein and 28 g carbohydrate); and (iii) a semiliquid fatty meal consisting of 50 ml Ensure (Abbott) and 50 ml of Calogen (SHS Pharma) which was calculated to contain 275 kcal; 26.7 g fat, 2 g protein, 6.7 g carbohydrate.

At 10 min after ingestion of the test meal or citric acid drink, a baseline exhaled breath sample was collected; thereafter, 75 mg of \(^{13}\text{C}\)-urea dissolved in 50 ml water was given orally (time 0). Further breath samples were taken at 15, 30, 45 and 60 min. The UBT-13 test was considered positive if the delta value was 4.

Of the patients, 48/80 \((60\%)\) were \(H. \text{pylori}\) positive and 32/80 \((40\%)\) \(H. \text{pylori}\) negative. All 3 breath test procedures were well tolerated, but the citric acid drink was generally considered to be the most pleasant to take. The citric acid drink was 24 times cheaper than any of the semiliquid meals and had the further advantage that it could easily be stored at 4°C for weeks on end. The study also confirmed it was not necessary to turn the patient on to each side or head down for a few minutes after ingestion of the labelled urea solution.

Figure 1 shows curves obtained in \(H. \text{pylori}\) positive patients by applying all 3 tests. The maximum delta value obtained with the citric acid drink was significantly higher than that obtained with Meritene or Ensure/Calogen (see Fig. 2) and, furthermore, the delta peak was obtained earlier with the citric acid drink than the two test meals (see Fig. 3). It is presumed that the citric acid solution delays gastric emptying because of its low pH \((3.0-3.5)\). In clinical practice, sampling at time 0 and 30 min seemed optimal and gave a high diagnostic accuracy.

In the case of the \(^{14}\text{C}\)-urea breath test, we originally took breath samples at 10, 20, 30, 40, 50, 60, 80, 90 and 120 min and computed the 2 h area under the curve \(2 \text{ h AUC}\). In recent years, we have opted to use the 40 min post \(^{14}\text{C}\)-urea breath sample, because this gave the best correlation with the 2h AUC. The cut-off we employ to separate \(H. \text{pylori}\)
positive from negative patients is a 2 h AUC of 40, which is equivalent to a 40 min value of 0.416% of administered dose/mmol CO$_2$ multiplied by body weight in kg$^{25}$. Using the single 40 min sample following $^{14}$C-urea, the Amsterdam group separated their $H.\ pylori$ positive and negative subjects with a sensitivity of 95% and specificity of 98%$^{19}$.

There is a commercially available $^{13}$C-urea breath test kit and the breath samples may be sent off to a central laboratory for analysis with a 48 h turnaround time for the results. A single UBT-13 test costs about £28 per patient compared with £14 per patient for the UBT-14 test.

**Clinical and research applications of urea breath testing**

*Assessment of different anti-Helicobacter eradication regimens*

The greatest role for the UBTs lies in the documentation as to whether or not a particular eradication regimen has or has not successfully eradicated a patient’s $H.\ pylori$ infection. It can take many months, and
in some cases years, for the serology tests to become negative post *H. pylori* eradication and, for this reason, breath testing and not serial serology testing is the best way to monitor the efficacy of different forms of eradication therapy. Using the UBT, it became rapidly clear that many so-called successful eradications were no more than a temporary clearance\(^3\) and many so-called 're-infections' following apparently successful eradication were examples of prolonged suppression of the organism followed by recrudescence and not true re-infection\(^26\).

The reader is reminded that *H. pylori* eradication is defined as a negative test taken at least 1 month after completing the candidate course of eradication therapy. The patient should not have had any antibiotics or bismuth containing compounds in the month prior to re-testing. All 3 of the PPIs currently available in the UK (omeprazole, lansoprazole and pantoprazole) can produce false negative results to the UBT test and so should be stopped at least 2 and preferably 4 weeks prior to breath testing\(^6,10,11,14\).

**Study of *H. pylori* acquisition and re-infection rates**

The two UBT tests are ideally suited to the task of studying the rate of re-infection or late recrudescence following apparently successful eradication therapy\(^26\). In Ipswich, we have been following a cohort of over 2000 adult patients with apparently successful eradication of their infection for periods of up to 8 years. After the first year, the re-infection rate was less than 0.25 % per patient per year\(^26\). As discussed elsewhere in this issue, re-infection rates in small children and mentally handicapped adults living in homes may be much higher.

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14C and 13C urea breath tests

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