Accurate Diagnosis of *Helicobacter pylori* Urease Tests

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Accurate Diagnosis of *Helicobacter pylori*
Urease Tests

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The presence of urease in the gastric mucosa of humans and animals was reported in the scientific literature in 1924 by Luck and Seth.[21] Those investigators assumed that the enzyme was part of the normal gastric mucosal epithelium because it was almost universally present in the animals they examined. This assumption was perpetuated in human studies, particularly by Fitzgerald and Murphy in 1950.[8] Hints that urease was secondary to bacterial infection of the mucosa came from an article by Lieber and Lefevre in 1959,[19] in which they observed that in humans, treatment with tetracycline could eliminate most of the gastric urease activity. Subsequent observations in gnotobiotic animals confirmed that normal gastric mucosa (bacteria-free) did not produce gastric urease.[4] Most of these observations were outside the mainstream of the gastroenterologic literature and were not followed up once the fiberoptic endoscope became available in the 1970s.

With the observation that *Helicobacter pylori* was a strong urease producer,[16] several groups[11] [12] [24] [25] began work on the use of urease as a marker for *H. pylori* in the human stomach. This work ultimately resulted in the rapid urease test and the urea breath test, both now widely used in the diagnosis of this common infection.[10] [23]

PHYSIOLOGY AND BIOCHEMISTRY

Detailed studies of the human urease enzyme were reported by Mobley et al in 1988.[26] These investigators found that the enzyme made up almost 10% of the total protein obtained from French press preparations of *H. pylori*. The KM (the value of KM is given by the substrate concentration at which one half of the maximum reaction velocity is obtained) of the enzyme was 0.8±0.1 mM urea with an optimal pH of 8.2. The enzyme was

active at physiologic temperatures, with an optimum of 45°C. The enzyme was found to be rapidly denatured in acid so that it was inactive at any pH less than 4.5. Mobley and others have confirmed that the amount of urease present in the superficial gastric mucosa is approximately the same as urease activity in the jackbean *(Canavalia ensiformis)*, the usual commercial source of urease as a biologic reagent.[21] Other background studies before the discovery of the rapid urease test included the observation by Marshall and Langton[23] that patients with *H. pylori* had virtually no urea in gastric juice (i.e., it had all been hydrolyzed by the *H. pylori* urease).

The aforementioned factors are responsible for the specificity and sensitivity of the rapid urease tests and urea breath tests as they stand today. Although the commensal flora of the oropharynx produces urease that is swallowed in the saliva, this weaker enzyme is denatured rapidly in the acidic lumen of the stomach (pH <2.0) so that after a few minutes swallowed material is rendered urease negative. Similarly, as *H. pylori* urease diffuses from within the mucus layer (pH 6.0) to the gastric lumen (pH <2.0), it also is denatured so that acidic gastric juice does not contain active urease. Active urease, if present in the stomach, is located only beneath the mucus layer where the pH is neutral and the *H. pylori* organism resides. Urease tests can be based on biopsy (the commonest method, i.e., CLOtest [Ballard Medical Products, Draper, UT][24]) or can be performed on samples of gastric mucus scraped and retrieved from the stomach at endoscopy. In the urea breath test, urea isotope present in the ingested urea comes into contact with the mucosa and diffuses through the mucus toward the *H. pylori* and the mucosal blood supply. If any urea hydrolysis occurs in the lumen (from bacteria swallowed with the isotope), its liberated carbon dioxide is more likely to remain in the lumen than to penetrate the gastric mucosa. Urea hydrolysis occurring within the mucus layer is far closer to the epithelial blood supply, however, where it is directed because of a solubility and concentration gradient (away from low pH) so that isotopic carbon dioxide appears in the breath within a few minutes.

FACTORS THAT IMPAIR ACCURACY OF RAPID UREASE TESTS

False-positive rapid urease tests are uncommon. When patients salivate excessively or have reflux of alkaline bile into the stomach, this liquid may contaminate a small gastric biopsy specimen such that the resulting surface pH is greater than 6.0. This situation can cause a weak positive reaction in some rapid urease tests, particularly if an acidic buffer (pH <6.0) is not present in the reagent.[28] Similarly, patients taking omeprazole often have achlorhydria with subsequent superficial colonization of the gastric mucus layer with urease-producing organisms (e.g., *Proteus mirabilis* or *Klebsiella*). These organisms can give a false-positive urease test after 24 hours of inoculation but generally are negative when the test is read 1 hour after biopsy insertion.[33] Patients taking proton-pump inhibitors may have false-positive rapid urease test results. Usually, patients are asked to cease these types of medications for at least 1 week before testing for *H. pylori*.

False-positive results may occur when non-*H. pylori Helicobacter* organisms infect the gastric mucosa. *Helicobacter heilmanni* (previously termed *Gastrospirillium heilmanni*), a cause of about 1% of gastritis, also is urease positive. Most authors report that urease reactions are less intense with non-*H. pylori Helicobacter* organisms and are more likely to be positive in the corpus rather than the antral mucosa. Nevertheless, a positive result in this circumstance indicates a gastric infection, and antibiotic therapy is the treatment of choice.

The presence of achlorhydria causes false-negative urease test results (biopsy and breath tests) because the luminal pH of 7.0 can lead to an extremely high pH adjacent to the organism, such that *H. pylori* is destroyed by the action of its own urease *(suicide)*. This situation has been studied best with the urea breath test; 30% of patients taking normal-dose proton-pump inhibitors revert to a negative breath test, although low numbers of organisms are still present in the gastric mucosa.[6]

Because urease is denatured in acid, active urease probably exists only within the mucus layer. It follows that the quantity of active urease in a biopsy specimen taken for a rapid urease test is not only proportional to the number of organisms present, but also proportional to the thickness of the mucus layer and the pH within the stomach. A patient with a few *H. pylori* organisms but a thick mucus gel could give a strongly positive urease reaction because of *stored* undenatured urease in the mucus. Similarly, patients with severe gastritis and heavy *H. pylori* colonization may not be able to accumulate undenatured urease in a deficient mucus layer and may give a weak urease reaction. These factors have ruled against urease detection as being an important way of quantitating *H. pylori* in the stomach.

Various medications may affect the presence of urease in the gastric mucosa. Within 24 hours of taking bismuth or antibiotic, most of the *H. pylori* organisms have disappeared, and urease tests in this situation are negative. Patients should be questioned about ingestion of antibacterial and inhibitory compounds before endoscopy if urease test is the only means of diagnosis. Data suggest that sucralfate, although not eliminating *H. pylori* completely, can suppress the numbers of organisms and decrease the amount of urease in the gastric mucosa. [13] Proton-pump inhibitors and H_2 -receptor antagonists have been shown to affect negatively the sensitivity of urease tests despite sampling from multiple stomach sites. [1] [18]

For maximal speed, urease tests should be performed at room temperature with prewarmed media because of the enzyme's higher activity at increased temperature. [29] Two other reasons for occasional misdiagnosis with the rapid urease test are related to slight patchiness of the infection in some patients. For example, one biopsy specimen can be colonized heavily with *H. pylori*, whereas a second biopsy sample 1 cm or so away can reveal hardly any organisms. Buffered urease tests require at least 1000 organisms to generate a positive reaction so that when the histology reveals only 1 or 2 organisms in the whole section, urease tests may be negative. All biopsy methods for *H. pylori* diagnosis suffer from this `patchiness' problem, including histology. Accuracy of histology usually is increased by taking multiple biopsy specimens, [2] however. [13] When intestinal metaplasia occurs in the stomach, extreme patchiness of the infection may be observed because *H. pylori* do not grow or adhere to areas of intestinal-type mucosa within the stomach. If the biopsy sample is taken from an area of intestinal metaplasia, all diagnostic tests fail. For unknown reasons, the sensitivity of urease tests in children may be lower (69% to 75%). Although not well studied, this lower sensitivity could be because of the fact that pediatric-size biopsy forceps were used. [5]

TYPES OF UREASE TESTS

Gel Tests

The original urease tests were based on Christensen's agar [3] used for the diagnosis of urease-positive infections, such as *P. mirabilis*. In Christensen's agar, the specimen is allowed to incubate overnight so that the color changes from clear to pink during a 12- to 24-hour period. Investigators soon modified this medium, recognizing that culture was not necessary for detection of *H. pylori* because it produces such large amounts of urease, sufficient to change the color of the medium within a few minutes. The *rapid urease test* was used as a laboratory identification procedure for *H. pylori* [16] and as the rapid urease biopsy test. The most widely used gel test is the CLOtest produced by Ballard Medical Products (Fig. 1 ; see also Color Plate 1, Fig. 2) . This test consists of a calibrated amount of buffered gel containing urea and a pH indicator with bacteriostatic agents to prevent growth of *H. pylori* or other urease-producing organisms. The buffering overcomes small amounts of alkaline bile or saliva in the stomach, which might cause a positive reaction. When tissue is inserted in the gel, it remains yellow and does not change color unless urease is present in the biopsy specimen. When urease from *H. pylori* is present, a color change is seen such that almost all positive reactions can be identified on the same day as the endoscopy. In the original evaluation of the CLOtest by Morris et al [27] in New Zealand and Marshall et al [24] in Australia, sensitivity of 95% and specificity of 98% were reported. Marshall et al [24] also reported use of the test as a *proof of cure* 2 to 4 weeks after completing antibiotic treatment for *H. pylori*. In that study of 18 treated patients, the positive and negative predictive values were 100%. In a larger, more recent study involving 134 patients, El-Zimaity and Graham [7] found that a negative urease test was an excellent proof of cure.

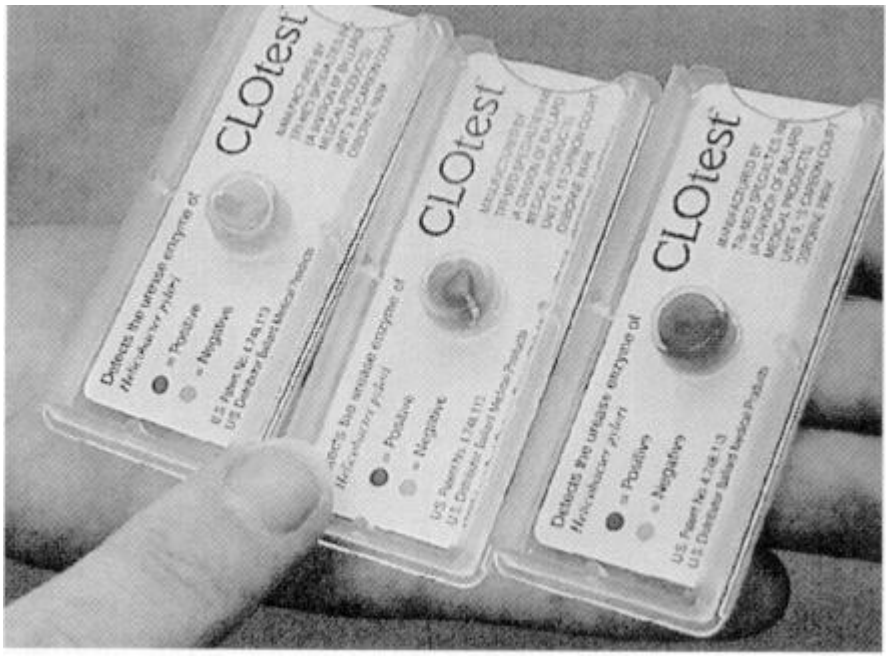

Figure 1. The *CLOtest* (Ballard Medical Products, Draper, UT) was so named when the initials "CLO" were used to describe *Helicobacters* as Campylobacter-like organisms. *Left*, the yellow test with inserted biopsy is negative. *Center*, an early positive reaction at 10 minutes. *Right*, a typical positive test at 3 hours. (See also Color Plate 1, Fig. 2.)

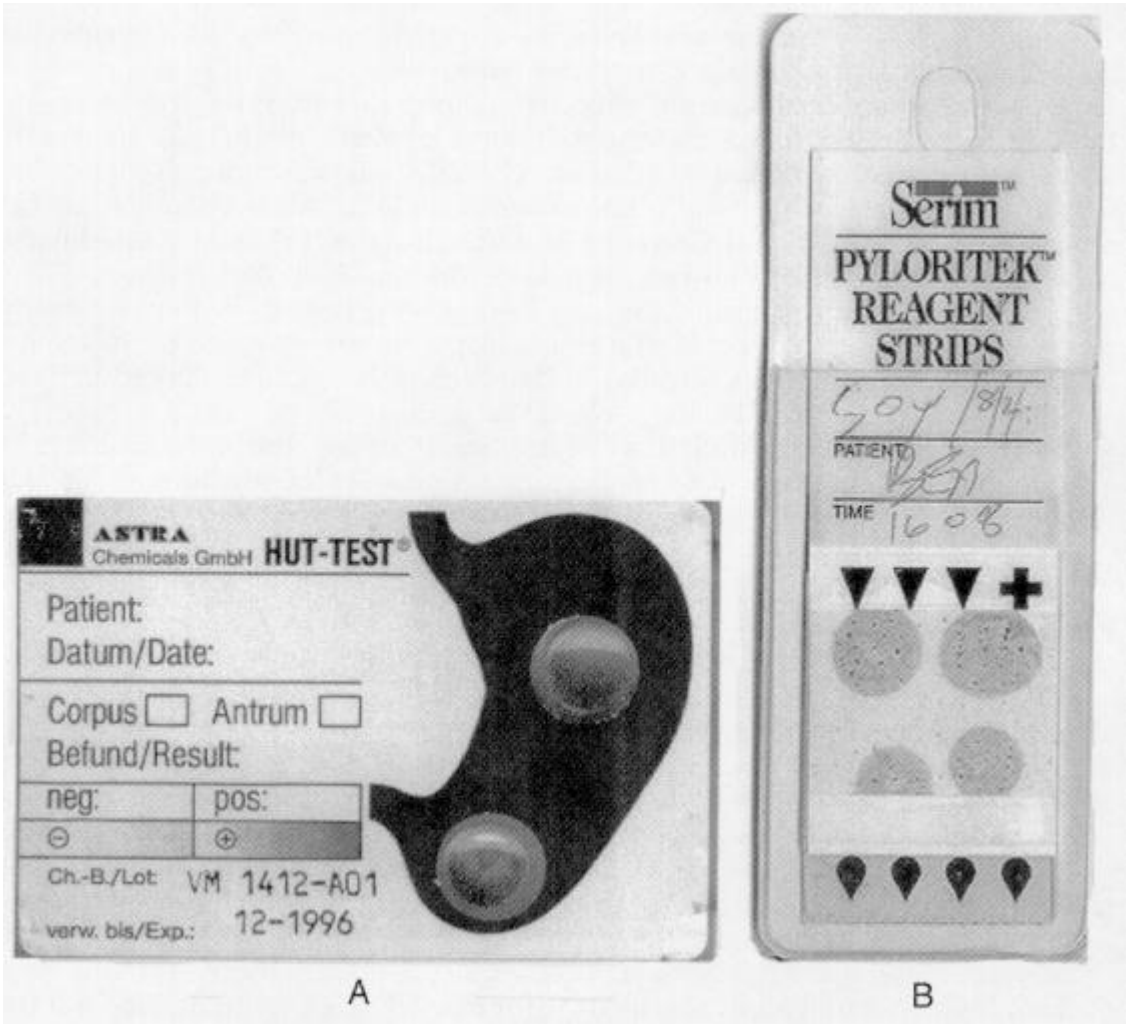

Figure 2. Other urease test formats: Hut-test *(A)* and PyloriTek *(B)*.

One further advantage of the bacteriostatic agent in the CLOtest gel is that if financial considerations are

paramount, a negative CLOtest can be reused on subsequent patients until a positive reaction occurs. [17] Gels without bacteriostatic agents would be likely to produce false-positive results if incubated more than 24 hours. The use of sodium azide as a bacteriostatic agent has been questioned by some investigators because of its potential toxicity. [9]

A few authors subsequently have maintained that *H. pylori* infections are slow to regrow after failed therapy, but most of these reports are related to anecdotal data not supported by adequate clinical studies. A more recent evaluation of use of the CLOtest and other urease tests in a follow-up situation was reported by El-Zimaity and Graham. [7] In their study of 134 biopsy specimens, correct diagnosis in the follow-up situation was made in 130 (accuracy 97%), with two false-negative results and one false-positive result. In the El-Zimaity study, the urease test was equal to a single biopsy specimen taken for histology. Histology was slightly more sensitive at detection of *H. pylori* because more than one biopsy specimen for histology was taken.

Various other gel formulations of the rapid urease test exist inside and outside the United States, most of them based on the CLOtest format. Table 1 lists the products and references describing their use in practice.

TABLE 1 -- GEL RAPID UREASE TESTS

Test	Sensitivity (%)	Specificity (%)	Reference
HUT-test (Astra Chemicals GmbH, Wedel/Holstein, Germany)	92	92	[20]
CLOtest (Ballard Medical Products, Draper, UT)	93	99	[15]
PyloriTek (Serim Research Corp., Elkhart, IN)	89	99	[15]
HpFast (GI Supply, Camp Hill, PA)	88	99	[15]
CLOtest	98	92	[34]
PyloriTek	98	68	[34]
CLOtest	80.5	93.4	[30]
CLOtest *	69	98	[5]
PyloriTek *	75	98	[5]

*Pediatric study.

Paper Tests

Paper tests were described first by Yu et al. [35] For their method, the usual urease reagents (urea, pH indicator, and buffer) were dried onto paper tape, and the biopsy sample was placed on the paper. This method has been resurrected in the United States as the PyloriTek (Serim Research Corp., Elkhart, IN) test and by various other generic paper tests around the world (Fig. 2) . Typically, one or more biopsy samples are placed on the paper. The samples are moistened with buffer or reagent containing urea and indicator so that the color change occurs in a small volume on the paper or on the biopsy sample. The small volume of liquid used leads to a rapid color change. Various authors have claimed high sensitivity and specificity with the PyloriTek, comparable with the gel-based rapid urease tests and with the potential advantage of more rapid color change. [15] [34] With the PyloriTek test, a positive control is incorporated into the slide. The downside of the positive control is that urease in the control spot of the test generates ammonia, which ultimately can contaminate the rest of the test, causing a false-positive reaction. It is recommended that paper tests with a control spot be read only at 1 hour because later time periods can be the source of false-positive reactions. [15] [34] This control spot may be a slight disadvantage if the test is not examined at exactly 1 hour.

Tablet Tests

In the tablet form of the test, initially pioneered by Rhom Pharma (West Germany) as the Jatrox (Procter & Gamble, Schwalbach, Germany) test, the usual components of a rapid urease test are incorporated into a tablet. To do the test, the biopsy specimen is placed in a small test tube, the tablet is dissolved in water, and the water is added to the biopsy specimen. These tests generally are not available outside Europe.

BIOPSY SITES

In most patients, biopsy specimens taken approximately 5 cm proximal to the pylorus on the lesser curve near the angulus or on the greater curve opposite the angulus give high sensitivity and specificity. According to Woo et al,[32] the area for heaviest colonization for *H. pylori* may be the lesser curve at the angulus, in the prepyloric region. A biopsy specimen is easier to obtain from the greater curve, however, and in less expert hands this may be a useful alternative site.

Some authors recommend taking two biopsy specimens to place into the urease gel. Laine et al[14] compared several urease tests and looked at the speed of reaction in the CLOtest with one or two biopsy samples. They concluded that the extra sample increased reaction speed but did not improve sensitivity significantly.

It has been reported that *H. pylori* can be cultured from biopsy specimens in rapid urease test agar if transported promptly to the laboratory.[31] This method would help to reduce the number of biopsy specimens needed to be taken for the diagnosis of infection and could reduce laboratory workload if positive urease test samples only were sent for culture.

SUMMARY

Because of their ease of use, rapidity, and cost-effectiveness, rapid urease biopsy tests continue to be an important diagnostic tool for the endoscopist. Various forms of these tests are available in different parts of the world, characteristically leading to sensitivities of 93% to 97% and specificities of 98%. When performing rapid urease tests, the endoscopist should be aware of factors that might lead to false-negative and false-positive results and should ensure that the biopsy specimens are of adequate size and taken from the correct location.

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