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 (RUT_0), by comparing this with a reading at 24 hours (RUT_{24}) 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 (H_2RAs) 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) (RUT_0), as per our current practice, and each specimen was re-read at 24 hours (RUT_{24}). 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_0 were 65.9% and 100% respectively, and 90.9% and 100% for RUT_{24}. After including the 25 patients with confounders, the sensitivity and specificity were 68.8% and 100% for RUT_0, and 90.1% and 100% for RUT_{24} respectively. Thirteen RUT_0 specimens (30.9%) that were initially negative became positive at the RUT_{24} reading. There were 6 (9.8%) RUT_0- and RUT_{24}-negative but histology-positive specimens. Four of these 6 false-negative RUT_{24} 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, H_2RAs and antibiotics preceding endoscopy did not impact significantly on the results.

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Recognition of the role of *Helicobacter pylori* gastric infection in human disease has dramatically changed the management of peptic ulceration, and the Nobel honours bestowed on Marshall and Warren in 2005 in acknowledgement of their landmark research has focused public attention on this organism.[1-3] Furthermore, the neoplastic potential of *H. pylori*, which is defined as a type 1 carcinogen, is well recognised and has resulted in some advocating the widespread, worldwide eradication of this infection.[2,4,5] A diagnosis of *H. pylori* infection is readily made using non-invasive tests, such as 13C and 14C urea breath testing, stool antigen analysis or *H. pylori* serology. Alternatively, infection can be detected in gastric biopsies obtained at endoscopy, through histological staining, tissue culture or polymerase chain reaction (PCR). Far simpler, however, is to diagnose the bacterium by detecting the presence of an *H. pylori*-derived enzyme, urease, within gastric biopsies. The urease-driven production of ammonia, when *H. pylori*-infected gastric tissue is added to a urea-containing medium, elicits a pH-dependent colour change that forms the essence of this diagnostic test.[5-8] Many versions of the 'urease test' have been developed, with varying reported sensitivities and specificities. A locally produced adaptation, the rapid urease test ((RUT), National Health Laboratory Services, Cape Town), is used at Groote Schuur Hospital and some centres across South Africa. The RUT is inexpensive (R4.40 per bottle excluding value added tax) and easy to perform; however, this in-house method has never previously been validated. In addition, while not evidence based, it is common practice in our clinic to use a single biopsy of the gastric antrum for this investigation and to analyse colour change within 5 minutes of RUT commencement and determine the need for eradication therapy based on this result. Other commercially available RUTs are read at 24 hours.

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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, RUT_0), by comparing this with a reading at 24 hours (RUT_{24}) 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 (H_2RAs) or antibiotics, was recorded and defined as study confounders (use of these agents suppresses *H. pylori* infection).[10] 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 10% urea solution (30 g urea and 300 ml de-ionised water) and 1% phenol red solution (1 g phenol red and 100 ml de-ionised water). In the presence of the *H. pylori* urease, urea is converted into ammonium and carbon dioxide triggering an alteration in pH. This results in a change from yellow to purple/magenta and denotes a positive test.

The RUT was read immediately (within 5 minutes of upper endoscopy, RUT_0), as per our current practice, and each specimen (stored at room temperature) was re-read at 24 hours (RUT_{24}).

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 Medicalc (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 RUT_0-positive and 55 (90.1%) were RUT_{24}-positive. Thirteen (30.9%) RUT_0 specimens that were initially negative became positive at the RUT_{24} 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)	
Age (years) (mean±SD, range)	55±14.9
	22 - 79
Gender (*N* (%))	
Male	35 (39)
Female	55 (61)
H. pylori gastritis on histological analysis (*N* (%))	61 (67.8)

Of the 90 patients in total, the sensitivity and specificity were 68.8% and 100% for RUT_0, and 90.1% and 100% for RUT_{24} 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 RUT_0-positive and 40 patients (90.9%) were RUT_{24}-positive. Eleven (37.9%) RUT_0 specimens that were initially negative became positive at the RUT_{24} 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 RUT_0 were 65.9% and 100%, and 90.9% and 100% for RUT_{24} respectively (Table II).

Twenty-five patients had received PPIs, H_2RAs 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 RUT_0-positive and 15 patients (88.2%) were RUT_{24}-positive. Two specimens (15.3%) that were initially negative became positive at the RUT_{24} reading. Two patients (11.7%) had false-negative RUTs with evidence of *H. pylori* infection on histological analysis. In the

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Table II. Performance characteristics of RUT_0 and RUT_{24}

	RUT_0	RUT_{24}
All patients (N=90)		
H. pylori on histological analysis (N=61)		
RUT-positive (N)	42	55
Sensitivity (%)	68.8	90.1
Specificity (%)	100	100
Positive predictive value (%)	100	100
Negative predictive value (%)	60.4	82.8
Excluding subjects with confounders* (N=65)		
H. pylori on histological analysis (N=44)		
Sensitivity (%)	65.9	90.9
Specificity (%)	100	100
Subjects with confounders* (N=25)		
H. pylori on histological analysis (N=17)		
Sensitivity (%)	76.5	88.2
Specificity (%)	100	100

*Confounders are defined as exposure to PPIs, H_2RAs or antibiotics within 2 weeks of rapid urease testing.

25 patients with confounders the sensitivity and specificity of the RUT_0 were 76.5% and 100% and 88.2% and 100% for RUT_{24} respectively (Table II).

On further analysis, the use of PPIs, H_2RAs or antibiotics did not alter the sensitivity and specificity outcomes, or impact significantly on the number of false-negative RUT_{24} results when compared with histologically proven *H. pylori* (p=1).

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.[8] 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[7]) the reading was done at 24 hours.

In our study 13 RUT_0 specimens (30.9%) that were initially negative became positive at the RUT_{24} 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 RUT_0 and RUT_{24}-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.[10] Four of the 6 subjects with false-negative RUT_{24} 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 H_2RAs did not significantly influence the number of false-negative RUT_{24}s, 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 RUT_{24}s detected in the subgroup with confounders.

The presence of blood in the stomach is also a well-described cause of false-negative urease tests.[9] 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.

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ORIGINAL ARTICLES

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