

Evaluation of Pyloriset Dry, a New Rapid Agglutination Test for *Helicobacter pylori* Antibody Detection

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We evaluated the performance of a new latex agglutination test, Pyloriset Dry (Orion Diagnostica, Espoo, Finland), in the simultaneous detection of immunoglobulin G (IgG), IgA, and IgM antibodies to *Helicobacter pylori* and compared it with that of the Pyloristat test (BioWhittaker, Fontenay-sous-Bois, France), an enzyme-linked immunosorbent assay detecting IgG to *H. pylori*, for 96 untreated dyspeptic patients who had undergone gastroduodenal endoscopy. Infection was diagnosed in 56 cases by positive culture and/or positive Giemsa stain and rapid urease test (antral biopsies) and was associated with chronic gastritis in 52 patients. Forty noninfected patients did not have chronic gastritis. The sensitivity of Pyloriset Dry was 91.1%. The sensitivity of Pyloristat was 91.1 or 82.1%, depending on whether equivocal results were considered positive or negative, respectively. Both tests had a specificity of 87.5%. Their performances were not statistically different. Thus, Pyloriset Dry is an alternative to serological tests for adults, particularly when a small number of serum samples has to be tested.

The diagnosis of *Helicobacter pylori* infections is achieved by both invasive (culture, rapid urease test, and/or histological staining of antral biopsy specimens) and noninvasive (urea breath test and serology) methods (6, 11). Available serological tests are mostly second-generation enzyme-linked immunosorbent assays (EIAs), which are highly sensitive and specific (20). However, these tests are time-consuming and require specialized equipment as well as trained technicians. Recently, several rapid serological tests for *H. pylori* have become available (4, 7–10, 12, 14, 16, 17, 21, 22). Pyloriset Dry is a new latex agglutination test, manufactured by Orion Diagnostica (Espoo, Finland) and distributed in France by Fumouze Diagnostics (Asnières, France), which simultaneously and specifically detects immunoglobulin G (IgG), IgA, and IgM to *H. pylori*. According to the manufacturer, Pyloriset Dry had a sensitivity of 97% and a specificity of 85% with culture and histology as the “gold standard” (10). The present study evaluated Pyloriset Dry with adult patients and compared it with Pyloristat (BioWhittaker, Fontenay-sous-Bois, France), an EIA that specifically detects IgG to *H. pylori*.

MATERIALS AND METHODS

Biopsy specimens and sera were obtained from 104 adult patients who had undergone gastroduodenoscopy for upper gastrointestinal symptoms. Before each endoscopy, the endoscopes and biopsy forceps were carefully cleaned and disinfected by immersion in a 2% glutaraldehyde solution (Cidex; Johnson Medical, Viroflay, France) for at least 10 min, rinsed in sterile distilled water, and dried. Patients treated with agents active against *H. pylori* (antibiotics, proton pump inhibitors, and bismuth salts) within the month before endoscopy were excluded from the study. Blood samples were collected immediately before endoscopy, and sera were stored at -80°C until tested. Three biopsy specimens were taken from the gastric antrum. One biopsy specimen was placed into a 2% urea-buffered broth for rapid detection of *H. pylori* urease activity. This test was read within 3 h. The second was fixed in 10% buffered formalin for histological examination, and the third was immediately placed in a semisolid agar transport medium (Portagerm pylori; bioMérieux, Marcy l'Etoile, France) for culture.

Ground biopsy specimens were inoculated onto both selective (Pylori agar; bioMérieux) and nonselective (chocolate agar supplemented with IsoVitaleX; bioMérieux) media within 24 h. The cultures were incubated at 37°C in a microaerobic atmosphere (5% O_2 , 10% CO_2 , and 85% N_2) and checked for growth at days 5 and 7. Identification of *H. pylori* was based on the presence of gram-negative spiral or curved bacilli that produced oxidase, catalase, and urease. Formalin-fixed specimens were sectioned and stained with hematoxylin and eosin to assess the severity of gastritis and with a modified Giemsa stain for detection of *H. pylori*. The intensity of gastritis was evaluated according to inflammation scores routinely used in pathology laboratories. Inflammation (number of whole inflammatory cells) and activity (number of polymorphonuclear leukocytes) were scored separately on the extent of inflammatory cell infiltration as absent, mild, moderate, or severe and classified in four different grades (from 0 to 3, respectively). Follicular gastritis was similarly scored on the basis of the additional presence of lymphoid follicles. The histopathologist was unaware of patients' clinical and biological findings. A patient with an inflammation grade of >1 and/or an activity grade of ≥ 1 and/or a follicular grade of ≥ 1 was considered to have chronic gastritis.

H. pylori status of patients was determined as either *H. pylori* positive, negative, or uncertain (Table 1). Eight patients of uncertain *H. pylori* status presented with active chronic gastritis ($n = 7$) or severe chronic gastritis ($n = 1$) without any evidence of infection. They were withdrawn from this study. Thus, 96 patients were retained in the study, of which 56 were considered *H. pylori* positive and 40 were considered *H. pylori* negative. These patients consisted of 57 males and 39 females (mean age, 46.4 years; range, 17 to 87 years). The ages of patients with *H. pylori*-positive (mean age, 44.8 years; range, 22 to 74 years) and -negative (mean age, 49.2 years; range, 17 to 87 years) status were of the same order. The male-to-female ratio was 0.6 in both groups. Seventy-eight patients were born in France, and 18 were born in North Africa.

The Pyloristat ELISA IgG kit was used according to the manufacturer's instructions as previously described (1). The sera were diluted 1:20 in buffer containing 0.1% sodium azide in duplicate wells of microtitration plates. The test consisted of three steps (serum distribution and addition of conjugate and substrate), with each step followed by a 15-min incubation at room temperature and shaking. After the enzymatic reaction was stopped, A_{550} was read (Microplate reader 2001; BioWhittaker, Fontenay-sous-Bois, France). Standard and control sera provided by the manufacturer were used to calculate predictive index (PI) values. According to the manufacturer, PI values of <0.80 are considered negative results, PI values between 0.80 and 0.99 are considered equivocal results, and PI values of ≥ 1.00 are considered positive results (1). Sera that gave equivocal results were retested. The Pyloriset Dry test was also used according to the manufacturer's instructions. The latex beads, sensitized with a purified antigen mixture enriched with *H. pylori* urease, are dried on a test card. Each test card contains three test circles. Sera were diluted 1:4 in phosphate-buffered saline (pH 7.2), and one drop was deposited onto one circle and mixed with the latex reagent. The card was tilted and rotated in a circular motion for 3 min. Then, if the serum sample was positive, agglutination was observed. Positive and negative controls of animal origin are provided with the kit.

The chi-square test with Yates correction was used to compare test results

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TABLE 1. Definition of *H. pylori* status

Chronic gastritis ^a	Result ^b of:			<i>H. pylori</i> status ^c	No. of patients
	Histology (Giemsa stain)	Rapid urease test	Culture		
+	+	+	+	HP+	18
+	+	+	-	HP+	18
+	+	-	+	HP+	7
+	+	-	-	HP+	0
+	-	+	+	HP+	2
+	-	+	-	HP+	0
+	-	-	+	HP+	7
+	-	-	-	HP±	8
-	+	+	+	HP+	4
-	+	+	-	HP+	0
-	+	-	+	HP+	0
-	+	-	-	HP±	0
-	-	+	+	HP+	0
-	-	+	-	HP±	0
-	-	-	+	HP+	0
-	-	-	-	HP-	40

^a +, with chronic gastritis; -, without chronic gastritis.

^b +, positive; -, negative.

^c HP+, *H. pylori* positive; HP-, *H. pylori* negative; HP±, *H. pylori* uncertain.

(Stat-ITCF software; Institut Technique des Céréales et des Fourrages, Paris, France).

RESULTS

Among *H. pylori*-positive patients, Pyloristat gave equivocal results in five cases even after retesting (Table 2). Four of these patients showed a positive result with Pyloriset Dry, two of whom had mild active gastritis, associated in one case with follicular gastritis. The third patient had a duodenal ulcer associated with moderate nonactive gastritis, and the fourth patient had normal endoscopy with mild nonactive gastritis associated with positivity of culture, Giemsa stain, and rapid urease test. The patient with a negative result by Pyloriset Dry and an equivocal result by Pyloristat presented with a duodenal ulcer. This suggests that 4 of the 5 EIA equivocal results were correctly identified as positive by the latex test, while this test gave one false-negative reaction. The sensitivity of Pyloriset Dry was 91.1%. For Pyloristat, the sensitivity was 91.1 or 82.1%, depending on whether equivocal results were considered positive or negative, respectively. This difference is not significant ($P > 0.05$).

Endoscopic diagnoses included gastric ulcer (1 of 96 or 1%), duodenal ulcer (17 of 96 or 17.7%), gastric cancer (1 of 96 or

TABLE 3. Endoscopy diagnoses according to serology

Diagnosis	No. of patients	No. of patients seropositive ^a (no. of <i>H. pylori</i> -positive patients) by test	
		Pyloriset Dry	Pyloristat
Healthy	61	29 (25)	31 (26)
Gastric erosions	11	5 (5)	6 (6)
Gastroduodenal erosions	2	1 (1)	1 (1)
Duodenal ulcer	15	15 (15)	13 (13)
Gastric ulcer	1	1 (1)	0
Gastric cancer	1	1 (1)	0

^a Patients with equivocal results by Pyloristat were excluded ($n = 5$).

1%), gastric erosions (11 of 96 or 11.4%), and gastroduodenal erosive lesions (2 of 96 or 2.1%). Duodenal ulcers showed a strong association with positive results of the tests compared with the other endoscopy observations except for gastric ulcer and cancer (Table 3). However, the number of these cases is too small to permit further analysis. The only gastric cancer patient had a positive result with the Pyloriset Dry test but a negative result with the Pyloristat test.

DISCUSSION

Pyloriset Dry replaces the previous Pyloriset test which used the liquid phase. Pyloristat has been shown to be an accurate method for detecting antibodies to *H. pylori*, with values in the range of values from commercially available EIAs (1, 9, 18). In our study, the sensitivity and specificity of Pyloriset Dry were slightly lower than those reported by the manufacturer. However, the sensitivity was similar to the values reported, with the adult population, for the other three available rapid serological tests, i.e., Pyloriset (Orion Diagnostica) (7, 9, 14, 16, 17, 22), QuickVue (Quidel, San Diego, Calif.) (21), and Flex-Sure (SmithKline Diagnostics Inc., Philadelphia, Pa.) (4). Discrepant results were reported by Hoek et al. (8), who found a sensitivity of only 68% for Pyloriset. Compared with these three tests, Pyloriset Dry showed a higher specificity.

In our hands, the performances of the latex test and Pyloristat were similar. In contrast, Midolo et al. found that Pyloriset Dry performed better than two other EIAs that also detect specific IgG: Pyloriset EIA (Orion Diagnostica) and Hel-p (Amrad, Kew, Australia) (12). However, a precise comparison between all these studies is difficult, since the gold standards used and the populations studied are different.

To evaluate the performance of a test in diagnosis of *H. pylori* infection, a gold standard must be used as a reference to

TABLE 2. Results of Pyloriset Dry and Pyloristat compared with *H. pylori* status

Method	Result	No. of patients		Sensitivity (CI) ^a (%)	Specificity (CI) (%)
		<i>H. pylori</i> positive	<i>H. pylori</i> negative		
Pyloriset Dry	Positive	51	5	91.1 (83.7-98.5)	87.5 (77.3-97.7)
	Negative	5	35		
Pyloristat	Positive	46	5	91.1 ^b (83.7-98.5) 82.1 ^c (72.1-92.1)	87.5 ^{b,c} (77.3-97.7)
	Negative	5	35		
	Equivocal	5	0		

^a CI, 95% confidence interval.

^b Sensitivity or specificity determined when Pyloristat borderline result was considered positive.

^c Sensitivity or specificity determined when Pyloristat borderline result was considered negative.

establish that the infection and gastritis exist (2, 20). As there is a patchy distribution of *H. pylori* and of gastritis in the antrum (3), the use of one biopsy for culture, rapid urease test, and histology may induce sampling errors. Unlike other studies (4, 7, 16, 18, 21, 22), we have considered both the microscopic appearance of the mucosa and the results of three methods for detecting *H. pylori* to define the *H. pylori* status, thus limiting sampling errors. In our series, all positive patients had positive culture and/or positive rapid urease test and Giemsa stain results (Table 1).

Only ulcerous patients with *H. pylori* infection require specific antimicrobial treatment (5, 13). Thus, to establish that there is an infection, upper gastrointestinal endoscopy is necessary for a direct diagnosis. Serology may be useful when endoscopy is not indicated, particularly with children, or to confirm a negative *H. pylori* status (5). Serology has also been proposed as preendoscopy screening to reduce endoscopy workload in young dyspeptic patients and in epidemiological studies (15, 19, 20). Both serological tests evaluated in our study may be used in such indications. Compared with the EIA, Pyloriset Dry gives more rapid results, is easier to perform, and has a lower absolute cost (ca. \$4 versus ca. \$8 per serum sample). With Pyloriset Dry, the card may easily be cut in thirds to perform one test at a time and is therefore more convenient for small numbers of serum samples.

In conclusion, Pyloriset Dry is a rapid, inexpensive, and convenient serological method that is useful in the diagnosis of *H. pylori* infection in adult patients. Its performance should be further evaluated in a larger study of adult patients to determine its place as a screening tool and with pediatric populations to establish its diagnostic value for children.

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