Evaluation of a locally produced rapid urease test for the diagnosis of *Helicobacter pylori* infection

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Background. The rapid urease test (RUT) is used at Groote Schuur Hospital for diagnosing *Helicobacter pylori* infection. This is an in-house method, which has not been validated.

Objective. To validate our practice of reading the RUT immediately after endoscopy (RUT0), by comparing this with a reading at 24 hours (RUT24) and with histological analysis.

Design. Ninety consecutive patients undergoing upper endoscopy over a 6-week period from October 2005 to November 2005, and in whom rapid urease testing was indicated, were included in the study. Patients with recent exposure (within 2 weeks of endoscopy) to proton pump inhibitors (PPIs), histamine receptor antagonists (H2RAs) and antibiotics (confounders) were noted and included in the cohort. Two antral and two body biopsies were taken for histological examination and a third antral biopsy was placed in the RUT bottle. Both haematoxylin and eosin and modified Giemsa staining methods were used to identify *H. pylori*. The RUT was read immediately (within 5 minutes of upper endoscopy) (RUT0), as per our current practice, and each specimen was re-read at 24 hours (RUT24). Sensitivity, specificity, positive and negative predictive values and the impact of confounders were calculated.

Results. Of the 90 patients undergoing rapid urease testing, 39% were male and 61% were female, with a mean age of 55 years (range 22 - 79 years). Histological examination revealed *H. pylori* in 67.8% (N=61) of the biopsy specimens. In the 65 patients without confounders, the sensitivity and specificity of the RUT were 65.9% and 100% respectively, and 90.9% and 100% for RUT24. After including the 25 patients with confounders, the sensitivity and specificity were 68.8% and 100% for RUT0 and 90.1% and 100% for RUT24 respectively. Thirteen RUT0 specimens (30.9%) that were initially negative became positive at the RUT24 reading. There were 6 (9.8%) RUT-normal and RUT-negative but histology-positive specimens. Four of these 6 false-negative RUT24 results could be accounted for by a low *H. pylori* density on histological analysis (2 patients were taking PPIs). Confounders did not alter the sensitivity and specificity outcomes or impact on the number of false-negative RUTs.

Conclusions. Our locally prepared RUT is a specific test for the detection of *H. pylori* infection. The sensitivity is greatly enhanced by reading the test at 24 hours. The use of PPIs, H2RAs and antibiotics preceding endoscopy did not impact significantly on the results.

The aim of this study was therefore to determine the validity of our current practice of reading the RUT early after endoscopy (at 5 minutes, RUT0), by comparing this with a reading at 24 hours (RUT24) and with histological examination.

Patients and methods

Patients

Ninety consecutive subjects undergoing upper gastrointestinal endoscopy over a 6-week period (1 October 2005 - 14 November 2005) in whom rapid urease testing was indicated based on standard clinical or endoscopic criteria, were included in the study. Patients with actively bleeding peptic ulcer disease were excluded, as this is a well-recognised cause of a false-negative urease test.9

Patient demographics were determined at the endoscopy visit. Recent medication use, in particular exposure within 2 weeks of endoscopy to proton pump inhibitors (PPIs), histamine-2 receptor antagonists (H2RAs) or antibiotics, was recorded and defined as study confounders (use of these agents suppresses *H. pylori* infection).9 Subjects with exposure to these drugs were included and analysed separately.

Diagnostic methods

Five gastric biopsies were taken during endoscopy. Four specimens, of which 2 were taken from the antrum within 3 cm of the pylorus and 2 from the body, were fixed with formalin and embedded in paraffin. Both haematoxylin and eosin and modified Giemsa staining methods were used for histological identification of *H. pylori*. Our in-house pathologists, who have experience with this technique, carried out the histological assessment. The accepted gold standard for histological diagnosis of *H. pylori* infection is 2 antral biopsies taken within 3 cm of the pylorus and evaluated by an experienced pathologist using the appropriate special stains.11 The fifth biopsy specimen, also taken from the antrum, was placed in the RUT bottle (National Health Laboratory Services, Cape Town). The ingredients of this in-house medium comprised a 1% phenol red solution (1 g phenol red and 100 ml de-ionised water), 10% urea solution (30 g urea and 300 ml de-ionised water) and 0.05% thymol solution. The RUT was read immediately (within 5 minutes of upper endoscopy, RUT0), as per our current practice, and each specimen (stored at room temperature) was re-read at 24 hours (RUT24).

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Statistical analysis

The chi-square test or Fisher’s exact test was used as appropriate to compare percentages. Estimates of sensitivity, specificity, as well as positive and negative predictive values, were calculated in the usual manner. Statistical analysis was performed using the computer software Medcalc (version 7.5, 2004, Medcalc Software, Belgium). Statistical tests were performed at the 5% level of significance.

This study was approved by the University of Cape Town Ethical Committee.

Results

Of the 90 patients undergoing rapid urease testing, 39% were male and 61% female, with a mean age (± standard deviation (SD)) of 55±14.9 years (range 22 - 79 years).

Overall, 61 patients (67.8%) had histological evidence of *H. pylori* infection on light microscopy (Table I). Of these, 42 patients (68.8%) were RUT0-positive and 55 (90.1%) were RUT24-positive. Thirteen (30.9%) RUT0 specimens that were initially negative became positive at the RUT24 reading. Six patients (9.8%) had false-negative RUTs at 24 hours, with confirmed evidence of *H. pylori* on histological analysis. No false-positive RUTs were detected.

Table I. Patient demographics (N=90)

<table>
<thead>
<tr>
<th>Age (years) (mean±SD, range)</th>
<th>55±14.9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (N (%))</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>35 (39)</td>
</tr>
<tr>
<td>Female</td>
<td>55 (61)</td>
</tr>
<tr>
<td><em>H. pylori</em> gastritis on histological analysis (N (%))</td>
<td>61 (67.8)</td>
</tr>
</tbody>
</table>

Of the 90 patients in total, the sensitivity and specificity were 68.8% and 100% for RUT0, and 90.1% and 100% for RUT24 respectively. The positive predictive values were 100% for both readings and the negative predictive values calculated were 60.4% and 82.8% respectively (Table II).

In the group of 65 patients without confounders, 44 patients (67.6%) had histological evidence of *H. pylori* infection. Twenty-nine patients (65.9 %) were RUT0-positive and 40 patients (90.9%) were RUT24-positive. Eleven (37.9%) RUT0 specimens that were initially negative became positive at the RUT24 reading. Four patients (9%) had false-negative RUTs with evidence of *H. pylori* on histological analysis. Of the 65 patients without confounders, the sensitivity and specificity of the RUT0 were 65.9% and 100%, and 90.9% and 100% for RUT24 respectively (Table II).

Twenty-five patients had received PPIs, H2RAs or antibiotics (confounders) within 2 weeks of undergoing endoscopy. Of these, 17 patients (68%) had histological evidence of *H. pylori* infection. Thirteen patients (76.5%) were RUT0-positive and 15 patients (88.2%) were RUT24-positive. Two specimens (15.3%) that were initially negative became positive at the RUT24 reading. Two patients (11.7%) had false-negative RUTs with evidence of *H. pylori* infection on histological analysis. In the
endoscopy to 24 hours increased both the sensitivity (from extending the RUT time interval from within 5 minutes of 91.9% respectively.8 The characteristics of these rapid urease data have found a sensitivity and specificity of 98.9% and respectively (Table II).

Almost 70% of the patients in this study had histological evidence of _H. pylori_ infection; this is in keeping with similar data from South Africa and other countries in Africa.12-13

Discussion

Almost 70% of the patients in this study had histological evidence of _H. pylori_ infection; this is in keeping with similar data from South Africa and other countries in Africa.12-13

Given the gastroduodenal pathology associated with _H. pylori_ infection, a rapid, safe and inexpensive test that has a high specificity and sensitivity is critical in our environment. The in-house RUT solution compares favourably with other RUTs for the detection of _H. pylori_, with an overall sensitivity and specificity of 90% and 100% respectively. A study comparing commercially available rapid urease tests, the CLO test (Delta West Bentley, WA, Australia), ProntoDry (Medical Instrument Corp., Solothurn, Switzerland) and HpONE (GI Supply, Camp Hill, Pa, USA) showed a sensitivity of 86.2%, 93% and 93% respectively.7 All three tests showed 100% specificity.7 Other data have found a sensitivity and specificity of 98.9% and 91.9% respectively.7 The characteristics of these rapid urease tests were reported using the appropriate commercially validated timing of reading the respective tests. In the case of the CLO test (the most widely used and best studied rapid urease test) the reading was done at 24 hours.

In our study 13 RUT₀ specimens (30.9%) that were initially negative became positive at the RUT₂₄ reading. Furthermore, extending the RUT time interval from within 5 minutes of endoscopy to 24 hours increased both the sensitivity (from 69% to 90%) and the negative predictive value of the test (from 60% to 83%). This suggests that the RUT should be read within 5 minutes of endoscopy, and, if negative, again at 24 hours, to ensure optimal detection of _H. pylori_ infection. This characteristic has been documented previously with other urease tests, in particular the CLO test, which was noted to have a false-negative rate of approximately 40% at 3 hours.14 We have not determined whether reading the RUT at other time intervals within the 24-hour period would yield an acceptable sensitivity.

Overall 6 specimens (9.8%) were both RUT₀ and RUT₂₄ negative, but positive for _H. pylori_ on histological examination. These cases can therefore be considered false-negatives. False-negative urease tests have been reported in association with several factors. The use of acid suppression medication, in particular PPIs, has been shown to reduce _H. pylori_ density and colonisation and suppress the urease activity of the bacterium, and, as such, reduce the accuracy of the test.16 Four of the 6 subjects with false-negative RUT₂₄ results in this study (of whom 2 had recent exposure to PPIs) had very low density of _H. pylori_ organisms on histological examination and, therefore, potentially low urease activity in the RUT solution. On further analysis, however, exposure to PPIs or H₂RAs did not significantly influence the number of false-negative RUT₂₄s, or alter the sensitivity and specificity outcomes. This may, however, represent a type 2 error as a consequence of the small number of false-negative RUT₂₄ detected in the subgroup with confounders.

The presence of blood in the stomach is also a well-described cause of false-negative urease tests.7 In this study, however, such subjects were excluded and this would not account for test inaccuracy.

Doubling the amount of tissue in the CLO test has been shown to hasten positivity of the test by up to 2 hours.14 In addition, biopsies taken from the gastric angulus maximise the probability of detecting _H. pylori_ using a RUT.15

Whether taking biopsies from the gastric angulus rather than the antrum and increasing the number of biopsy samples placed in the bottle improves time to positivity and sensitivity, remains unanswered by this study.

Unanswered questions notwithstanding, what this study does highlight are the deficiencies with using an early negative RUT reading to determine the presence of _H. pylori_ infection. However, it is comforting to know that in the resource-conscious environment within which we practise medicine in South Africa, there is an inexpensive, sensitive and specific test that compares very favourably with the industry standard. The recommendation to those practitioners doing gastroscopy and using the RUT solution as detailed in this study, is that their practice should change from reading the RUT within 5 minutes of endoscopy to include a reading of the RUT at 24 hours, should the initial test be negative.

Table II. Performance characteristics of RUT₀ and RUT₂₄

<table>
<thead>
<tr>
<th></th>
<th>RUT₀</th>
<th>RUT₂₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients (N=90)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>H. pylori</em> on histological analysis (N=61)</td>
<td>42</td>
<td>55</td>
</tr>
<tr>
<td>RUT-positive (N)</td>
<td>76.5</td>
<td>88.2</td>
</tr>
<tr>
<td>Sensitivity (%)</td>
<td>68.8</td>
<td>90.1</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Positive predictive value (%)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Negative predictive value (%)</td>
<td>60.4</td>
<td>82.8</td>
</tr>
</tbody>
</table>

Excluding subjects with confounders* (N=65)

| _H. pylori_ on histological analysis (N=44) | 65.9 | 90.9 |
| Sensitivity (%) | 65.9 | 90.9 |
| Specificity (%) | 100  | 100   |

Subjects with confounders* (N=25)

| _H. pylori_ on histological analysis (N=17) | 76.5 | 88.2 |
| Sensitivity (%) | 76.5 | 88.2 |
| Specificity (%) | 100  | 100   |

*Confounders are defined as exposure to PPIs, _H₂RAs_ or antibiotics within 2 weeks of rapid urease testing.

25 patients with confounders the sensitivity and specificity of the RUT₀ were 76.5% and 100% and 88.2% and 100% for RUT₂₄ respectively (Table II).

On further analysis, the use of PPIs, _H₂RAs_ or antibiotics did not alter the sensitivity and specificity outcomes, or impact significantly on the number of false-negative RUT₂₄ results when compared with histologically proven _H. pylori_ (p=1).
References


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