Minitek Urea Disk Test, a Sensitive and Cost-Effective Method To Screen for Campylobacter pylori in Gastric Biopsies

LINDA SWEENEY,1* LUIS P. GARCIA,2† MICHAEL TALBERT,3 MARK SILVERMAN,4 AND CYNTHIA A. NEEDHAM1

Departments of Laboratory Medicine,1 Gastroenterology,2 and Anatomic Pathology,4 Lahey Clinic Medical Center, 41 Mall Road, Burlington, Massachusetts 01805, and Department of Pathology, Massachusetts General Hospital, Boston, Massachusetts 02114

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One hundred fifty-five biopsy specimens from the gastric mucosa of 81 patients undergoing routine endoscopy procedures were tested for the presence of Campylobacter pylori by three methods: Gram stain, culture, and modified Minitek, a rapid urea disk test (BBL Microbiology Systems, Cockeysville, Md.). Twenty-nine patients were infected with C. pylori. Sensitivities and specificities of detection were 100 and 94% with the Minitek test and 93 and 100% with Gram stain, respectively. Rapid testing by the urea disk is a simple, cost-effective, and accurate method for detecting the presence of C. pylori in gastric biopsy specimens.

Campylobacter pylori has recently been established as the etiologic agent of chronic active antral gastritis (type B) and may play a role in gastric and duodenal ulcers. In 1983, Marshall and Warren (8) observed organisms resembling campylobacters on the surface of biopsy specimens of gastric mucosa from patients with chronic active gastritis. One year later, they reported the successful cultivation of these organisms in a prospective study of 100 consecutive patients (9). Of the patients studied who had chronic active gastritis, 95% harbored the organism. Langenberg et al. (7) subsequently reported that C. pylori produced high levels of urease, an enzyme not present in mammalian cells.

Several investigators have evaluated tests designed to detect the urease produced by C. pylori in gastric biopsy specimens. Marshall et al. (10) reported a sensitivity of 98% with the CLOtest (Delta-West Ltd., Perth, Western Australia, Australia), a pellet of buffered gel containing urea and a bacteriostatic agent. Hazell et al. (6) used a microdilution method with buffered urea broth and noted a sensitivity of 91%. A one-minute urease test, which utilizes freshly prepared 10% (wt/vol) urea solution in deionized water and the indicator phenol red, has also been used successfully in an endoscopy room (1). These methods are not commercially available in the United States. Slants of Christensen urea agar and broth have been evaluated by several investigators (2-5, 11, 12). Although early reports seemed promising, only small numbers of patients were evaluated. As larger groups of patients were tested, the sensitivity of this method proved to be unacceptably low, ranging from 50 to 88%. In addition, the test is subject to false-positive results at 24 h because of the growth of other urease-producing bacteria, such as Proteus species. Recently, Westblom et al. (14) reported the use of urea broth incubated at 37°C and inspected at 1 h. This approach improved the specificity of the assay to 100%, but the sensitivity remained low (67%). In the present study, a 0.6-mg urea disk test (Minitek; BBL Microbiology Systems, Cockeysville, Md.) was evaluated for its ability to detect preformed urease produced by C. pylori in gastric tissue obtained at biopsy.

MATERIALS AND METHODS

Eighty-one patients undergoing routine endoscopy procedures were included in the study. Preendoscopic diagnoses included gastric ulcer, duodenal ulcer, unexplained abdominal pain, and nonulcerative dyspepsia. Antral and fundal biopsy specimens were obtained from 74 patients, antral specimens only were obtained from 4 patients, and fundal specimens only were obtained from 3 patients. The anatomic site of each biopsy was confirmed by histopathology. Specimens were placed in 0.5 ml of sterile nonbacteriostatic saline and transported to the laboratory within 30 min.

An imprint of each tissue specimen was made on a sterile glass slide, which was stained by the Gram method and examined for the presence of spiral gram-negative rods consistent with Campylobacter species. The tissue was then used to inoculate a series of three plates: chocolate agar, brucella agar with 5% horse blood, and Skirrow agar (BBL Microbiology Systems). The plates were incubated at 35°C in an environment of 5% carbon dioxide–10% oxygen–85% nitrogen. Plates were examined for 7 days for bacterial growth consistent with C. pylori. Colonies were identified on the basis of morphologic results of Gram stain and positive reactions for catalase, oxidase, and rapid urease. Immediately after the original tissue was cultured, it was placed in a sterile tube containing 0.2 ml of bacteriostatic saline and a 0.6-mg urea disk (Minitek) and was left at room temperature. The test was examined by one observer after 1- and 24-h incubation periods. A positive result for urease was indicated by a color shift from yellow to purple resulting from a pH rise caused by hydrolysis of urea to ammonia. Additional biopsy specimens were submitted for histopathologic examination. These specimens were fixed in Formalin and embedded in paraffin, and sections were stained with hematoxylin and eosin.

Biopsy specimens were categorized as demonstrating no evidence of gastritis, chronic gastritis, or chronic active gastritis. Active gastritis is defined by the presence of neutrophils in the epithelium. When chronic active gastritis
TABLE 1. Sensitivity and specificity of culture, Gram stain, and rapid urease test in detecting patients colonized with C. pylori

<table>
<thead>
<tr>
<th>Test</th>
<th>Colonized No.</th>
<th>Noncolonized No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive results</td>
<td>Negative results</td>
</tr>
<tr>
<td>Culture</td>
<td>23</td>
<td>6</td>
</tr>
<tr>
<td>Gram stain</td>
<td>27</td>
<td>2</td>
</tr>
<tr>
<td>Urease</td>
<td>29</td>
<td>0</td>
</tr>
</tbody>
</table>

was present, patients were further categorized into four groups according to the relative degree of activity present: focal activity, mild activity, moderate activity, or severe activity.

RESULTS

In this study, a patient was considered to be colonized with C. pylori when the organism was isolated in culture or when results of Gram stain indicated the presence of bacteria consistent with Campylobacter sp. or both. Twenty-nine patients were considered to have C. pylori by these criteria. All 29 patients had both fundal and antral biopsies submitted. The results of each test, considered individually, are presented in Table 1.

The rapid urease test detected C. pylori in 29 of 29 patients (sensitivity, 100%). Both antral and fundal specimens were positive in 25 of the patients. The time at which specimens tested positive for urease was available for 24 patients. Eighteen (75%) of these patients had positive results after 1 h of incubation. The remaining six patients (25%) did not have positive results until the second examination at 24 h.

The three patients with false-positive urease tests each had both fundal and antral biopsy specimens submitted. Only one of the two biopsy specimens from each patient was positive for urease activity. Cultures from these three patients did not recover any other bacteria, and thus it seems unlikely that other organisms producing urease were present.

Cultures were positive for C. pylori in 23 of the 29 patients, yielding a sensitivity of 79%. We observed no advantage of the selective medium (Skirrow agar) compared with the nonselective media. Most strains grew more luxuriantly on the brucella plate with 5% horse blood, but no difference in recovery was observed between the chocolate and horse blood agars.

Of the 29 patients confirmed to have colonization by C. pylori, 28 had evidence of chronic active gastritis. Three patients had focal activity only, and the remainder were categorized as having mild (9 patients), moderate (13 patients), or severe (3 patients) chronic active gastritis. Of the 52 patients without bacteriologic evidence of C. pylori, 39 had either no histologic evidence of active gastritis or evidence of chronic inflammation. Thirteen patients had evidence of chronic active gastritis; six of these patients had only focal activity, and seven patients were categorized as having mild (five patients), moderate (one patient), or severe (one patient) chronic active gastritis. Two of the three patients with false-positive results of urease tests were included in one of the latter groups with chronic active gastritis.

DISCUSSION

C. pylori has convincingly been established as the agent of chronic active gastritis. Whether the organism causes nonulcerative dyspepsia or gastric and duodenal ulcers remains controversial. Nevertheless, the laboratory clinician is being asked to provide diagnostic data for patients in whom colonization is suspected.

The fastidious nature of C. pylori makes its isolation time-consuming and expensive. Positive results on culture are usually not detected until 3 to 5 days after the specimen has been inoculated onto a supportive growth medium. In our laboratory, cultural techniques detected C. pylori in 79% of colonized patients. Thus, an alternative method that is rapid, sensitive, and inexpensive is highly desirable.

The Gram stain represents one such test. A recent report (13) claims a sensitivity of 100%, using an impression smear of both antral and fundal biopsy specimens.

A modified Minitek urea disk test, as described in this report, offers an alternative or adjunct to the Gram stain. The test is simple to perform, inexpensive, and highly sensitive and specific. Although a sensitivity of 100% was achieved during the formal evaluation of the urea disk, our ongoing experience suggests that the sensitivity is approximately 98%. This value, however, represents a substantial improvement over other approaches. The specificity of the test is superior to that of similar tests that have been reported (4, 5, 11). We believe the use of bacteriostatic saline, which permits the detection of preformed enzyme only, is the key difference.

Three false-positive reactions were observed during the formal study, resulting in a specificity of 94%. However, all three patients whose specimens yielded an isolated positive result for urease had evidence of chronic active gastritis. Because other bacteria were not recovered from these patients, the origin of the urease activity is unknown. It is possible that small numbers of C. pylori were present but were not detected by Gram stain or culture.

The Minitek urea disk test can also be used in conjunction with Gram stain and oxidase for rapid confirmation of C. pylori from culture. Results are positive within 5 min of inoculation with an isolated colony. Inoculating loops made of nichrome generate an immediate, spuriously positive test result; a platinum loop or wooden stick should be used.

In conclusion, the Minitek urea disk test offers a rapid, highly reliable, and inexpensive method for detecting the presence of C. pylori in gastric biopsies.

LITERATURE CITED


