

Validation of a new, commercially available dry rapid urease test for the diagnosis of *Helicobacter pylori* infection in gastric biopsies

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ABSTRACT

Background: To compare the accuracy and reaction time of a new dry rapid urease test (GUT test) with the CLO test and an independent gold standard in the diagnosis of *Helicobacter pylori* infection. To determine whether this new test can replace the CLO test in routine clinical practice.

Methods: We included consecutive patients in whom normal-sized gastric biopsies were taken in routine practice. Six antral and three corpus biopsies were taken for determination of *H. pylori* infection. Results of the GUT test were monitored after 15, 60 and 120 minutes of incubation. Results were compared with the standard CLO test and an independent gold standard (bacterial culture and histology). The results of the CLO test were also compared with the gold standard.

Results: 116 patients were recruited in the study: 60 were males and 56 females. The mean age was 59.3 years (range 14-89 years). Compared with the CLO test, the GUT test had a sensitivity of 76.7% and a specificity of 100% in 15 minutes. After 60 minutes the sensitivity of the GUT test increased to 95.3%, the specificity remained 100%. All positive results of the GUT test occurred before 60 minutes of incubation. Compared with the gold standard, the GUT test had a sensitivity and specificity of 97.4 and 96.1% respectively. The CLO test had a sensitivity of 97.4% and a specificity of 93.5%, when compared with the gold standard.

Conclusion: The GUT test appeared to be a good and reliable alternative for the widely used CLO test in diagnosing *H. pylori* infection. The GUT test results were not yet reliable after 15 minutes, but all positive results occurred before 60 minutes of incubation. The test can best be read 60 to 120 minutes after endoscopy.

KEYWORDS:

Diagnosis, dry rapid urease test, GUT test, *H. pylori*

INTRODUCTION

Helicobacter pylori is a spiral-shaped Gram-positive bacterium which produces the enzyme urease. These bacteria are found in human gastric mucosa, wherever this is situated in the human body. *H. pylori* bacteria are usually found under the mucus layer in the gastric pits and in close apposition to gastric epithelial cells.¹ *H. pylori* infection causes chronic active gastritis in the antrum (antral gastritis), the corpus (corpus gastritis) or in both (pangastritis). It is a major aetiological factor in peptic ulcer disease.¹ Haemorrhage and perforation are the most frequent complications of peptic ulcer disease and are associated with substantial morbidity, mortality and health care costs. Patients with recurrent haemorrhage, particularly the chronically ill and elderly, have excessive morbidity and mortality.² Peptic ulcer disease can be cured by eradicating *H. pylori* so that complications no longer occur.¹ *H. pylori* infection is also a major aetiological factor in gastric cancer and B cell lymphoma.^{1,3-5}

H. pylori infection can be diagnosed by biopsy-based tests but these require an endoscopy. There are also some noninvasive tests such as serology, faecal antigen testing and urea breath testing. However, no single test is currently available that can provide the definite diagnosis by itself. Due to this lack of a true gold standard, biopsy-based tests are still considered to be the reference method for diagnosing *H. pylori* infection and monitoring eradication treatment.⁶ The most widely used biopsy-based tests are histology, culture and rapid urease tests.

We have previously shown that a combination of these three tests is a very reliable method for the diagnosis of *H. pylori* infection.⁷ With this combination a calculated sensitivity of 98.3% and a specificity of 99.7% can be reached. This translated into a positive predictive value (PPV) of 99.6% and a negative predictive value (NPV) of 98.9% in 869 patients.⁷

Biopsy urease tests can determine the presence or absence of urease activity in a gastric biopsy. This enzyme is only produced by *H. pylori*. Presence of urease activity in a biopsy can therefore be considered as proof of the presence of this infection.⁸ A biopsy urease test container carries urea and the biopsy is immersed in the fluid or gel. If *H. pylori* is present the urease activity will break down urea, thereby generating ammonia. The resulting elevation of pH (decrease in acidity) can be detected by an indicator, usually phenol red, and this will change the colour from yellow to red.⁹ Rapid urease tests have the advantage that they are not operator dependent, they have a high reproducibility worldwide and they are cheaper than culture or histology.⁸ The CLO test is the most widely used commercial biopsy urease test. We have a wide experience with this test. In 468 pretreatment endoscopies the CLO test had a sensitivity, specificity, PPV, NPV and accuracy of 91.4, 99.4, 99.7, 85 and 94% respectively. In 244 post-treatment endoscopies this was 93.3, 100, 100, 99.1 and 99.2% respectively.⁸ There are a few well-validated wet rapid urease tests commercially available: CLO test,¹⁰⁻¹⁷ HUT test,¹⁵ Helicocheck¹⁸ and HPfast¹⁷ and also one dry rapid urease test: PyloriTek.^{10,12,14,16-20} In order to save costs, urease tests can also be produced locally by the hospital pharmacist.^{21,22} The new and commercially available gastroscopic urease test (GUT test) is a cheap alternative for the currently available tests.

The aim of this study was to compare the accuracy and reaction time of the newly available GUT test with the CLO test and with the gold standard in the diagnosis of *H. pylori* infection. We wanted to determine whether this new test can reliably replace the CLO test in routine clinical practice.

MATERIALS AND METHODS

In this study we included consecutive patients in whom normal-sized biopsies for determination of *H. pylori* status were taken by three experienced endoscopists in a Dutch community hospital. The use of acid-suppression therapy was allowed. A standard biopsy protocol was used for *Helicobacter* diagnosis at all times. At baseline, gender and age were recorded. In patients being examined for *H. pylori* infection, six antral and three corpus biopsies were taken. One antral biopsy was sent for bacterial culture. Two antral and two corpus biopsies were used for histological examination. One antral and one corpus biopsy were used

for two separate CLO tests and two antral biopsies were used for the GUT test. Test outcome for each method was assessed independently from the other test results: culture results by a microbiologist, histology results by a pathologist and urease test results by the endoscopist. Because the GUT test biopsies were only taken from the antrum of the stomach, their results were compared with the antral CLO test results and the antral histology and culture results. In this way we can make a good comparison of the different methods and the results are not biased by patients in whom *H. pylori* is only present in antrum and not the corpus or *vice versa*.^{13,23}

Antral histology and antral culture together were considered the independent gold standard for the diagnosis of *H. pylori* infection in this study. A positive diagnosis of infection was made when one of these two tests was positive or when both these tests were positive.

Culture

One biopsy specimen for bacterial culture was placed in 1 ml of thioglycolate broth and transported to the microbiological laboratory within six hours of upper gastrointestinal endoscopy. Culture was done with Belo-Horizonte medium containing brain-heart infusion agar (35 g/ml), sheep blood (10%), vancomycin (10 mg/l), trimethoprim lactate (5 mg/l), cefsoludin (5 mg/l), and amphotericin (5 mg/l). The plates were incubated microaerobically at 36°C for seven days. Identification was confirmed by Gram staining, catalase, oxidase activity, and hydrolysis.

Histology

For histological examination, two biopsy specimens were fixed in neutral buffered 4% formaldehyde. *H. pylori* identification was performed on Giemsa-stained sections of paraffin-embedded tissue.

CLO test

To measure urease activity in our biopsy we performed the CLO test (Delta West, Bentley, Western Australia). One antral and one corpus biopsy specimen were placed in two separate plastic cups of two CLO tests, both containing a urea agar gel with phenol red buffer. After immersing the biopsy in the test it was kept at body temperature in the pocket of the endoscopist for up to ten hours to speed up the chemical reaction. The CLO test was read after 24 hours.

GUT test

Two extra antral biopsy specimens were used in the GUT test (Lencomm Trade International, Warschau, Poland/Lansmedical, Huissen, the Netherlands) for this study. The label was peeled off, exposing the test well which is a dry filter paper. With a sterile needle, the specimens were removed from the biopsy forceps and placed into this well. After resealing the test the label was pressed over the test dot with

the finger to squeeze the tissue juice out of the specimens and this is absorbed by the filter paper. Results were monitored at room temperature after 15, 60 and 120 minutes. When urease was present in the tissue, an expanding magenta colour zone was noted around the biopsy.

RESULTS

Altogether, 116 patients were recruited in the study. Of these patients, 61 were males and 56 were females. The mean age was 59.3 years with a range of 14 to 89 (table 1). The diagnosis of *H. pylori* infection was made, according to the gold standard, in 39 patients, while the other 77 patients were regarded as negative.

Interpretation of the GUT test appeared to be easy, we had no equivocal results. Compared with the CLO test, the GUT test had a sensitivity of 76.7% (95% CI: 63.9 to 89.6%) and a specificity of 100% (95% CI: 98.4 to 100%) in 15 minutes. After 60 minutes the sensitivity of the GUT test increased to 95.3% (95% CI: 88.9 to 100%), the specificity remained at 100% (95% CI: 98.4 to 100%). After 60 minutes incubation no additional positives were found. The results after 120 minutes were therefore the same as after 60 minutes. Compared with the gold standard the GUT test had a sensitivity of 79.4% (95% CI: 66.6 to 92.4%) and a specificity of 97.4% (95% CI: 93.8 to 100%) after 15 minutes incubation. After 60 minutes incubation the sensitivity increased to 97.4% (95% CI: 92.4 to 100%), the specificity became 96.1% (95% CI: 91.7 to 100%). This remained the same after 120 minutes.

When compared with the same gold standard, the CLO test had a sensitivity of 97.4% (95% CI: 92.4 to 100%) and a specificity of 93.5% (95% CI: 87.9 to 99.1%).

The results of the tests are presented in table 2.

Table 1. Patient characteristics

N	116
Male : female	60 : 56
Mean age	59.3 years
Age range	14-89

DISCUSSION

The GUT test appeared to be a good and reliable alternative for the widely used CLO test in diagnosing *H. pylori* infection.

The GUT test results were not yet reliable after just 15 minutes. The test slide only changed colour within a time span of 15 minutes when a high bacterial load was present. Our results demonstrate that there are still many false-negative results after 15 minutes. All positive test results (colour change) occurred between 15 and 60 minutes. No additional positives were found thereafter. This indicates that the test can best be read after at least 60 minutes. Results at 60 and 120 minutes were similar, but we did not investigate the stability of the colour change thereafter. We do not know if the test can still be trusted if it is read longer than 120 minutes after the procedure. This information, however, might be important in a situation when the test is forgotten and left overnight.

Biopsy rapid urease testing is the most simple and rapid method for identifying *H. pylori* infection in endoscopic practice.^{6,8} Moreover, these tests are not dependent on the experience and accuracy of individual laboratories as is the case for histological examinations or culture. False-positive rapid urease tests are rare.¹⁰⁻¹⁷ When patients salivate excessively or have reflux of alkaline bile into the stomach, this liquid may contaminate a small gastric biopsy specimen such that the resulting surface pH is >6.0. In theory, this situation could cause a weak positive reaction in some rapid urease tests. Similarly, patients taking a proton pump inhibitor chronically may develop achlorhydria with subsequent superficial colonisation of the gastric mucus layer with urease-producing organisms (e.g., *Proteus mirabilis* or *Klebsiella*). These organisms can give a false-positive urease test after 24 hours of inoculation but generally tests are still negative when the test is read one hour after biopsy insertion.⁹

Other authors have shown that acid-suppressing medication prolongs the time to positivity for the rapid urease tests.²⁴ Use of proton pump inhibitors increases the numbers of false-negative tests. Two possible mechanisms by which acid-suppressing medication delays positivity in the CLO test are known. First, the medication may directly inhibit

Table 2. The results of the GUT test compared with those of the CLO test and the gold standard

		CLO test		Gold standard	
		Positive	Negative	Positive	Negative
15 minutes incubation	GUT positive	33	0	31	2
	GUT negative	10	73	8	75
60 minutes incubation	GUT positive	41	0	38	3
	GUT negative	2	73	1	74
120 minutes incubation	GUT positive	41	0	38	3
	GUT negative	2	73	1	74

H. pylori urease. Secondly, CLO test positivity may be delayed due to changing patterns of *H. pylori* colonisation after acid suppression. *H. pylori* often only resides in the corpus during long-term use of proton pump inhibitors and can therefore not be detected in antral biopsies.²⁴

The presence of blood may adversely affect the performance of all biopsy urease tests. This is explained by the buffering effect of serum albumin on the pH indicator, rather than by a direct inhibition of the urease activity.²⁵

The GUT test gives a very distinct colour change in all cases. A positive reaction is noted when the yellow ring in the test well turns a distinct magenta, which is clearly distinguishable from contamination by blood. In the CLO test blood around the biopsy sample may give an initial false impression of a positive test. Therefore, the CLO test can only be interpreted as positive with confidence when the colour changes the total volume of the agar gel.²⁶

The advantage of this new GUT test is that the results are available faster than the results of the CLO test (after one hour and 24 hours respectively). Another economic advantage is that the GUT test is cheaper than the CLO test. The CLO test has an incubation period of 24 hours,²⁴ requires refrigeration for storage and needs to be warmed to room temperature prior to use. Another advantage of the GUT test is therefore that it does not require any special storage conditions. It can be stored at room temperature in the endoscopy suite and is therefore readily available.

A disadvantage of the GUT test is that so far it has only been validated with the use of two biopsies in one test slide; taking an extra biopsy is time-consuming. In contrast the CLO test has been widely validated with only one biopsy in the test slide. Some authors, however, have also recommended putting two biopsies in one CLO test slide. Adding a second biopsy in the same agar cup speeds up the reaction and may partly eliminate the problem of sampling error. Theoretically this may improve sensitivity.⁶ Some found *H. pylori* in a patchy distribution throughout the stomach.^{13,23} The most reliable diagnosis of infection is therefore achieved by testing multiple sites.^{6,27} *H. pylori* is not present in intestinal metaplasia and this may indeed be a reason for missing the diagnosis if only a site of metaplasia is sampled. In the GUT test we used two biopsies in one test slide. This may also have improved the sensitivity of the GUT test. Further research is therefore needed with one biopsy in the GUT test. Until this research has been done users of the GUT test need to add two biopsies in order to know that they have a reliable test.

Two possible explanations can be given for the more rapid reaction time of the GUT test. First the biopsy tissue is squeezed with the finger as recommended by the manufacturer. By doing this the tissue 'juice' and the urease enzyme reacts with the test substrate. Second, the urease enzyme can be absorbed very quickly because of the presence of the dry filter paper ring around the test well, causing a

rapid colour change. In contrast the CLO test depends on the slower diffusion of urease into the agar gel which contains the urea substrate. In the CLO test the speed of the reaction also depends on the way the biopsy is placed in the agar gel.²⁶ If the CLO test is used properly the biopsy needs to be immersed completely into the gel with a sterile needle. However, in real life the biopsy is sometimes only put on top of the agar gel and the lower contact surface delays the colour change.

The GUT test has been validated once before by Said *et al.*²⁶ They compared the GUT test, which in their study is called the Pronto Dry, with the CLO test in 208 patients. In this study the results for both the Pronto Dry and the CLO test were completely concordant. The Pronto Dry had a sensitivity, specificity, PPV, NPV and diagnostic accuracy of 98.1, 100, 100, 98.1 and 99%, respectively. The Pronto Dry showed a faster reaction time to positive compared with the CLO test. With Pronto Dry 96.2% of all positive reactions occurred before 30 minutes *vs* 70.8% for the CLO test. Pronto Dry had a 100% positive reaction time by 55 minutes *vs* 83% for the CLO test.

Tseng *et al.*²⁸ have investigated the accuracy and positive reaction time of two new rapid urease tests (Pronto Dry and Hp One) in 49 patients. In their study the sensitivities, specificities, PPVs and NPVs of the three rapid urease tests were not significantly different.

Our results are therefore in agreement with these earlier validation studies.

CONCLUSION

From our study we conclude that the GUT test with two gastric biopsies is highly accurate for the diagnosis of *H. pylori* infection. Compared with the CLO test, the GUT test gives the endoscopist a more rapid test result and it is much cheaper. Because it can be stored at room temperature in the endoscopy room it is always easily available. The GUT test results were not yet reliable after 15 minutes. However, all positive results occurred before 60 minutes of incubation. Test results did not change between 60 and 120 minutes. This indicates that the test can best be read 60 to 120 minutes after endoscopy. Based on our data we believe that this is a reliable, very attractive and affordable biopsy urease test for the diagnosis of *H. pylori* infection.

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