

## Evaluation of the one-minute ultra-rapid urease test for diagnosing *Helicobacter pylori*

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### Summary

To determine the diagnostic accuracy of the one-minute ultra-rapid urease test for diagnosing *Helicobacter pylori* infection, two biopsies were taken from both the gastric corpus and antrum from 1000 patients undergoing upper gastrointestinal endoscopy. All the biopsies were subjected to the one-minute ultra-rapid urease test before imprint smears were prepared from them. Thereafter, the biopsies were fixed in 10% formalin and histological sections were examined for the presence of *H pylori* by a pathologist who was not aware of the clinical details or the results of the urease test. The prevalence of *H pylori* in the gastric antrum and corpus was 86.7% and 53.3%, respectively. The sensitivity, specificity, positive and negative predictive value and the overall diagnostic accuracy of the ultra-rapid urease test to diagnose *H pylori* infection in the gastric antrum were 92%, 100%, 100%, 66%, and 93%, respectively. The corresponding figures for the gastric corpus were 83%, 100%, 100%, 85%, and 91%, respectively. It is concluded that the one-minute ultra-rapid urease test has a high sensitivity and specificity and may be used as a rapid and cheap method to diagnose *H pylori* infection.

**Keywords:** diagnosis; *Helicobacter pylori*; urease testing

There are several methods of diagnosing *Helicobacter pylori* infection.<sup>1</sup> The fact that the bacterium produces urease that splits urea has been exploited to develop several rapid urease tests. Although several of these are available commercially,<sup>2</sup> they are relatively expensive and may therefore not be available to all clinicians, especially in developing countries. However, there are several locally made urease tests that are very easy to prepare and are inexpensive.<sup>3-5</sup> There are, however, doubts about the sensitivity and specificity of these tests. We have been using the one-minute ultra-rapid urease test (URUT)<sup>3</sup> for most of our studies. This study was undertaken to assess the reliability of the URUT compared to histology.

We first carried out a pilot study on 20 gastric biopsies to see whether the same gastric biopsy could be used for URUT, imprint smears and histology. The results of this pilot

study showed that the same biopsy tissue could be used to perform the URUT and prepare imprint smears without adversely affecting the tissue for subsequent histological examination.

### Patients and methods

One thousand patients undergoing upper gastrointestinal endoscopy were studied. Patients who were receiving proton pump inhibitors, antibiotics or bismuth-containing agents and patients who had received therapy aimed at eradication of *H pylori* were excluded from the study. Four gastric mucosal biopsies were taken: two from the antrum within 4 cm of the pylorus (one from the greater and the other from the lesser curvature) and two from the corpus of the stomach.

The one-minute ultra-rapid urease test was freshly prepared as described by Thillainayagam *et al.*<sup>3</sup> Briefly, each biopsy tissue was placed immediately into a capped Eppendorf tube containing 0.5 ml of a freshly prepared solution of 10% urea in deionized water, to which had been added two drops of 1% phenol red as a pH indicator. A positive result was indicated by a change in the colour of the solution from orange to pink within the first minute.

After reading the results of the URUT the biopsy tissue was removed from the urea solution and imprint smears were made by lightly rolling it on a clean glass slide, using a hypodermic needle. The imprint smear was air-dried and fixed in absolute alcohol. The biopsy was then fixed in 10% formalin and sent for histopathological examination.

Imprint smears were stained by the Loeffler's methylene blue stain as described earlier.<sup>6</sup> The smears were read by a pathologist who was not aware of the identity of the patient, the clinical diagnosis, or the result of the URUT.

The biopsy material was processed routinely and 3-5 µm sections were made. The sections were stained with the Loeffler's methylene blue stain<sup>6</sup> and coded. These were read by the same pathologist who had read the imprint smears.

The imprint smears and histologic sections were carefully examined for the presence of *H pylori*. The severity of *H pylori* colonization was graded as: 0, no bacteria seen; 1, sporadic bacteria seen; 2, many bacteria seen in most microscopic fields; 3, bacteria seen in clusters in all the fields examined.

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## Results

The mean ( $\pm$  SD) age of the patients was 31 ( $\pm$  10.2) years and there were 664 (66%) males.

### ANTRAL BIOPSIES

Both biopsies were positive for *H pylori* in 858 patients. One of the antral biopsies was positive while the other was negative in nine patients and both the biopsies were negative for presence of *H pylori* in 133 patients. The prevalence of *H pylori* in the gastric antrum was thus 86.7%. Imprint smears showed a near-perfect correlation with histology and all except one of the biopsies showing presence of *H pylori* also showed *H pylori* on imprint smears. In one biopsy specimen with grade 1 *H pylori*, the imprint smear was false negative; in this patient, the other imprint made from the other antral tissue showed *H pylori*. None of the imprint smears were false positive.

Of the 1725 biopsies positive for *H pylori* by histology, 1583 (91.8%) tested positive by the URUT. Of 858 patients in whom both biopsies (total 1716) were positive, URUT was positive in 1576 biopsies. It was positive in seven of the nine patients in whom one of the biopsies was positive. All the biopsies that did not show presence of *H pylori* at histology tested negative by URUT. The sensitivity, specificity, positive and negative predictive value and the overall diagnostic accuracy of the URUT to diagnose *H pylori* infection in the gastric antrum were 92%, 100%, 100%, 66%, and 93%, respectively. Of the 142 false negative URUTs, 140 had grade 1 *H pylori* and the remaining two had grade 2 *H pylori*. None of the biopsies with grade 3 colonization tested false negative by the URUT.

### BIOPSIES FROM THE GASTRIC CORPUS

Both biopsies were positive for *H pylori* in 470 patients. One of the two was positive in 63 and both biopsies were negative in the remaining 467 patients. The prevalence of *H pylori* in the corpus was 53.3%. Imprint smears were false negative in two biopsies from two patients. Both the biopsies showed presence of grade 1 *H pylori*. None of the imprint smears tested false positive.

Of the 1003 biopsies showing *H pylori*, 832 (83%) were positive by the URUT. The URUT was positive in 776 (82.5%) of the 940 biopsies from 470 patients in whom both the biopsies showed *H pylori* and 56 (44%) of the 126 biopsies in 63 patients in whom only one of the two biopsies showed *H pylori*. None of the biopsies that did not show *H pylori* on histology tested positive by the URUT. The sensitivity, specificity, positive and negative predictive value and the overall diagnostic accuracy of the URUT for diagnosing *H pylori* infection in the corpus of the stomach were 83, 100%, 100%, 85%, and 91%, respectively. Of the 171 false-negative URUTs, 168 had grade 1 *H pylori* and the remaining three had grade 2 *H pylori*. None of the biopsies with grade 3 colonization tested false negative by the URUT.

## Discussion

For comparison of biopsy-based tests, most studies have used different sets of gastric biopsies to compare the various tests. In an earlier study comparing the CLO test, imprint smears and histology, we too used different sets of biopsies for the CLO test and different sets for imprint and histology.<sup>7</sup> In another recent study, different sets of gastric biopsies were used for comparing a locally made RUT with histology.<sup>5</sup> This could have led to fallacious results as it is well known that the distribution of *H pylori* is patchy in nature.<sup>8-10</sup> To avoid this confusion we planned the present study so that the same biopsy specimen was studied by the URUT, imprint cytology, and histology, thus giving an ideal comparison.

The results of this study show that the URUT is a useful test for detection of *H pylori* with high sensitivity and 100% specificity, especially for the presence of *H pylori* in the gastric antrum. These observations are similar to that by the original workers<sup>3</sup> and that noted in another recent study that used a slightly modified version of the test.<sup>5</sup> The sensitivity of the test would have improved further had we read the URUT for a longer duration of time, but that may also have led to some false positive results. Moreover, the design of our study (preparing imprints from the same biopsy specimen) did not allow us to read the results of the URUT for a longer duration of time. The advantage of the one-minute URUT is that the result is available before the patient even leaves the endoscopy room. The clinician can therefore plan the appropriate therapy almost immediately and the patient is spared another visit to the hospital to receive treatment.

Although not evaluated in this study, it is well known that the urease test has markedly decreased sensitivity in detecting the presence of *H pylori* in patients receiving drugs aimed at eradicating the organism.<sup>5</sup> In an earlier study we noted the sensitivity of the CLO-test to be only 9% in patients who had received anti-*H pylori* treatment.<sup>7</sup>

The prevalence of *H pylori* in our group of patients undergoing upper gastrointestinal endoscopy, appears very high. However, this is the trend in developing countries where the infection is seen in nearly all people by middle age.<sup>11</sup> In earlier studies we have observed a prevalence of 78% in a group of 90 healthy volunteers belonging to the middle class.<sup>12</sup> The prevalence in patients undergoing upper gastrointestinal endoscopy was noted to be 90%.<sup>7</sup>

In this study imprint cytology was noted to be nearly as sensitive and specific as histology in detecting *H pylori*. This is similar to earlier observations made by several workers, including ourselves.<sup>7 13-14</sup> We have earlier shown that, by carefully preparing imprint smears, the quality of the biopsy material is not adversely affected, and it may be used for histology.<sup>15</sup>

Considering the high sensitivity, low cost and the ease of the URUT we recommend it for situations in which a patient has to undergo endoscopy and *H pylori* status needs to be inves-

tigated. Since biopsies have already been obtained, examining imprint smears from the tissue would lead to a further improvement in the sensitivity of the URUT. The result of

imprint smears can also be obtained fairly quickly.<sup>6</sup> Histological examination of the biopsy material may, however, also be performed if insight into other histologic details is required.

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### Medical Anniversary

#### Georges Fernand Widal, 9 March 1862

Georges Fernand Widal (1862–1929) was born in Algiers, the son of a physician who was a medical inspector in the army. He studied medicine in Paris, became professor of pathology and was eventually appointed to a chair of clinical medicine at Hôpital Cochin; this training made him a ready link between the bedside and the clinical laboratory. His article on the sero-diagnosis of typhoid fever appeared in the *Lancet*, 1896;2:1371–2. He also contributed with distinction on haemolytic anaemia, paroxysmal cold haemoglobinuria, anaphylaxis and urticaria. — *DG James*