Chapter 9
Urea Breath Test and Rapid Urease Test

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Abstract The urea breath test (UBT) and the rapid urease test (RUT) are the most commonly used diagnostic methods for *H. pylori* infection and rely on detecting the presence of urease produced by *H. pylori*. The sensitivity of the UBT is excellent (often exceeding 95 %). The majority of false-positive results after eradication occur when the results are near the cutoff value and are most frequent in areas where atrophic gastritis is common and citric acid is not used as an adjuvant. The sensitivity of RUT is slightly less (approximately 80–95 %). Increasing the number and size of biopsy fragments, collecting them from the antrum and corpus, or sampling gastric mucus more widely instead of biopsy samples achieves better results with the RUT. Negative results of the either test should not be taken as evidence of the absence of the infection especially in PPI users as well as in patients with bleeding or a history of partial gastrectomy.

Keywords Urea breath test • Rapid urease test • Diagnosis • *Helicobacter pylori* • Proton pump inhibitor • Gastrectomy • Citric acid • Bleeding • False-negative results

9.1 Introduction

The vast numbers of infected patients worldwide and the severe clinical outcomes have made *Helicobacter pylori* (*H. pylori*) infection a public health issue. Because at least 20 % of those with clinically latent *H. pylori* infections eventually develop serious clinical diseases, the infection should be eradicated whenever it is discovered unless there are compelling reasons [1, 2]. If a simple and effective therapy or

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Table 9.1 Accuracy of diagnostic tests for *H. pylori* infection

	Rapid urease test		Urea breath test		Stool antigen test [5]	
	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
Before eradication	○	◎	◎	◎	○	◎
After eradication	X	○	◎	○	◎	◎
PPI use	X	◎	△	◎	△	◎
Upper GI bleeding	X	△	○	○	△	○
Gastrectomy	△	◎	△	○	○	◎

◎ more than 95 %, ○ 85 ~ 95 %, △ more than 75 %, X 75 % and less

vaccine were available, *H. pylori* would likely be targeted for a worldwide elimination campaign. The recommended approach is a three-part strategy of test, treat, and confirm cure. The decision to treat must be based on an accurate diagnosis, and the test of choice among a variety of available tests depends on the clinical condition and the indication for testing. Noninvasive testing includes the urea breath test (UBT), stool antigen test, and serologic tests. Biopsy-based tests include the rapid urease test (RUT), histological evaluation, culture, and molecular tests using the polymerase chain reaction (PCR) [3, 4].

Tests differ in specificity and sensitivity, and choice of which test to use is often influenced by the pretest probability of infection (local prevalence of infection). Additional important factors are availability, cost, clinical setting, and factors that might influence the accuracy of testing such as post-eradication, the use of proton pump inhibitors (PPIs), bismuth or antibiotics, upper gastrointestinal (GI) bleeding, past history of partial gastrectomy, etc. (Table 9.1). The choice between noninvasive and invasive testing is dependent on whether there are other indications to perform GI endoscopy. In this paper, we review the RUT and UBT which are the most commonly used diagnostic methods for *H. pylori* infection. Both rely on detecting the presence of urease produced by *H. pylori*.

9.2 Urea Breath Test (UBT)

The UBT is a preferred test for the diagnosis and confirmation of cure because it is a noninvasive, simple, and accurate method for diagnosis of active infections [3]. Testing requires the patient to ingest a small quantity of urea in which the carbon is labeled with either the stable isotope ^{13}C or the radioactive isotope ^{14}C [3, 6]. If *H. pylori* is present, *H. pylori* breaks down orally ingested ^{13}C- or ^{14}C-labeled urea into CO_2 and ammonia. $^{13}CO_2$ or $^{14}CO_2$ diffuses into the blood, is exhaled via the lungs, and can be measured in the exhaled air. Both ^{13}C- and ^{14}C-UBTs are sensitive and specific for *H. pylori* detection; however, ^{13}C is generally preferred because it is not radioactive and thus avoids the potential problems

associated with the use of radioactive substances, especially in children and in pregnant women.

Most previous studies report similar high sensitivity and specificity of the UBT exceeding 95 % [7]. Positive and negative UBT results tend to cluster outside of the range between 2‰ and 5‰ such that change in cutoff value within this range would be expected to have little effect on the clinical accuracy of the test (see below) [7, 8].

The simplicity, good tolerance, and economy of the citric acid test meal probably make its systematic use advisable. Some protocols use a test meal to delay gastric emptying and attempt to spread the urea within the stomach. Over time the choice of test meal switched from an actual test meal such as a pudding to citric acid. A study comparing a pudding test meal, ascorbic acid, and two doses of citric acid in 11 volunteers showed that the increased intragastric urease activity associated with citric acid could not be attributed only to gastric emptying. Citric acid and malic acid both enhance urease activity possibly via an effect on Urel, a proton-gated urea channel, thus making urea more accessible to the intra-bacterial urease [9]. The dose of urea varies from essentially none with the ^{14}C test to mg quantities of ^{13}C-urea. Originally, the ^{13}C-UBT was studied with a dose of ^{13}C-urea of 5 mg/kg of body weight [10]. Subsequently, doses of 125 and 100 mg were validated, and more recently 75 mg, 50 mg, or less and possibly even 10 mg of ^{13}C-urea have proved to be sufficient [11–15]. The UBT protocol containing citric acid test meal can 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 [16].

It is frequently asked whether fasting before UBT is required. Most have found no significant differences between tests performed under fasting and non-fasting conditions. Thus fasting prior to testing seems to be not necessary and non-fasting may be more applicable in the routine setting. In the USA, fasting from solid food for one hour is required. Because the issue remains controversial, it would seem prudent to perform UBT in fasting condition from solid food for one hour until new data will definitively clarify this issue [7, 17–20].

9.2.1 Assessment for Eradication

Overall, the UBT is generally the best noninvasive method for detection of active *H. pylori* infections and for confirmation of cure of the infection. The excellent sensitivity of the UBT, especially after eradication therapy, may be explained by the fact that the UBT is more likely to produce positive results than biopsy-based tests including RUT in cases of moderate colonization or patchy distribution of *H. pylori*. The sensitivity and specificity were previously reported to be greater than 95 %, and it is widely recommended as the primary method for confirmatory testing after *H. pylori* eradication therapy [7, 12].

The accuracy of UBT for eradication assessment is markedly affected by two factors: the timing of the assessment and the cutoff value used for the assessment. After eradication treatment, the values resulting from the ^{13}C-UBT have converged around the cutoff value. A cutoff value of 5‰ for ^{13}C-UBT is widely used for eradication assessment worldwide. In Korea and Japan, the optimum cutoff value of the ^{13}C-UBT performed using the UBiTkit (Otsuka Pharmaceutical; measurement at 20 min after the administration of 100 mg of urea) for the detection of *H. pylori* infection before and after eradication is 2.5‰ [15]. The cutoff value of 2.5‰ was based on a multicenter trial conducted in patients prior to treatment for eradication of *H. pylori* in Japan [15], while the cutoff value is 2.4‰ in the USA.

Early experience with a low proportion of false-positive results has been recently challenged by studies from Spain and Korea [21, 22]. In Korea they reported a low specificity of 47.1 % instead of high sensitivity of 99.3 % using the film-coated urea tablet without citric acid after *H. pylori* eradication [22]. False-positive results after eradication tend to occur in studies using cutoff in patients with atrophic gastritis. The majority of false-positive results occur when the results are near the cutoff value and are most frequent in areas where atrophic gastritis is common and citric acid is not used. In those locations we recommend that tests with results between the cutoffs (e.g., 2.5‰ and 10‰) should be considered indeterminate and confirmed with a different test. In Korea this accounted for up to 10 % of cases of post-*H. pylori* eradication therapy. It is unknown if the addition of citric acid which increases the urease activity of *H. pylori* and inhibits non-*H. pylori* ureases would prevent this problem. After failure of *H. pylori* therapy, the infection may recover slowly such that testing should be delayed for at least 4 weeks after the end of therapy and 2 weeks after treatment with PPI to allow any remaining *H. pylori* to achieve a sufficient density to be detected [23].

The stool antigen test is also useful for confirming *H. pylori* eradication; however, stool testing is inconvenient and has low diagnostic accuracy when polyclonal antibodies are used. Both the UBT and stool antigen test have similar accuracy if one ensures one is using a validated stool antigen test based on the use of monoclonal antibodies [24].

9.2.2 Diagnosis in Children

Citric acid has demonstrated good performance as an adjuvant to the UBT in adults and is well accepted in children when a sweetener is included as in the USA. Apple, orange, or grape juices are often used as alternatives in UBT of children although their suitability has not been evaluated critically. The standard ^{13}C-UBT is less accurate for the diagnosis of *H. pylori* infection in young children, especially under 6 years old, because the test is assessed by measuring the relative amounts of natural and $^{13}CO_2$. Attempts to adjust based on cutoff value, pretest meal, and urea dose are gross attempts to this adjustment and are not recommended as CO_2 is

highly dependent on size which varies greatly in different populations. The problem can be overcome by adjusting for the CO_2 production rate [25].

A systematic review and meta-analysis showed that stool antigen tests using a monoclonal antibody are also highly accurate for the diagnosis of *H. pylori* infection in children [26]. The specificity of UBT was less than 90 % in young children aged 6–30 months in the developed countries, while both monoclonal stool antigen test and UBT have proven to be reliable in South American developing countries [27].

9.3 Rapid Urease Test (RUT)

The diagnosis of *H. pylori* can be established by endoscopy to obtain specimens for RUTs, histology, or culture. Choosing among these tests depends upon the clinical circumstance, the accuracy of the tests, and their relative costs and especially their local availability. Endoscopy is generally not indicated only to establish *H. pylori* status, and rather, the primary use of endoscopy is to evaluate for the presence of upper GI diseases, many of which are consequences of *H. pylori* infections, or to obtain biopsies for culture to perform antimicrobial susceptibility testing. As noted above, endoscopy is expensive and not without risk, and it is prudent to combine visual inspection with diagnostic testing for *H. pylori*. Japan is one of the countries with the highest incidence of gastric cancer, and the Japanese Minister of Health, Labour and Welfare has recently approved the application of medical insurance of *H. pylori* eradication in patients with chronic gastritis diagnosed by endoscopy based on the strategy for the elimination of gastric cancer deaths. Therefore in Japan, endoscopy for screening of gastric cancer and evaluation of gastric cancer risk is required prior to *H. pylori* eradication for chronic gastritis.

The RUT is inexpensive, rapid, widely available, and highly specific. In 1998, American College of Gastroenterology suggested that the RUT is the test of first choice when endoscopy is indicated [28]. Additional biopsy specimens should also be taken from normal appearing mucosa and placed into formalin.

RUTs are based on the fact that *H. pylori* urease splits urea into ammonia and CO_2. When a gastric biopsy containing *H. pylori* is placed into a urea-containing medium, the ammonia produced by bacterial urease will increase the pH, and this is detected by the color change of a pH indicator. In regions where cost is an important factor in terms of whether testing can be done, any laboratory can produce "home-made" tests for pennies. These tests are made from a solution containing 2 g urea, 10 mL of 0.5 % (w/v) phenol red, and 20 mg sodium azide in 100 mL of 0.01 M sodium phosphate buffer, pH6.5. Approximately ½ to 1 mL of this solution is placed in a dram vial and the biopsy is immersed in this solution. The pH indicator is initially yellow, and the addition of *H. pylori*-positive biopsy specimens will change the solution from yellow to pink. Many commercial RUTs are available, including tests that use urea-impregnated agar (e.g., *hp*fast, GI Supply, Camp Hill,

PA; CLO test, TriMEd Specialties, Inc., Lenexa, Kans), liquid-based tests (Helicocheck, Otsuka Pharmaceutical, Tokyo, Japan), or dry filter-paper tests using a urea-impregnated semipermeable membrane (PyloriTek, Serim Research Co., Elkhart, IN). They typically provide a result within 1–24 h, depending on the format of the test and the bacterial density in the biopsy specimens. The speed of the reaction in the agar tests can be increased by the use of a warmer (e.g., Helicoview, GI Supply, PA), the use of large biopsy specimens, or adding several specimens to the agar [29–31]. A study comparing the two types of RUTs, a liquid-based test and a dry filter-paper test, showed they had significantly faster reaction times than an agar test such as the CLO test [32]. Some RUTs marketed in Europe are reported to give accurate results within minutes. However, clinical experience has not shown rapid or ultrarapid results to be advantageous clinically.

The specificity for these RUTs varies from 90 % to 100 %, but their sensitivity is slightly less (approximately 80–95 %) [21, 30, 31, 33, 34]. Positive results should generally prompt *H. pylori* treatment. However, false-positive results may occur if the test is not interpreted within 24 h because of the growth of urease-containing mouth bacteria. We recommend RUTs be discarded after 24 h. Importantly, a negative RUT should not be taken as evidence of the absence of the infection especially in PPI users and those with atrophic gastritis (see below).

9.4 False-Negative Results

Because the RUT samples such a tiny fraction of the gastric mucosa, there is a high possibility of false-negative results with RUT due to the presence of a low-level, suppressed, or patchy infection. This problem with false-negative tests is responsible for the abovementioned dictum not to accept a negative RUT as the sole criterion for either the absence of the infection. Extensive atrophy or intestinal metaplasia leads to patchy distribution of the infection and may be associated with low *H. pylori* density and false-negative RUT results. Corpus-predominant gastritis or achlorhydria can also lead to false-negative UBTs, as like RUT. The number of bacteria present in the biopsy specimen is the main cause in reducing the test's sensitivity. It is estimated that densities lower than 10^4–10^5 organisms may result in false-negative tests [35]. The false-negative result could also be caused by the use of *H. pylori*- and urease-suppressive therapies, such as PPIs, antibiotics, or bismuth compounds [36, 37].

Preimmersion of the biopsy forceps in formalin does not adversely affect viability of the organisms, although it has been suggested that formalin contamination of forceps used to collect the biopsy may possibly contribute to reduced sensitivity [38–41].

9.4.1 PPI Users

False-negative rates greater than 30 % have been reported when PPI is used just prior or at the time of testing, and this problem is present with histology, culture, RUT, UBT, and stool antigen testing. Gatta et al. [13] reported that the sensitivity of both UBT and stool antigen test was significantly decreased (UBT range, 77.1–85.4 %; stool test, 83 %) after 14 days of PPI treatment, while it was unchanged in those that took antacids. PPIs should be stopped 2 weeks (at least 1 week) before the UBT [42]. H_2 receptor antagonists have no effect on *H. pylori* and can be continued up to the day of testing for histology, RUT, and stool antigen testing. However, high pH caused by H_2 receptor antagonists may reduce the accuracy of the UBT, especially the ^{14}C-UBT. Citric acid can overcome the problem with the ^{13}C-UBT.

9.4.2 Diagnosis in Patients with Upper GI Bleeding

The false-negative result in RUT or culture may often occur in bleeding patients [43, 44]. The biological mechanisms for the false-negative results in the setting of acute upper GI bleeding are poorly understood and may indeed vary depending on the test. Blood adversely affects the performance of the RUT. One suggestion is the pH-buffering effect of the blood or serum albumin rather than a direct inhibition on the urease activity. The color change of the pH indicator was progressively suppressed by higher concentrations of serum albumin regardless of the presence of anti-*H. pylori* antibody [45]. Velayos et al. [46] investigated the accuracy of UBT performed immediately after emergency endoscopy in patients with peptic ulcer bleeding by comparing the results with those of UBT performed after hospital discharge. The sensitivity and specificity of the early UBT were 86 and 66 %, respectively, with a negative predictive value of 50 %. In contrast, Tu et al. [47] compared invasive and noninvasive methods for detecting *H. pylori* infection in bleeding peptic ulcer and reported a higher sensitivity of UBT compared to RUT (95 % vs. 46 %). In reality, there is no compelling reason to diagnose *H. pylori* in a bleeding patient. Since *H. pylori* infections are acquired in childhood, most patients have been infected for decades. There is thus no rush to diagnose and diagnostic testing can be delayed until after the acute problems have settled.

9.4.3 Diagnosis in Patients with History of Partial Gastrectomy

The studies using UBT performed in patients with a history of partial gastrectomy indicated that the sensitivity is decreased in patients with partial gastrectomy. One

problem is that bile inhibits *H. pylori* and thus the density of the organism is often low. The accuracy of UBT in patients' post-distal gastrectomy was reported to be lower than that of RUT [48]. The UBT may become unreliable because of rapid gastric emptying and/or enterogastric alkaline reflux. A cutoff of 4.0‰ using the commercially available test has been recommended [49]. A meta-analysis indicated that the sensitivity and specificity of UBT were 0.77 (95 % CI, 0.72–0.82) and 0.89 (95 % CI, 0.85–0.93) compared to the RUT (0.79 (95 % CI, 0.72–0.84) and 0.94 (95 % CI, 0.90–0.97)) and histology (0.93 (95 % CI, 0.88–0.97) and 0.85 (95 % CI, 0.73–0.93)) [50]. In contrast, a Japanese trial used the UBT protocol that included ingestion of 100 mg ^{13}C-urea, the use of mouthwash, and the body in a horizontal position on the left side. Using that approach the sensitivity of ^{13}C-UBT in patients with a remnant stomach was improved to 95.7 % [51]. Overall, histology is often the best choice for the diagnosis of *H. pylori* infection after partial gastrectomy [50]. However, stool antigen test may also be reliable, and the specificity of stool antigen test in Japanese patients who underwent distal gastrectomy was reported to be 90.5 % in contrast of a standard UBT of only 59 % [52].

9.5 False-Positive Results

In contrast, urease-producing bacteria (*Streptococcus*, *Staphylococcus*, *Gardnerella*, *Lactococcus*, and *Enterococcus*) could cause false-positive results of urease-dependent tests [53–55]. Although some members of the microbiota in the oropharynx produce urease which is swallowed in the saliva, most non-*H. pylori* urease enzymes have a pKa greater than seven and are thus inactive in the acidic conditions of the stomach. However, in the achlorhydric patient, their presence may prove to be a problem. A previous Japanese study demonstrated that five bacterial species with urease activity (*Proteus mirabilis*, *Citrobacter freundii*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, and *Staphylococcus aureus*) were subsequently isolated from the oral cavity and/or stomach and all of the patients with a false-positive UBT result were suffering from atrophic gastritis [55]. The use of the citric acid adjuvant will also lower the intragastric pH below the pH optimum of non-*H. pylori* ureases and thus reduce the chance of false-positive results seen in patients with hypochlorhydria and overgrowth of non-*H. pylori* urease-containing organisms. False-positive RUT is infrequent but may occur if the sample is kept beyond 24 h [56].

9.6 Improvement of the UBT

Mouthwashing prior to a standard UBT has been recommended to reduce the interaction of the urea with mouth urease-containing organisms to reduce frequency of false-positive UBT results; however, it has not been critically examined

[57]. Alternative methods have been to use a straw to drink the urea solution or the use of film-coated ^{13}C-urea tablets to decrease the interaction of the urea and urease-positive bacteria in the oral cavity. The simplest method is to use citric acid and to delay the first breath sample to at least 10 min to allow the low pH of the citric acid solution to inhibit non-*H. pylori* ureases and to enhance *H. pylori* urease activity. One potential problem with urea tablets or capsules is that they can potentially empty from the stomach without exposing the *H. pylori* to the labeled urea. Clearly, more head-to-head studies are needed to compare different delivery methods.

9.7 Improvement of the RUT

Increasing the number and size of biopsy fragments and/or collecting them from various regions of the stomach (e.g., combining biopsies for the antrum and body in one well) achieves better results with the RUT [58]. Combining tissues not only increased the detection of *H. pylori* compared with testing separate specimens but also produces faster results [59]. Obtaining two samples one from the antrum and one from the corpus avoiding areas of ulceration and obvious intestinal metaplasia is sufficient to obtain optimal results and provide the highest yield [56].

The tissue sample contained in the agar of an RUT can be used for other purposes. For example, the sample can be removed from the agar gel of positive tests and used for molecular testing for *H. pylori* and/or for the presence of clarithromycin resistance [60]. Since the sample contains host tissue, it could be used for other testing such as the CYP2C19 genotype of the host as well as 23S rRNA of *H. pylori* [61].

To increase the sensitivity of RUT, authors have recommended sampling gastric mucus more widely instead of biopsy samples. We assessed whether adherent gastric mucus to biopsy forceps instead of biopsy samples was suitable for diagnosis. Gastric mucus was obtained by gently scraping gastric mucosa from the antrum greater curvature to corpus greater curvature using biopsy forceps and put into RUT tube. The accuracy of RUT using gastric mucus was superior to that using combined two biopsy specimens in 494 subjects including 300 *H. pylori*-positive patients. The sensitivity and specificity of using gastric mucus were 0.93 (95 % CI, 0.90–0.96) and 0.93 (95 % CI, 0.89–0.96) compared to using biopsy specimens (0.87 (95 % CI, 0.80–0.94) and 0.92 (95 % CI, 0.86–0.99)). (The data is not published.) An alternative is to use an endoscopy brush to collect mucus. As noted previously, the use of the RUT seems to have declined in Western countries possibly because of the desire to obtain the additional information available with histology.

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