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


**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.
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. Suspicious 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 Altman 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 medications 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 1

<table>
<thead>
<tr>
<th>Kit</th>
<th>Number of tests/kit</th>
<th>Solid phase</th>
<th>Enzyme conjugate (incubation time)</th>
<th>Substrate (incubation time)</th>
<th>OD450 nm cut off</th>
</tr>
</thead>
<tbody>
<tr>
<td>Premier Platinum Hp SA</td>
<td>96</td>
<td>Rabbit polyclonal antibody</td>
<td>HP labelled rabbit polyclonal (60 mins)</td>
<td>TMB (10 mins)</td>
<td>Negative, OD&lt;0.140</td>
</tr>
<tr>
<td>Femtolab Cnx</td>
<td>96</td>
<td>Monoclonal antibody</td>
<td>HP labelled monoclonal (60 mins)</td>
<td>TMB (10 mins)</td>
<td>Negative, OD&lt;0.16</td>
</tr>
<tr>
<td>Dia. Pro Hp Ag</td>
<td>48</td>
<td>Affinity purified monoclonal antibody</td>
<td>Several monoclonals labelled with HP (120 mins)</td>
<td>TMB (20 mins)</td>
<td>Negative control, + 0.150</td>
</tr>
</tbody>
</table>

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

Table 2

<table>
<thead>
<tr>
<th>Group A (n=72)</th>
<th>Number of equivocals</th>
<th>Number of true positives</th>
<th>Number of true negatives</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>NPV</th>
<th>PPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gold standard diagnosis</td>
<td>0</td>
<td>25</td>
<td>47</td>
<td>100%</td>
<td>92.6%</td>
<td>82.6%</td>
<td>82.4%</td>
</tr>
<tr>
<td>Premier Platinum Hp SA</td>
<td>8</td>
<td>14</td>
<td>38</td>
<td>63.6%</td>
<td>90.1% to 98.5%</td>
<td>68.6% to 92.2%</td>
<td>56.6% to 96.2%</td>
</tr>
<tr>
<td>Femtolab Cnx</td>
<td>0</td>
<td>22</td>
<td>46</td>
<td>88.0%</td>
<td>97.6%</td>
<td>93.6%</td>
<td>95.7%</td>
</tr>
<tr>
<td>Dia. Pro Hp Ag</td>
<td>0</td>
<td>14</td>
<td>46</td>
<td>56.0%</td>
<td>87.7%</td>
<td>93.6%</td>
<td>93.8%</td>
</tr>
</tbody>
</table>

For sensitivity, specificity, NPV, and PPV the percentages are given with 95% confidence intervals in parenthesis. 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 3

<table>
<thead>
<tr>
<th>Group B (n=39)</th>
<th>Number of equivocals</th>
<th>Number of true positives</th>
<th>Number of true negatives</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>NPV</th>
<th>PPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gold standard diagnosis</td>
<td>0</td>
<td>8</td>
<td>31</td>
<td>100%</td>
<td>96.0%</td>
<td>60.0%</td>
<td></td>
</tr>
<tr>
<td>Premier Platinum Hp SA</td>
<td>2</td>
<td>6</td>
<td>24</td>
<td>85.7%</td>
<td>96.0%</td>
<td>96.0%</td>
<td></td>
</tr>
<tr>
<td>Femtolab Cnx</td>
<td>0</td>
<td>7</td>
<td>28</td>
<td>87.5%</td>
<td>93.0%</td>
<td>96.6%</td>
<td></td>
</tr>
<tr>
<td>Dia. Pro Hp Ag</td>
<td>0</td>
<td>7</td>
<td>26</td>
<td>87.5%</td>
<td>93.0%</td>
<td>93.0%</td>
<td></td>
</tr>
</tbody>
</table>

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

The three kits tested had lower sensitivity and specificity than previously published data.1 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 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.

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

REFERENCE