Evaluation of Commercial Immunoassays for Detection of Antibody against *Helicobacter pylori* in Thai Dyspeptic Patients

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The performance of five immunoassays for detection of immunoglobulin G antibody against *Helicobacter pylori* in 191 dyspeptic patients was evaluated. The sensitivities, specificities, accuracies, positive predictive values, and negative predictive values ranged from 86.32 to 97.89%, 57.95 to 72.22%, 77.02 to 83.76%, 71.54 to 77.42%, and 83.33 to 96.23%, respectively. The immunoglobulin A test kit also gave a high sensitivity and negative predictive value (95.79 and 91.40%, respectively), while the specificity was relatively low (51.14%).

Serological assay for *Helicobacter pylori* antibody is a noninvasive method to detect *H. pylori* infection. It has been reported to have sensitivity and specificity in predicting the status of *H. pylori* infection in untreated patients as accurately as invasive tests (11, 12). However, it has been suggested that serological tests for *H. pylori* should be locally validated (7), because assays validated in one region may yield variable diagnostic performances in others. These variations may be attributed to many factors, including the source of antigen used, the prevalence of infection in each population studied, and the reference method used to determine true *H. pylori* infection status. Therefore, reevaluation is needed before implementing a test in different populations. In Thailand, the seroprevalence of *H. pylori* infection has been reported to be higher than that in industrialized countries (10), and commercially available enzyme immunoassay (EIA) test kits have been reported to have lower sensitivities and specificities compared to in-house EIAs in Thai dyspeptic patients (1). We therefore evaluated the performance of five commercial test kits for detecting of immunoglobulin G (IgG) antibody to *H. pylori*. Three of them use a standard enzyme-linked immunosorbent assay (Cobas Core anti-*H. pylori* EIA [Roche, Mannheim, Germany]; Pyloriset EIA-GIII [Orion, Espoo, Finland]; and Enzygnost anti-*H. pylori* II/IgG [Dade Behring, Marburg, Germany]), and two are rapid assay test kits (Pyloriset Dry [Orion] and anti-*H. pylori* IgG Immunocomb [Orgenics, Yavne, Israel]). One *H. pylori* IgA antibody test kit (Pyloriset EIA-AIII) was also evaluated.

A total of 191 patients (57 males and 134 females; age range, 16 to 83 years [mean, 39 years]) were studied. Endoscopy was performed in all patients, and 183 (95.81%) of them were diagnosed as having nonulcer dyspepsia while the remaining 8 patients (4.19%) had a duodenal ulcer. Patients who received antibiotic therapy, bismuth treatment, or a proton pump inhibitor or H_2 blocker within 1 month prior to the study were excluded. Written informed consent was obtained from all patients before the study. Five milliliters of clotted blood was

obtained on the day of endoscopy. Sera were kept at -20°C until analyzed. The biopsy specimens from the antrum and stomach body were obtained for rapid urease (CLO) test and histological and cultural examination. All the tests were performed according to the manufacturer's instructions and without the knowledge of the status of the patient's infection. The results of these examinations were described previously (4).

In this study, a patient was considered infected with *H. pylori* when either culture was positive or both rapid urease (CLO) test and histological analysis were positive.

Statistic analyses for sensitivity, specificity, accuracy, positive predictive value, and negative predictive value were calculated against the status of *H. pylori* infection. As shown in Table 1, the three standard EIAs for IgG antibody gave a similar higher sensitivity (95.95 to 97.89%) and negative predictive value (92.06 to 96.23%) when compared to the rapid immunoassays. The specificity of all tests was considered low (57.95 to 69.57%), while the accuracy was similar, with the highest at 83.76% by Cobas Core anti-*H. pylori* EIA. The agreement between each test as analyzed by kappa statistic was relatively high among the standard immunoassays. Pyloriset Dry gave the lowest agreement with other tests, especially with Immunocomb (Table 2).

As reported by other investigators who have found that Western antigen-based serology has relatively poor performance with samples from Asian groups (5, 6, 8), we also found a low specificity of these tests in our study. The possible reasons may be due to the high prevalence rate of *H. pylori* infection in the Thai population (10). Therefore, the presence of antibody in some sera may reflect past infection. Furthermore, the results of validation are highly dependent on the reliability of the reference method used, and it is generally accepted that all the tests for *H. pylori* have their pitfalls and limitations that may affect the status of infection. In this study, the status of infection depended on the results of culture or histology and rapid urease (CLO) test. We observed that 10 out of 35 seropositive (as demonstrated by at lease two serological tests used in this study) patients in the 92 noninfected groups were concomitantly positive by histology or urease test. Most of them had higher antibody levels than those who were

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TABLE 1. Performance of immunoassays for the detection of antibody to *H. pylori*

Kit	No. tested	No. of *H. pylori*-positive (No. of true positive)[a]	No. of *H. pylori*-negative (no. of true negative)[a]	Sensitivity (%)	Specificity (%)	Accuracy (%)	Positive predictive value (%)	Negative predictive value (%)
Cobas Core anti-*H. pylori* EIA	191	124 (99)	67 (92)	96.97	69.57	83.76	77.42	95.52
Enzygnost anti-*H. pylori* II/IgG	191	129 (99)	62 (92)	95.95	63.64	80.64	73.44	92.06
Pyloriset EIA-G III	183	130 (95)	53 (88)	97.89	57.95	78.69	71.54	96.23
Anti-*H. pylori* IgG Immunocomb	161	111 (86)	50 (75)	93.02	58.67	77.02	72.07	88.00
Pyloriset Dry	185	107 (95)	78 (90)	86.32	72.22	79.46	76.64	83.33
Pyloriset EIA-A III	183	134 (95)	49 (88)	95.79	51.14	74.32	67.91	91.40

[a] Numbers of true positive and true negative were determined by the results of culture or CLO test and histology.

positive by serology alone, as shown in Fig. 1. Therefore, it is possible that some patients of the group may have had *H. pylori* infection during the study period. Figure 1 also demonstrates the distribution of *H. pylori* IgG antibody levels in the *H. pylori*-infected and noninfected patients.

The performance of the rapid tests was similar to that reported previously (2) in that they were slightly inferior to the standard EIA tests. Although better results have also been reported (9, 14), the use of rapid tests has not been recommended (7). However, these tests are easy to perform and can be finished within a few minutes without the need of sophisti-

cated equipment. By using these tests and with careful interpretation, the physician can determine *H. pylori* infection of a patient at the first consultation.

Anti-*H. pylori* IgA antibody was found in 91 out of 95 (95.79%) *H. pylori*-infected patients (Table 1). Almost all, except one, were also found to have IgG antibody (by Pyloriset EIA-GIII) in their serum. Combining the results of this Pyloriset EIA-AIII and Pyloriset EIA-GIII slightly increased the sensitivity and the negative predictive value, but the specificity was markedly decreased (data not shown). Although anti-*H. pylori* IgA antibody has been reported to have diagnostic values
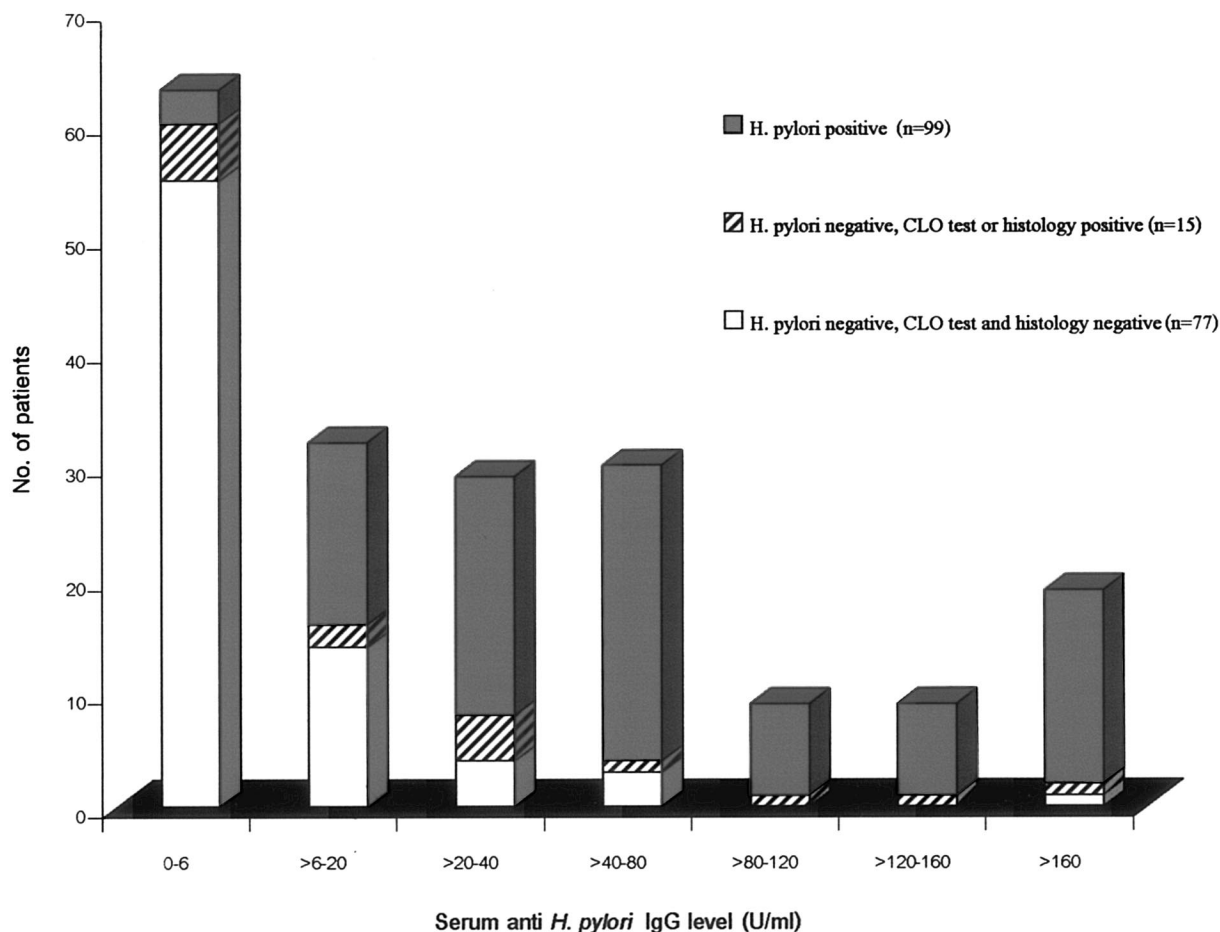

FIG. 1. Distribution of anti-*H. pylori* IgG antibody levels in *H. pylori*-infected and uninfected patients (*n* = 191). Results of the Cobas Core anti-*H. pylori* EIA test, with serum antibody levels of <6 U/ml, were considered negative.

TABLE 2. Agreement between test kits by kappa statistic

Kit	Agreement[a] (kappa) with:			
	Enzygnost	Pyloriset EIA-G III	Immunocomb	Pyloriset Dry
Cobas Core anti-*H. pylori* EIA	0.79	0.78	0.79	0.67
Enzygnost anti-*H. pylori* II/IgG		0.76	0.71	0.64
Pyloriset EIA-G III			0.77	0.59
Immunocomb				0.55

[a] The degree of agreement between each test kit was significant ($P < 0.001$) by kappa statistic analysis.

when used in conjunction with IgG (3, 13), we agree with a previous report (12) that IgA had no additional diagnostic value in our clinical settings.

With regards to the high sensitivity and negative predictive values of the commercial *H. pylori* IgG antibody kits used in this study, we concluded that, with careful interpretation, these tests may be used as an alternative test for determining *H. pylori* infection, especially in those for whom gastroscopy cannot be performed.

REFERENCES

1. **Bodhidatta, L., C. W. Hoge, S. Churnratanakul, W. Nirdnoy, P. Sampathanukul, C. Tungtaem, S. Raktham, C. D. Smith, and P. Echeverria.** 1993. Diagnosis of *Helicobacter pylori* in a developing country: comparison of two ELISAs and a prevalence study. J. Infect. Dis. **168:**1549–1553.
2. **Chen, T. S., F. Y. Chang, and S. D. Lee.** 2002. No difference of accuracy between capillary and venous blood in rapid whole blood test for diagnosis of *Helicobacter pylori*. Dig. Dis. Sci. **47:**2519–2522.
3. **Granberg, C., A. Mansikka, O. P. Lehtonen, H. Kujari, R. Gronfors, H. Nurmi, I. Raiha, M. R. Stahberg, and R. Leino.** 1993. Diagnosis of *Helicobacter pylori* infection by using Pyloriset EIA-G and EIA-A for detection of serum immunoglobulin G (IgG) and IgA antibodies. J. Clin. Microbiol. **31:**1450–1453.
4. **Hanvivatvong, O., K. Tatiyakavee, D. Thong-Ngam, A. Pongpanich, C. Chirathaworn, P. Suwanagool, P. Nunthapisud, and P. Kullavanijaya.** 2002. Detection of antibody in serum and secretion for the diagnosis of *Helicobacter pylori* infection. J. Med. Assoc. Thai. **85**(Suppl. 1)**:**S383–S388.
5. **Kim, S. Y., J. S. Ahn, Y. J. Ha, H. J. Doh, M. H. Jang, S. I. Chung, and H. J. Park.** 1998. Serodiagnosis of *Helicobacter pylori* in Korean patients using enzyme-linked immunosorbent assay. J. Immunoassay **19:**251–270.
6. **Leung, W. K., E. K. Ng, F. K. Chan, S. C. Chung, and J. J. Sung.** 1999. Evaluation of three commercial enzyme-linked immunosorbent assay kits for diagnosis of *Helicobacter pylori* in Chinese patients. Diagn. Microbiol. Infect. Dis. **34:**13–17.
7. **Malfertheiner, P., F. Megraud, C. O'Morain, A. P. Hungin, R. Jones, A. Axon, D. Y. Graham, G. Tytgat, et al.** 2002. Current concepts in the management of *Helicobacter pylori* infection—the Maastricht 2–2000 consensus report. Aliment. Pharmacol. Ther. **16:**167–180.
8. **Matsuo, K., N. Hamajima, S. Tominaga, T. Suzuki, T. Nakamura, and A. Matsuura.** 2000. *Helicobacter pylori* IgG antibody test established in the United States showed a substantially lower sensitivity for Japanese population. Am. J. Gastroenterol. **95:**1597–1598.
9. **Oksanen, A., L. Veijola, P. Sipponen, K. O. Schauman, and H. Rautelin.** 1998. Evaluation of Pyloriset Screen, a rapid whole-blood diagnostic test for *Helicobacter pylori* infection. J. Clin. Microbiol. **36:**955–957.
10. **Perez-Perez, G. I., D. N. Taylor, L. Bodhidatta, J. Wongsrichanalai, W. B. Baze, B. E. Dunn, P. D. Echeverria, and M. J. Blaser.** 1990. Seroprevalence of *Helicobacter pylori* infections in Thailand. J. Infect. Dis. **161:**1237–1241.
11. **Talley, N. J., D. G. Newell, J. E. Ormand, H. A. Carpenter, W. R. Wilson, A. R. Zinsmeister, G. I. Perez-Perez, and M. J. Blaser.** 1991. Serodiagnosis of *Helicobacter pylori*: comparison of enzyme-linked immunosorbent assays. J. Clin. Microbiol. **29:**1635–1639.
12. **B. A. M. van de Wouw, W. A. de Boer, A. R. Jansz, R. T. J. M. Roymans, and A. P. G. Staals.** 1996. Comparison of three commercially available enzyme-linked immunosorbent assays and biopsy-dependent diagnosis for detecting *Helicobacter pylori* infection. J. Clin. Microbiol. **34:**94–97.
13. **Von Wulffen, H., and H. J. Grote.** 1988. Enzyme-linked immunosorbent assay for detection of immunoglobulin A and G antibodies to *Campylobacter pylori*. Eur. J. Clin. Microbiol. Infect. Dis. **7:**559–565.
14. **Wong, W. M., S. K. Lam, H. H. Xia, V. S. Tang, K. C. Lai, W. H. Hu, D. K. Chan, K. L. Chung, and B. C. Wong.** 2002. Accuracy of a new near patient test for the diagnosis of *Helicobacter pylori* infection in Chinese. J. Gastroenterol. Hepatol. **17:**1272–1277.