Evaluation of a novel monoclonal enzyme immunoassay for detection of *Helicobacter pylori* antigen in stool from children

S Koletzko, N Konstantopoulos, D Bosman, A Feydt-Schmidt, A van der Ende, N Kalach, J Raymond, H Rüssmann

**Background:** Reliable non-invasive methods for detection of *Helicobacter pylori* infection are required to investigate the incidence, transmission, and clearance of infection in childhood.

**Aim:** To evaluate a new monoclonal enzyme immunoassay (EIA) [FemtoLab H pylori Cnx] for detection of *H pylori* antigen in stool in a large cohort of children compared with invasive diagnostic methods and the 13C urea breath test.

**Patients and methods:** A total of 302 symptomatic previously untreated children (aged 0.5–18.7 years; 148 girls) were recruited at three centres. *H pylori* status was defined by results of culture, histology, the rapid urease test, and the 13C urea breath test. Stool samples were investigated locally by the EIA using two different production lots. According to the manufacturer’s recommendations, an optical density (OD) of 0.150 was used as a cut off value.

**Results:** OD values clearly differentiated between the 92 *H pylori* infected and the 210 non-infected children (median (5th–95th percentiles) 2.729 (0.232–4.000) vs 0.021 (0.009–0.075)). Only two false positive and two false negative results occurred, giving a sensitivity, specificity, positive predictive value, and negative predictive value of 98%, 99%, 98%, and 99%, respectively. No significant relation was found between age and OD values in infected or non-infected children.

**Conclusions:** The monoclonal stool antigen EIA was excellent in diagnosing *H pylori* infection in symptomatic children. Accuracy was independent of the laboratory, production lot used, or the child’s age. Because only 18/116 children <6 years of age were infected with *H pylori*, further validation of the test is needed in young infected children.
RESULTS

Two patients from Munich had false positive results; one was infected by Campylobacter jejuni at the time of endoscopy. The other two patients with false negative results came from Amsterdam. One of the four false negative results in all diagnostic tests performed and were therefore considered H pylori negative.

Age of the children ranged from 0.5 to 18.7 years. A total of 116 patients were <6 years of age (18 were positive and 98 negative for H pylori), 106 were ≥6–<12 years (42 positive, 64 negative), and the remaining 80 children were ≥12 years of age (32 positive, 48 negative) (table 1). The proportions of infected to non-infected children in the three centres were as follows: in Munich, 55 to 118; in Amsterdam, 27 to 70; and in Paris, 10 to 22. The geographical background of the children's families was Northern or Western Europe in 188 cases, 23 families came from Southern Europe, three from Eastern Europe, 45 from Turkey, nine from Asia, 27 from Africa, and seven from America.

Figure 1  Optical density (OD) values in relation to age in 92 children with a positive Helicobacter pylori status. The cut off value was set at an OD of 0.150.

Figure 2  Optical density (OD) values on a log scale in relation to age in 210 children with a negative Helicobacter pylori status. The cut off value was set at an OD of 0.150.

<table>
<thead>
<tr>
<th>Age group (y)</th>
<th>n (Hp+)</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Accuracy</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;6</td>
<td>116</td>
<td>94.4 (62.7–99.9)</td>
<td>98.0 (92.6–99.8)</td>
<td>97.4 (92.6–99.9)</td>
<td>89.5 (66.9–98.7)</td>
<td>99.0 (94.4–99.9)</td>
</tr>
<tr>
<td>≥6–≤12</td>
<td>106</td>
<td>97.6 (87.4–99.9)</td>
<td>100 (94.1–100)</td>
<td>99.1 (94.9–99.9)</td>
<td>100 (91.4–100)</td>
<td>98.5 (91.7–99.9)</td>
</tr>
<tr>
<td>&gt;12–&lt;18</td>
<td>80</td>
<td>100 (89.1–100)</td>
<td>100 (92.6–100)</td>
<td>100 (95.5–100)</td>
<td>100 (89.1–100)</td>
<td>100 (92.6–100)</td>
</tr>
<tr>
<td>All children</td>
<td>302</td>
<td>97.8 (92.4–99.7)</td>
<td>99.0 (96.6–99.9)</td>
<td>98.7 (96.6–99.8)</td>
<td>97.8 (92.4–99.7)</td>
<td>99.0 (96.6–99.9)</td>
</tr>
</tbody>
</table>

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

Values are median [5th–95th percentiles].
results had an OD value that was close to the cut off value (fig 1). Sensitivity, specificity, accuracy, and positive and negative predictive values (with 95% confidence intervals) are presented in table 1 according to the three different age groups and globally for the total cohort. The likelihood ratio for a positive test result was calculated as 103.

**DISCUSSION**

To the best of our knowledge, this is the first prospective multicentre based study of a novel monoclonal EIA stool test used to establish a diagnosis of *H pylori* infection in children. For every child, *H pylori* status was assessed using three different tests. In fact, culture, which is considered to be 100% specific, was successful in 88/92 children with a positive *H pylori* status. The monoclonal EIA on stool samples correctly classified 298 of 302 children, giving an accuracy of 98%.

To date, only one study has been published using this monoclonal EIA in children prior to treatment. Makristathi et al used a developmental kit provided by the manufacturer at a time when the test was not yet marketed. The authors performed the test in 79 children, 39 of whom were considered to be *H pylori* positive according to positive results from UBT and serology. The test yielded a sensitivity of 98% and a specificity of 97%, which is similar to our results of 98% and 99%, respectively. In our study, these excellent results were obtained in spite of the fact that the test was performed in three different laboratories using two different production lots. In contrast, the HpSA, which is of polyclonal origin, seems to have problems with lot to lot variability. This variability is reflected by the wider range of sensitivity and specificity values, reported in some studies to be as low as 63%, even in patients before therapy. In a recent multicentre European trial involving non-invasive tests in 316 children with a biopsy based *H pylori* status, HpSA achieved a sensitivity of only 72–77%.

Differentiation between positive and negative results (figs 1, 2) is valuable. In contrast with the HpSA test, no grey zone is necessary. To improve the accuracy of the HpSA, some investigators have suggested adapting the cut off value. In our study, the HpSA showed 98% sensitivity and 99.5% specificity, with a positive predictive value for positive test results of 99.9%.

In accordance with our previous experience with the polyclonal test, we did not find any relation between OD values and patient age. This is particular advantageous in paediatric clonal test, we did not find any relation between OD values and patient age. This is particular advantageous in paediatric clinical practice.

In conclusion, the monoclonal stool test is easy to perform and provides excellent differentiation between positive and negative test results. In symptomatic children the test is well suited for evaluation of *H pylori* status. The high accuracy seems to be independent of the laboratory, production lots, and age of the child. An age specific cut off value is not required, even in young children. Therefore, if further studies in children confirm our results, this test may become an excellent tool to study the incidence, spontaneous clearance of *H pylori* infection, and effect of preventive measures such as vaccination.

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