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Evaluation of Eight Enzyme Immunoassays for Detection of Immunoglobulin G against *Helicobacter pylori*

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Eight commercial enzyme-linked immunosorbent assays (ELISAs) were used to test sera taken from 102 patients in whom *Helicobacter pylori* infection status had been determined by means of biopsy culture, PCR, histology, and urease production and by ^{13}C urea breath test. By those means, 61 patients had been found to be infected. Assays were compared by receiver operating characteristic analysis. Sensitivities ranged from 86 to 98%; specificities ranged from 83 to 98%. In a group consisting of the assays by Bio-Whittaker, Meddens Biotech, Orion (Pyloriset EIA G, new version), and Enteric Products, Inc. (HM Cap), differences in performance were not statistically significant. Sensitivities in this group ranged from 93 to 98%; specificities ranged from 95 to 98%. Assays from this group may be useful in addition to biopsy-based methods in diagnosing *H. pylori* infection.

Helicobacter pylori is well established as a major cause of type B gastritis and ulcer of the stomach and the duodenum (7), and it is associated with gastric carcinoma and lymphoma (1, 6, 8, 11, 17). Consequently, detection and cure of *H. pylori* infection have become important.

The diagnosis has so far most often been made by endoscopy-based methods, but serology can be a useful alternative (9, 14). Recently Marshall proposed a diagnostic protocol that does not include endoscopy in all cases (7).

In a subset of cases, a serologic test may be used as an alternative to invasive techniques, provided sensitivity and specificity are adequate for primary diagnosis and the test can be used for serological follow-up. It has been shown that serology can be used to evaluate therapy aimed at eradication of *H. pylori* (4, 5, 12, 16). The present study evaluates eight commercial enzyme-linked immunosorbent assays (ELISAs) for serodiagnosis of *H. pylori* infection.

TABLE 1. ELISA kits used

Kit	Commercial name	Supplier	Result type[a]
Orion (old)	Pyloriset EIA G	Orion, Espoo, Finland	Titer
Orion (new)	Pyloriset EIA G[b]	Orion, Espoo, Finland	Titer
Meddens		Meddens Biotech, Brummen, The Netherlands	Titer
Whittaker	PyloriStat	BioWhittaker, Walkersville, Md.	O. D. index
Porton	Helico-G	Porton, Newmarket, United Kingdom	Titer
HM Cap	HMCAP	EPI, Westbury, N.Y.	O. D. index
FlexSure	FlexSure	EPI	Quick positive/negative
μPLATE	μPlate Helicobacter IgG	Boehringer Mannheim, Germany	O. D. index

[a] Titer, estimate of antibody concentration; O. D. index, standardized result, depending linearly on optical density.
[b] The new version was introduced in 1995.

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Patients and *H. pylori* status. A total of 107 patients were seen by the gastroenterologist, presenting with upper gastrointestinal complaints necessitating gastroscopy. Patients who had received antibiotic therapy or bismuth treatment aimed at eradication of *H. pylori* up to 2 years previously were excluded. Furthermore, exclusion criteria were partial or complete gastrectomy in the past and therapy with a proton pump inhibitor in the preceding month. All patients gave informed consent.

In the remaining 102 evaluable patients, 42 were female and 60 were male; age ranged from 20 to 79 years; median age was 51 years. To establish the diagnosis of *H. pylori* infection, a ^{13}C urea breath test was done, and histologic examination for *H. pylori*, culture, PCR (15), and rapid detection of urease activity were performed on antral biopsy samples. A patient was considered infected if two or more of those five tests were positive.

Serology. ELISA kits used are summarized in Table 1. All tests were performed according to their respective manufacturers' instructions. For the FlexSure assay, negative readings were coded as 0, dubious readings were quantified as 0.5, and positive results were graded 1 to 4. According to the FlexSure kit insert, readings of 0 and 0.5 would be negative and readings of 1 and above would be positive. Tests of all sera were performed without knowledge of the patients' infection status.

TABLE 2. Numbers tested and Wilcoxon's *W*

Assay	No. of serum samples			*W* (standard error)
	Not infected	Infected	Not done	
Whittaker	41	61	0	0.998 (0.002)
Meddens	41	61	0	0.994 (0.005)
Orion (new)	41	61	0	0.993 (0.005)
HMCap	41	59	2	0.991 (0.006)
FlexSure	41	59	2	0.970 (0.016)
Porton	41	61	0	0.963 (0.016)
Orion (old)	41	61	0	0.956 (0.016)
μPlate	39	59	4	0.951 (0.022)

TABLE 3. Sensitivity (Se), specificity (Sp), and accuracy at 50% prevalence (Acc) at several cutoff values[a]

Assay	Optimal				Recommended (lower)				Recommended (upper)			
	Cutoff[b]	Acc (%)	Se (%)	Sp (%)	Cutoff[b]	Acc (%)	Se (%)	Sp (%)	Cutoff[b]	Acc (%)	Se (%)	Sp (%)
Whittaker	0.77	98	60/61 (98)	40/41 (98)	0.80	98	60/61 (98)	40/41 (98)	1.00	96	58/61 (95)	40/41 (98)
Meddens	14.9	97	60/61 (98)	39/41 (95)	15.0	97	60/61 (98)	39/41 (95)	20.0	95	58/61 (95)	39/41 (95)
Orion (new)	445	94	57/61 (93)	39/41 (95)	300	92	60/61 (98)	35/41 (85)				
HMCap	2.12	96	56/59 (95)	40/41 (98)	1.8	96	56/59 (95)	40/41 (98)	2.2	96	56/59 (95)	40/41 (98)
FlexSure	1	95	54/59 (92)	40/41 (98)	1	95	54/59 (92)	40/41 (98)				
μPlate	0.51	93	54/59 (93)	36/39 (92)	0.8	90	52/59 (88)	36/39 (92)	1	92	52/59 (88)	37/39 (95)
Porton	13.0	91	55/61 (90)	38/41 (93)	10.0	87	56/61 (92)	34/41 (83)				
Orion (old)	393	86	54/61 (89)	34/41 (83)	500	87	50/61 (82)	38/41 (93)				

[a] Some manufacturers specify two cutoff values, results in between being equivocal. At the expense of some indeterminate results, both sensitivity and specificity are increased.
[b] Readings at or above the cutoff value are counted positive.

Statistics. Receiver operating characteristic (ROC) analysis (2, 10) was applied to each assay. As a general measure of assay quality we used Wilcoxon's *W* as described by Hanley and McNeil (3). Eccentricity (*z*) tests were used to compare *W* values. If readings are split into two categories (high and low) by introducing a cutoff value, *W* becomes the accuracy of the test at a prevalence of 0.5. Following the method of Trautmann and colleagues (13), we used maximum accuracy to select an optimal cutoff value for each test, for which we recorded sensitivity and specificity (10).

Discrepancies. Given the theoretically optimal cutoff, we recorded false-positive and false-negative sera.

By the reference methods, 61 patients were considered infected. In all infected patients but one, culture was positive. In the one patient with a negative culture result, all other biopsy-based tests were positive. In all noninfected patients but one, all of the diagnostic tests were negative. In one of the noninfected patients the pathologist was uncertain whether *H. pylori* was present, but all other tests were negative.

Results of Wilcoxon's *W* are in Table 2. For Wilcoxon's *W*, the Bio-Whittaker assay had a significantly higher value than Porton Cambridge ($P < 0.05$), Orion (old version) ($P < 0.02$), and μPlate ($P < 0.05$). Meddens Biotech and Orion (new version) gave a higher value for *W* than Orion (old version) ($P < 0.05$ for both comparisons). Because in the μPlate assay four infected patients tested below the detection limit, the ROC theoretical optimum is placed unrealistically low. In further computations we used a value just above the detection limit as the optimal cutoff.

Sensitivity and specificity for recommended cutoff values and for those we found to be optimal are listed in Table 3. For the FlexSure, following the manufacturer's reading instructions yielded sensitivity and specificity of 92 and 98%, respectively. Table 4 contains results on false-positive or -negative sera. The one serum sample that was false positive in six assays was from a patient with extensive gastric metaplasia. This patient may well have been infected with *H. pylori.*

In this study, assay quality is quantified by two different methods. The first, Wilcoxon's *W* for the original readings, is statistically most informative but cannot be used to compare assays that differ greatly in the number of tied readings. The other, accuracy at the optimal cutoff, can be applied to all assays at the expense of some statistical information. Tables 2 and 3 order the assays by decreasing performance according to both methods. The Whittaker, Meddens, Orion (new), and HMCap assays score highest; differences in this group are not statistically significant. All others except Orion (old) have accuracies at or above 90%.

The FlexSure procedure could be used in a physician's office. Quick diagnosis with this test has to be balanced against the lack of a quantitative result that could be used in serological follow-up on therapy. If, in addition, serum is stored for subsequent reanalysis by a quantitative method such as the Orion or Meddens ELISA, both fast diagnosis and serological follow-up can be achieved.

For serological follow-up on therapy, assay results that are proportional to specific antibody concentration are most convenient. The Meddens Biotech, Orion (new), and Porton assays produce such results; the first two assays are also among the best for primary diagnosis. It should be possible to adapt the other ELISAs so that their results will become proportional to antibody concentration.

In conclusion, the tests from Bio-Whittaker, Meddens, Orion (new), and EPI (HM-CAP) are probably useful additions to the diagnosis of *H. pylori* infection. Their main advan-

TABLE 4. Discrepant results

Assay	Result[a] for serum no.																			
	4	20	3	10	43	676	698	17	41	701	719	848	685	877	677	35	840	853	5	23
Whittaker		−											+							
Meddens	−												+		+					
Orion (new)	−		−	−	−								+	+						
HMCap		−	−				−									+				
FlexSure		−	−				−		−		−					+				
μPlate	−	−	−		−								+	+			+			
Porton	−	−		−		−						−	+	+	+					
Orion (old)	−	−		−	−	−		−		−			+	+	+		+	+	+	+

[a] −, false negative; +, false positive.

tages are the low cost and the fact that serology is noninvasive. For quick diagnosis, the FlexSure is a useful alternative.

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