Gastric Urease, *Campylobacter pylori* and the Interpretation of CLOtest®

SUSIE R. HOFFMAN, RN, BSN
BARRY J. MARSHALL, MD
KEVIN R. DYE, MD
STEPHEN H. CALDWELL, MD

Campylobacter pylori infection of the stomach may be an etiological factor in non-ulcer dyspepsia, gastric and duodenal ulcer disease. Herein is described the use of a rapid urease test (CLOtest®) to detect *C. pylori* infection of gastric mucosal biopsies. The CLOtest, a simple endoscopy room biopsy test, will detect most *C. pylori* infections on the day of endoscopy, allowing the physician to prescribe treatment before the patient leaves the hospital.

Introduction

Campylobacter pylori has been shown to cause active chronic gastritis and has been implicated as a primary etiologic factor in duodenal ulcer disease, gastric ulcer and non-ulcer dyspepsia. By causing chronic inflammation, *C. pylori* weakens mucosal defense and allows acid and pepsin to disrupt mucosal integrity.

C. pylori produces prodigious amounts of urease enzyme. Although the enzyme primarily allows *C. pylori* to utilize urea as a nitrogen source, the breakdown of urea also produces high local concentrations of ammonia, which enables the organism to withstand the low gastric pH. Although the presence of *C. pylori* can be diagnosed by culture and histologic examination, simple tests for the presence of urease have become the cornerstone of diagnosis.

Background

The presence of urease enzyme in the gastric mucosa of animals was first noticed by Luck and Seth[1] in 1924 and its presence in the resected antrums of ulcer patients in Ireland was studied extensively by Fitzgerald and Murphy[2] in 1950. They observed that gastric mucosa had the ability to split urea and concluded that the gastric mucosa generated ammonia by the action

About the authors: The authors practice at the University of Virginia, Division of Gastroenterology, in Charlottesville, Virginia.

Send correspondence to: Susie R. Hoffman, RN, Department of Internal Medicine, Division of Gastroenterology, Box 145, University of Virginia Health Sciences Center, Charlottesville, VA 22908.

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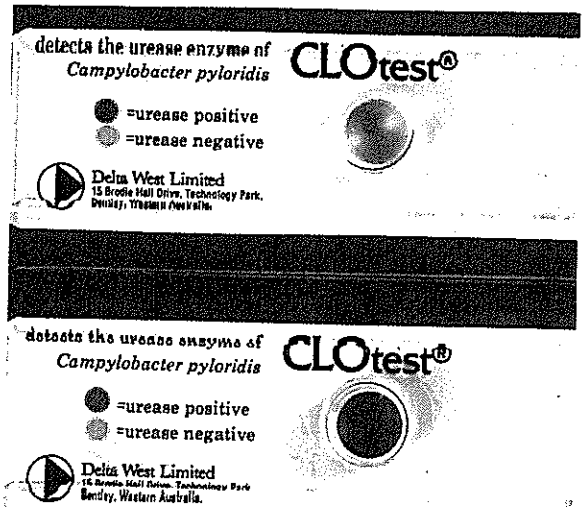

Figure 1. Two CLOtests. The one on the top is the color of a non-used CLOtest. It remains this yellow color in a CLO-negative patient. The red colored CLOtest on the bottom is that of a CLO-positive patient 1 hour after insertion of the biopsy.

of urease in order to protect itself from the luminal gastric acid. We now know that only patients infected with *C. pylori* have gastric urease activity and it may be the organism rather than the muscosa which is protecting itself from acid.

Three different possible mechanisms can account for the presence of the enzyme urease in the stomach, the most important of which is the production of urease by *C. pylori*.

CLOtest®[3] is designed to detect the presence of urease in gastric mucosal biopsies (Fig. 1). It is an agar gel, which contains urea, phenol red (a pH indicator), buffers and bacteriostatic agents. If the urease enzyme of *C. pylori* is present in the biopsy sample, the resulting degradation of urea causes the pH to rise and the pH indicator turns a bright red color.

Another possible but less common source of urease is from commensal organisms of the oropharynx swallowed into the stomach. Interestingly, urease is irreversibly denatured in strong acid. Thus, urease which reaches the stomach from organisms in the mouth will be inactivated by the gastric acid. *C. pylori*, however, burrows itself into the gastric mucous layer therefore protecting the urease it produces from gastric acid.

A third possible source is from the production of urease by bacteria other than *C. pylori* present in the stomach. Unlike *C. pylori*, these bacteria are able to survive in the stomach only in low acid states. In a patient with achlorhydria, or perhaps in patients taking large doses of H_2-receptor antagonists or omeprazole, urease generated in the gastric mucosa is not denatured in the lumen of the stomach. Likewise, urease reaching the stomach from commensal flora in the mouth, or because of bacterial overgrowth in the stomach, is not denatured in the absence of acid. In this latter circumstance false positive results for urease test can occur because of commensal organisms such as *Proteus mirabilis*, rather than *C. pylori*. *C. pylori* produces 10–100 times more urease than does *P. mirabilis* so these false positive tests react very slowly and do not give the rapid color change usually seen when *C. pylori* is present. Although these false positives results lead to an occasional incorrect diagnosis of infection with *C. pylori*, it should be remembered that the normal stomach is sterile, so the presence of urease, in a patient not receiving acid-reducing medication, always signals a pathologic state of some type.

How to Use the CLOtest

Before use, the CLOtest should be inspected to make sure that the well is full and is of a yellow color. CLOtests are buffered to approximately pH 6.0, but there may be gradual decomposition of urea with time which generates ammonia and raises the pH slightly. If a CLOtest has an orange color, it should be used with caution as it may give a false positive result.

CLOtests which are not completely full should also be used with caution. The CLOtest contains a bacteriostatic agent so that contaminating organisms cannot grow in the gel. If the biopsy specimen cannot be completely immersed in the gel, the bacteriostat may not penetrate the tissue and overgrowth of urease-producing commensal bacteria may occur. Normally, CLOtest detects only preformed urease in the gastric biopsy. Before endoscopy, the CLOtest may be placed on a warming plate at 37–45° C or placed in the endoscopist's pocket (approximately 30°C). This warming will help speed the chemical reaction.

Preparation of the Patient

Because CLOtest is a microbiological test, patients should not have taken antibacterial agents (antibiotics or bismuth) for at least 3 weeks prior to endoscopy. Suppression of *C. pylori* by these agents makes the organism difficult to detect by any means, and regrowth of the organism may be patchy, leading to false negative results in the first few weeks after treatment. For this reason we routinely postpone endoscopy for 3–4 weeks after antibacterial therapy.

The Biopsy Specimen

For immediate diagnosis the CLOtest biopsy specimen

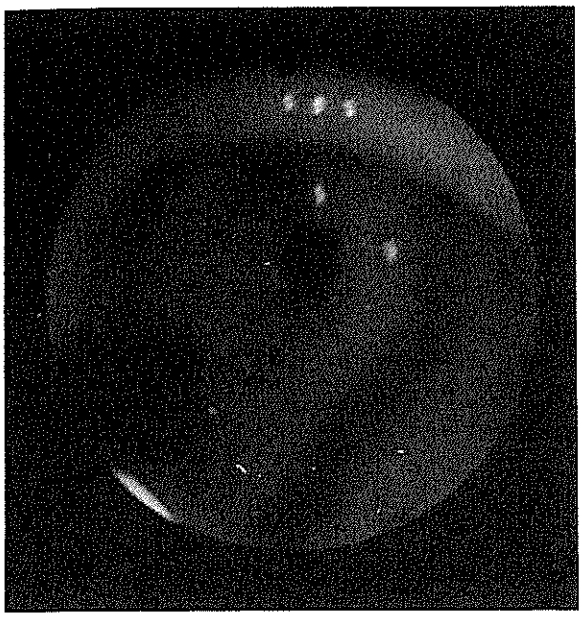

Figure 2. Taking a biopsy of antrum for identification of *C. pylori.* The lesser curve angularis lies under the notch at 12 o'clock. The biopsy should be taken opposite the angularis along the greater curvature aspect of the antrum roughly 5 cm from the pyloris.

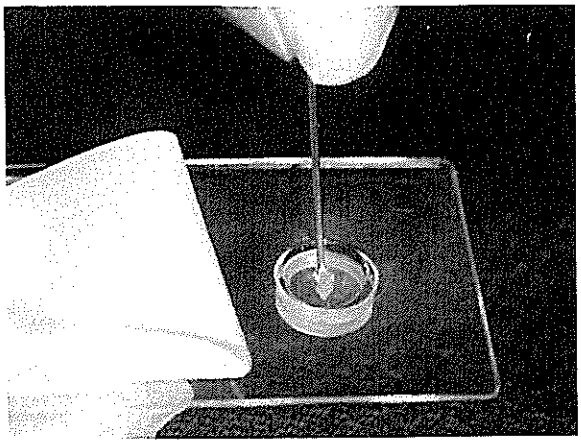

Figure 3. Insertion of 2-mm pinch biopsy into the CLOtest gel. A 19-G needle is preferred. The CLOtest should be immediately resealed.

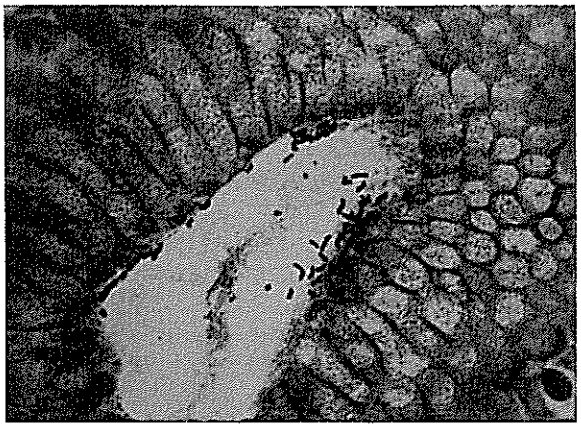

Figure 4. Gastric biopsy showing the presence of *Campylobacter pylori.*

may be taken as soon as the endoscopist has briefly examined the stomach and duodenum. The specimen should be taken in the dependent portion of the antrum, along the greater curvature. This helps to avoid any islands of intestinal metaplasia where *C. pylori* may not be present. These islands are more prevalent near the pylorus and along the lesser curve. The biopsy specimen should also be taken in an area of normal looking tissue rather than in an area effected by erosions or ulceration because the organism may be present in smaller numbers after an area has become eroded and the mucus layer denuded.

The biopsy specimen can be taken with a standard biopsy forceps (Fig. 2); thus, there is no need to use a jumbo forceps. If the specimen appears to be very small, it may be worthwhile taking a second specimen and inserting both specimens into the CLOtest sample. Be careful not to get too much blood on a specimen because blood may give an orange tinge to the CLOtest, making it difficult to read.

As soon as the samples have been inserted deep into the gel (Fig. 3), reseal the CLOtest, and write the time of insertion of the specimen on the label along with the patient's name and the date.

Reading the CLOtest

When first inserted in the gel, biopsies may have a very slight pink tinge, particularly if blood or bile is present on the biopsy specimen. Note the tinge present at 5 minutes and reexamine the CLOtest at 15 minutes. Only if the redness clearly expands in size from the 5-minute to the 15-minute examination will the results of the CLOtest be called positive. Usually the CLOtest is examined *after* the endoscopy report has been completed. This allows the endoscopist to perform a "prospective, blinded study" by comparing his own endoscopic diagnosis of gastritis with the results of the CLOtest (which is usually far more accurate) (Fig. 4).

After the 15-minute reading, the CLOtest should be reexamined at 1 hour, 3 hours and finally at 24 hours (Fig. 5). During this time, it is convenient to carry the CLOtest in one's pocket. In at least 85% of patients, diagnosis will be made at 1 hour, in sufficient time to prescribe therapy before their departure from the recovery room. If only a small number of *C. pylori* organisms are present, then instead of seeing a definite

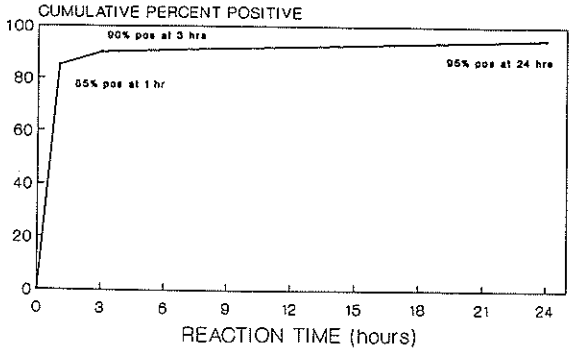

Figure 5. Graph of CLOtest results versus reaction time (0–24 hours).

red color around the biopsy, the color of the whole CLOtest gel will gradually change. An orange gel at 3 hours is almost certainly a positive reaction and will usually turn to a red color overnight.

CLOtest will diagnose 75% of *C. pylori* infections in the endoscopy room with no false positive results at that time.[3] By 1 hour 85% of patients with infections should be detected by the CLOtest. At 3 hours 90% are detected. Between 3 and 24 hours another 5% of patients with infections will be detected by the CLOtest. A few of these late reactions may only proceed to a deep orange color and an occasional false positive reaction will occur during this time, particularly if the patient is achlorhydric. Thus, correctly used, the CLOtest will detect 90% of patients with *C. pylori* on the day of the endoscopy. Another 5% will be detected within 24 hours. The false positive rate for CLOtest is about 2%, but false positive results never cause a rapidly positive CLOtest (within the first 3 hours). On a single biopsy, CLOtest appears to be as sensitive as histology and more sensitive than culture. If more than one CLOtest is performed, the sensitivity should improve.

Occasionally, patients have *C. pylori* present in the body of the stomach but not in the antrum (approximately 2% of patients). It may be useful therefore to do a second CLOtest on body mucosa. Data suggest that patients with duodenal ulcer have *C. pylori* predominantly concentrated in the antrum, whereas patients with gastric ulcer or other kinds of dyspepsia have more widespread *C. pylori* infection, occasionally including the gastric body.

As current treatment regimens for *C. pylori* are only 50–75% effective,[4] it is important to reassess the patient's condition after therapy to be sure the infection has been eradicated. Remember to wait at least 28 days after completion of therapy to perform a retest. Biospies taken before this time may be falsely negative as the organism may have merely been suppressed rather than eradicated. Because regrowth of the organism can be patchy, it may be wise to perform two CLOtests at follow-up after therapy. Once eradication has been achieved and properly confirmed (i.e., 1 month after therapy), reinfection appears to be uncommon (preliminary data, pending publication).

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