

ORIGINAL ARTICLE

Comparison of three stool antigen tests for *Helicobacter pylori* detection

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Background: Active *Helicobacter pylori* infection can be diagnosed by invasive (biopsy based) or non-invasive methods, such as stool antigen testing.**Aims:** To compare three stool antigen enzyme immunoassay kits—Premier Platinum Hp SA, FemtoLab Cnx, and Hp Ag—with biopsy based methods for the detection of *H pylori* in previously undiagnosed patients.**Methods:** One hundred and eleven adults with dyspepsia referred for endoscopy provided a stool sample for testing and had biopsies taken. Patients were considered *H pylori* positive if two out of three invasive tests were positive or if culture alone was positive.**Results:** The sensitivities and specificities of the Premier Platinum Hp SA, FemtoLab Cnx, and Hp Ag stool antigen kits when compared with biopsy based diagnosis were, 63.6%, 88.0%, and 56.0% and 92.6%, 97.6%, and 97.6%, respectively.**Conclusions:** FemtoLab Cnx may be considered as an alternative to urea breath testing in the initial diagnosis of patients with dyspepsia who do not require immediate endoscopy. Stool testing has the potential advantages of being simple to perform, relatively cheap, and samples can be submitted directly from primary care.

Infection with *Helicobacter pylori* is causally associated with peptic ulceration, gastric adenocarcinoma, and some types of gastric lymphoma.¹ Diagnosis of current infection may be by biopsy based tests (invasive tests), such as culture, histology, or rapid urease testing, or by non-invasive tests, such as urea breath testing (UBT) and stool antigen testing (SAT). Current serological tests are unable to distinguish active from past infection.²

All patients over 45 and those with "alarm" symptoms of weight loss or bleeding require an endoscopy to exclude gastric malignancy, and require an invasive test to be performed.³ Non-invasive tests are useful for primary diagnosis, when a treatment indication already exists, or to monitor treatment success or failure. They are also useful in patients who cannot tolerate endoscopy, children, and in epidemiological population studies.

In 2000, a consensus report⁴ stated that two non-invasive tests, UBT and SAT, could be used both safely and cost effectively to screen for *H pylori* positive patients (below the age of 45) without alarm symptoms.

"Non-invasive tests are useful for primary diagnosis, when a treatment indication already exists, or to monitor treatment success or failure"

UBT has excellent performance data but is expensive, may involve a visit to the hospital, and may be complicated for children to perform. SAT has advantages in that the sample can be referred from primary care and tests are technically simple and relatively cheap to perform.

The Premier Platinum Hp SA (Meridian Diagnostics, Cincinnati, Ohio, USA), a polyclonal antibody based stool enzyme immunoassay, has been evaluated extensively. In 2001, a meta analysis of 4769 untreated patients in 43 separate studies using the Premier Platinum Hp SA kit showed a weighted mean sensitivity of 92.1% and 91.9% for specificity.⁵

Other *H pylori* faecal antigen kits are now available. FemtoLab Cnx (Dako, Ely, Cambridgeshire, UK) is a monoclonal antibody kit that has been tested principally in initial

diagnosis. A study of untreated children (n = 79) found the sensitivity and specificity to be 98% and 96.7%, respectively, when compared with UBT and serology.⁶ Hp Ag (Dia.Pro, Milan, Italy) is also a kit that uses monoclonal antibodies, which at the time of writing had no published performance data.

The aim of our study was to compare the above three stool antigen tests against a "gold standard" of combined culture, histology, and rapid urease testing in a busy district general hospital setting and to determine the kit(s) with the best performance data and practicability.

MATERIALS AND METHODS

Patients

The local research and ethics committee approved the study in full. All adults with dyspepsia undergoing an elective upper gastrointestinal endoscopy during a four month period were invited by post to participate in our study. Questionnaires were completed, to ascertain whether patients had received proton pump inhibitors, bismuth containing agents, H₂ antagonists, or antimicrobials in the four weeks before endoscopy, because these medications can destroy *H pylori* antigens and thus interact with SATs. Each patient was asked to provide a stool sample up to 48 hours before endoscopy; samples were stored at -20°C and the assays performed in batches.

Histological biopsy

Antral and corpus biopsies of the stomach mucosa were taken at endoscopy, formalin fixed, and stained using fast crystal violet, as described by Burnett *et al.*⁷ A single histopathologist (DB) reviewed each specimen blind.

Rapid urease testing

Rapid urease testing was performed in the endoscopy suite using CLOtest (Ballard Medical Products, Utah, USA) and read at 18-24 hours.

Abbreviations: SAT, stool antigen testing; UBT, urea breath testing

Table 1 Characteristics of stool antigen enzyme immunoassay kits

Kit	Number of tests/kit	Solid phase	Enzyme conjugate (incubation time)	Substrate (incubation time)	OD450 nm cut offs
Premier Platinum Hp SA	96	Rabbit polyclonal antibody	HP labelled rabbit polyclonal (60 mins)	TMB (10 mins)	Negative, OD<0.140 Positive, OD>0.16
FemtoLab Cnx	96	Monoclonal antibody	HP labelled monoclonal (60 mins)	TMB (10 mins)	Negative, OD<0.19 Positive, OD>0.19
Dia. Pro Hp Ag	48	Affinity purified monoclonal antibody	Several monoclonals labelled with HP (120 mins)	TMB (20 mins)	Negative control, + 0.150

HP, horseradish peroxidase; OD450 nm, optical density at 450 nm; TMB, tetramethylbenzidine.

Culture

One biopsy sample from each patient was plated on Columbia blood agar (CM331; Oxoid Ltd, Basingstoke, UK) and Columbia blood agar with Dents *H pylori* selective supplement (SR147, Oxoid Ltd), then incubated at 37°C in microaerophilic jars for up to 10 days. Suspect colonies were confirmed as *H pylori* by characteristic morphology on Gram staining and positive urease and oxidase reactions.

Gold standard diagnosis

Histology, culture, and rapid urease testing were performed on all patient samples. Patients were considered *H pylori* positive if culture alone was positive or if two out of three invasive tests were positive.

Enzyme immunoassays

Stool specimens were tested in batches, and manufacturers' cut off points were used throughout (table 1). No equivocal results were repeated and no repeat specimens were requested. Equivocal results were excluded from the calculation of sensitivity and specificity.

Statistics

For each antigen kit the sensitivity, specificity, positive, and negative predictive values were determined and compared

with the gold standard results using exact binomial 95% confidence intervals, as described by Gardner and Altman⁸ using Epi-table (Epi-info Version 6).

RESULTS

An invitation to participate in our study was sent to 422 adult patients undergoing upper gastrointestinal tract endoscopy; 148 (35.2%) submitted a stool sample. Stool samples and biopsies were available from 111 patients (58 women and 53 men; age range, 23–86 years; mean age, 55). These patients were analysed in two groups. Group A contained those 72 patients who had not taken interacting medications in the four weeks before endoscopy. Group B contained 39 patients who had taken one or more interacting medication in the preceding four weeks.

Compared with combined CLO and histology results, the sensitivity of *H pylori* culture in our study was 79.4%. In Group A, the prevalence of *H pylori* colonisation overall was 25 of 72 (36.1%). In Group B, containing those patients who had taken interacting medications, the prevalence of *H pylori* was lower, at eight of 39 (20.1%). Results of the three enzyme immunoassays are presented in tables 2 and 3.

Table 2 Performance data for the three stool antigen kits for group A (n=72): patients who had received no interacting medications in the past 4 weeks

Group A	Number of equivocals	Number of true positives	Number of true negatives	Sensitivity	Specificity	NPV	PPV
Gold standard diagnosis	0	25	47	100%	100%		
Premier Platinum HpSA†	8	14	38	63.6% (40.4% to 82.8%)	92.6% (80.1% to 98.5%)	82.6% (68.6% to 92.2%)	82.4% (56.6% to 96.2%)
FemtoLab Cnx	0	22	46	88.0% (68.8% to 97.5%)	97.6% (88.7% to 99.9%)	93.6% (83.1% to 98.7%)	95.7% (78.1% to 99.9%)
Dia.Pro Hp Ag	0	14	46	56.0% (34.9% to 75.9%)	97.6% (88.7% to 99.9%)	80.7% (68.1% to 90.0%)	93.6% (68.1% to 99.8%)

For sensitivity, specificity, NPV, and PPV the percentages are given with 95% confidence intervals in parenthesis.

Gold standard diagnosis: patients were considered *Helicobacter pylori* positive if culture alone was positive or if two of three invasive tests were positive; †1 less stool was tested with this kit.

NPV, negative predictive value; PPV, positive predictive value.

Table 3 Performance data for all three stool antigen kits for group B (n=39): patients who were on one or more interacting medication(s)

Group B	Number of equivocals	Number of true positives	Number of true negatives	Sensitivity	Specificity	NPV	PPV
Gold standard diagnosis	0	8	31	100%	100%		
Premier Platinum Hp SA	2	6	24	85.7% (42.1% to 99.6%)	85.7% (67.3% to 96.0%)	96.0% (79.7% to 99.9%)	60.0% (26.2% to 87.8%)
FemtoLab Cnx	0	7	28	87.5% (47.4% to 99.7%)	90.3% (74.3% to 98.0%)	96.6% (82.4% to 99.9%)	70.0% (34.75% to 93.3%)
Dia.Pro Hp Ag	0	7	26	87.5% (47.4% to 99.7%)	89.7% (72.7% to 97.8%)	96.3% (81.0% to 99.9%)	70.0% (34.8% to 93.3%)

For sensitivity, specificity, NPV, and PPV the percentages are given with 95% confidence intervals in parenthesis.

Gold standard diagnosis: patients were considered *Helicobacter pylori* positive if culture alone was positive or if two of three invasive tests were positive; ‡2 less stools were tested with this kit.

NPV, negative predictive value; PPV, positive predictive value.

DISCUSSION

The three kits tested had lower sensitivity and specificity than previously published data.^{3,6} Each test was performed to the manufacturer's instructions, with quality control requirements being met, in a routine diagnostic laboratory setting.

The most promising kit appeared to be FemtoLab Cnx, which had a sensitivity of 88.0% and a specificity of 97.6% when tested on the 72 patients who had not received medications in the preceding four weeks. The sensitivity was similar, at 87.5%, and the specificity lower, at 90.3%, for group B patients. The data sheet for FemtoLab Cnx clearly states that patients should not be on antibiotics, proton pump inhibitors, or bismuth preparations for four weeks before stool antigen testing. Therefore, group B patients were excluded from the final results.

In group A patients, the proportion of patients with gold standard diagnosed *H pylori* infection was 25 of 72 (36.1%), giving FemtoLab Cnx a positive predictive value of 95.7% and a negative predictive value of 93.6% in this group of patients.

Both the Premier Platinum Hp SA and Hp Ag had lower sensitivities of 63.6% and 56.0%, respectively, and similar specificities of 92.6% and 97.6%, respectively, in the 72 patients not on medication (group A). Both kits had high false negative rates—eight of 71 for Premier Platinum Hp SA and 11 of 72 for Hp Ag—which is of concern, because this test would primarily be used as a screening test. Patients would be falsely reassured that they were not infected with *H pylori* if these tests were used. Both kits had high specificity results so patients who did test positive would probably be infected and warrant treatment. In addition to low sensitivity and negative predictive values, the Premier Platinum Hp SA kit had a high rate of equivocal results, 10 of 109 tests (10.9%), which would have required repeating. The need for repetition would increase the cost of testing and delay results. If a second equivocal result were obtained, a further stool sample would need to be requested. We did not repeat equivocal results in our study.

“All three kits were easy to use, but FemtoLab Cnx was both the quickest and simplest test to perform”

Storage of stools at -20°C instead of -70°C may have contributed to the lower sensitivities of the kits in our study when compared with other published results. However, most of the other studies stored faeces at -20°C before testing, and this is the recommended temperature in the kit inserts.

All three kits were easy to use, but FemtoLab Cnx was both the quickest and simplest test to perform. Dia.Pro Hp Ag had a lengthy sample preparation stage. The kit inserts were easy to follow, with both detailed instructions and quick reminders for the more experienced users. The FemtoLab Cnx insert was thought to be the clearest and contained more information on

Take home messages

- FemtoLab Cnx could be used as an alternative to urea breath testing (UBT) in the initial diagnosis of patients with dyspepsia who do not require immediate endoscopy
- Stool testing has the potential advantages of being simple to perform, relatively cheap, and samples can be submitted directly from primary care
- Because UBT is difficult to perform in children it may be reasonable to replace UBT by FemtoLab Cnx stool testing

exclusion criteria than the other two kits. The Premier Platinum Hp SA kit had the most detailed previous performance data available. The Hp Ag had no performance data within the kit information sheet.

In summary, our study cannot recommend the use of stool testing with Premier Platinum Hp SA or Hp Ag because of poor performance. The Premier test kit performed least well because of the high proportion of equivocal results. The FemtoLab Cnx test performed reasonably well in the screening of previously untreated patients, but not as well as published pretreatment UBT results.

FemtoLab Cnx has a high negative predictive value, suggesting that it might be useful in screening out uninfected patients. Because UBT is difficult to perform in children it may be reasonable to replace UBT by FemtoLab Cnx stool testing.

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