A Rapid Serologic Test and Immunoblotting for the Detection of *Helicobacter pylori* Infection in Children

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Summary

The gold standard for the diagnosis of *Helicobacter pylori* infection requires an endoscopic biopsy of gastric mucosa for histological examination, urease test and culture. Noninvasive serological tests are useful as a screening test for *H. pylori* infection. The aim of this study was to evaluate the performance of a rapid office-based serologic test, using immunochromatography (ICM), and the immunoblotting for the diagnosis of *H. pylori* infection in Thai children. Eighty-two symptomatic children, 30 boys and 52 girls (mean age 9.2 ± 3.8 years; range, 1.2–16.0 years) who had no previous treatment for *H. pylori* underwent upper endoscopy. Biopsies were obtained from the gastric body and antrum for histopathology and rapid urease test. Serum samples collected from all patients were tested for *H. pylori* IgG antibodies using ICM (Assure™ *H. pylori* Rapid Test, Genelabs® Diagnostics, Singapore). Immunoblotting (HelicoBlot 2.1, Genelabs® Diagnostics, Singapore) was tested in sera of 75 patients to detect antibodies to specific antigens of *H. pylori*. Positive *H. pylori* status was defined as positive for both histology and rapid urease test. Of 82 patients, 25 (30.5%) were *H. pylori* positive, 56 (68.3%) were *H. pylori* negative and one was equivocal. ICM assay yielded a positive result in 24 of the 25 *H. pylori*-positive patients (96.0%) and 3 of the 56 *H. pylori*-negative patients (5.4%). The immunoblotting yielded a positive result in all of 22 *H. pylori*-positive patients (100%) and in 2 of the 52 *H. pylori*-negative patients (3.8%). Obtained ICM’s sensitivity, specificity, positive predictive value, negative predictive value and accuracy were 96.0, 94.6, 88.9, 98.1 and 95.1%, with immunoblotting 100.0, 96.2, 91.6, 100.0, and 97.3%, respectively. The immunochromatographic and immunoblot tests are non-invasive, reliable and useful for the diagnosis of *H. pylori* infection in Thai children.

Introduction

*Helicobacter pylori* (*H. pylori*) is one of the most common pathogens associated with chronic gastritis, peptic ulcer disease, mucosal-associated lymphoid tissue (MALT) lymphoma and gastric carcinoma [1, 2]. Diagnosis of *H. pylori* is therefore important for the management of gastroduodenal diseases. The gold standard criteria for the diagnosis of *H. pylori* infection require endoscopically obtained gastric tissue to demonstrate the organisms by histopathology and/or culture and its enzyme activity by urease test [3, 4]. However, endoscopy is not always possible especially in developing countries where pediatric endoscopic facility is lacking in many parts of the countries. The alternative practical option is serologic testing for the detection of antibodies in the blood or serum. Available serologic techniques include enzyme-linked immunosorbent assay (ELISA), latex agglutination, immunochromatography (ICM) and immunoblotting [5]. The performance of commercial serological tests for *H. pylori* varies in different populations, mainly due to strain heterogeneity and variations in antigenic preparations [6, 7]. Therefore, local validation is needed before implementing a serological test into

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different populations. In children, the cutoff value for serodiagnosis of *H. pylori* infection has been shown to be different from adults [8], suggesting the different immune responses between children and adults. Thereby, validation of the tests in both children and adults is mandatory.

The aim of this multi-center, cross-sectional study is to evaluate the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of the serologic tests using ICM and immunoblotting for the diagnosis of *H. pylori* infection in Thai children.

**Patients and Methods**

**Patients**
Children who underwent upper gastrointestinal (GI) endoscopy for upper GI symptoms (such as recurrent abdominal pain, chronic vomiting and dyspepsia) at Ramathibodi Hospital, Siriraj Hospital, Queen Sirikit National Institute of Child Health and Maharat Nakhon Ratchasima Hospital were included in the study. The study was approved by the Ethic Committee of each of the participating institutions. Written informed consent was obtained from parents or legal guardians. Exclusion criteria included patients previously treated for *H. pylori* and patients received proton pump inhibitors, antibiotics or bismuth within 2 weeks before upper endoscopy.

**Histology and urease test**
During the endoscopic examination, two biopsy specimens, obtained from two sites, gastric antrum and body, were placed into the rapid urease test kit (CLOtest, Ballard Medical Products, USA.) to detect urease activity produced by the bacterium. A positive result was defined as a color change from yellow to pink within 24 h of incubation in the room temperature.

Two additional biopsy specimens, one from the antrum and the other from the body, were immediately fixed in 10% formalin for histological examination. The histological studies were blindly examined by the single investigator (KA) to identify the organism using hematoxylin and eosin (H&E), Giemsa and immunohistochemical stains.

**Determination of antibodies by immunochromatography**
Blood was obtained at the time of endoscopy and sera were kept at −20°C until analyzed. The ICM test was performed at Ramathibodi Research Center using a commercial kit, Assure™ *H. pylori* Rapid Test (Genelabs® Diagnostics, Singapore), following the manufacturer’s instruction. Antibodies in the test samples, if present, formed antibody-antigen complexes with immobilized *H. pylori* antigen on the membrane of the kit. The bound antibody-antigen complexes were subsequently detected by anti-human IgG conjugated to colloidal gold resulting in the appearance of the pink-colored bands.

**Determination of antibodies by immunoblotting**
The immunoblot assay was performed at Ramathibodi Research Center using a commercial kit, HelicoBlot 2.1 (Genelabs® Diagnostics, Singapore). The kit consists of Western Blot using a bacterial lysate, including a recombinant antigen of *H. pylori*, designated as current infection marker (CIM). This protein was constructed by immunological screening of the genomic DNA library of *H. pylori* (ATCC strain 43526) [9]. The test was performed according to the manufacturer’s instruction. Briefly, the membrane strips were incubated in washed buffer and then a 1/100 dilution of serum in blocking buffer was added to each strip. An anti-human IgG antibody conjugated with alkaline phosphatase was used as the probe. Specifically bound antibody was detected by addition of the substrate, 5-bromo-4-chloro-3 indolyl phosphate and nitroblue tetrazolium. A serum sample was considered to have tested positive for *H. pylori* by Western Blot if it reacts with the bands as follows (Fig. 1):

![Fig. 1. Nitrocellulose strips representative of immunoblot results: lane1, positive control; lane2, and 3, positive sera; lane4 and 5, negative sera; lane6, negative control.](image-url)
The ICM and immunoblot tests were performed blindly, each by different authors without the information of *H. pylori* status of the serum samples.

The gold standard for diagnosing *H. pylori* was identification of the organism from histological section and positive for rapid urease test.

### Statistical analysis

The sensitivity, specificity, PPV and NPV of the two commercial kits were calculated with reference to the gold standard for diagnosing *H. pylori* infection. The performance of the two tests was compared by McNemar’s test. Statistical significance was taken at $p<0.05$.

### Results

A total of 82 children, 30 boys and 52 girls, aged 1.2–16.0 years (mean 9.2 ± 3.8) were included into the study. *H. pylori* infection was diagnosed in 25 children (30.5%) based on positive results in both the histology and rapid urease test. One patient was excluded from the analysis due to an equivocal result which showed a positive histology but negative rapid urease test. Of the 81 patients, ICM assay detected *H. pylori* antibodies in 24 of the 25 *H. pylori*-positive patients (96.0%) and 3 of the 56 *H. pylori*-negative patients (5.4%). Immunoblot assay was performed in 75 patients, 22 *H. pylori*-positive and 52 *H. pylori*-negative patients and one with an equivocal *H. pylori* status as described above. The immunoblotting yielded a positive result in all of the 22 *H. pylori*-positive patients (100%) and in 2 of the 52 *H. pylori*-negative patients (3.8%). Of the 22 patients with *H. pylori* infection, CagA and VacA antibodies were positive in 21 cases (95.5%) and 16 cases (72.7%), respectively. The sensitivity, specificity, PPV, NPV and accuracy of ICM and immunoblotting for the diagnosis of *H. pylori* infection are shown in Table 1. The sensitivity and specificity of the two tests, using McNemar’s test, were not significantly different ($p>0.05$). The endoscopic findings in 25 *H. pylori*-infected patients included gastritis (11), antral nodularity (10), duodenal ulcer (1) and normal (3).

### Table 1

*The performance of immunochromatographic (ICM) and immunoblot tests for the diagnosis of *H. pylori* infection compared to gold standard (positive histology and rapid urease test)*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ICM (95%CI)</th>
<th>Immunoblot (95%CI)</th>
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<tbody>
<tr>
<td>Sensitivity (%)</td>
<td>96.0 (79.6, 99.8)</td>
<td>100.0 (87.3,100.0)</td>
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<tr>
<td>Specificity (%)</td>
<td>94.6 (85.1, 98.9)</td>
<td>96.2 (86.8, 99.5)</td>
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<td>Positive predictive value (%)</td>
<td>88.9 (70.8, 97.6)</td>
<td>91.6 (73.0, 98.9)</td>
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<tr>
<td>Negative predictive value (%)</td>
<td>98.1 (90.1, 99.9)</td>
<td>100.0 (94.1, 100.0)</td>
</tr>
<tr>
<td>Accuracy (%)</td>
<td>95.1 (87.8, 98.6)</td>
<td>97.3 (90.6, 99.7)</td>
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CI, confidence interval.

(i) Presence of 116 kDa (CagA) band with one of the following bands: 37 kDa, 35 kDa, 30 kDa (UreA) and 19.5 kDa together, or with CIM; (ii) Presence of any of the band 89 kDa (VacA), 37 kDa or 35 kDa; (iii) Presence of both the 30 kDa and 19.5 kDa bands.

Serological test is a non-invasive method for the diagnosis of *H. pylori* infection, carried out by detecting *H. pylori*-specific antibodies, primarily IgG antibodies. The advantages of serologic tests include simplicity, low cost and usefulness for a screening test and an epidemiologic study [5]. In comparison with biopsy-based method, they are not influenced by sampling errors. However, the disadvantages of the tests are the limitation in differentiating the past and present infection and the inaccuracy in assessing the success of eradication therapy.

The most often used serologic test is ELISA. Previous studies on commercially available ELISA tests showed conflicting results in children [8, 10]. Khanna *et al.* reported the low sensitivity of three commercial ELISA kits (62, 70 and 78%) although all kits showed good specificity of more than 90% [8]. Sim *et al.* demonstrated the good performance of commercial ELISA with more than 90% sensitivity and specificity [10]. However, ELISA is usually laboratory based, requiring some technical competence. A rapid office-based serologic test is of interest because it is simple and provides a result within minutes. Therefore it is more practical for clinical use. Elitsur *et al.* previously demonstrated that a rapid office-based serologic test using FlexSure HP kit (SmithKline Diagnostics, Palo Alto, CA, USA) provided results comparable to those of standard enzyme immunoassay [11]. The study from Canada by Day *et al.* using the two rapid tests, MedMira rapid *H. pylori* Antibody Test (MedMira Laboratories, Halifax, NS, Canada) and FlexSure HP (SmithKline Diagnostics, Palo Alto, CA, USA) in children, showed the sensitivity of 71 and 59 and the specificity of 98 and 92%, respectively [12].
In contrast, Kindermann et al. evaluated the other rapid test, BM-Test (Boehringer Mannheim, Germany) in German children and found the outcome to be of poor sensitivity of 54%, albeit good specificity of 90% [13]. To our knowledge, studies with Assure™ H. pylori Rapid Test in children have not been previously reported. Our study using Assure™ H. pylori Rapid Test shows a satisfactory performance with sensitivity and specificity of 96 and 94%, respectively. The results are comparable to the study in Chinese adults which showed the sensitivity and specificity of 94% and 90%, respectively [14]. The good performance of the test in different populations suggests the highly conserved nature of the prepared antigen. However, more investigations in different population are required for supporting this speculation. Hanvivatvong et al. reported the results of the two rapid tests, anti-H. pylori IgG Immunocomb (Orgenics, Yavne, Israel) and Pyloriset Dry (Orion, Espoo, Finland), in Thai adults and demonstrated relatively poor results with the sensitivity of 93% and 86%, specificity of 58% and 72%, and accuracy of 77% and 79%, respectively [15]. The performance of the commercial serologic tests varies in different population, mostly due to geographic variation of H. pylori strains, host immune response [8] and the difference in prevalence of the disease [12]. It is likely that serological tests developed and validated for a specific population may not be applicable universally.

The immunoblotting detects antibodies to specific proteins of H. pylori including virulence factors, CagA and VacA [16]. Its performance has been shown to be superior to conventional ELISA [17]. Moreover, it can provide the possible relationships between disease presentations and the presence of H. pylori specific antigens [18]. CagA and/or VacA have been shown to be the markers of peptic ulcer disease and gastric cancer in many studies [19–21] but not in Asian population in whom the majority are infected with CagA and VacA-positive strains [22]. Our study showed that most Thai children are infected with CagA and VacA-positive H. pylori, similar to previous studies [23, 24]. The majority of the H. pylori-infected patients in this study had gastritis demonstrated by endoscopy. Only one patient had a duodenal ulcer. It is unlikely that CagA and VacA are the markers for the severe disease in Thai children with H. pylori infection.

Previous studies from Turkey and Portugal [25, 26] have demonstrated a satisfactory performance using Helicoblot 2.1 for the diagnosis of H. pylori in children. To date this kit has not been evaluated in Asian children. This study showed a good performance of Helicoblot 2.1 for the diagnosis of H. pylori in Thai children with the sensitivity and specificity of 100% and 96%, respectively. Although immunoblotting was not performed in all patients due to the limited resource, the test covered more than 90% of the patients. Moreover, the percentage of H. pylori infection of the tested group was not significantly different from the whole group (29.3% vs. 30.5%, p > 0.05). Therefore the result is reliable.

The accepted standard criteria for the diagnosis of H. pylori infection require endoscopically obtained gastric tissue for histological examination and/or culture and urease test. Endoscopic examination also provides the information of underlying mucosal diseases. Unfortunately, in most parts of Thailand the pediatric endoscopic facility is lacking resulting in problematic clinical decision making. Our results suggest that ICM and immunoblotting may be used as alternative methods for the diagnosis of H. pylori infection in Thai children when endoscopic facility is not available. However, the immunoblotting is more expensive than ICM and requires more complicated laboratory facilities. Our suggestion therefore is to use ICM as the first line and immunoblotting in those whose ICM is equivocal.

Conventionally, culturing for H. pylori forms an essential diagnostic step. This test, however, is not routinely performed in our country due to financial constraint. The CLOtest, a commercial rapid urease test used in this study, has been shown to have a sensitivity and specificity of 100% for detecting H. pylori infection in Thai adults while histology yielded sensitivity and specificity of 100% and 72%, respectively [27]. For histological examination in this study, we also performed immunohistochemical stain in addition to routine H&E and Giemsa stains. Immunohistochemical stain has been shown to increase the sensitivity and specificity and had a lower inter-observer variation [28, 29]. There was a disagreement in only one patient between histological examination and rapid urease tests in this study.

Conclusions
The immunochromatographic and immunoblot tests are non-invasive, reliable and useful for the diagnosis of H. pylori infection in Thai children.

References


