

# Gut

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## Leading article

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### The urea breath test for *Helicobacter pylori*

The urea breath test (UBT) is the most important non-invasive test for *Helicobacter pylori*. It is easy to perform, reliable, comparatively cheap, and without risk to patients. Its main indication is in follow up after attempted *H pylori* eradication and hence it has important advantages over the other main non-invasive test, serology. The UBT is already widely used as a research tool and deserves wider use in routine clinical practice.

*Helicobacter pylori* produces large amounts of a powerful urease enzyme the detection of which forms the basis of the UBT. Gastric urease was first reported in 1923 by Luck who described hydrolysis of urea in the stomachs of dogs and other animals<sup>1</sup> and it was not long before humans were added to the list.<sup>2</sup> In the 1950s Kornberg used a forerunner of the UBT in anaesthetised cats to show that antibiotics destroyed urease activity and thus concluded that urease was bacterial in origin.<sup>3</sup> The discovery of *H pylori* by Warren and Marshall in 1983<sup>4</sup> was not at first linked to gastric urease (the bacterium was thought to be urease negative!<sup>5</sup>) but the relation soon became obvious.<sup>6</sup> The benefit of urease to *H pylori* is still much debated but its usefulness in diagnosis was quickly recognised and in 1985 the biopsy urease test was first described in a modified form by McNulty.<sup>7</sup> In 1987 Graham described the first UBT specifically designed to detect *H pylori* using urea labelled with the stable isotope <sup>13</sup>C,<sup>8</sup> and his description was quickly followed by reports of UBTs using the radioactive isotope <sup>14</sup>C from the UK,<sup>9</sup> Australia,<sup>10</sup> and The Netherlands.<sup>11</sup>

The principle of the UBT is simple. Urea isotopically labelled with <sup>13</sup>C or <sup>14</sup>C is given orally and if *H pylori* is present in the stomach its urease enzyme hydrolyses the urea producing isotopically labelled carbon dioxide. This diffuses into blood, is excreted by the lungs, and can be detected in breath. Many refinements to this essentially simple test have been described, the importance of which depends upon the information required from the test and the situations in which it is to be used.

In the primary diagnosis of gastroduodenal pathology, endoscopy based tests are indicated rather than UBTs. Endoscopy gives much more information than just whether *H pylori* is present in the stomach and this helps when making treatment decisions, including whether or not to treat the infection. In situations, however, where knowledge of *H pylori* status alone is needed UBTs have obvious advantages; they are without risk, well tolerated, comparatively cheap, and do not require experienced operators. Thus the main indication for UBT is after attempted *H pylori* eradication in those situations where follow up endoscopy is

unnecessary. Checking eradication is important as treatment failure is comparatively common and the success or failure of treatment has important implications for further treatment and follow up and for patient advice or reassurance. Follow up after treatment is not, however, the only indication for breath testing. Others include checking for infection when an ulcer is found on barium meal and checking for infection when an ulcer is found at endoscopy but biopsy specimens cannot be taken because of anticoagulant treatment.

The information needed from a UBT is whether or not *H pylori* infection is present. In some research situations it may be desirable to quantify urease activity but in clinical situations this is not necessary and UBT protocols should be kept simple and not made more complicated to try to obtain this information. The aim should be to discriminate accurately between *H pylori* positive and negative patients while minimising inconvenience and cost. What are the causes of inaccuracies in the test? The commonest reason for false negative tests is breath testing too soon after a course of antibiotics,<sup>10</sup> bismuth<sup>8,9</sup> or omeprazole<sup>12</sup> whether given specifically as a treatment for *H pylori* or not. Breath testing should not be performed until at least one month after the end of such a course of treatment.<sup>10,13</sup> False negative tests can also occur after gastric surgery,<sup>13</sup> presumably because of rapid emptying of urea from the stomach, and false positive tests are possible if other urease producing bacteria are present in the stomach such as may occur in patients with achlorhydria from gastric atrophy.<sup>14</sup> Breath testing should be used with caution in these situations. Suboptimal UBT protocols are another potential cause of inaccurate results. In practice, however, most published protocols give similar accuracy with sensitivity ranging from 90–100% and specificity from 78–100%<sup>11,15–24</sup> when compared with biopsy based tests. The low specificity for UBT in some of these trials is misleading and merely reflects the low sensitivity of tests with which UBT is compared. As most UBT protocols give acceptable accuracy the decision about which to use must depend largely on other factors such as safety, cost, convenience to the patient and operator, and speed at which results can be available. These factors in turn depend mainly on whether the <sup>13</sup>C or <sup>14</sup>C UBT is used, as both have practical problems and advantages arising to a large extent from the theoretical problems and advantages of <sup>13</sup>C and <sup>14</sup>C as isotopic markers.

The main theoretical problem for the <sup>13</sup>C UBT is that <sup>13</sup>C occurs naturally and at somewhat variable concentrations, usually being present in about 1.11% of expired carbon dioxide. In a positive breath test this may, for example, increase by just 0.01% to reach 1.12% – a tiny increase on a

high and variable baseline. The need to optimise and detect this increase leads to the main practical problem – cost. For a typical  $^{13}\text{C}$  UBT the patients attend fasting, are given a high energy liquid test meal to delay gastric emptying, and soon afterwards a fixed amount of  $^{13}\text{C}$  urea dissolved in water. Early UBTs used 250 mg or more,<sup>8</sup> but  $^{13}\text{C}$  urea is expensive and amounts of 100 mg<sup>23</sup> or even 75 mg<sup>17</sup> have been shown to give consistent results. Breath samples are collected before  $^{13}\text{C}$  urea is given and at regular time intervals afterwards. As  $^{13}\text{C}$  is a stable isotope, analysis is by isotope ratio mass spectrometry. This is advantageous in that its extreme accuracy permits detection of the tiny increase over baseline that the test produces, but a disadvantage is that most hospitals do not have access to a mass spectrometer and commercial analysis is expensive. To reduce costs there is much interest in single sample protocols, for example a single sample taken 30 minutes after urea administration,<sup>17,23</sup> and such protocols do not seem to adversely affect the accuracy of the test. Some workers, however, continue to prefer the reassurance of analysing samples from at least two time points.<sup>25</sup>

The most variable part of the  $^{13}\text{C}$  UBT, from one protocol to another, is the test meal. This is given to delay gastric emptying of urea and so avoid false negative results but it also increases the discriminative value of the test<sup>17</sup> by increasing all but the earliest breath test values in *H pylori* positive subjects.<sup>11</sup> Unfortunately this is partly offset by the fact that high energy meals increase the proportion of  $^{13}\text{C}$  carbon dioxide in expired breath in everyone.<sup>17,26</sup> For this reason low or non-carbohydrate meals are sometimes used, the most discriminating results between *H pylori* positive and negative subjects are reported after a 0.1N citric acid meal.<sup>17</sup> Whether the test meal could be omitted altogether, as has been done in the  $^{14}\text{C}$  UBT, has not been tested although there are suggestions that sensitivity and specificity may not be affected.<sup>17</sup> In the absence of further studies, however, test meals should continue to be used in  $^{13}\text{C}$  UBT protocols.

The optimal protocol for a  $^{14}\text{C}$  UBT is controversial. One way of performing the test is by using essentially the same protocol as for  $^{13}\text{C}$ .<sup>11,16,18</sup> Only tiny amounts of  $^{14}\text{C}$  urea are needed so 'cold' ( $^{12}\text{C}$ ) urea is added to avoid the  $^{14}\text{C}$  urea being used up too quickly. An exact amount of expired carbon dioxide (usually 1 or 2 mmol) is collected by blowing through a solution containing a fixed amount of the carbon dioxide trapping agent hyamine hydroxide and an indicator. Analysis is by liquid scintillation counting,  $^{14}\text{C}$  being a  $\beta$  emitter. This  $^{13}\text{C}$  equivalent  $^{14}\text{C}$  UBT gives values interchangeable with  $^{13}\text{C}$  UBTs,<sup>27</sup> but Marshall in particular has argued that the protocol can be simplified because  $^{14}\text{C}$  does not occur naturally and this gives it an intrinsic advantage over  $^{13}\text{C}$  as an isotopic label.<sup>21</sup> Simplified  $^{14}\text{C}$  UBT protocols do not use a test meal or cold urea and thus permit earlier breath sampling and a quicker, easier test.<sup>20,21</sup> The theoretical problem of isotopic urea exhaustion does not seem to occur but timing of breath samples does become more critical. Sampling too early may produce false positive results resulting from urease activity of oral bacteria and sampling too late may produce false negative results because of emptying of urea from the stomach. Sampling at 20 minutes<sup>21</sup> is a seemingly successful compromise and sampling at 10 minutes<sup>20</sup> has also given apparently successful results. The problem of oral urease contributing to results can be further avoided by mouth washing, and the best effect seen is with a 0.1N citric acid mouthwash<sup>17</sup> although an antiseptic mouthwash would be expected to be as good. In practice, performance of the  $^{14}\text{C}$  UBT as a diagnostic test seems to be very similar whether meal and cold urea,<sup>11,16,18</sup> meal and no cold urea,<sup>19</sup> or no meal and no cold urea<sup>15,20,21</sup> are used, and in the absence of direct comparative evidence using a simple version of the test seems preferable.

When deciding which isotope to use the main consideration

is whether facilities for using  $^{14}\text{C}$  are available. From the patients' point of view radiation exposure in the  $^{14}\text{C}$  UBT is negligible: a typical test in which 185 kBq of  $^{14}\text{C}$  urea is given gives radiation exposure to gonads and bone marrow of just  $3 \times 10^{-6}$  Sv, equivalent to roughly one day's background radiation,<sup>21</sup> and low dose  $^{14}\text{C}$  UBTs, which seem to be equally discriminative, give one quarter of this exposure.<sup>28</sup> Put another way, about 10 standard or 40 low dose  $^{14}\text{C}$  UBTs would be needed to give the same bone marrow exposure as a chest x ray.<sup>21</sup> The only patient groups therefore, in which  $^{14}\text{C}$  UBTs should be avoided are pregnant women and children. The problem with using radioactive isotopes like  $^{14}\text{C}$ , however, is the strict regulations governing such matters as licensing, training of staff, recording and moving of stock, security and spillage, and for this reason  $^{14}\text{C}$  UBTs are only manageable in the context of a medical physics department or a fully equipped research department. In most other respects  $^{14}\text{C}$  UBTs are preferable; they are cheaper because of the low cost of  $^{14}\text{C}$  urea, trapping solution, and analysis, they are quicker to perform because simplified protocols are used, and they can give results almost immediately. Performance of the two isotopes as markers in the test seems similar when direct comparisons are made,<sup>27,28</sup> and reported sensitivity and specificity for  $^{13}\text{C}$  and  $^{14}\text{C}$  UBTs are also very similar. Performance in the test, therefore, seems not to be an important consideration compared with others. We would recommend the  $^{13}\text{C}$  UBT in hospitals without a liquid scintillation counter; there are no overheads, it is extremely simple to perform, and the only disadvantage is expense (still low compared with endoscopy). For hospitals with scintillation counting available and a medical physics or research department willing to run the test,  $^{14}\text{C}$  UBTs will work out cheaper and more convenient.

In research situations it may sometimes be desirable to quantify bacterial load in *H pylori* infection. The UBT cannot do this as urease concentrations between strains vary by up to eightfold,<sup>29,30</sup> but it has been suggested that it could quantify total gastric urease activity.<sup>31</sup> The idea is that if the enzyme can be saturated with substrate (as in the  $^{13}\text{C}$  UBT or the  $^{14}\text{C}$  UBT with cold urea) and gastric emptying delayed with a test meal until a steady state is reached, the resultant UBT value can be converted to total urease activity providing carbon dioxide production rate is known. There are likely to be many inaccuracies in practice. Firstly, carbon dioxide production varies widely between subjects and is further varied by a test meal<sup>22</sup> so calculations of its value are likely to be inaccurate. Secondly, the contribution of endogenous urea to the substrate pool is unknown and presumably may vary between subjects. Thirdly, not all carbon dioxide produced in the stomach is excreted by the lungs.<sup>17</sup> Fourthly, although the test meal encourages body and fundal as well as antral coating by urea, the urea empties fairly quickly and variably from these sites<sup>33</sup> so the contribution to UBT values of *H pylori* in body and fundus is unclear and is likely to be variable. Despite these drawbacks there have been attempts to correlate UBT values with semiquantitative biopsy measures of bacterial numbers and not surprisingly a modest correlation can be shown between two vaguely quantitative tests.<sup>11</sup> Some groups, however, have failed to show even that<sup>8,22</sup> and simple UBTs without meal or cold urea are no worse at showing this modest correlation.<sup>20</sup> The UBT probably functions better as a quantitative tool when repeated in the same subject as the same strain of *H pylori* is present (with therefore the same urease activity) and many of the expected inaccuracies might be constant between tests. Published reproducibility figures,<sup>8,13,22,23</sup> however, show that even in this situation the UBT is at best a semiquantitative tool. Gross reductions in value after, for example, bismuth or antibiotics, are clearly meaningful.<sup>11,31</sup> Smaller changes should be interpreted with caution.

Some hospitals have delayed setting up a breath testing

service aware that serology, an even simpler non-invasive test, is improving fast. Although impressive results are being reported for serology it is less accurate than breath testing when direct comparisons are made.<sup>22-24</sup> It is also considered unreliable in the elderly<sup>34</sup> and suffers the intrinsic problem that a small group of patients do not have a systemic antibody response to *H. pylori*.<sup>35</sup> The biggest practical problem for serology, however, is that it is unreliable in treatment follow up before six months.<sup>36-38</sup> As the main clinical indication for a non-invasive test is in follow up after attempted eradication, the fact that breath testing is performed one month after the end of treatment<sup>10,13</sup> gives it important advantages; the risk of ulcer recurrence before treatment outcome can be determined is lower, recurrent symptoms can be treated with the knowledge of *H. pylori* status, and the shorter wait is preferable to the patient. Serology is already the investigation of choice in population studies and may become reliable enough for routine use in situations where antibiotics have not been given. After treatment, however, whether intentional or not, it will probably not replace breath testing.

So what should be the place of the UBT in research and clinical practice? In research it is a useful non-invasive tool, although of limited application in quantification of *H. pylori* load. In clinical practice it deserves to be much more widely used especially in follow up after attempted *H. pylori* eradication. Hospitals with a cooperative medical physics department or a research department with facilities for liquid scintillation counting should use the cheaper, more convenient and quicker <sup>14</sup>C UBT. Others should use the <sup>13</sup>C UBT; it requires no specialist equipment, is extremely simple to perform, and is inexpensive when compared with endoscopy. Whichever option is chosen the UBT will prove itself to be a useful test in routine clinical practice.

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