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Review article: ^{13}C-urea breath test in the diagnosis of Helicobacter pylori *infection – a critical review*

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SUMMARY

The urea breath test is a non-invasive, simple and safe test which provides excellent accuracy both for the initial diagnosis of *Helicobacter pylori* infection and for the confirmation of its eradication after treatment.

Some studies have found no differences between urea breath test performed under non-fasting conditions. The simplicity, good tolerance and economy of the citric acid test meal probably make its systematic use advisable. The urea breath test protocol may be performed with relatively low doses (<100 mg) of urea: 75 mg or even 50 mg seem to be sufficient. With the most widely used protocol (with citric acid and 75 mg of urea), excellent accuracy is obtained when breath samples are collected as early as 10–15 min after urea ingestion.

A unique and generally proposed cut-off level is not possible because it has to be adapted to different factors, such as the test meal, the dose and type of urea, or the pre-/post-treatment setting. Fortunately, because positive and negative urea breath test results tend to cluster outside of the range between 2 and 5‰, a change in cut-off value within this range would be expected to have little effect on clinical accuracy of the test.

INTRODUCTION

Helicobacter pylori infection is the most important aetiological factor in chronic gastritis and gastroduodenal ulcer disease, and its presence is closely related to the development of gastric cancer. Diagnostic methods for *H. pylori* infection have been traditionally divided into direct (invasive) and indirect (non-invasive). The first are based on the direct identification of the microorganism by means of the study of samples obtained by gastric biopsy. They are, therefore, techniques which require an endoscopy and they are consequently aggressive or uncomfortable for the patient, and carries a small but finite risk. Furthermore, upper gastrointestinal endoscopy is expensive, and biopsy-based tests for *H. pylori* add to this expense. On the contrary, indirect or non-invasive methods are based on the study and detection of certain characteristics of the bacteria (their capacity to hydrolyse the urea, for example) or on the reaction of the immune system of the host to the infection (quantification of specific antibodies by means of various serological tests). These indirect techniques do not necessitate an endoscopy and are therefore more convenient for the patient and cheaper, and thus should be preferred in situations where the extra information yielded by an endoscopy is not needed.

Breath tests are used in gastroenterology practice to study (patho)physiological and metabolic processes in an indirect way.[1] These tests have been used to measure gastric emptying, small-bowel bacterial overgrowth, exocrine pancreatic function, liver metabolic capacity, and finally, the presence of *H. pylori* in the stomach.[1] Urea breath test (UBT) has proved to be one of the most accurate methods for assessing *H. pylori* status.[2–14] This test has become, during recent years, increasingly popular in clinical practice both for screening patients

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before endoscopy and for assessing the success of *H. pylori* eradication therapies. The UBT is simple, innocuous, easy to repeat and highly accurate. It is particularly suitable in all clinical conditions where endoscopy is not strictly necessary, and to check the success of eradication regimens.

The principle of the UBT relies upon the capacity of *H. pylori*, when present in the stomach, to hydrolyse orally administered labelled urea to produce isotopically labelled CO_2 which diffuses into the blood, is excreted by the lungs and can be detected in breath samples by means of measuring equipment. Since *H. pylori* is the most common urease-containing gastric pathogen, urea hydrolysis can generally be equated with the presence of *H. pylori* infection.[7]

Urea can be labelled with two different isotopes, ^{14}C (the radioactive isotope) or ^{13}C (the non-radioactive stable isotope). Labelling urea with ^{13}C has become increasingly popular because the non-radioactive isotope is innocuous, so the test can be repeated as often as required in the same patient and can also be safely performed in children, pregnant women, and women of child-bearing age. On the contrary, the breath test which uses ^{14}C, a technique which is not authorized by most Health Authorities for the diagnosis of *H. pylori*, is associated with a dose of radiation for which, although considerably low, a licence to handle is necessary; it needs adequate storage and its use is not permitted for pregnant women or children. The main inconvenience of the breath test with ^{13}C-urea is that it usually requires a mass spectrometer to obtain the results, with a high initial economical investment. However, since the isotope is not radioactive, just one spectrometer shared by several centres permits the samples to be sent to the reference centre, thereby considerably decreasing the cost of the technique.

The non-invasive methods in general, and the UBT in particular, have a variety of uses in clinical practice. First, the strategy denominated 'test and treat' (diagnose the *H. pylori* infection and treat it) was recently recommended for patients with dyspeptic symptoms, based on the initial use of an indirect test.[15] Consequently, it has been suggested that the eradication treatment for *H. pylori* might be a valid alternative for a young dyspeptic patient in whom infection has been demonstrated, as long as no 'alarm' signs exist and no ingestion of gastroerosive drugs has taken place.[16] In this situation, both the UBT and serology might be useful, although the latter has a lower diagnostic accuracy and, in addition, needs to be validated locally before proceeding to use it regularly.[5] On the contrary, the UBT test indicates the actual *H. pylori* status, as it diagnoses the active infection, differentiating it from serology (which indicates only exposure to *H. pylori*).[5] Finally, another diagnostic alternative of recent appearance, which does not need an endoscopy, is based on the detection of *H. pylori* antigen in stools, a technique which can be considered an accurate non-invasive method for the diagnosis of the infection in untreated patients.[17]

The ^{13}C-UBT is ideally suited for the determination of *H. pylori* status in children, although until very recently there was a paucity of adequate data to validate its routine use. However, several recent studies have confirmed that the UBT has in children the same accuracy as in adults.[18–42] In the other extreme of life, some studies have confirmed, in conditions of real clinical practice that the UBT is also a reliable test of *H. pylori* diagnosis in elderly patients.[43]

To confirm eradication of *H. pylori* after treatment, the test to be used will depend on the underlying disease. For uncomplicated duodenal ulcer or dyspepsia – in the circumstance that administration of treatment is decided upon – the UBT test is the diagnostic method of choice. In such circumstances, the breath test rapidly confirms the disappearance of *H. pylori* after treatment, unlike the serological techniques, which need a prolonged period of time to confirm the eradication effect.[5] Thus, because antibody titres can take up to 6 months to fall after successful treatment, serological tests cannot readily be used to assess the efficacy of *H. pylori* eradication regimens shortly after treatment. Finally, it has been demonstrated in various studies that the detection of antigen in stools may be a reliable technique to confirm the eradication of *H. pylori*, but some other authors have not confirmed these encouraging results. In this respect, a recent systematic review on the role of stool antigen test for the diagnosis of *H. pylori* showed relatively low accuracy in some post-treatment studies with the polyclonal stool antigen test, suggesting that its use in clinical practice is yet to be defined.[17]

The diagnostic accuracy of the UBT is very high. In practice, most published protocols give similar high sensitivity and specificity, ranging from 90 to 100%, and being >95% in most cases, especially when well-designed studies are considered.[2–14] The high sensitivity obtained with this test might be due to the

fact that the UBT evaluates the totality of the gastric mucosa, unlike the diagnostic methods based on the analysis of the sample obtained by gastric biopsy, which are subject to the heterogeneous distribution of the *H. pylori* in the gastric cavity. Nevertheless, reasons for false-negative results are low intragastric bacterial load, fast gastric emptying (shorter contact time between ingested urea and infected mucosa), previous gastric surgery and concomitant administration of urease-inhibiting drugs, such as recent use of antibiotics, bismuth or proton pump inhibitors (PPI). On the contrary, although it has been suggested that, at least in theory, a false-positive result can be obtained as a consequence of the existence in the oral cavity or in the stomach of other urease-producing bacteria, the clinical relevance of this fact seems to be very limited. Urease-producing oropharyngeal bacteria may rarely cause false-positive results if breath samples are taken within 10 min of urea administration. To overcome this problem, some investigators have encapsulated the urea or administered it as a tablet.[44–47] The presence of other urease-producing bacteria in the stomach may be due to bacterial overgrowth in patients with achlorhydria from gastric atrophy[48] or after protracted PPI therapy.[12]

It should 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 standard and it is well known that this latter test is susceptible to sampling error (because of the discontinuous *H. pylori* colonization of the gastric mucosa). For example, the low specificity (e.g. the high rate of false-positives) of the UBT in some of the comparative studies could be due, in fact, to the low sensitivity of the test with which the UBT has been compared.[7] As confirmation of this fact, some authors have found 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.[49] In summary, the UBT is a very accurate diagnostic technique and it may therefore be considered the non-invasive method of choice to determine *H. pylori* status.

Since the original description by Graham *et al*. of the ^{13}C-UBT to specifically diagnose *H. pylori* infection,[50] several modifications have been proposed. These changes have included the dose of labelled urea used, the type of test meal, the time of breath collection, the cut-off values and the equipment adopted to measure isotope enrichment. Numerous modifications have been proposed for the UBT technique and a multitude of papers regarding its methodology have been published, in spite of which a definitive standardization of this test does not yet exist. Therefore, the aim of the present article will be to review the several modifications that have been suggested for the UBT, trying to answer the question of which is the best protocol for this test. This review will be focused on ^{13}C-UBT (i.e. the non-radioactive stable isotope), as it is completely innocuous and it has become the most widely used. The following aspects of the UBT protocol will be reviewed: (i) Which UBT measuring equipment may be used? (ii) Is fasting before UBT testing necessary? (iii) Is a test meal necessary? Which test meal is better? (iv) Which dose of substrate (urea) should be used? (v) Are basal breath samples (before urea ingestion) necessary? (vi) Which is the optimal breath sampling time after urea ingestion? and (vii) Which is the best cut-off point for discriminating between positive and negative tests?

SEARCH STRATEGY AND SELECTION CRITERIA

Bibliographical searches were performed in MEDLINE (up to May 2004) electronic database, looking for the following words (all fields): '*Helicobacter pylori*' or '*H. pylori*' on one hand, and 'breath test' or 'urea breath test' or '^{13}C-urea', on the other. Articles published in any language, except Japanese, were included. References of reviews on diagnostic methods for *H. pylori* infection, and from the articles selected for the study, were also examined in search of articles meeting inclusion criteria, i.e. those studies dealing with the following aspects of the UBT protocol: (i) UBT measuring equipment, (ii) necessity of fasting before UBT testing, iii) necessity and type of test meal, (iv) dose of urea, (v) necessity of basal breath samples, (vi) breath sampling time after urea ingestion, and (vii) cut-off point for discriminating between positive and negative tests.

WHICH UBT MEASURING EQUIPMENT MAY BE USED?

After hydrolysis of ^{13}C-labelled urea by *H. pylori* urease in the stomach and the formation of $^{13}CO_2$, which is transported to the lungs, ^{13}C must be detected in exhaled air by specific measuring equipment. ^{13}C analysis in the breath sample, which is always

measured as a ratio of ^{13}C–^{12}C ($\delta^{13}CO_2$/mL), may be carried out by three different types of equipment: isotope ratio mass spectrometer (IRMS), non-dispersive isotope-selective infrared spectroscope (NDIRS) and laser-assisted ratio analyser (LARA) equipment.[14] The first equipment to be used was IRMS; later on, in an effort to render the test cheaper and easier to perform, new instruments capable of measuring $^{13}CO_2$ have been developed such as the NDIRS and LARA systems.

The IRMS requires a gas chromatograph, and also requires a helium supply as carrier gas. The precision of IRMS measurements is very high, so very low isotopic enrichment can be detected, thus permitting the use of small samples of exhaled air (a 10 mL tube sample is enough to obtain reliable measurements). This, in addition to the capacity of this machine to sequentially process more than 200 samples in an automated manner, makes the system ideal for referral gastroenterological centres performing several analyses per day. The major disadvantages of IRMS are the high cost and the relatively long analysis time, which is longer than those required by the other two systems.[14]

The NDIRS seems to be similar in terms of accuracy to mass spectrometry and has the great advantage of being much cheaper than IRMS. Several non-comparative and comparative studies (vs. other measuring systems) have demonstrated excellent results with this equipment.[22, 42, 44, 51–72] However, an important disadvantage of this equipment is that it can sequentially process only a few breath samples. NDIRS also requires large breath bags to be connected directly to the spectrometer for measurement, which greatly limits the possibility of storing and transporting breath samples to a measuring laboratory. All these operating characteristics make NDIRS particularly suitable for laboratories where the daily number of assays is small or for use in the doctor's office.[14]

Finally, the LARA system is a novel technology based on laser spectroscopy. In brief, the method employs CO_2 lasers to excite a breath sample, producing an optogalvanic effect, which on analysis provides a measure of the ratio of $^{13}CO_2$–$^{12}CO_2$. Several studies using this equipment have confirmed the initial encouraging results.[73–78] The LARA system has the quickest analysis time compared with the other two machines, whereas other technical characteristics (the number of samples it can sequentially process, the volume of breath sample required and the cost of maintenance) are more similar to the IRMS than to the NDIRS system. The price of this device is slightly lower than IRMS but higher than NDIRS; this makes it attractive for laboratory settings but not for doctors' practices.[14]

IS FASTING BEFORE UBT TESTING NECESSARY?

To date, there are conflicting opinions as to whether patients should fast for a few hours before testing in order to avoid any interference between substrate and food. Some authors advocate fasting before UBT testing since in *H. pylori*-positive patients non-fasting test conditions may induce a systematic shift towards lower UBT results. A possible explanation for this may be that the tracer is mixed with the gastric chyme, which may partly prevent contact between tracer and the *H. pylori*-infected mucosal surface.[49] In contrast, in *H. pylori*-negative patients non-fasting test conditions may induce a systematic shift towards higher UBT results, which could be due to ^{13}C-urea in the food, since ^{13}C-urea is enriched in many plants.[49] A high baseline UBT value associated with false-positive results could be attributable, in theory, to the consumption of corn or cane products within the previous 2 h.[79] Therefore, it has been postulated that dietary restrictions limiting corn or cane product consumption for 6 h before testing should reduce the frequency of false results.[80] In summary, it has been suggested that non-fasting conditions may be associated with a higher risk of both false-positive and -negative results.[49] In this way, some authors have observed that feeding causes a significant change in the delta over baseline (DOB) values compared with the fasting ones and an increase in false-positive and -negative results, therefore suggesting that fasting before testing should be mandatory.[49, 63] Finally, Mana *et al.*[52] performed two successive ^{13}C-UBTs (with and without fasting), with an interval of 48–72 h, to a group of healthy volunteers; the concordance between the two protocols was perfect with respect to *H. pylori*-positive cases, but 33% of those *H. pylori*-negative patients in fasting conditions were considered infected after performing the test using the non-fasting protocol.

However, the difference between DOB values in fasting and non-fasting conditions in some studies has been very small[52, 81] or even inexistent.[82] Some authors have demonstrated that relaxation of the fasting state does not reduce the accuracy of the UBT, making this test more convenient for patients.[81–87] Furthermore, some of these authors have demonstrated the

equivalence of both protocols, in fasting and non-fasting conditions, in the same group of patients.[81, 84, 85]

The use of citric acid as the test meal may result in a wider separation in DOB values between *H. pylori*-positive and *H. pylori*-negative patients (see later the appropriate section). Thus, it has been suggested that a citric acid test meal may make the UBT more robust allowing a relaxation of the fasting state. In this respect, a randomized trial has recently shown that fasting before testing was unnecessary when citric acid was used instead of the pudding test meal.[86] For UBT protocols without prior fasting, it has been shown that the determination of the best cut-off value for DOB depends on whether a test meal is used.[82] In this respect, it has been suggested that, in case of performing the test in non-fasting conditions (and without any test meal), it would be recommendable to decrease the cut-off point to consider a patient infected with the UBT.[82, 85]

In conclusion, while some studies suggest that fasting before UBT should be mandatory, others have not found any significant differences between tests performed under fasting and non-fasting conditions and therefore do not recommend fasting before the UBT, thus making it even more applicable in the routine setting. Therefore, although prior fasting may not be essential, this methodological aspect still remains controversial, and thus it would seem prudent to perform UBT in fasting conditions until new data definitively clarify this issue.

IS A TEST MEAL NECESSARY? WHICH TEST MEAL IS BETTER?

Test meals have often been incorporated to improve diagnostic performance of ^{13}C-UBT for the diagnosis of *H. pylori* infection, as it has been suggested that administration of the urea with no additional test meal may lead, in theory, to gastric emptying of the substrate before sufficient reaction with *H. pylori* urease can take place, with the risk of having false-negative results. Thus, test meals have been designed in order to slow gastric emptying and to maximize the distribution of the substrate within the stomach so as to increase the area and time of contact between the bacteria and the substrate.

Different nutrient meals have been used, including mixtures of carbohydrates, proteins and fats, with quite similar results.[6, 7, 11, 14] A citric acid solution is currently one of the most widely used, and it has been stated that, thanks to its use, higher and faster maximum concentrations of $^{13}CO_2$ are obtained in the breath in comparison with other semiliquid test meals previously used.[45, 88–93] Consequently, a citric acid test meal may increase the discrimination between positive and negative UBT values. Several explanations for this observation have been suggested, but the exact mechanism for the beneficial effect is still controversial.[89, 90, 94–96] The total costs for the citric acid test meal are very low and can be ignored in comparison with the costs of the ^{13}C-urea. Other test meals such as commercially available, semiliquid nutritional drinks are much more expensive. In consequence, citric acid solution is also advantageous from an economic point of view.

In addition to citric acid, other test drinks-like orange or apple juice have been used because of a better taste. Several studies have included orange juice as a test meal,[47, 51, 53, 91, 97] but the diagnostic accuracy of the UBT with either orange juice or citric acid solution has only been exceptionally compared.[47, 91] In one of these comparative studies, it was shown that both citric acid and orange juice were equally effective.[47] However, other authors[91] showed significantly higher DOB values and higher area under the curve in *H. pylori*-positive patients when citric acid solution was administered compared with orange juice. Furthermore, sensitivity of the UBT was 100% when citric acid was used as a test drink and only 88% with orange juice. In summary, it seems that UBT may partially lose diagnostic accuracy when orange juice instead of citric acid is used as a test drink, and that the faster gastric emptying of orange juice might be responsible for the lower diagnostic accuracy of the UBT. Finally, apple juice, which contains a variety of organic acids, also increases $^{13}CO_2$ excretion. Nevertheless, in one published study the use of apple juice as the test drink was associated with unsatisfactory results, with diagnostic accuracy below 90%.[66]

Malfertheiner's group firstly described an optimize test protocol for the UBT based on the administration of urea 10 min after ingestion of citric acid solution.[88] Citric acid induces an immediate relaxation of the gastric fundus and a marked inhibition of antral motility through duodenogastric reflexes.[98] This German group afterwards demonstrated that UBT could be further simplified with no loss of diagnostic accuracy by administrating the substrate (urea) dissolved in the citric acid solution rather than 10 min after administration of the test drink.[98] Finally, in a third study, those

authors evaluated and confirmed the accuracy of this modified UBT for both primary and post-treatment diagnosis of *H. pylori* infection in a large patient population in clinical practice.[99] This modification makes it possible to prepare the total volume of solution (substrate dissolved in the test drink) required for all patients to be tested on 1 day. Furthermore, as ^{13}C-urea is stable in the citric acid solution at room temperature for at least 2 weeks,[98] the required amount of test solution can be prepared, for example, once every 2 weeks and preparation of one test for each patient is no longer required.

The elimination of the test meal in UBT protocol would have several advantages, such as better tolerance and higher simplicity, speed and economy of the test. The higher DOB values obtained with citric acid (when compared with those without citric acid) does not necessarily imply that it is associated with a better discrimination between infected and non-infected patients. Thus, some authors have also evaluated and obtained encouraging results without using citric acid (or any other test meal) in UBT.[55, 82, 85, 93, 100–105] Furthermore, some studies have demonstrated the equivalence between both protocols – with and without test meal – in the same study with the same group of patients.[82, 85, 93, 104, 105] Furthermore, additional studies have even reported similar DOB values with and without (citric) test meal, although this has been suggested to be a unique observation in the Chinese population and is possibly related to the difference in the distribution, delivery and emptying of ^{13}C-urea.[104, 105] In fact, a similar observation was reported with regard to the parietal cell mass and acid secretory capacity of Asian patients with duodenal ulcer, which are only slightly more than half the values of Caucasian patients despite correction for body stature.[106] Furthermore, ethnic differences in gastric emptying have been reported previously.[107]

One explanation for the lack of negative consequences of eliminating citric acid, in spite of the theoretical aforementioned advantages of this test meal, is suggested by the study by Atherton *et al.*[108] In that study, the test meal did not affect UBT results at 10 min, but increased values at 30 min and thereafter. When no test meal was given, delivery of urea solution to the body and fundus of the stomach was extremely poor, so the UBT value reflected mainly antral urease activity. In the subjects in that study, however, the contribution of urease in the body and fundus to UBT values seemed to be small. One possible interpretation of this would be that, as the authors suggest, antral urease is the major contributor to ^{13}C-UBT values.

The optimal cut-off point may differ depending on the test meal. In that respect, it has been suggested that, in case of performing the test without any test meal, it would be recommendable to decrease the cut-off point to consider a patient infected with the UBT.[82, 85, 93] In this respect, several authors have reported encouraging results when UBT was performed without test meal and using a cut-off for DOB lower than 5.[56, 82, 100, 105, 109–112]

Some of the studies reporting favourable results without a test meal included only patients who had not received eradication treatment[93] or a very reduced number of post-treatment patients.[104, 105] Perhaps, citric acid might not be necessary in cases of high *H. pylori* urease activity, as in patients with no eradication therapy being administered. However, the accuracy of the diagnosis of the UBT test should be evaluated and confirmed not only before receiving treatment but also after administration of antibiotics for *H. pylori*. The accuracy of any diagnostic method in the assessment of eradicating efficacy after *H. pylori* therapy may be lower than in the diagnosis of the infection in untreated patients, as the density of the microorganism in gastric mucosa, when the infection persists after antibiotic therapy, is usually lower. Menegatti *et al.*[113] demonstrated that the sensitivity of the UBT with citric acid for the diagnosis of *H. pylori* infection after eradication treatment was 100%, whilst this figure decreased to 80% when citric acid was not used.

It is well known that PPIs should be stopped some time before performing UBT. However, patients with ongoing dyspeptic symptoms are often unwilling to withhold PPIs while awaiting breath testing. Or perhaps the doctor may forget to advise the patient about the cessation of PPIs prior to breath testing. When subjects taking PPIs undergo UBTs with meals-containing citric acid, the diagnostic accuracy of the test is improved.[45, 91, 92] It has been argued that a false-negative UBT in subjects on PPI therapy is due to the pH increase induced by PPIs and a decrease in intrabacterial urease activity because of inactivation of urea entry into the organism.[96] Acidification of the test meal seems therefore desirable for accurate UBT results, particularly in subjects on PPI therapy.[96]

In conclusion, in the absence of further studies, test meals (and especially citric acid) should probably continue to be used in UBT protocols. Although it is possible that the advantage of administering a test meal may be restricted to specific circumstances (e.g. patients with particular parietal cell mass, acid secretory capacity, or gastric emptying related with their ethnic origin, patients taking PPIs, confirmation of *H. pylori* eradication after treatment, etc.), the simplicity, good tolerance, and the economy of the citric acid test meal probably makes its systematic use advisable in clinical practice.

WHICH DOSE OF SUBSTRATE (UREA) SHOULD BE USED?

The dosage of urea used in the UBT has not proven to be a critical factor in achieving accurate results. Theoretically, there is a lower limit below, which the proportion of false-negative tests will increase. However, the minimum dose of substrate is not clearly established. Thus, the dose of urea employed to perform the test has been progressively reduced in order to reduce cost. Whilst the first tests were performed with 350 mg of urea,[50] later a dose of 125 mg[114, 115] or 100 mg has been used with success. More recently, it has been suggested that 75 mg of urea might be sufficient to obtain good results with the UBT, and this has been the dose more widely used. Finally, several studies have confirmed that even 50 mg of urea can be employed to obtain high accuracy with the UBT.[46, 47, 64, 105, 116, 117] Urease-producing oropharyngeal bacteria may theoretically cause false-positive UBT results. As previously mentioned, to overcome this problem, some investigators have encapsulated the urea or administered it as a tablet.[44–47, 105, 117] With this form of administration, some authors have reported excellent results with only 50 mg of urea[46, 47, 105, 117] or even with 38 mg.[44] As the endogenous CO_2 excretion by children is less than in adults, less urea is required for children. Accordingly, it has been demonstrated that a dose of 50 mg of urea is sufficient to achieve excellent results with UBT in this population.[18, 20, 22, 27, 42]

The selection of the optimal dose of urea may depend, at least in part, on the test meal used. Thus, the citric acid test meal produces a more rapid increase in labelled CO_2 in the breath,[45, 88–92] suggesting that a lower dose of urea might be used for categorization of *H. pylori* status.[89] In this respect, Graham *et al.*[86] compared, in a randomized study, the standard US UBT (which uses a pudding test meal and 125 mg of urea) with a new protocol (using citric acid as the test meal and only 75 mg of urea), demonstrating excellent agreement between the two versions of the UBT.

It has been suggested that when low doses of urea are used (e.g. 50 mg), the cut-off value for DOB should be reduced.[105] In this respect, as shown in Table 1, almost all studies employing this low dose of urea have considered a cut-off point between 2.5 and 3.5‰, i.e. relatively low.

In conclusion, UBT protocol may be performed with relatively low doses (<100 mg) of urea. Thus, when considering studies that have employed <100 mg of urea (see Table 1), excellent mean sensitivity (98%) and specificity (97%) is calculated. Even when excluding those studies prescribing the urea in tablet or capsule form, the accuracy of the test was very high, both with 75 mg (mean sensitivity and specificity of 97%) but also with only 50 mg of urea (mean sensitivity and specificity of 98%).

ARE BASAL BREATH SAMPLES (BEFORE UREA INGESTION) NECESSARY?

Basal breath samples are usually obtained, in addition to those obtained after urea intake, as it has been suggested that basal values may oscillate among population (e.g. depending on diet).[80] The result of UBT for the diagnosis of *H. pylori* is usually expressed as DOB at a fixed time, i.e. as the algebraic difference between $^{13}C/^{12}C$ value at time t (for example, 30 min) and $^{13}C/^{12}C$ value at baseline. The $^{13}C/^{12}C$ ratio is usually expressed as part per thousand (‰) relative to an international standard, the Pee Dee Belemnite (PDB) calcium carbonate. By expressing the results as DOB, possible confusion and variations between mass spectrometers in their standardization with the PDB are thought to be avoided.[118] However, the superiority of this strategy has not been confirmed by all authors. The elimination of basal samples in UBT protocol would have the advantages of higher simplicity and speed. Thus, some authors have reported encouraging results when basal samples are omitted. For example, Klein and Graham[79] found comparable specificity and slightly reduced sensitivity when only ^{13}C-posturea values were used, yielding only a 2% of false-positive and false-negative results. Nevertheless, Ensure solution was used as the test drink in this study (instead of a citric acid solution, the most common test drink at present), and a

Table 1. Diagnostic accuracy of the ^{13}C-urea breath test with the following protocols: (i) <100 mg of urea, or (ii) samples obtained <30 min after urea ingestion, or (iii) considering a cut-off point <5‰ for discriminating between positive and negative results

Author (reference)	Indication	Measuring equipment	Urea dose (mg)	Test meal	Time (min)	Cut-off point (δ‰)	Gold standard	Sensitivity (%)	Specificity (%)
Braden *et al.*[81]	Pre	IRMS	75	–	20	5	120 min UBT	99	100
Dominguez *et al.*[88]	Pre	IRMS	75	Citric acid	30	4	RUT, H, C	100	100
Eggers *et al.*[90]	Pre	IRMS	75	Citric acid	30	2	RUT, C, S	94	78
Epple *et al.*[49]	Pre/post	IRMS	75	Citric acid	30	1.3	H	96	100
Gatta *et al.*[46]	Pre	IRMS	50†	Citric acid	10	1.6–3.1	RUT, H, C	100	100
Gatta *et al.*[46]	Post	IRMS	50†	Citric acid	10	1.5–1.6	RUT, H, C	100	100
Gatta *et al.*[46]	Pre	IRMS	75†	Citric acid	10	3.1–6.8	RUT, H, C	100	100
Gatta *et al.*[46]	Post	IRMS	75†	Citric acid	10	4.9–7	RUT, H, C	100	100
Gatta *et al.*[46]	Pre	IRMS	100†	Citric acid	10	1.5–3	RUT, H, C	100	100
Gatta *et al.*[46]	Post	IRMS	100†	Citric acid	10	1.4–7.4	RUT, H, C	100	100
Gatta *et al.*[47]	Pre	IRMS	75	Citric acid	30	4.5	RUT, H, C	100	–
Gatta *et al.*[47]	Pre	IRMS	75	Orange juice	30	4.5	RUT, H, C	100	–
Gatta *et al.*[47]	Pre	IRMS	50†	Citric acid	10	1.5	RUT, H, C	100	–
Gisbert *et al.*[93]	Pre	IRMS	100	–	30	3.3–3.9	Citric acid UBT	100	100
Gisbert *et al.*[123]	Post	IRMS	100	Citric acid	30	4.6	RUT, H	100	97
Hamlet *et al.*[45]	Pre/post	IRMS	100†	Citric acid	10	1.8	RUT, H, C	95	100
Hamlet *et al.*[45]	Post	IRMS	100†	Citric acid	10	1.3	RUT, H, C	95	100
Kato *et al.*[109]	Pre	IRMS	100	–	10	3.5	RUT, H, C	99	100
Kato *et al.*[109]	Post	IRMS	100	–	10	3.5	RUT, H, C	87	99
Kato *et al.*[110]	Post	IRMS	100	–	20	3.5	H, C	67	99
Kato *et al.*[110]	Post	IRMS	100	–	20	2.5	H, C	100	96
Klein *et al.*[114]	Pre/post	IRMS	125	Milk	30	2.4	H	100	100
Klein *et al.*[114]	Pre/post	IRMS	250	Milk	30	4.2	H	100	98
Koletzko *et al.*[58]	Pre	IRMS	75	Citric acid	15	5	NDIRS UBT	100	100
Labenz *et al.*[97]	Pre	IRMS	75	Orange juice	30	4	H, C	98	100
Leodolter *et al.*[99]	Pre	IRMS	75	Citric acid	30	4	RUT, H, C	92	99
Leodolter *et al.*[99]	Pre	IRMS	75	Citric acid‡	30	4	RUT, H, C	95	98
Leodolter *et al.*[99]	Post	IRMS	75	Citric acid	30	4	RUT, H, C	95	100
Leodolter *et al.*[91]	Pre	IRMS	75	Citric acid	30	4	RUT, H, C	100	100
Leodolter *et al.*[91]	Pre	IRMS	75	Orange juice	30	4	RUT, H, C	88	100
Liao *et al.*[116]	Pre	IRMS	50	Milk	10	2.5	RUT, H	99	95
Liao *et al.*[116]	Pre	IRMS	50	Milk	15	2.5	RUT, H	99	97
Liao *et al.*[116]	Pre	IRMS	50	Milk	30	2.5	RUT, H	99	97
Malaty *et al.*[100]	Pre	IRMS	125	–	20	2.4	RUT, H, C	96	100
Miwa *et al.*[102]	Pre/post	IRMS	100	–	20	5	H	96	97
Ng *et al.*[82]	Pre	IRMS	75	Citric acid	30	5	RUT, H	97	96
Ng *et al.*[82]*	Pre	IRMS	75	Citric acid	30	5.5	RUT, H	93	97
Ng *et al.*[82]	Pre	IRMS	75	–	30	3.5	RUT, H	96	94
Ohara *et al.*[111]	Pre	IRMS	100	–	20	2.5	RUT, H, C	98	98
Peng *et al.*[125]	Pre	IRMS	100	Milk	15	4.8	RUT, H, C	94	89
Savarino *et al.*[63]	Pre	IRMS	75	Citric acid	15	5	RUT, H	98	100
Savarino *et al.*[63]	Pre	IRMS	75	Citric acid	30	5	RUT, H	100	100
Savarino *et al.*[76]	Pre/post	IRMS	75	Citric acid	30	5	RUT, H	98	97
Sheu *et al.*[64]	Pre	IRMS	50	Citric acid	15	3.5	H, C	96	99
Sheu *et al.*[122]	Pre	IRMS	100	–	15	4	H, C	97	97
Sheu *et al.*[122]	Post	IRMS	100	–	15	2.5	H, C	94	95
Wong *et al.*[104]	Pre	IRMS	75	Citric acid	30	5	RUT, H	96	98
Wong *et al.*[104]	Pre	IRMS	75	–	30	5	RUT, H	95	98
Wong *et al.*[105]	Pre/post	IRMS	50	–	20	7	RUT, H	100	96
Wong *et al.*[105]	Pre/post	IRMS	50	–	30	3	RUT, H	100	100
Wong *et al.*[105]	Pre/post	IRMS	75	–	20	4.5	RUT, H	100	92

Table 1. Contiuned

Author (reference)	Indication	Measuring equipment	Urea dose (mg)	Test meal	Time (min)	Cut-off point (δ‰)	Gold standard	Sensitivity (%)	Specificity (%)
Wong *et al.*[105]	Pre/post	IRMS	75	–	30	3.5–4.5	RUT, H	100	100
Wong *et al.*[105]	Pre/post	IRMS	50	Citric acid	20	3	RUT, H	100	98
Wong *et al.*[105]	Pre/post	IRMS	50	Citric acid	30	2.5	RUT, H	100	98
Wong *et al.*[117]	Pre/post	IRMS	50†	Citric acid	10	1.2	RUT, H	100	90
Wong *et al.*[117]	Pre/post	IRMS	50†	Citric acid	20	2.1	RUT, H	100	100
Wong *et al.*[117]	Pre/post	IRMS	50†	Citric acid	30	1.2	RUT, H	100	98
Bielanski and Konturek[44]	Pre	NDIRS	38†	–	–	5	RUT, H	97	95
Chang *et al.*[56]	Pre	NDIRS	20	–	20	3.5	RUT, H, C, SAT	100	100
Chen *et al.*[55]	Pre	NDIRS	100	–	20	5	RUT, H, C	97	99
Coelho *et al.*[51]	Pre	NDIRS	75	Orange juice	30	4	RUT, H, ^{14}C-UBT	100	100
Ellenrieder *et al.*[66]	Pre	NDIRS	75	Apple juice	30	3.5	RUT, H, C	91	89
Gisbert *et al.* [72]	Pre/post	NDIRS	100	Citric acid	30	11	RUT, H	100	89
Koletzko *et al.*[58]	Pre	NDIRS	75	Citric acid	15	5	IRMS UBT	100	100
Mana *et al.*[54]	Pre	NDIRS	75	Citric acid	10	4–5	H	100	95
Riepl *et al.*[53]	Pre	NDIRS	75	Orange juice	15	6.5	H, C	92	94
Savarino *et al.*[63]	Pre	NDIRS	75	Citric acid	15	5	RUT, H	98	93
Savarino *et al.*[63]	Pre	NDIRS	75	Citric acid	30	5	RUT, H	97	95
Sheu *et al.*[64]	Pre	NDIRS	50	Citric acid	10	3.5	H, C	96	99
Sheu *et al.*[64]	Pre	NDIRS	50	Citric acid	15	3.5	H, C	96	99
Taniguchi *et al.*[59]	Pre	NDIRS	100	–	15	?	H	98	74
Savarino *et al.*[76]	Pre/post	LARA	75	Citric acid	30	5	RUT, H	98	97
Tanahashi *et al.*[112]	Pre/post	LARA	100	–	20	2.5–3	H, C	98	100

Pre, before eradication treatment; post, after treatment to assess *Helicobacter pylori* eradication success; IRMS, isotope ratio mass spectrometer; NDIRS, non-dispersive isotope-selective infrared spectroscope; LARA, laser-assisted ratio analyser; time: breath sampling time after urea ingestion.
* Test in non-fasting conditions.
† Urea in tablet form (instead of in water solution form).
‡ Urea disolved in citric acid.
Gold standard – RUT, rapid urease test; H, histology; C, culture; UBT, ^{13}C-urea breath test; ^{14}C-UBT, ^{14}C-urea breath test; SAT, stool antigen test; S, serology.
Studies including only children were excluded.

high dose (125 mg) of urea was given (instead of the more usual dose of 75 mg). Lotterer *et al.*,[119] using a citric acid solution and lower doses of urea (75 mg), concluded that a reduction of samples to one single breath sample taken 30 min after the ingestion of the tracer showed no significant differences in the quality parameters when compared with the standard UBT protocol. Oksanen *et al.*,[101] using a simplified UBT without a test meal, and a single-point breath evaluation after ingestion of ^{13}C-labelled urea, reported a sensitivity and specificity of 92% and 95%, respectively. Labenz *et al.*[97] performed a UBT using 75 mg ^{13}C-labelled urea and orange juice as test meal, and showed exactly the same accuracy results (98% sensitivity and 100% specificity) with the standard protocol (two-point measurements) and the single-point measurement (at 30 min). Finally Gisbert *et al.*[120] have confirmed these promising results when basal samples are omitted using 100 mg or ^{13}C-urea and citric acid as test meal.

Nevertheless, as the baseline ^{13}C-UBT value of fasting subjects varies between populations with different dietary habits,[80] this simplified procedure may not always be applicable. Thus, the baseline abundance of $^{13}CO_2$ in breath CO_2 is not constant for all individuals, but reflects recent diet history, in particular, the proportion of carbohydrates derived from wheat, rice and beet sugar products as opposed to those from corn and cane sugar.[80] A high baseline UBT value associated with false-positive results could be attributable to the consumption of corn or cane products within the previous 2 h, and might be expected from non-compliant adolescents.[79] In a recent study,[120] almost all patients that were misdiagnosed by omitting basal samples had ^{13}C-posturea results very close to the cut-off point, suggesting that basal values should also be

taken into account when borderline results are obtained with ^{13}C-posturea values only. Therefore, a strategy of not analysing baseline samples unless borderline results are obtained deserves evaluation.

The explanation for the excellent results in aforementioned studies despite eliminating basal samples may be that basal UBT values are very similar in all patients, at least within a geographical area. In this respect, mean values of basal UBT values oscillated within a very narrow range in some of aforementioned studies.[79, 120] Nevertheless, in patients with low basal UBT levels (due, for example, to Asian dietary habits with a preponderance of rice in the diet) false-negative results could be obtained.[79] Although in some European geographical areas this seems not to be a problem, it could be that more false-negative results appear if the suggested strategy would be applied in other populations. Therefore, previous encouraging results without basal samples should be confirmed in other geographical areas before they can be generally recommended.

WHICH IS THE OPTIMAL BREATH SAMPLING TIME AFTER UREA INGESTION?

In the original protocol by Graham *et al.*,[50] breath samples were collected every 10 min for 3 h. Subsequently, the test has been progressively simplified. At present, there is general agreement on the use of two breath samples, one collected before and another collected, in most cases, 20–30 min after urea ingestion. It has been shown that sampling too early (at 5 or 10 min) may produce false-positive results because of urease activity of oral bacteria.[7, 27] Conversely, sampling too late may produce false-negative results because of the emptying of urea from the stomach.

When administered urea as a drink, interference from urease-producing bacteria in the oropharynx may cause false-positive results in early breath samples. Therefore, most UBT protocols do not obtain the diagnostic breath sample until 20–30 min after dosage and usually include a test meal to prevent premature emptying of urea from the stomach. In contrast, when using a capsule or tablet (instead of the generally used water solution), the problem of oropharyngeal bacteria seems to be eliminated, and the test can be performed already after 10 min.[45–47]

The selection of the optimal sampling time may depend on the time needed for the hydrolysis of ^{13}C-urea by contact with the *H. pylori* urease. Thus, the citric acid test meal produces a more rapid increase in labelled CO_2 in the breath,[45, 88–92] which suggests that a postdose breath collection period as short as 10–15 min might be used for categorization of *H. pylori* status.[89] Furthermore, the use of citric acid has been shown to markedly reduce the interfering effect of the oral urease through an acid-induced stimulation of saliva production.[90] Graham *et al.*[86] compared, in a randomized study, the standard US UBT (which uses a pudding test meal and obtain breath sample 30 min after urea ingestion) with a new protocol (using citric acid as the test meal and breath sample taken earlier, at 15 min), and there was excellent agreement between the two versions of the UBT with >98% of subjects having concordant results.

Studies evaluating diagnostic accuracy of the UBT with samples obtained <30 min (from 10 to 20) after urea ingestion are included in Table 1. Accuracy with these 'short' protocols was very high, with both sensitivity and specificity of 97%. When only protocols without test meal (nor citric acid or any other test meal) and with urea in the usual form of water solution (instead of capsule or tablet) were considered, accuracy of the UBT was very similar (mean sensitivity and specificity of 95% and 96% respectively), which suggests that it is probably unnecessary to wait 30 min before obtaining breath samples after urea ingestion. In conclusion, when UBT is performed following the most widely used protocol (i.e. with citric acid and 75 mg of urea solution), excellent accuracy results (sensitivity of 99% and specificity of 98%) are obtained when breath samples are collected as early as 10–15 min after urea ingestion (Table 1).

WHICH IS THE BEST CUT-OFF POINT FOR DISCRIMINATING BETWEEN POSITIVE AND NEGATIVE TESTS?

The determination of the appropriate cut-off value for discriminating between positive and negative tests is critical. The results of each UBT are issued in δ units, which include a corrective factor (a gas of known composition and concentration, which is analysed in the same manner and at the same time as the samples under study). This δ-value is standardized and is internationally expressed as delta per thousand of the $^{13}C/^{12}C$ ratio of the patient compared with the standard. Thereby two δ-values are obtained for each patient, one baseline and one posterior, and the algebraic difference

between them will result in the final value supplied by the laboratory.

The precise choice of the cut-off point to define whether the UBT is positive or negative represents a controversial issue. The cut-off value for the UBT was originally determined as 5.0‰ based on the normal distribution of excess $\delta^{13}CO_2$ values for *H. pylori*-negative subjects who have never been infected.[118] This cut-off point was proposed by Logan *et al.*[118] in their European standard protocol, and it has been traditionally the most widely recommended. Later, by using receiver operator characteristic (ROC) curves, some authors showed that this cut-off value could be lowered to 3.0 or 3.5‰ without compromising the sensitivity and specificity of the test, and even improving its accuracy.[84, 100] Cluster analysis was used by Mion *et al.*,[121] who also suggested adopting 3.0‰ as the optimal cut-off point. Other authors have recommended even lower values for the cut-off point, of about 2.5‰, although they have employed lower doses of urea (as it will be reviewed later, the urea dose may influence, in turn, the best cut-off point).[90, 114, 119, 121, 122]

Some authors have considered a 'grey zone' in which the results of UBT are inconclusive, to account for the spontaneous variation of $^{13}CO_2$ in breath and the limits of the IRMS analytical precision,[45, 54, 121] This grey zone has been varied among the different studies, but it usually includes values between 2.0 and 5.0‰, or sometimes between 2.5 and 3.5‰.[121] The grey zone includes just a narrow spectrum of DOB values and a markedly low number of corresponding patients, which indicates that when the UBT is positive, DOB figures are usually much higher than the cut-off point; on the other hand, when the UBT is negative, DOB values are usually very close to zero, and far away from the cut-off point. In this respect, some authors have found that only a remarkably low percentage of the patients (approximately 1–2%) evaluated with the UBT has DOB values in the indeterminate or grey zone.[45, 55, 84, 91, 99, 121] These findings suggest that a borderline DOB value (e.g. very close to the selected cut-off point) should be cautiously interpreted, and the result should probably be confirmed (either by repeating the UBT or by other diagnostic methods).

As previously mentioned, the selected cut-off point may depend on the dose of urea administered. Klein *et al.*[114] reported a sensitivity and specificity of 100% when 125 of urea were administered and the DOB cut-off point was set at 2.4‰; however, when the same patients received a higher urea dose of 250 mg, the best cut-off point was increased up to 4.4‰. Similarly, Ellenrieder *et al.*,[66] when administering 75 mg of urea confirmed that reduction of the DOB cut-off level from 5.0 to 3.5‰ led to significant improvement in accuracy of the UBT. As shown in Table 1, most of the protocols administering 75 mg of urea select a cut-off point lower than 5.0‰ (usually between 3.5 and 5.0‰); and this cut-off point was between 2.5 and 3.5‰ in almost all protocols employing 50 mg of urea. Furthermore, the urea presentation and not only the dose may also influence on the appropriate protocol, as it has been suggested that the optimal cut-off point for the tablet-based UBT should be lower owing to its much lower levels of background ^{13}C-urea hydrolysis in uninfected subjects.[45]

Finally, the best cut-off point may also depend on the indication of the UBT (i.e. before or after eradication therapy has been administered). The gastric density of microorganisms appears to be lower (in comparison with the pre-treatment situation) in patients in which, in spite of receiving eradication treatment, infection persisted. In addition, after the eradication treatment including a PPI *H. pylori*, migration from the antrum to the corpus has been described, which could perhaps decrease the contact between the organism and the urea solution. In a recent study,[122] the sensitivity and specificity of the first UBT achieved most optimally was 97.5% and 96.7%, respectively, by setting the cut-off point at 4.0‰; in contrast, using the same cut-off point of 4.0‰, the sensitivity and specificity of the second UBT in patients after therapy achieved just 80% and 97.6% of sensitivity and specificity, respectively. By applying cut-off points downward of 4.0, 3.5, 3.0 and 2.5‰ for the second UBT, the sensitivity was elevated to 80%, 82.8%, 88.6% and 94.3%, respectively, while the specificity was preserved at more than 95.2%. Seven of 35 patients with failure of therapy had 'equivocal' DOB results at the second UBT (range: 2–5‰), and this accounted for the false-negative result. All seven patients had low bacterial densities, and three patients had bacteria distributed only in high body near the cardia.[122] Other studies have also suggested that the cut-off point which was associated with the greatest diagnostic accuracy post-treatment is slightly lower than the corresponding for the pre-treatment evaluation.[45, 123] From the studies included in Table 1 it can be deduced that a cut-off of approximately 4‰ was considered in most pre-treatment studies, while this figure was closer to 3‰ when the post-treatment studies were considered.

Therefore, selection of a lower cut-off value (in comparison with the pre-treatment setting) may be helpful to maintain the diagnostic accuracy of UBT for monitoring the *H. pylori* eradication.

In conclusion, a unique and generally proposed DOB cut-off level is not possible because it has to be adapted to different factors, such as the test meal, the dose and type of urea, or the pre-/post-treatment setting in which the test is employed. Fortunately, because positive and negative UBT results tend to cluster outside of the DOB range between 2 and 5‰, a change in cut-off value within this range would be expected to have little effect on clinical accuracy of the test.[124]

CONCLUSIONS

The UBT is based on the simple principle that a solution of isotopically labelled urea will be rapidly hydrolysed by the abundantly expressed urease of *H. pylori*. The UBT is a non-invasive, simple and safe test, which provides excellent accuracy both for the initial diagnosis of *H. pylori* infection and for the confirmation of its eradication after treatment. Consequently, UBT is, at present, the test of choice for screening patients before endoscopy and in the non-invasive evaluation of the efficacy of eradication regimens. This method can and should be used in place of invasive methods in all those conditions not requiring endoscopy, while, in other cases, it can be used along with the invasive tests to improve diagnostic accuracy. The UBT can be used as the sole method for evaluating the effectiveness of treatment of *H. pylori* infection. In summary, the UBT is an accurate, practical and readily available test that provides a 'gold standard' against which other tests for *H. pylori* can be compared.

Numerous modifications have been proposed for the UBT technique and a multitude of papers regarding its methodology have been published, in spite of which a definitive standardization of this test does not yet exist. ^{13}C analysis in the breath sample can be carried out by three different types of equipment: IRMS, NDIRS and LARA. While some studies suggest that fasting before testing should be mandatory, others have not found any significant differences between tests performed under fasting and non-fasting conditions and therefore do not recommend fasting before the test, thus making it even more applicable in the routine setting. Therefore, although prior fasting may not be essential, this methodological aspect still remains controversial, and thus it would seem prudent to perform UBT in fasting conditions until new data clarify this issue. Test meals (and especially citric acid) should probably continue to be used in UBT protocols; although it is possible that the advantage of administering a test meal may be restricted to specific circumstances (e.g. patients with particular parietal cell mass, acid secretory capacity, or gastric emptying related with their ethnic origin, patients taking PPIs, confirmation of *H. pylori* eradication after treatment, etc.), the simplicity, good tolerance, and the economy of the citric acid test meal probably makes advisable its systematic use in clinical practice. The UBT protocol may be performed with relatively low doses (<100 mg) of urea: 75 mg or even 50 mg seem to be sufficient. Basal breath samples are usually obtained, in addition to those obtained after urea intake; however, some authors have reported promising results when basal samples are omitted. Nevertheless, these last results should be confirmed in other geographical areas before they can be generally recommended. At present, there is general agreement on the use of two breath samples, one collected before and another collected, in most cases, 20–30 min after urea ingestion; however, when UBT is performed following the most widely used protocol (i.e. with citric acid and 75 mg of urea solution), excellent accuracy results are obtained when breath samples are collected as early as 10–15 min after urea ingestion. A unique and generally proposed DOB cut-off level is not possible because it has to be adapted to different factors, such as the test meal, the dose and type of urea, or the pre-/post-treatment setting in which the test is employed. Fortunately, because positive and negative UBT results tend to cluster outside of the DOB range between 2 and 5‰, a change in cut-off value within this range would be expected to have little effect on clinical accuracy of the test.

In conclusion, although definitive standardization of the protocol for UBT does not yet exist, it can be suggested the following recommendations: (i) UBT can be carried out by different types of equipment, such as IRMS, NDIRS or LARA. (ii) It would seem prudent to perform UBT in fasting conditions until new data definitively clarify this issue. (iii) Citric acid should probably be used as the test meal. (iv) 75 mg or even 50 mg of urea seem to be sufficient to perform UBT with high accuracy. (v) It would seem recommendable to obtain basal breath samples (in addition to those obtained after urea intake), until promising results

reported by some studies omitting basal samples are confirmed. (vi) The use of two breath samples, one collected before and another collected 10–30 min after urea ingestion, is associated with excellent accuracy results. (vii) A unique and generally proposed cut-off point is not possible because it has to be adapted to different factors, but all figures included in the range between 2 and 5‰ seem reasonable.

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