Comparison of Three Commercially Available Enzyme-Linked Immunosorbent Assays and Biopsy-Dependent Diagnosis for Detecting *Helicobacter pylori* Infection

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We evaluated the performance of three enzyme-linked immunosorbent assays (ELISAs) in detecting serum immunoglobulin G (IgG) and IgA antibodies to *Helicobacter pylori*; two were new ones from Pyloriset (Pyloriset EIA-G update and Pyloriset EIA-A update; Orion Diagnostica, Espoo, Finland), and the third was the Malakit EIA-G (Biolab, Limal, Belgium). Serum samples from 154 dyspeptic patients were collected. As a reference method, multiple biopsy specimens from different anatomical areas of the stomach were obtained by endoscopy and were analyzed by culture and/or histology and direct urease testing. Accordingly, 126 patients (82%) were found to be *H. pylori* positive and 28 patients (18%) were found to be *H. pylori* negative. To validate serology as a predictor of *H. pylori* infection, sensitivity, specificity, positive and negative predictive values, and accuracy of the assays were calculated against the *H. pylori* status as determined by the reference method. The corresponding data for the different ELISAs were 100%, 79%, 95%, 100%, and 96% for the Pyloriset EIA-G update, 81%, 89%, 97%, 52%, and 82% for the Pyloriset EIA-A update, and 87%, 86%, 96%, 60%, and 87% for the Malakit EIA-G, respectively. We conclude that the Pyloriset EIA-G update is a reliable and accurate test and that because of its 100% sensitivity, conjunctional IgA testing is not necessary. Its 100% negative predictive value makes it a very useful screening test. For purposes of excluding infection with *H. pylori*, the performance of the Malakit EIA-G is moderate but can be improved by conjunctional IgA testing. The Pyloriset EIA-A update can be useful as such a conjunctional test.

After an individual becomes infected with *Helicobacter pylori*, a chronic, active, antral gastritis that persists for life in most individuals occurs (3). Currently, *H. pylori* is considered to be the major etiological factor in peptic ulcer disease (17, 20, 27) and a probable initiating factor in gastric carcinoma (3, 23) and gastric lymphoma (30). Successful eradication of the bacteria cures (chronic) peptic ulcer disease. It is therefore generally agreed that all patients with proven ulcer disease need antibiotic treatment, so long as infection with *H. pylori* has been established (3, 8, 20). Detecting the infection by analysis of endoscopically obtained biopsy specimens by culture and/or histology and/or direct urease test is the investigational method of choice (1), assuming that multiple biopsy specimens from different gastric areas are taken to minimize sampling errors (2, 19). However, endoscopy is an invasive and rather uncomfortable as well as expensive method. Therefore, diagnostic alternatives to detect this infection have been developed. One attractive alternative is serology; it is cheap, simple, and relatively noninvasive and causes less patient discomfort than endoscopy. It is known that infection with *H. pylori* elicits both a systemic as well as a local immune response involving predominantly immunoglobulin G (IgG) and IgA antibodies. This immune response probably offers no protection against *H. pylori*, but it can nevertheless be of diagnostic value (7). Several methods to detect this response are available, including hemagglutination, bacterial agglutination, complement fixation, indirect immunofluorescence, and enzyme-linked immunosorbent assay (ELISA) (25). ELISA is attractive, because it is cheap, quick, simple, and capable of determining class-specific immunoglobulins. Most people, however, have a predominately IgG immune response to infection with *H. pylori*. We previously validated the commercially available Pyloriset EIA-G (Orion Diagnostica, Espoo, Finland) in the same study population as is investigated in the present study (28). The test yielded 81% sensitivity and 89% specificity. This result was comparable to findings by others using the same assay (10–12, 16, 18). Because these results demonstrated that the test needed improvement, a new generation was developed. In this study, we evaluate these new-generation ELISAs, Pyloriset EIA-G update and Pyloriset EIA-A update (Orion Diagnostica), and another commercial assay, the Malakit EIA-G (Biolab, Limal, Belgium) (4–6, 9), and compare them with conventional biopsy-based tests. We were able to directly compare these assays with one another and also, because we used the same study population, to directly compare the results of the old Pyloriset EIA-G with the results of the new Pyloriset EIA-G update.

MATERIALS AND METHODS

Biopsy specimens and serum samples were collected from 170 patients. All these patients suffered from upper abdominal complaints which necessitated upper endoscopy, and they either had ulcers or their referring physician had requested the collection of biopsy specimens. Patients who had received antibiotics in between biopsy analysis and serological analysis (13 patients) as well as patients whose biopsy specimens were analyzed by only one diagnostic method (3 patients) were excluded. The study population therefore consisted of 154 patients (103 male, 51 female) with a mean age of 49 years (range, 18 to 82 years). In a subgroup of 136 patients, nine biopsy specimens each were obtained. Six of these specimens, two from the antrum, two from the corpus, and two from the cardia, were analyzed by histology. These six biopsy specimens were fixed in neutral-buffered 4% formaldehyde. Giemsa-stained sections of paraffin-embedded tissue were studied. Histology was considered positive if *H. pylori* was found...
TABLE 1. Definition of H. pylori status

<table>
<thead>
<tr>
<th>Histology</th>
<th>Bacterial culture</th>
<th>CLO test</th>
<th>Definition*</th>
<th>No. of patients</th>
</tr>
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<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>HP+</td>
<td>92</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>HP+</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>HP+</td>
<td>2</td>
<td></td>
</tr>
<tr>
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<td>+</td>
<td>HP+</td>
<td>4</td>
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<td>HP+</td>
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</tr>
<tr>
<td>-</td>
<td>-</td>
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<td></td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>HP−</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>NPb</td>
<td>HP+</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>NP</td>
<td>HP+</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>NP</td>
<td>HP−</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>NP</td>
<td>HP−</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

* HP+, H. pylori positive; HP−, H. pylori negative.

b NP, not performed.

in any of the six biopsy samples. Histology was considered negative if H. pylori was found in none of the six biopsy samples. Two antral biopsy specimens were taken for bacteriological examination (of which only one was processed), and one antral biopsy specimen was analyzed by a CLO test (Delta-West). The CLO test was read after 24h. In a subgroup of 18 patients, three biopsy specimens were observed. Two antral biopsy specimens were analyzed by histology (as described above), and one antral biopsy specimen was analyzed by a CLO test. On the basis of the biopsy results, all patients were classified as either H. pylori positive or H. pylori negative. Table 1 shows which diagnostic techniques were performed, their results, and how we interpreted the various test results. According to this classification, 126 patients (82%) were H. pylori positive and 28 patients (18%) were H. pylori negative.

For all these 154 patients, a serum sample from each was tested for the presence of serum IgG antibodies to H. pylori (Pyloriset EIA-G update and Malakit EIA-G) and/or serum IgA antibodies to H. pylori (Pyloriset EIA-A update). The investigations were not aware of the H. pylori status of the patients whose blood they investigated. Samples were processed on a single day by a single operator.

**Culture.** Biopsy specimens for bacteriological examination were transported in thioglycolate broth, kept at 4°C, and processed within 4h. Incubation took place for 7 days on selective (Oxoid SR 147 E supplement) and nonselective columbia agar (Oxoid CM 331) with 7% lysed horse blood at 37°C and under a microaerobic atmosphere (Anoxomat 80% N2, 5% CO2, 5% H2, and 8% O2). Typical colonies were selected for identification by Gram stain, hirrurate reaction, and urease and catalase activity.

**Serology.** Blood samples were taken from the patients. The serum was separated, divided in two tubes, and stored at −20°C. The Pyloriset EIA-G update and EIA-A update (Orion Diagnostica) assays were performed according to the manufacturer’s instructions. Briefly, the sera were diluted 1:200 in a serum dilution buffer. Diluted samples (100 μl) and four diluted reference serum samples were pipetted onto the strips, which were coated with inactivated H. pylori antigens. The plates were incubated for 60 min at room temperature. The wells were washed three times and tapped dry. An enzyme conjugate, alkaline phosphatase (100 μl of swine anti-human IgG for EIA-G or swine anti-human IgA for EIA-A), was pipetted into each well. The wells were covered with plastic tape, and the plates were incubated for 30 min at room temperature. The wells were washed twice with 200 μl of washing buffer and twice with distilled water. A fresh substrate solution, p-nitrophenyl phosphate (100 μl), was added, and the plates were incubated for 30 min at room temperature. The enzyme reaction was stopped with 100 μl of 1 M NaOH. A405 readings were determined with a spectrophotometer. The titer of each patient’s serum was read from a graph based on the standard curve obtained with a semilogarithmic paper, with the mean A405 readings of the reference sera with known titers being used. A sample was considered positive for IgG antibodies to H. pylori or IgA antibodies to H. pylori if the absorbance of the serum samples was equal to or higher than the absorbance of the second reference. If it was not, the sample was considered negative.

The Malakit test (Biolab) was performed according to the manufacturer’s instructions. The sera were diluted 1:200 in a diluent buffer. The diluted samples (100 μl) and six calibrators, including one cutoff calibrator, were pipetted into wells coated with purified H. pylori antigens. The wells were covered and incubated for 30 min at room temperature, washed three times, and tapped dry. An enzyme conjugate, peroxidase-labeled anti-human IgG (rabbit), was pipetted into each well. The wells were incubated and washed as described before. A substrate, tetramethylbenzidine, was added. Incubation took place in the dark at room temperature for 30 min. The enzyme reaction was stopped with 25 μl of sulfuric acid. A405 readings were determined. A graph was made by plotting the absorbance value of the calibrators against the known arbitrary units of the calibrators per milliliter. Titers were read from the graph. A serum sample was considered positive for IgG antibodies to H. pylori if the absorbance of the serum sample was equal to or higher than the absorbance value of the cutoff calibrator. If it was not, the sample was considered negative.

**Statistical methods.** To validate serology as a predictor of H. pylori infection, the sensitivity, specificity, positive predictive value, negative predictive value, and accuracy were calculated against the H. pylori status as determined by culture, histology, and direct urease test results.

**RESULTS**

The results shown in Table 2 reveal that the Pyloriset EIA-G update had an optimal sensitivity of 100%. The differences between the sensitivity, negative predictive value, and accuracy of the Pyloriset EIA-G update and the corresponding values of the other two tests were statistically significant (Table 2). The Pyloriset EIA-A update performed best in specificity (although this difference was not statistically significant), with the Malakit EIA-G falling in between. In one patient, all three ELISAs were false-positive; histology, culture, and the direct urease test were negative in this patient, but these could be false-negative biopsy results. This patient was a 83-year-old woman who suffered from a nonsteroidal antiinflammatory drug-induced gastric ulcer. Cross-reactivity with other organisms might be an alternative explanation (13, 14, 21, 24). She might also have had H. pylori infection earlier in her life, but although the immune response had sustained, she might have cleared the infection. All four patients with a false-positive Malakit EIA-G test result also had a false-positive test result with the Pyloriset EIA-G update. Two out of three patients with a false-positive result with the Pyloriset EIA-A update also showed a false-positive result with the Pyloriset EIA-G update.

The Pyloriset EIA-A update yielded the most false-negative test results, thus strengthening the former assumption that an IgA immune response is not apparent in many patients with H. pylori infection and that most, though not all, people have a predominantly IgG immune response to infection with H. pylori.

Combining the results of the Pyloriset EIA-G update and the Pyloriset EIA-A update (Table 3) did not improve the sensitivity and/or specificity achieved with the Pyloriset EIA-G update alone. In fact, such a combination lowered the speci-
The very high incidence of infection makes it necessary to reduce sampling errors and thus optimize the reference method (2, 19). The previous version of the Pyloriset EIA-G in the same population (28), we can directly compare the performances of the Pyloriset EIA-G update and the older version. It can be concluded that the Pyloriset EIA-G update is indeed an improvement. Because of its high positivity of 100%, the negative predictive value will be 100% in all populations. The positive predictive value will be high in population with high frequency of H. pylori infection, whereas it will be low in a population in which H. pylori infection is uncommon. This high negative predictive value makes it a very useful screening test to exclude H. pylori infection.

The previous version of the Pyloriset EIA-A was found to have sensitivity values ranging from 39 to 82% and specificity values ranging from 65 to 96% (10–12, 16). The results we found with the Pyloriset EIA-A update were highly comparable to those found by Granberg et al. (12) with the older version, suggesting that this new assay may not be an improvement compared with the previous one. However, the same restrictions in comparing the two assays, which were tested on different populations with different age distributions and for which different reference methods were used, as was described above for the Pyloriset EIA-G, occur. In general, one can expect an IgA ELISA to have lower sensitivity values than an IgG ELISA because most individuals exhibit a predominantly IgG immune response to infection with H. pylori. Some authors have found (12, 15) that about 2% of the investigated patients produce an IgA response in the absence of an IgG response. We can’t confirm this observation for the Pyloriset EIA-G update. In our population, only 1 out of 154 patients was IgA positive and IgG negative (Table 3), but this was a false-positive IgA test result. Although an IgA ELISA can be of diagnostic value when used in conjunction with an IgG ELISA (12, 16, 29), in the case of the Pyloriset EIA-G update, the IgA ELISA had no additional diagnostic value. Furthermore, we think that since the Pyloriset IgG alone yielded a sensitivity of 100% in a large population of dyspeptic patients, the use of a conjunctival IgA ELISA is redundant, especially when cost aspects are taken into account. This conclusion does not mean that a conjunctival IgA ELISA in general can’t be of (additional) diagnostic value.

The previous version of the Pyloriset EIA-G was found to have sensitivity values ranging from 76 to 92% and specificity values ranging from 75 to 100% (10–12, 16, 18). The results we achieved with the Pyloriset EIA-G update suggest that this new version has a greatly improved sensitivity (100%) but a specificity that is at the lower end of previously published results. However, it must be noted that the reference method to which a test is compared, the chosen cutoff values, and differences in the age distribution of the population studied can influence the results. It is, for example, known from the literature that serology becomes less reliable in the elderly (22). We also found that the older version of the Pyloriset EIA-G gained in specificity when older patients were excluded and furthermore that sensitivity was improved by lowering the cutoff value, which was recommended by the manufacturer (28). Since we tested the previous version of the Pyloriset EIA-G in the same population (28), we can directly compare the performances of the Pyloriset EIA-G update and the older version. It can be concluded that the Pyloriset EIA-G update is indeed an improvement. Because of its high sensitivity of 100%, the negative predictive value will be 100% in all populations. The positive predictive value will be high in population with high frequency of H. pylori infection, whereas it will be low in a population in which H. pylori infection is uncommon. This high negative predictive value makes it a very useful screening test to exclude H. pylori infection.

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Blecker et al. reported that the Malakit EIA-G has very high sensitivity (96 to 100%) and specificity (96 to 98%) (4–6). Also, some other authors found high sensitivity and specificity values with the Malakit EIA-G (9). Like Taha et al., who also evaluated the Malakit EIA-G (26), we could not confirm these high values in our study. As can be seen from Table 4, in the case of the Malakit IgG, a conjunctival IgA ELISA can be of additional diagnostic value because of increased sensitivity. Thirteen patients were IgA positive (of which two were false positive) and IgG negative.

From our results, we conclude that the Pyloriset EIA-G update performed better than the previous version and can, because of its high sensitivity, reliably serve to exclude H. pylori infection (as a screening test) and thus serve as an alternative to endoscopy. The conjunctival use of the Pyloriset EIA-A update did not improve performance. The Pyloriset EIA-A update can be useful as a conjunctival test when an IgG ELISA with lower sensitivity values is used. In the case of the Malakit EIA-G, which in our study only had moderate sensitivity and specificity values, the use of a conjunctival IgA ELISA can offer some additional diagnostic value.

**ACKNOWLEDGMENT**

We thank Zambon/Imphos b.v., Amersfoort, The Netherlands, for providing the Pyloriset EIA-G update and the Pyloriset EIA-A update.

### Table 3. Combined results of Pyloriset EIA-G update and EIA-A update compared with results of the reference method

<table>
<thead>
<tr>
<th>Detection of antibodies</th>
<th>No. H. pylori positive</th>
<th>No. H. pylori negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG+ and IgA+</td>
<td>102</td>
<td>2</td>
</tr>
<tr>
<td>IgG+ and IgA–</td>
<td>24</td>
<td>4</td>
</tr>
<tr>
<td>IgG– and IgA+</td>
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<td>1</td>
</tr>
<tr>
<td>IgG– and IgA–</td>
<td>0</td>
<td>21</td>
</tr>
</tbody>
</table>

*The combined results of histology, direct urease testing, and (when performed) culture.

### Table 4. Combined results of Malakit EIA-G and Pyloriset EIA-A update compared with results of the reference method

<table>
<thead>
<tr>
<th>Detection of antibodies</th>
<th>No. H. pylori positive</th>
<th>No. H. pylori negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG+ and IgA+</td>
<td>91</td>
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</tr>
<tr>
<td>IgG+ and IgA–</td>
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<td>3</td>
</tr>
<tr>
<td>IgG– and IgA+</td>
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</tr>
<tr>
<td>IgG– and IgA–</td>
<td>5</td>
<td>22</td>
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*The combined results of histology, direct urease testing, and (when performed) culture.
REFERENCES