

Antral brushings and biopsies for gastric *Campylobacter pylori*: A comparative study of a rapid urease test, culture and histology

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ABSTRACT: A prospective study was conducted to assess the sensitivity and specificity of diagnostic tests for *Campylobacter pylori* in 60 unselected patients referred for gastroscopy. Urease testing was performed on samples of gastric mucus obtained by a disposable cytology brush and on gastric mucosal biopsies. The combination of the brush urease test and biopsy urease test had a sensitivity of 78% and a specificity of 96%. The combination of the brush urease test and examination of gastric biopsies for curved bacilli had a sensitivity of 96% and a specificity of 96%. The combination of the brush urease and biopsy urease tests will rapidly identify the majority of patients with *C pylori*. This test is rapid, easy, sensitive and inexpensive. If a pathologist is available, the addition of histologic review for *C pylori* in combination with the brush urease test, will identify virtually all patients with *C pylori* without the need for culturing this fastidious organism. *Can J Gastroenterol* 1989;3(3):91-94. *Pour résumé voir page 92*

Key Words: *C pylori*, Diagnosis, Urease test

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IN 1982, WARREN AND MARSHALL (1-3) successfully cultured the Gram-negative bacterium *Campylobacter pylori* from human gastric mucosa and first established an association between this organism and gastroduodenal disease. Since then, a number of investigators have confirmed the association of *C pylori* with gastritis, gastric ulcers and duodenal ulcers (4-15). Recent studies have shown that standard treatment for peptic ulcer disease does not eradicate *C pylori* from gastric mucosa (16-18). Whether more specific therapy for *C pylori* will change the long term prognosis of peptic ulcer disease is presently under investigation.

Multiple modalities have been used to detect the presence of *C pylori*. An inoculum of this fastidious organism from gastric mucosal biopsies will yield visible colonies on agar plates in five to seven days (19). Curved bacilli can be seen on gastric mucosa stained with hematoxylin and eosin, Warthin-Starry sil-

Biopsies et brossages antraux pour la détection de *Campylobacter pylori* gastrique: Etude comparative d'un test rapide d'uréase, de la culture et de l'histologie

RESUME: Nous avons effectué une étude prospective pour établir la sensibilité et la spécificité des tests diagnostiques destinés à détecter *Campylobacter pylori* chez 60 patients non sélectionnés devant subir une gastroscopie. Le test d'uréase a été effectué sur plusieurs échantillons de mucus gastrique obtenus au moyen d'une brosse de cytologie jetable et sur des biopsies mucoales gastriques. La combinaison du test d'uréase par brossage et sur biopsies avait une sensibilité de 78% et une spécificité de 96%. La combinaison du test d'uréase par brossage et l'examen des biopsies gastriques destinées à détecter les bacilles à bâtonnets incurvés avait une sensibilité de 96% et une spécificité de 96%. La combinaison des tests par brossage et par biopsies permet la détection rapide de *C pylori* chez la majorité des patients. La procédure est rapide, facile, sensible et peu coûteuse. Si un pathologiste est disponible, ajouter une revue histologique de *C pylori* au test d'uréase par brossage permet d'identifier virtuellement tous les patients avec *C pylori* sans qu'il soit nécessaire de procéder à une culture de cet organisme exigeant.

ver stain, Giemsa stain or acridine orange (1-14, 20, 21).

The organism can also be seen with phase contrast microscopy and by electron microscopy (4, 6, 10, 14, 22). Preformed urease of *C pylori* will cause a rapid colour change in urea containing media such as Stuart's test broth (23), Christensen's 2% urea broth (24-30) and the CLOtest of Marshall et al (31). Urea breath tests and serologic tests can also be used to diagnose the presence of *C pylori*.

The purpose of this study was to assess the use of urea broth tests as a simple inexpensive technique available to all endoscopy laboratories to detect the presence of *C pylori*. Gastric biopsies as well as a disposable cytology brush were used to obtain samples of gastric mucus for culture and urease testing in Stuart's modified urea broth. These tests were compared to previously established methods for the detection of *C pylori*.

PATIENTS AND METHODS

A prospective study was conducted in which each investigator was blinded to the results of the other diagnostic tests. The study was approved by the Cleveland Veterans Administration Hospital Investigation Review Board. All patients gave written informed consent.

Patient population: The study group consisted of 60 hospitalized and outpatient adult males referred for upper

gastrointestinal endoscopy. The patient's ages ranged from 26 to 77 years with a mean age of 61 years. Patients were excluded for: active gastrointestinal bleeding; therapeutic endoscopy (sclerotherapy, electrocautery, dilatation); prothrombin time more than 15 s; platelets less than 75,000/mm³. The presence of *C pylori* was established by either a positive culture for *C pylori* from the biopsy or gastric brushings, or identification of *C pylori* by microscopic examination. Bacteria recovered at endoscopy were identified as *C pylori* based on colony morphology, Gram stain, and the production of urease, catalase and oxidase.

Endoscopy: One investigator performed all upper gastrointestinal endoscopies using either the Olympus XQ10 or Olympus Q endoscope. Each quadrant of the gastric antrum was randomly brushed with a single disposable cytology brush until the mucosa became erythematous. The brush was then swished in a tube containing 1 mL of urease test broth. Four random antral biopsies were obtained. Two were placed in a tube containing 1 mL of urease test broth and two were placed in buffered formalin and sent for histologic examination. Endoscopic findings were recorded prior to checking the urease test broth results.

Urease test broth: The Stuart's urease test broth (23) was modified by the addition of 200 g glucose to each litre (19). Urease test broth containing the antral

brushings and the tubes containing the antral biopsies were each observed at room temperature for up to 6 h. A minimum of 1000 organisms will cause a change in colour from yellow to pink, defined as a positive urease test. Incubation at 37°C or 42°C did not hasten the reaction. Test results were recorded by a microbiologist who was unaware of the endoscopic findings.

Microbiology: All samples were kept at room temperature for 2 to 6 h before culture and the presence or absence of colour change was recorded. Gastric antral biopsies, first ground with a sterile mortar and pestle in 0.4 mL of brain-heart infusion broth (BBL, Cockeysville, MD) and antral brushings were inoculated into Columbia blood agar plates (Difco Labs, Detroit, MI). The plates were then incubated in 5% oxygen and 10% carbon dioxide at 37°C for up to 10 days. Colonies were identified as *C pylori* on the basis of Gram stain and chemical testing for the presence of urease (23), catalase and oxidase (Difco oxidase differentiation disk). The microbiologist recorded the results without knowledge of the endoscopic or histologic findings.

Histologic studies: Two antral biopsies were routinely processed and stained with hematoxylin and eosin. Hematoxylin and eosin have been shown to be as effective as the Giemsa stain for detecting *C pylori* (32). One investigator reviewed each biopsy for the presence of *C pylori* and gastritis. The degree of gastritis was graded as mild, moderate or severe by the following criteria: amount of inflammatory infiltrate; relative number of acute inflammatory cells; presence or absence of ulceration or other changes involving the epithelial lining. The degree of polymorphonuclear infiltration was characterized as being absent, rare, mild, moderate or severe. The pathologist recorded the results without knowledge of the endoscopic, urease test broth or microbiologic studies.

Statistical analysis: Sensitivity, specificity, positive predictive value and negative predictive value were calculated for each of the various diagnostic tests. The association between histologic gastritis, polymorphonuclear cell infiltrate and the presence of *C pylori* was compared using χ^2 with the Yate continuity correction.

TABLE 1
Campylobacter pylori diagnostic test results

Test	<i>C. pylori</i>		Sensitivity	Specificity	Predictive value	
	Positive	Negative			Positive	Negative
Brush urease						
Positive	20	1	62%	96%	95%	69%
Negative	12	27				
Biopsy urease (Bx)						
Positive	22	0	68%	100%	100%	74%
Negative	10	28				
<i>C. pylori</i> histology (CP Bx)						
Positive	23	0	72%	100%	100%	76%
Negative	9	28				
Brush or biopsy						
Culture positive	24	0	75%	100%	100%	78%
Culture negative	8	28				
Either brush or biopsy						
Urease positive	25	1	78%	96%	96%	79%
Both brush and Bx						
Urease negative	7	27				
Urease Bx positive						
or CP Bx positive	29	0	90%	100%	100%	90%
Urease Bx negative						
and CP Bx negative	3	28				
Urease brush positive						
or CP Bx positive	31	1	96%	96%	97%	96%
Urease brush negative						
and CP Bx negative	1	27				
Brush urease, Bx urease						
or CP Bx positive	31	1	96%	96%	97%	96%
Brush urease, Bx urease						
and CP Bx all negative	1	27				

RESULTS

Diagnosis: The presence of *C. pylori* was established in 32 of 60 patients (53%) as defined by a positive culture from either brush or biopsy specimens or identification of organisms on histologic examination.

The results of the diagnostic tests are listed in Table 1. The brush urease and biopsy urease tests have a diagnostic sensitivity of 62 and 68%, respectively. *C. pylori* histology has a sensitivity of 72% and culture has a sensitivity of 75%. The combination brush urease and biopsy urease test has a sensitivity of 78%. By using a combination of *C. pylori* histology with either the biopsy urease or the brush urease test, diagnostic sensitivity increases to 90 and 96%, respectively. The combination of brush urease, biopsy urease and histologic review will not increase the overall detection of *C. pylori* but will increase the number of patients detected by the rapid urease test.

Histology: All patients who were positive for *C. pylori* had some degree of histologic gastritis. There was a significant

association between moderate and severe gastritis as well as the presence of polymorphonuclear cell infiltration and the diagnosis of *C. pylori*.

DISCUSSION

The findings of the present study confirm the previously reported relationship of *C. pylori* to peptic ulcers. Six of the nine patients with duodenal ulcers and six of the eight patients with gastric ulcers were positive for the organism. These results compare with previous studies which have reported the presence of *C. pylori* in 75 to 100% of patients with duodenal ulcers and 35 to 81% of patients with gastric ulcers (6-12). *C. pylori* was present in 87% of patients with severe gastritis and 75% of patients with moderate gastritis.

The purpose of this study was to assess the use of Stuart's modified urea broth as a medium for detecting the presence of *C. pylori* from gastric biopsies and cytologic brushings.

This is the first study to report the use of the 'brush urease test' to aid in

the diagnosis of *C. pylori*. The use of a cytology brush increases the sampling area and can increase the diagnostic yield. The authors selected the modified Stuart's test broth (21) because it is inexpensive and available in any microbiology laboratory. This broth will detect as few as 1000 organisms.

In the authors' experience, careful histologic examination of mucosal biopsies, stained with hematoxylin and eosin under high magnification (100 ×), will identify *C. pylori* as effectively as the Giemsa stain. This test alone yields a diagnostic sensitivity of 72%.

The combination of the brush urease test and the biopsy urease test has a diagnostic sensitivity of 78% and specificity of 96%. The combination urease test is an easy, rapid and specific test that will detect the majority of patients infected with *C. pylori* within hours of completion of gastroscopy.

The authors found that the most sensitive test was the combination of the brush urease test and histologic review of the mucosal biopsies for the presence of curved bacilli. The advantage of this combination is that it seems to detect almost all patients with *C. pylori* associated gastroduodenal disease. The brush urease test will diagnose the majority of the patients within hours of endoscopy while the remaining patients will be diagnosed at the time of histologic review. The addition of the biopsy urease test to the combination of brush urease and *C. pylori* histology will increase the number of patients diagnosed within hours of gastroscopy, but will not increase the overall diagnostic sensitivity.

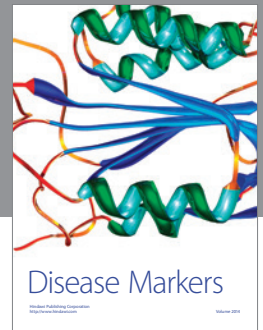
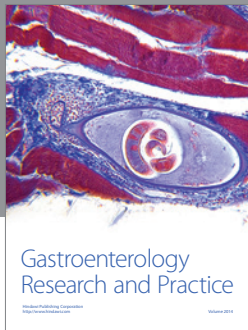
In summary, this study confirms the association of *C. pylori* with significant gastroduodenal disease. Recently a number of reviews have highlighted the importance of *C. pylori* in the pathogenesis of gastroduodenal disease. Although optimal therapy for *C. pylori* gastric disease is not yet available, the presence of *C. pylori* may alter standard medical management. Therefore, a simple sensitive and specific test for the presence of *C. pylori* would be a useful addition to the endoscopic evaluation. These data support the use of the combination brush test and biopsy urease test to identify the majority of patients with *C. pylori* associ-

ated gastroduodenal disease without the necessity for histologic or microbiologic assessment. This test is rapid, easy, sensitive, inexpensive and readily available for use in all gastrointestinal laboratories. When available, histologic identification of *C pylori* in antral biopsies, in combination with the brush urease test, will identify virtually all patients with *C pylori* without the need for culturing this fastidious organism.

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