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Comparison of Commercial Diagnostic Tests for *Helicobacter pylori* Antibodies

M. A. SCHEMBRI, S. K. LIN, AND J. R. LAMBERT*

Department of Medicine, Monash Medical Centre, Monash University, Clayton 3168, Victoria, Australia

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A number of serological tests measuring the presence of *Helicobacter pylori*-specific serum immunoglobulin G (IgG) are now commercially available. The aim of this study was to evaluate the clinical accuracy of five commercial *H. pylori* antibody tests: GAP-IgG (Biomerica), HELpTEST (AMRAD, Kew, Victoria, Australia), HELICO-G (Porton Cambridge), Pyloriset (Orion Diagnostica), and ROCHE (Roche Diagnostics). A total of 162 subjects presenting for routine upper endoscopy were studied. *H. pylori* was diagnosed if culture, histology, or both were positive. Ten milliliters of venous blood was collected at the time of endoscopy for serological assessment. The sensitivity and specificity of each test (GAP-IgG, HELpTEST, HELICO-G, Pyloriset, and ROCHE) were as follows: 83 and 79%, 92 and 77%, 86 and 65%, 89 and 56%, and 98 and 69%, respectively. Positive and negative predictive values were 97 and 83%, 90 and 91%, 76 and 83%, 68 and 84%, and 86 and 97%, respectively. The specificity of most tests increased by approximately 10% when sera from subjects less than 45 years old were examined. The number of sera falling into the grey zone for each test (an indeterminate result with respect to *H. pylori* status) varied between 2.5 and 19%. This study highlights the need for all serological kits to be independently evaluated on the population to be studied by testing against a microbiologically defined panel of *H. pylori*-positive and -negative sera.

Helicobacter pylori is the causative agent of type B active chronic gastritis (2, 3), and there is increasing evidence to support a pathogenic role in duodenal ulcer disease (8). The current "gold standard" tests for diagnosing *H. pylori* infection involve histological staining and/or culture of antral biopsy specimens (1). However, these methods are invasive, and alternative, noninvasive methods such as the urea breath test and serological tests are available (6). Various serological methods have been employed, including the complement fixation test, the bacterial agglutination test, the passive hemagglutination test, the hemagglutination assay, immunoblotting techniques, and enzyme-linked immunosorbent assays (ELISAs) (7). All of these techniques have demonstrated a correlation between the titers or level of reactivities and the presence of *H. pylori* in the gastric antrum. A number of different serological tests to determine *H. pylori* infection are now commercially available and are being used routinely in diagnostic laboratories. The aim of this study was to evaluate the clinical accuracy of five commercial *H. pylori* antibody tests, HELpTEST (AMRAD, Kew, Victoria, Australia), GAP-IgG (Biomerica), HELICO-G (Porton Cambridge), Pyloriset (Orion Diagnostica), and ROCHE (Roche Diagnostics), against a well-documented panel of 162 subjects diagnosed as either positive or negative for *H. pylori* infection.

(The results of this study were presented in part at the Fourth Workshop on Gastroduodenal Pathology and *Helicobacter pylori*, Bologna, Italy, 28 to 30 November, 1991.)

MATERIALS AND METHODS

Subjects. Serum samples were collected from 190 consecutive subjects presenting for upper endoscopy because of upper gastrointestinal symptoms. Endoscopic diagnoses included gastric ulcer (4.8%), duodenal ulcer (9%), gastric erosion (22.4%), reflux esophagitis (28%), and non-ulcer dyspepsia (35.2%). No subject had undergone previous gastroduodenal surgery or had taken antibiotics or bismuth preparations within the previous three months. Twenty-eight samples could not be tested with all five kits owing to an insufficient volume of sample, leaving a total of 162 subjects in the study. Blood samples were collected at the time of endoscopy and stored at −20°C until assayed. Approval for the study was obtained from the Monash Medical Centre Human Ethics Committee.

***H. pylori* assessment.** *H. pylori* status was assessed by culture and histology (6). Diagnosis of *H. pylori* infection was made if culture, histology, or both were positive. *H. pylori*-negative subjects were negative for both tests.

For culture, one antral biopsy was transported to the laboratory in 0.1 ml of sterile saline and processed within 3 h. It was finely minced in approximately one drop of saline, and about a quarter of this homogenate was pressed firmly between two glass microscope slides, one of which was Gram stained.

The remainder of the sample was cultured on chocolate (horse blood) agar (Columbia agar, Oxoid no. CM331) and on brain heart infusion agar (Oxoid no. CM375) supplemented with 7% horse blood, 3 μg of vancomycin per ml, 50 μg of nalidixic acid per ml, and 5 μg of trimethoprim per ml. The plates were incubated for up to 7 days in a humid atmosphere of 5% oxygen–10% carbon dioxide–85% nitrogen.

Isolates were considered to be *H. pylori* if they grew as 0.5- to 1-mm translucent greyish colonies after 3 days on chocolate (horse blood) agar and were gram-negative curved or S-shaped rods which were positive for oxidase, catalase, and urease. Growth occurred under microaerophilic conditions but not in air.

For histological analysis, one antral biopsy was fixed in 10% neutral buffered formalin and processed. Histological staining with hematoxylin and eosin and with modified Giemsa stain of antral biopsies was performed. Histological

* Corresponding author.

gastritis was defined as chronic inflammatory change with or without activity (6).

***H. pylori* antibody tests.** The HELpTEST, GAP-IgG, HELICO-G, and ROCHE ELISA evaluated in this study were of conventional design comprising the absorption and immobilization of antigen onto the wells of a 96-well microtiter plastic plate to capture *H. pylori* antibodies from sera that are then detected with an anti-human immunoglobulin G (IgG) coupled to horseradish peroxidase (HRPO), followed by reaction with a chromogenic enzyme substrate, tetramethylbenzidine. The Pyloriset test is a latex agglutination test for the rapid detection of *H. pylori* antibodies in serum. *H. pylori* antigen is incorporated into the latex reagent, and when this is added to a drop of diluted serum the agglutination of latex particles is indicative of the presence of *H. pylori* antibodies.

Each test was carried out, and its results were interpreted, according to the manufacturer's instructions. All tests except HELICO-G used a grey-zone region, which was an indeterminate result with respect to *H. pylori* status. Sensitivity was defined as the ability of the test to detect a *H. pylori*-positive specimen. Likewise, specificity was defined as the ability of the test to detect a *H. pylori*-negative specimen. The test results of specimens within the grey zones were excluded from sensitivity and specificity calculation. Positive and negative predictive values reflect the likelihood of a specimen identified as positive or negative being a *H. pylori*-positive or -negative specimen as defined by gold standard tests.

(i) HELpTEST. In the HELpTEST, each plate contained four negative and four positive control samples (provided by the manufacturer). Serum specimens were diluted 1:200 (5 μl of sample and 1 ml of specimen diluent), with 100 μl being added to each well. After incubation for 30 min at 37°C, each plate was washed six times with the wash buffer provided (approximately 250 μl per well per wash). After being dried, 100 μl of freshly prepared HRPO-IgG conjugate reagent was added to each well, and the plates were incubated for 30 min at 37°C. After repeat washings, 100 μl of freshly prepared substrate reagent was added to each well and the plates were incubated in the dark for 30 min at room temperature. Stopping solution (100 μl) was then added to each well to terminate the enzymatic reaction. The absorbance was read within 10 min with a 450-nm filter, with a 615- to 620-nm filter as the reference.

The cutoff value for the assay was calculated by multiplying the mean of the positive controls by 0.1 (lower cutoff) or 0.3 (upper cutoff). Values falling in between this range were regarded as intermediate (grey-zone) results. Both positive and negative controls were used to ensure that all reagents were working properly. The sensitivity and specificity of the test were claimed by the manufacturer to be 92 and 86%, respectively.

(ii) GAP-IgG test. In the GAP-IgG test, each plate contained three negative and four positive control samples (provided by the manufacturer). Serial twofold dilutions were made with the positive control. Serum specimens were diluted 1:200 (25 μl of sample and 5 ml of specimen diluent), with 100 μl being added to the wells in duplicate. After incubation for 1 h at room temperature, each plate was washed three times with the wash buffer provided (approximately 250 μl per well per wash). After being dried, 100 μl of HRPO-IgG conjugate reagent (exact volume not specified) was added to each well and the plates were incubated for 1 h at room temperature. After repeat washings, 100 μl of freshly prepared substrate reagent (exact volume not specified) was added to each well and the plates were incubated in the dark for 30 min at room temperature. Fifty microliters of stopping solution (exact volume not specified by the manufacturer) was then added to each well to terminate the enzymatic reaction. The A_{405} was read within 10 min against an air blank. The readings were expressed as positive/negative ratios by subtracting the mean negative control reading from the mean sample reading and then dividing this value by the mean negative control reading.

The cutoff value and grey zone for the assay were determined from the serial twofold dilutions of the positive control. Both positive and negative controls were used to ensure that all reagents were working properly. The sensitivity and specificity of the test were claimed by the manufacturer to be 78 and 86%, respectively.

(iii) HELICO-G test. In the HELICO-G test, each plate contained two positive control samples (calibrator sera provided by the manufacturer contained 10 U of specific antibody per ml). Serum specimens were diluted 1:200 (5 μl of sample and 1 ml of specimen diluent), with 100 μl being added to the wells in duplicate. After incubation for 1 h at 37°C, each plate was washed three times with the wash buffer provided (approximately 250 μl per well per wash). After being dried, 100 μl of freshly prepared HRPO-IgG conjugate reagent was added to each well and the plates were incubated for 30 min at 37°C. After repeat washings, 100 μl of freshly prepared substrate reagent was added to each well and the plates were incubated in the dark for 30 min at room temperature. Stopping solution (50 μl) was then added to each well to terminate the enzymatic reaction. The A_{450} was read within 10 min against an air blank.

The 10-U/ml calibrator serum positive control was used to determine the cutoff value of the assay. Mean sample absorbance readings greater than this value were considered positive for *H. pylori* infection. The sensitivity and specificity of the test were claimed to be 93 and 91%, respectively.

(iv) Pyloriset. In the Pyloriset test, positive and negative controls were provided by the manufacturer. Serum specimens were diluted 1:2 (25 μl of sample and 25 μl of specimen diluent), and 40 μl was pipetted onto one circle of the test card. One drop of the latex reagent was then added to the circle containing the diluted serum sample, and the droplets were mixed. The test slide was tilted and rotated in a circular motion for 3 min, during which time evidence for agglutination was observed.

The result was positive when agglutination was detected in the test circle within 3 min and negative if no agglutination was detected. The test is highly subjective and thus was carried out by one person, with the results being interpreted according to a scale ranging from 3+ to −. A grey zone (±) was incorporated owing to the nature of the test. Positive and negative controls were run along with each test sample to monitor the performance of the test reagent. The sensitivity and specificity of the test were claimed by the manufacturer to be 92.8 and 78.8%, respectively.

(v) ROCHE test. In the ROCHE test, each plate contained three negative control samples and one positive control sample (provided by the manufacturer). Serum specimens were diluted 1:101 (10 μl of sample and 1 ml of specimen diluent), with 100 μl being added to each well. After incubation for 30 min at 37°C, each plate was washed four times with the wash buffer provided (approximately 250 μl per well per wash). After being dried, 100 μl of freshly prepared HRPO-IgG conjugate reagent was added to each well and the plates were incubated for 30 min at 37°C. After repeat washings, 100 μl of freshly prepared substrate reagent was

TABLE 1. Summary of endoscopy findings: histology, serology, and culture results

Endoscopy diagnosis	No. of subjects with histologic gastritis/total no. (%)	No. of subjects seropositive/total no. (%)[a] by:					No. of subjects culture positive/total no. (%)
		GAP-IgG	HELp	HELICO-G	Pyloriset	ROCHE	
Normal	40/61 (66)	29/57 (51)	29/47 (62)	37/63 (59)	36/52 (69)	42/60 (70)	29/61 (48)
Esophagitis[b]	11/22 (50)	3/16 (19)	3/16 (19)	10/22 (45)	7/17 (41)	7/22 (32)	5/22 (23)
Gastritis[b]	19/32 (59)	14/30 (47)	14/30 (47)	16/32 (50)	20/29 (69)	16/30 (53)	12/32 (36)
Gastric ulcer	7/7 (100)	5/6 (83)	6/6 (100)	7/7 (100)	6/6 (100)	7/7 (100)	7/7 (100)
Duodenal ulcer	13/14 (93)	13/15 (87)	11/12 (92)	11/14 (79)	11/14 (79)	13/14 (93)	13/14 (93)
Duodenitis[b]	17/26 (65)	14/24 (58)	12/20 (60)	17/26 (65)	13/22 (57)	17/25 (68)	14/26 (54)

[a] Grey-zone results were excluded.
[b] Edema, erythema, or erosions.

added to each well and the plates were incubated in the dark for 10 min at room temperature. Stopping solution (100 μl) was then added to each well to terminate the enzymatic reaction. The absorbance was read within 10 min with a 450-nm filter against a reagent blank.

The cutoff value for the assay was calculated by adding 0.2 to the mean value of the absorbances of the negative controls. The grey zone was calculated as ±10% of this value. Both positive and negative controls were used to ensure that all reagents were working properly. The sensitivity and specificity of the test were claimed to be 95 and 97%, respectively.

RESULTS

In total, 162 subjects presenting for routine upper endoscopy were studied. The mean age of this group was 48 years (range, 16 to 86 years), with a male-to-female ratio of 70:92. These values remained similar when the population group was divided into *H. pylori*-positive and -negative subjects.

A summary of the endoscopic findings with reference to histology, serology, and culture results is presented in Table 1. Of the total 162 subjects, 80 were positive for *H. pylori* by culture or histology. Of the 13 culture-positive duodenal ulcer subjects, one was negative in the Pyloriset test only, one was negative in the HELICO-G test and intermediate (±) in the HELpTEST, and the other was positive only in the ROCHE test. The remaining 10 culture-positive duodenal ulcer subjects were positive in all tests. The one culture-negative duodenal ulcer subject was also negative in all serological tests. Of the seven gastric ulcer subjects (all culture positive), one was intermediate in the GAP-IgG test, one was negative in the GAP-IgG test and intermediate in the Pyloriset test, and the remaining five were positive in all tests.

The serology results for the total 162 subjects are shown in Table 2. When the less-than-45-year-old age group subpopulation was examined, the specificity and positive predictive value of most tests increased by approximately 10%, while the sensitivity and negative predictive value did not change significantly (Table 3).

Complete agreement was observed between the gold standard tests and all serological kits in 43% (69/162) of sera (45 positive and 24 negative). Disagreement between gold standard tests and more than one serological kit was found in sera from 57 subjects (16 positive and 41 negative). Thirteen sera from *H. pylori*-negative subjects were positive by four or more serological kits.

H. pylori was identified in 77 of 80 positive subjects by histology. In the 13 subjects found positive by four or more serological kits, *H. pylori* was not detected by culture or histology. Chronic active inflammation was observed in four subjects negative by culture or histology. This would suggest that *H. pylori* was not identified by culture or histology owing to technical reasons or that recent eradication or clearance of the bacteria had occurred.

DISCUSSION

In this study we have evaluated the clinical accuracy of five commercially available diagnostic tests for *H. pylori*. Subjects were considered positive for *H. pylori* only if

TABLE 2. Serology results for the total population (n = 162)

Assay	Serological result for subjects with:									
	H. pylori present[a]					*H. pylori* absent[b]				
	No. +	No. ±[c]	No. −	Sensitivity	PPV[d]	No. +	No. ±	No. −	Specificity	NPV[e]
GAP-IgG	63	4	13	83%	81%	15	10	57	79%	81%
HELp	60	15	5	92%	80%	15	16	51	77%	91%
HELICO-G	69	NA	11	86%	70%	29	NA	53	65%	83%
Pyloriset	62	10	8	89%	67%	31	12	39	56%	83%
ROCHE	78	0	2	98%	77%	24	4	54	69%	96%

[a] Positive by culture or histology.
[b] Negative by culture and histology.
[c] Grey zone. NA, not applicable.
[d] PPV, positive predictive value.
[e] NPV, negative predictive value.

TABLE 3. Serology results for the less-than-45-year-old age group (n = 78)

Assay	Serological result for subjects with:									
	H. pylori present[a]					*H. pylori* absent[b]				
	No. +	No. ±[c]	No. −	Sensitivity	PPV[d]	No. +	No. ±	No. −	Specificity	NPV[e]
GAP-IgG	29	2	7	81%	97%	1	6	33	97%	83%
HELp	28	7	3	90%	90%	3	8	29	91%	91%
HELICO-G	32	NA	6	84%	76%	10	NA	30	75%	83%
Pyloriset	28	6	4	88%	68%	13	6	21	62%	84%
ROCHE	37	0	1	97%	86%	6	2	32	84%	97%

[a] Positive by culture or histology.
[b] Negative by culture and histology.
[c] Grey zone. NA, not applicable.
[d] PPV, positive predictive value.
[e] NPV, negative predictive value.

culture, histology, or both were positive and were considered negative if both tests were negative. In this way we have attempted to reduce the number of false-negative subjects in the negative population group.

Each of the tests evaluated had the manufacturers' quoted sensitivities and specificities well above those determined in this study. The sensitivity and specificity of a particular test is highly dependent upon the population group examined. The vast differences in *H. pylori* prevalence among different ethnic groups may account for this, and it may be that cutoff values need to be readjusted for different populations.

The performance of the kits evaluated in this study was comparable to that of others reported in the literature (both commercial and in-house ELISAs). The type of antigen preparation used (either crude or purified) did not produce any significant differences in the overall results. Interestingly, the ROCHE kit had quite a low specificity despite its use of a purified antigen. A recent study by Hoek and colleagues comparing four commercially available kits found low specificities of the Pyloriset, GAP-IgG, and HELICO-G tests (4).

Thirteen of the sera from *H. pylori*-negative patients were positive by four or more of the kits. It is most likely that the serology results for these patients were correct and could be explained by either past or recently acquired infection. In addition, sampling problems from the antral mucosa may occur when areas of intestinal metaplasia are biopsied, giving a false-negative result. It would be of interest to follow up this group of patients with a ^{14}C- or ^{13}C-urea breath test.

The use of a grey zone was incorporated into all tests except for HELICO-G. The number of sera whose readings fell into this grey zone varied according to the test used and ranged from 2.5 to 19% of the total number of samples. This area of uncertainty appears to be a problem for all serological tests and is an area where further attention is required. In general, it was found that titers falling into the grey zone of one test also fell in or close to the grey zone of another test.

The correlation between *H. pylori* infection and seropositivity was greater in the less-than-45-year-old age group. The specificity of most tests improved approximately 10% when only this age group was examined. Reduced immunological responses in the elderly have been reported (5), and it is therefore important to consider age profiles when assessing the accuracy of serological kits. These findings differ from those of other studies, which show lower sensitivities and predictive values in subjects younger than 45 years (4). The explanation for these differences is unclear.

The use of serology as a diagnostic tool for the detection of *H. pylori* infection presents itself as a rapid and noninvasive technique. Our comparison of five commercially available serological kits has revealed most to have greater than 80% positive and negative predictive values in subjects less than 45 years of age. All serological kits need to be independently evaluated on the population to be studied by testing them against a microbiologically defined panel of *H. pylori*-positive and -negative sera. On the basis of this study, serology with the appropriate kit of choice offers a noninvasive, convenient, and inexpensive technology applicable to epidemiological studies and in the diagnosis of *H. pylori* infection. In choosing a serological kit one must consider cost, ease of use, sample numbers to be tested, and diagnostic accuracy.

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