Diagnosis of *Helicobacter pylori* using the rapid urease test

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Abstract: *Helicobacter pylori* (*H. pylori*) is a major human pathogen which causes progressive gastroduodenal damage. Guidelines recommend that, unless there are compelling reasons to delay, treatment is indicated for all patients in whom the infection is diagnosed. The rapid urease test (RUT) is a popular diagnostic test in that it is a rapid, cheap and simple test that detects the presence of urease in or on the gastric mucosa. The sensitivity and specificity are generally high and many versions have been approved for use in humans. Best results are obtained if biopsies are obtained from both the antrum and corpus. The tissue sample embedded in the RUT gel can also be utilized for other tests such as for molecular based tests of microbial susceptibility or for host factors. False-positive results are rare if the RUT contains an antibacterial agent to prevent growth of urease-containing contaminants and the tests are discarded at 24 hours. The use of antimicrobial drugs and proton pump inhibitors as well as the presence of intestinal metaplasia may result in false-negative results. A negative test should not be used as the criteria for cure or in cases where accuracy is important for patient management such as in upper gastrointestinal bleeding. Interpretation of the test should take into account the pretest probability and the prevalence of *H. pylori* in the population. The test can also be used to provide an informal assessment of the accuracy of the histopathology result and discrepancies should prompt a review of the histopathology and discussions with the pathologist.

Keywords: Diagnosis; *Helicobacter pylori* (*H. pylori*); urease; rapid urease test (RUT); review; campylobacter-like organism test

Introduction

Helicobacter pylori (*H. pylori*) is an important human pathogen involved in the pathogenesis of atrophic gastritis, gastroduodenal ulcer, gastric cancer, MALT lymphoma, idiopathic thrombocytopenic purpura, iron deficiency anemia and vitamin B12 deficiency (1). Despite an overall decline in the prevalence of *H. pylori* infections in western countries, 30-40% of the USA population remains infected (2,3). *H. pylori* is not a commensal organism in that the infection always causes gastric mucosal inflammation and damage. The basic lesion is progressive mucosal inflammation which may result in preneoplastic atrophic changes (1). Although *H. pylori* vary in virulence (e.g., whether the cag pathogenicity island is present), there is a risk of a significant clinical outcome as the difference in risk between the least and the most virulent is only approximately twofold (1). Because the rate of progression of the mucosal damage is unpredictable, and the infection is always transmissible, it has been recommended that whenever an *H. pylori* infection is found it should be cured unless there are compelling reasons that would mitigate that choice (e.g., very elderly with extensive comorbid diseases) (4). In February on 2013, the Japanese government approved a universal test and treat strategy as part of Japan's goal to eliminate gastric cancer (5).

H. pylori infections like other major chronic infectious diseases (i.e., syphilis and tuberculosis) are associated with a long latent period before presenting clinically. As such, many infections will be discovered during this latent period. A number of methods to *H. pylori* infection have been developed and they are generally grouped as being "invasive" meaning that they require gastric tissue or mucus, or "non-invasive" requiring only blood, breath or stool or analysis.

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Here, we discuss the rapid urease test (RUT) or RUT which is an invasive test in that it requires sampling of the gastric mucosa. The test provides indirect evidence of the infection by identifying the presence of a non-mammalian enzyme, urease, in or on the gastric mucosa.

History

H. pylori was first cultured in 1983. The initial report described it as urease-negative. However, other laboratories attempting to replicate the initial finding correctly identified the organism was urease positive (6). McNulty *et al.* adapted a standard laboratory test for urease activity in which a loop of culture is placed in a urea containing substrate with a pH indicator such as Christensen's 2% urea broth; Urease hydrolyzes the urea to produce ammonia and carbon dioxide (7). The ammonia increases the pH leading to the color change (from brown to pink). McNulty *et al.* showed that urease could be identified directly from gastric biopsies using this test thus eliminating the need for culture or histology. The original observation was followed with a large clinical trial published in 1989 in which 1,445 patients underwent testing for *H. pylori* using modified Christensen's urea broth (8). They reported almost 100% specificity and 96% sensitivity in comparison with histology and culture. Barry Marshal added an antibacterial agent to an agar gel containing urea and a pH indicator and patented the first test RUT called the campylobacter-like organism (CLO) test which rapidly has become a standard for clinical use and for clinical trials.

Gastric urease

Gastric urease had a long history prior to the discovery of *H. pylori* (9). For decades it was believed that urease was a mammalian enzyme and it was hypothesized that urease was produced by the stomach in order to protect the stomach from digestion by gastric acid (9). Urease was even the basis for peptic ulcer therapy. For example, Oliver Fitzgerald, an Irish physician, treated peptic ulcers with both oral and intravenously administered urea to neutralize gastric acidity and heal peptic ulcers (10). The first urea breath test equivalent using ^{13}C-urea was actually reported in 1951 and used to measure urease activity in a frog's stomach (11). When it became evident that urease was microbial in origin, interest in urease waned as it was clear it could not be responsible for protecting the gastric mucosa against damage by acid

and because most of the pertinent experiments had already been done. The discovery that *H. pylori* produced abundant urease allowed these many experiments to assume new meaning (6).

The RUT is an indirect test of the presence of *H. pylori* based on the presence of urease in or on the gastric mucosa. It has an advantage over serology in that it only detects the presence of an active infection. The test requires a sample of gastric mucosa or mucus that is added to a tube, gel, or other device which brings that sample into contact with urea and a method to detect the products of urea hydrolysis, ammonia or carbon dioxide. Different tests have been developed to assess the presence of gastric urease activity and have been based on probably every possible component of the reaction including those that assess changes in labeled CO_2 in serum or breath, breath ammonia, and pH change induced by the ammonia either as a color change or directly using a pH meter. The initial test used phenol red in which the color indicator which changes from yellow to pink or red as the pH increases. Other tests have used different indicators each with a potential advantage such as being able to start the reaction at a lower pH such as 5.4 and thus reduce the activity of contaminating mouth bacteria many of which also contain abundant urease (e.g., HP*fast*®) (*Figure 1*). The simplest tests are home-made and contain a small quantity of substrate in a tube with a pH indicator and can be produced of one or two cents each (12).

As for any enzymatic reaction, one must consider the parameters that affect the reaction such as substrate concentration, enzyme concentration and activity, time, and temperature and studies have been done to test many of the different parameters so as to provide reliable results. The important variables include sample collection, sample size, time before scoring the test as negative, and whether warming is beneficial. A positive RUT requires approximately 10^5 *H. pylori* in the biopsy sample to change the color using an agar-based test such as the CLO test (13). This is generally not a problem as the concentration of *H. pylori* typically exceeds that minimum. The sensitivity of various RUT tests as primary diagnostic tests is high and has been reported to vary between approximately 80% and 100% and specificity between 97% and 99% (14-17).

The first requirement is that the biopsy sample must come from a site where the organisms are present. Patients with duodenal ulcer typically have non-atrophic mucosa and *H. pylori* are especially abundant throughout the antrum. For those patients a single sample from the antrum or gastric angle will have a high yield, typically greater than

Figure 1 Examples of three popular commercial rapid urease tests (RUTs) available in the United States including the CLO test and two second-generation tests, one using a pH controlled gel and the other a urea saturated membrane. One of each pair is the control, and the other is after the color change. CLO, campylobacter-like organism.

85% and often in the range of 95% to 100%. However, if the patient has atrophic gastritis and large areas of intestinal metaplasia, which contain few *H. pylori* organisms, the results will often be disappointing. Numerous studies of biopsy site have been published. For example, one study took two biopsy specimens from the antrum and reported a sensitivity and specificity of 95% and 100% (18). Another study compared the results from a biopsy taken at the gastric angle, a prepyloric site and a corpus site with results of 100%, 87% and 84.4%, respectively and no false-positive results (i.e., specificity 100%) (19). Overall, for best results, two samples, one from the antrum avoiding areas of ulceration and obvious intestinal metaplasia, and one from normal appearing corpus are sufficient and provide the highest yield. The two samples can be placed into the same gel or media or into separate containers depending on physician preference and whether other uses are planned for the samples.

Biopsy size

The sensitivity and specificity of jumbo cup forceps (3.3 mm diameter) and tiny cup forceps (1.8 mm diameter) were compared using antral biopsies from the same patients (20). The sensitivity and specificity with jumbo forceps were 92.1% and 95.5% compared to 88.5% and 98.8% for the tiny forceps, respectively. Thus, for practical purposes biopsy cup size seems to make little difference. Because larger forceps also provide more information for histologic examination, we recommend that the largest forceps that

will fit through the insertion channel of the endoscopy be used. If a truly tiny endoscopy is used, such as an ultra-slim endoscope, samples for RUT can be taken using either the tiny forceps supplied or by brushing the mucosa and placing the brush in the RUT media.

Reaction speed of a RUT depends in part of the temperature of the reaction. A comparison was done in which antral biopsies were placed at 38 ℃ in a warmer or kept at room temperature (~21 ℃). The ability to make a diagnosis within 30 minutes was 20% higher using the warmer (21) but overall the results were the same. Many endoscopists place the RUT device in their pocket to warm them. Overall, the use of warmer makes little difference except it may allow for a diagnosis to be confirmed more often before the patient leaves the endoscopic area.

Commercially available RUT kits suggest that the decision be made (positive *vs.* negative) within 24 hours. The time the test turns positive depends on the concentration of bacteria and the temperature. Most will turn positive within 120 to 180 minutes but it is best to hold those that appear negative for 24 hours (22,23). After 24 hours the test may turn positive from the presence non-*H. pylori* urease containing organisms (24). Positive results after 24 hours are most often false positive and should not be used for treatment decisions. We routinely discard the samples after 24 hours. There are a number of different commercially available RUT kits that primarily differ depending on the platform (e.g., gel, liquid, membrane, etc.). Choice depends on availability and local preference as none has proven to be superior.

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False-negative and false-positive results

As noted above, the RUT is a test for the presence of the urease enzyme. The actual results will however depend on the gastric disease and the likelihood of atrophic changes or exogenous factors that reduce the bacterial load and thus produce false negative results. False positive results can occur if other urease containing organisms are present in sufficient quantity or if one allows contact of the specimen and the media for a prolonged period, typically longer than 24 hours. As noted before, approximately 10^5 bacteria must be present in the biopsy sample for a positive result (13) and anything that reduces the bacterial density such as the use of antibiotics, bismuth-containing compounds, or proton pump inhibitors may result in false-negative results (25-27). The two most common reasons for false negative results are the recent use of proton pump inhibitors and the presence of intestinal metaplasia. H_2-receptor antagonists do not reduce the bacterial density and can be used up to the day of the test (28). It is unclear how long one must wait after stopping proton pump inhibitors before the possibility of a false negative result becomes unlikely. The recommendation of two weeks is commonly given based on being on the safe side; however the organisms typically recover rapidly from inhibition with a proton pump inhibitor (26,29). Nonetheless, all of the tests for active infection including RUT, histology, urea breath test, and culture may become false negative during proton pump inhibitor use or after bismuth or antibiotic use (28). It is unlikely that a false negative RUT will also be accompanied by histologically uninflamed and normal gastric mucosa. When in doubt and the result is important, it is best to obtain a noninvasive test (urea breath test or stool antigen) after discontinuation of the PPI.

False-positives are rare and when present may be due to the presence of other urease containing organisms such as *Proteus mirabilis*, *Citrobacter freundii*, *Klebsiella pneumonia*, *Enterobactor cloacae* and *Staphylococcus aureus* (22). However, unless the patient has achlorhydria or hypochlorhydria, non-*H. pylori* organisms are unlikely to be present in sufficient concentration to produce a positive test unless the RUT substrate lacks an inhibitor to bacterial growth in which they may possibly overgrow during the 24 hours observation period.

Clinical interpretation of the RUT results

The interpretation of RUT, like any diagnostic test, depends in part on the pretest probability of an infection (30). Thus, in a patient with a duodenal ulcer, a single positive RUT would be considered confirmative of the diagnosis whereas a negative test would need to confirm by the results of another test such as histology (e.g., no evidence of gastric inflammation). In contrast, in an elderly patient undergoing endoscopy for gastro-esophageal reflux disease, one would be hesitant to start therapy based only on the basis of a positive RUT. Thus, interpretation depends on the pretest probability and the importance of the result in relation to the subsequent management of the patient. In order to save the patient money, some physicians have suggested taking biopsies for RUT as well as for histology from normal appearing mucosa and then, if the RUT is positive, discarding the histology specimens as unnecessary (23,31,32). While this may make sense in the presence of a high pretest possibility (e.g., active duodenal ulcer), it would not be prudent in a patient with non-ulcer dyspepsia where the histologic findings themselves may be important (e.g., presence of atrophic changes, dysplasia, etc.). It has also been recommended that RUT should not be used as the sole arbiter of the results of *H. pylori* eradication therapy because the sensitivity of the RUT is not 100% and thus a negative test does not fully exclude the presence of an active infection (4,28). This is particularly important when the reason for the examination is for test-of-cure. In reality, endoscopy is infrequently done for test of cure except in patients with lesions where further endoscopic and/or histologic evaluation is needed such as gastric ulcer or after resection of adenomatous polyps and the question of the role of RUT post therapy rarely arises. For those patients it is probably most cost effective to rely on histologic examination of antral and corpus biopsies.

Generally speaking, upper endoscopy is an expensive test associated with a small but definite risk and unless there are specific contraindications, biopsy for examination of the mucosal histology is generally indicated. RUT testing can also be done and is especially helpful in difficult diagnostic situations when the physician would like to start treatment soon. As such biopsies for RUT are often taken in patient with recent upper gastrointestinal bleeding. In this situation, there may be an increased prevalence of false negative results. It has been postulated that blood leads to decrease sensitivity of RUT possibly related to the presence of albumin (33), *H. pylori* killing factors in human plasma (34), or blood in gastric lumen (35,36). However, other studies report that blood does not influence the test (37,38). False negative tests are also common after partial

gastrectomy probably because of reduced bacterial load often related on the presence of bile (39,40). In none of these situations, it is critical to diagnose or start treatment for an *H. pylori* infection immediately. As such false negative results have little clinical importance unless they are accepted as proof of the absence of an *H. pylori* infection. One must always fall back on considering what one plans to do with the information (i.e., positive tests are generally to be believed but negative tests require histologic or other confirmation when the presence of an *H. pylori* infection is likely responsible for the reason for the endoscopy such as with a bleeding peptic ulcer). Since most *H. pylori* infections were acquired in childhood, there is little need to treat immediately and one can always wait until the acute conditions have resolved.

Use of the RUT sample for additional purposes

The tissue sample contained in the agar of an RUT test can be used for another purpose. 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 (41). Since the sample contains host tissue, it could also be used for other testing such as the CYP2C19 genotype of the host (42).

Summary

The RUT is a rapid, cheap and simple test that is used frequently in clinical practice. The RUT is best considered as a screening test and not as the gold standard for *H. pylori* infection. For clinical trials the diagnosis of an *H. pylori* infection requires that two different types of test be positive or a positive culture. *H. pylori* negative is typically defined as all tests negative. The key to proper use is to consider any test in terms of pretest probability as well as in the importance of the information in terms of patient management. False negative tests are more frequent than false positive tests and thus a negative result should not be used to exclude *H. pylori* when a wrong diagnosis would be detrimental to patient management (e.g., in a patient with recent upper gastrointestinal bleeding or after *H. pylori* eradication therapy). A positive culture is considered the only true gold standard for the diagnosis of *H. pylori* such that additional testing is needed when one suspects a false-negative result. Finally, the test can also be used as an informal assessment of the accuracy of the pathology laboratory and discrepancies between the RUT and histology especially a positive RUT and negative histology should prompt review of the histopathology and discussions with the pathologist.

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