

# Histology compared with chemical testing for urease for rapid detection of *Helicobacter pylori* in gastric biopsy specimens

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## Abstract

Gastric biopsy specimens from 283 patients with ulcer and non-ulcer dyspepsia attending five gastroenterology clinics in the northern region of the United Arab Emirates (UAE) were tested by the agar gel test (n = 115) or the ultra-rapid endoscopy room test (n = 168) for the presence of *Helicobacter pylori* urease. Results were compared with a histological technique using the Romanowsky type (Diff-3) stain for detecting *H pylori* in both antral and body type gastric mucosa. A sensitivity of 94% and specificity of 100% using the agar gel test compared with 87% sensitivity and 99.3% specificity for the ultra-rapid endoscopy room test. Grading of *H pylori* in gastric biopsy specimens showed that the higher the histological grade, the more likely that the urease test would be positive.

Both forms of urease tests have high specificity for detecting *H pylori* in gastric biopsy specimens, although the urea agar test has a higher sensitivity than the ultra-rapid test. Low numbers of *H pylori* in gastric biopsy specimens are the most important determinant of a false negative urease test.

(J Clin Pathol 1993;46:684-685)

A large body of evidence indicates that *Helicobacter pylori* is involved in the pathogenesis of chronic gastritis and perhaps in peptic ulcer disease. Several invasive and non-invasive techniques are currently used for detecting *H pylori* infection. Of the invasive techniques, histology, culture, and the endoscopic biopsy urease test are the most widely used methods.<sup>1</sup> The endoscopic biopsy urease test is a rapid diagnostic test based on the presence of the pre-formed enzyme in *H pylori* in the specimens. Different forms of the test have been described including a CLO gel,<sup>1,2</sup> a microtitre test,<sup>1,3</sup> and urea broth and liquid forms.<sup>4-7</sup> Each method has its advantages and disadvantages and the sensitivity has been reported to be in the region of 90%.

This study aimed to assess the value of two endoscopic biopsy urease tests and to identify the possible factors responsible for their lower sensitivity than histological techniques which use the Sydney system for the classification of gastritis.<sup>8</sup>

## Methods

Five gastric biopsy specimens were taken routinely from 283 patients (89 females) with ulcer and non-ulcer dyspepsia from five hospitals.

The mean age of the patients was 37.4 years (range 12-100 years). One antral biopsy specimen was used for the urease agar gel test (n = 115) or for the ultra-rapid endoscopy room test (n = 168). For the former, the biopsy specimen was inserted into the gel of a urea agar slope (Biomérieux, France). A positive result was indicated by a colour change to pink, usually after two hours at room temperature (up to nine hours). For the ultra-rapid test, a large batch of 10% (weight/volume) urea solution in deionised water at a pH of 6.8 was prepared and tested with an *H pylori* strain isolated from an antral biopsy specimen before use. A 0.5 ml aliquot of urea solution was placed in a capped tube and stored in a freezer at -20°C. Urea solution tubes were then dispatched to all five hospitals and only thawed before use. Two drops of a 1% phenol red solution were added to the test tube and an antral biopsy specimen was placed immediately into the urea solution. A positive result was recorded by the endoscopist if the colour changed from yellow to pink 1-15 minutes after insertion of the biopsy specimen.<sup>7</sup>

Four gastric biopsy specimens from the anterior and posterior wall of the antrum and corpus<sup>8</sup> were placed in 10% formol-saline. The specimens were then processed and sections 4 µm thick were cut and stained by haematoxylin and eosin and Diff-3 stains.<sup>9</sup> The density of spiral bacteria was assessed blind according to the method of Wyatt<sup>10</sup> in both antral and body type gastric biopsy specimens.

Each gastric biopsy was scored separately and the mean score of four biopsy specimens was used as the final score: 0 = no curved bacteria seen; 1 = one or two mucosa-associated bacteria seen; 2 = *H pylori* in less than 50% of the gastric pits and surface area; 3 = *H pylori* in more than 50% of the pits or surface area; 4 = dense *H pylori* forming a carpet of bacteria.

Statistical evaluation was performed using the  $\chi^2$  test with the Yates correction.

## Results

Of the 115 patients with ulcer and non-ulcer dyspepsia, 14 had a negative urease gel test, six of which were found to have a false nega-

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Accepted for publication 27 January 1993

Comparison of urease gel test and ultra-rapid endoscopy biopsy urease test with *H pylori* grades obtained histologically from 283 patients with ulcer and non-ulcer dyspepsia

	<i>H pylori</i> grade					Total	Sensitivity %	Positive predictive value %
	0	1	2	3	4			
<i>Urease gel test</i>								
Test negative 14	8	†	1	0	0	115	94	100
Test positive 101	0	‡	56	26	13			
<i>Ultra-rapid test</i>								
Test negative 36	14	▲	8	1	0	168	87	99.3
Test positive 132	1	☆	65	35	19			

p value: † v ‡, ▲ v ☆ p < 0.0001; \* v Δ p < 0.05

tive test compared with histology (table). The remaining 101 patients had positive urease gel tests which were confirmed histologically. *H pylori* grades in gastric biopsy specimens stained by the Diff-3 stain are also shown in the table. Five out of six (83%) patients with false negative urease tests had grade 1 *H pylori* compared with six out of 101 (6%) with positive urease tests ( $p < 0.0001$ ). For *H pylori* grade 2, one patient had a false negative urease test compared with 56 patients with positive urease tests.

Thirty six of the 168 patients examined using the ultra-rapid urease test were found to be negative and 22 were false negative when compared with histology (table). Thirteen out of 22 had *H pylori* grade 1 (59%) in comparison with 9% with a positive ultra-rapid test ( $p < 0.0001$ ). Only one patient out of 168 had a false positive ultra-rapid test when histological examination did not show any organisms. The sensitivity of the urease gel test and the ultra-rapid urease test were 94% and 87%, respectively ( $p < 0.05$ ). The positive predictive values for both urease tests were similar (100 v 99.3%).

## Discussion

In this study, biopsy specimens were obtained following the recommendations of the Sydney system of classification of gastritis.<sup>8</sup> Although the gastric antrum is the preferred site of biopsy for demonstration of *H pylori* infection using the urease technique, histology, and culture,<sup>1</sup> sampling both anterior and posterior walls of the body and antrum will effectively eliminate any false negative results caused by the patchiness of colonisation of gastric mucosa by *H pylori*. The histological technique described in this study can be considered the gold standard for detecting *H pylori* infection with which the urease test can be compared.

Most other authors have reported the sensitivity of the urease test to be comparable with the results found in this study.<sup>2-7</sup> A 100% specificity of the urease test has been reported by other workers.<sup>3-7</sup> The false positive result in one patient in the present study tested by the ultra-rapid test may be explained by the presence of other urease producing bacteria in the gastric biopsy specimen.

This study has shown that the higher the

grade of *H pylori* colonisation in the gastric biopsy specimen the more likely that the biopsy specimen will be positive on urease testing. There was a significant difference between false negative and true positive urease tests. This indicates that for patients with *H pylori* grade 1 infection a significant number will have false negative tests.

Another possible cause of false negative urease tests is the complete absence of *H pylori* in antral biopsy specimens due to the patchiness of organisms in patients who have intestinal metaplasia.<sup>11</sup> Diff-3 stained smears have a sensitivity similar to the histological technique and can be performed and interpreted within minutes of endoscopic examination.<sup>9</sup>

It is concluded that both the urease gel and ultra-rapid urease tests have specificities of about 100% and sensitivities near 90%. Grading of *H pylori* in gastric biopsy specimens has shown that the higher the grade the more likely the biopsy urease test is to be positive and vice versa. The presence of a small number of *H pylori* in the gastric biopsy specimen is the main determinant of a false negative urease test.

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