THE RAPID UREASE TEST IN THE DIAGNOSIS OF *HELICOBACTER PYLORI* INFECTION

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ABSTRACT

With the increasing recognition of the importance of H. pylori in gastrointestinal disease, there is a need for a reliable, efficient and yet inexpensive diagnostic test. The performance of the rapid urease test (RUT) as an endoscopy suite diagnostic test was compared to the established methods of culture, histology and Gram stain of tissue smear, in 274 gastric biopsy samples. Histology had the highest sensitivity of 99.3% followed by the RUT (96.6%). Culture and Gram stain of tissue smear had 100% specificity, while the rapid urease test had 99.2% specificity. The RUT had a positive predictive value of 99.3% and a negative predictive value of 96.2%. The RUT is an inexpensive, rapid and reliable diagnostic test of H. pylori infection.

Keywords: rapid urease test (RUT), H. pylori infection

INTRODUCTION

Helicobacter pylori (H. pylori) infection can be diagnosed by several methods[1-3]. Culture and histological examination of gastric biopsies are established methods of diagnosis as are examination of a Gram stain of fresh tissue smear and detection of urease activity in the biopsy sample. Non invasive tests such as serology and carbon urea breath tests are being increasingly used[2,3]. The role of serology in the management of *H. pylori* infected patients is still being defined. The C^{14} breath test while indicating the presence of the bacterium in the stomach requires a scintillation counter[3] which may restrict its use to larger gastroenterology centres. With the increasing recognition of the role of *H. pylori* in gastrointestinal diseases, there is a need for a reliable, efficient and yet inexpensive diagnostic test. In 1988, Arvind et al[4] described a rapid one minute urease test (RUT) for the diagnosis of *H. pylori* infection in the endoscopy suite and in this report we describe our experience with this test.

PATIENTS AND METHODS

Patients were requested to fast overnight or for at least 4 hours before endoscopy and consent for the procedure was obtained. The endoscopic examination was performed using either an Olympus GIF Q10 fibreoptic panendoscope or a Fujinon

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videoendoscope EVG-F. The stomach and duodenum were examined visually. Four to five antral biopsies were taken from areas uninvolved by any local lesion such as an ulcer. Biopsy specimens were placed in a urease test medium, sterile universal bottle for culture and in formalin for histological examination.

An endoscopic diagnosis was recorded and the endoscope and biopsy forceps were disinfected with glutaraldehyde.

The specimens for bacteriology were kept refrigerated at 4°C prior to transport to the laboratory and processed within two hours of collection.

The endoscopist, microbiologist and histopathologist were blind to each other's findings until the final analysis.

Culture

The biopsy specimen was ground with 0.3 ml of a 20% glucose solution using either a glass tissue grinder or a porcelain pestle and mortar. The homogenate was plated on 5% ox blood agar, chocolate agar and a selective medium (Oxoid Blood Agar Base No. 2 containing Skirrow's formula for selective supplement). The cultures were incubated for up to seven days at 37°C, microaerophically in an anaerobic jar using anaerobic gas generating kits (Oxoid, BBL) but without the catalyst. Colonies of *H. pylori* were provisionally identified by their colonial morphology, characteristic Gram-stain appearance and positive oxidase, catalase and urease reactions[1].

Gram-stain of tissue smear

An impression smear of a specimen was made and stained by Gram's method to visualise the typical Gram-negative curved bacteria.

Histology

Two antral biopsies were fixed in formalin, processed and embedded in paraffin. Four micron thick sections were stained with Warthin-Starry silver stain and haematoxylin and eosin (H&E) and examined for the presence of the organism and gastritis. All specimens were read by one investigator.

Urease tests

A rapid one minute urease test (RUT) as described by Arvind et al[4] was used. An antral biopsy sample was placed in an eppendorf tube containing 1 cc of a freshly prepared 10% urea (w/v) in unbuffered deionised water at pH of about 6.8. Phenol red was used as the pH indicator. A positive result was recorded when the colour of the urea solution changed from yellow to magenta. The colour change occurred very rapidly within one minute. The bacteria produce large amounts of urease which hydrolyse the urea substrate to ammonia resulting in a rise in the pH of the solution. This pH change is detected by the phenol red indicator

which changes colour from yellow at pH 6.8 to magenta at pH 8.0.

As a comparison the commercially available CLO test (Delta West Ltd, W. Australia) which is a gel pellet containing urea, phenol red and a bacteriostatic agent mounted on a plastic slide was used in 41 biopsies. It makes use of the same principle as the rapid urease test described above. The biopsy was placed in the CLO test slide which was then kept in the endoscopist's pocket where the temperature approximates 30°C as recommended by the manufacturer.

RESULTS

A total of 274 endoscopies performed were entered into the study. Culture, histology, rapid urease test and Gram-stain of a tissue smear were performed in all 274 biopsies but the CLO test was performed only on 41 biopsies.

Patients were considered positive for *H. pylori* infection if they had a positive culture, or if two of the following tests were positive: urease test (RUT), histological examination or Gram stain of a fresh tissue smear. By this criteria 146 endoscopies were considered positive and 128 negative. The results of the various tests are summarised in Table I.

Table I – Comparison of 5 methods in the diagnosis of *H. pylori* infection. (n = 274)

	True positive (n)	False positive (n)	True negative (n)	False negative (n)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Culture	118	0	128	28	80.8	100	100	82.1
Gram-stain	135	0	128	11	92.5	100	100	92.1
Histology	145	6	122	1	99.3	95.3	96.0	99.2
Rapid urease test	141	1	127	5	96.6	99.2	99.3	96.2
CLO test (n=41)	23	0	16	2	92.0	100	100	88.9

Histological examination had the highest sensitivity (99.3%) and culture, the lowest (80.8%) whilst specificities of 100% were observed with culture, tissue Gram stain and the CLO test. The rapid urease test gave highly comparable results with a sensitivity of 96.6% and a specificity of 99.2%. A positive predictive value of 100% was recorded with culture, tissue Gram stain and the CLO test. Culture gave a negative predictive value of 82.1% while histology, a negative predictive value of 99.2%. The RUT had a positive predictive value of 99.3% and a negative predictive value of 96.2%.

DISCUSSION

Diagnosis of *H. pylori* infection using gastric biopsies is now well established. While culture is the "gold standard" of identifying *H. pylori* infection, we recorded a sensitivity of 80% for culture which is comparable to results in other laboratories[1]. A high sensitivity and specificity was observed with histology as well.

However, with both these tests as with other gastric biopsy tests, false negative results are inevitable because of the patchy distribution of the bacteria and consequent sampling error. A further possible confounding factor with histological examination is the presence of other spiral bacteria such as *Gastrospirillum hominis* which may be mistakenly diagnosed as *H. pylori*.

Examination of a Gram stained fresh tissue smear also yielded high sensitivity and specificity when performed by the microbiologist. It is not a difficult test nor an expensive one to perform and could presumably be performed in an endoscopy side room. However, expertise is required in reading the smear.

Culture and histological examination of a biopsy sample are generally considered to be essential tests to perform during treatment trials aimed at eradication of *H. pylori*. There are several drawbacks with these tests; firstly, a delay in the availability of results and secondly, especially in a developing country, the need for good microbiology and histopathology laboratory support.

We have found in our experience that the rapid urease test overcomes these shortcomings while at the same time recording high sensitivity and specificity rates. As the name denotes, results are immediate, allowing prompt treatment, when appropriate, to be instituted whilst the patient is still in the clinic. The test is inexpensive, easy to prepare and according to Thillainayagam et al[5] could in fact be prepared in large batches, frozen and thawed before use. Most importantly in our local context is the usefulness of such a test in the majority of endoscopy units in the country where laboratory facilities are not always available. A field trial in India using a similar modified RUT has shown that the test is both reliable and robust to use[6].

A commercially available urease test, the CLO test has been in use for more than 5 years. We found the test to have high sensitivity and specificity rates and easy to use; results although not as prompt as the RUT are generally available within 2 hours. The prohibitive factor, however, is the relatively high cost of the test.

We therefore recommend that the RUT be more widely used in endoscopy units where diagnosis of *H. pylori* is routine practice.

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