A low cost rapid urease test to detect *Helicobacter pylori* infection in resource limited settings

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Abstract

The aim of the study was to evaluate the suitability of a modified one minute rapid urease test (one day rapid urease test) as a low cost *H. pylori* detection method. A sample of 205 patients clinically suspected of having *H. pylori* infection was tested. One day rapid urease test and histology based *H. pylori* tests (the gold standard) were performed on endoscopic antral biopsies. There were 6 true positives, 191 true negatives, 8 false positives and zero false negatives. The sensitivity, specificity and positive (PPV) and negative predictive values (NPV) of the test were 100%, 96%, 42.9%, and 100% respectively. The cost per patient was 0.3US$. High sensitivity, specificity and NPV, low cost and simplicity of method were the advantages of the test and the main limitation was low PPV. Hence, one day rapid urease test can be considered as a suitable low cost method to detect *H. pylori* infection in resource limited settings.

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Introduction

Empirical treatment of patients with suspected *Helicobacter pylori* gastritis with triple therapy is discouraged because of emergence of antibiotic resistance and test and treat strategy has been advocated as a proven management strategy [1, 2]. However, in developing countries, lack of an affordable *H. pylori* diagnostic test is a major limitation in implementing this strategy. *H. pylori* detection methods such as histology, culture and urease breath test require expensive infrastructure and trained personnel. Although commercially available methods such as CLO test, stool antigen test and serology are easy to carry out, they are too expensive for routine use in developing countries. Therefore, an inexpensive *H. pylori* detection method with reasonable validity is required for use in these countries.

One minute rapid urease test described by Arvind *et al* is an inexpensive test carried out using locally prepared solutions and has a sensitivity and specificity of 91% and 100% respectively [3]. We conducted the following study to evaluate the suitability of a modified version of one minute rapid urease test (one day rapid urease test) as a *H. pylori* detection method in patients undergoing upper gastrointestinal endoscopy, in resource limited settings.

Methods

This cross sectional study was conducted on 205 patients who underwent upper gastrointestinal endoscopy and were clinically and endoscopically suspected of having *H. pylori* gastritis. Study was conducted at the Teaching Hospital, Peradeniya, Sri Lanka from 2012 to 2013. These patients had dyspeptic symptoms and endoscopic evidence of antral mucosal inflammation such as erythema, erosions or peptic ulcers. None of them had been treated with triple therapy during the past one year.

All patients underwent a standard biopsy protocol which included three biopsies from the antral mucosal lesion (erythema, erosions or ulcer). One biopsy was immediately utilised to perform one day rapid urease test in the endoscopy room and the rest were collected in 10% formalin solution for histology and immunohistochemistry. Formalin fixed and paraffin embedded tissue sections were stained with haematoxylin and eosin and toluidine blue. *H. pylori* specific immunohistochemistry (DAKO B0471) was performed using peroxidase method. Diagnostic efficacy of one day rapid urease test was evaluated by calculating sensitivity, specificity negative predictive value (NPV) and positive predictive value (PPV) using histology based methods as the gold standard. Positive results with one or more of the three histology based tests were considered as evidence for presence of *H. pylori* infection.

The procedure for the original one minute rapid urease test described by Arvind *et al* is as follows, ‘‘an antral mucosal biopsy is placed immediately into a capped Eppendorf tube containing 0 5 ml freshly prepared 10% urea (w/v) in deionised water at a pH of around 6.8, to

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which two drops of 1% phenol red (free acid) is added as a pH indicator [3]. A positive result is recorded if there is a colour change from yellow to pink within the first minute''. We followed the same procedure with the following modifications. 1. Instead of freshly prepared 10% urea solution, we prepared a stock of urea solution once a week and stored in a domestic type refrigerator and aliquots of solution were brought to the room temperature before use; 2. Distilled water was used instead of deionised water; 3. Solution was observed for a colour change up to 24 hours and interpreted as follows: an obvious colour change from yellow to pink involving the entire test solution within 24 hours was regarded as a positive reaction. Weak colour changes such as, a subtle colour change giving rise to a cloudy appearance or presence of a pinkish smoke like line emanating from the biopsy and no colour change were regarded as negative reactions.

Venous blood was drawn from each patient to screen for presence of anti *H. pylori* antibodies using a qualitative immunochromatographic method (SD bioline *H. pylori*).

Results

The mean age of the sample was 56.9 ± 14 years and 56.6% were males. One day rapid urease test was positive in 14 cases and in 6 at least one histology based method demonstrated presence of *H. pylori* organisms (Table 1). Accordingly, one day rapid urease test had 100% sensitivity (95% CI 0.54 - 1.0); 96% specificity (95% CI 0.92 - 0.98); 42.9% PPV (95% CI 0.18 -0.71), and 100% NPV (95% CI 0.98 - 1). Accuracy of the test was 96.1% and 2.9% of the sample were *H. pylori* positive.

Of the 14 one day rapid urease test positive cases, 12 became positive within one minute and the two showed positivity later, one within one hour and the other within 24 hours. All these were true positives. Twenty one cases showed a weak colour change and all of them were true negatives. In 10/205 (4.9%) anti *H. pylori* antibodies were present. Cost of the test per patient was about 30 Sri Lankan Rupees (0.3 US dollars).

Table 1. Comparison of one day rapid urease test with the gold standard method

	H. pylori positive	*H. pylori negative*	*Total*
One day rapid urease test positive	6	8	14
One day rapid urease test negative	0	191	191
Total	6	199	205

Discussion

One day rapid urease test has a high sensitivity (100%) and a reasonable specificity (96%) in detecting *H. pylori* infection. The original version had a sensitivity of 91% and a specificity of 100% [3].

Superior sensitivity and the NPV of one day rapid urease test are due to observation of the test up to 24 hours; the two late positives were true positives. Therefore, we recommend observing the solution up to 24 hours as in CLO test. Furthermore, absence of true positives in the 21 cases with weak colour changes justify treating weak reactions as negative. Interpretation of weak colour changes as positive would have reduced the specificity of the test to 85.4%.

The main limitation of the study was the low prevalence of *H. pylori* infection in the sample tested (2.9%) which can influence the predictive values of the detection methods. This could explain the low PPV (42.9%) of the present test. The low seroprevalence of anti *H. pylori* antibodies (4.9%) confirms that the *H. pylori* prevalence in the sample is truly low and the study sample had low exposure to the organism. In contrast, with the one minute rapid urease test. PPV was 100% and the disease prevalence in the sample tested was 52.5%.

Although the urea solution should ideally be prepared fresh, the authors of the original study had observed that pre-prepared frozen solutions could also be used by thawing just before use [4]. In our experience, storage of pre-prepared urea solution in the door compartment of a domestic type refrigerator up to 7 days did not significantly change the test efficacy. The optimal action of urease enzyme is at 45°C and therefore, it is important to bring the solution to room temperature before testing to prevent false negatives.

In conclusion, high sensitivity, specificity and NPV, low cost and simple methodology were the advantages of one day rapid urease test and the main limitation was low PPV. Hence, one day rapid urease test can be considered as a suitable low cost method to detect *H. pylori* infection in gastric biopsies in resource limited settings.

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Declaration of Interest

There are no conflicts of interest.

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