

## Diagnosis of *Helicobacter pylori* Infection in Adult and Pediatric Patients by Using Pyloriset, a Rapid Latex Agglutination Test

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Received 28 June 1991/Accepted 15 October 1991

**Pyloriset (Orion Diagnostica, Espoo, Finland) is a rapid antibody test using latex particles coated with acid-extracted antigen of *Helicobacter pylori*. We evaluated its ability to predict infection in 100 adult patients and 50 pediatric patients referred for gastric endoscopy. Sixty of 65 *H. pylori*-infected adults were correctly identified by the test. There were 12 false-positive and 5 false-negative reactions seen. Pyloriset had a sensitivity of 92% and a specificity of 66%. The positive predictive value was 83% and the negative predictive value 82%. In contrast, sensitivity dropped to 36% in the pediatric patients and the positive predictive value was only 40%. Pyloriset could become an important alternative to other more time-consuming diagnostic tests for *H. pylori*-infected adult patients but is inadequate for diagnosis of pediatric *H. pylori* infection.**

Culture (3), histological examination (6), and rapid urease tests (12) are established means of diagnosing *Helicobacter pylori*. They are highly accurate but have the drawback of requiring gastroduodenoscopy. Current noninvasive procedures for diagnosis include serology (2, 9) and urea breath (7) testing. Serology tests can have high sensitivity and specificity but have the disadvantage of needing a laboratory set up to be performed. Turnaround time for results can become long when the test has to be sent out to another institution. The urea breath test calls for equipment that may not be readily available at all institutions and uses radioactive material. A latex agglutination test for antibodies to *H. pylori* combines the advantages of a noninvasive test with speed and low cost.

Pyloriset (Orion Diagnostica, Espoo, Finland) is a latex agglutination test for rapid diagnosis of *H. pylori* antibodies in serum. The test uses latex particles coated with acid-extracted antigen of *H. pylori*. In a pilot study by the manufacturer, Pyloriset had a sensitivity of 97% and a specificity of 95% by using enzyme immunoassay as the "gold standard" (5). We now have evaluated Pyloriset in adult and pediatric patients in the United States.

### MATERIALS AND METHODS

The first patient sample consisted of 100 adult patients who had undergone gastroduodenoscopy for dyspeptic symptoms. Their ages ranged from 26 to 93 years, with a mean age of 57.4 years. All the patients were males. At the time of endoscopy, we collected biopsies for culture and histology tests and blood for enzyme-linked immunosorbent assay (ELISA). The second group consisted of 50 pediatric patients referred for gastroduodenoscopy because of symptoms consistent with acid peptic disease, such as chronic abdominal pain, vomiting, or hematemesis. Their ages ranged from 4 to 21 years, with a mean age of 12.8 years. Forty patients were Caucasians and ten were Afro-Americans. Twenty-four of the 50 patients were males.

We defined a patient as infected with *H. pylori* when either

the culture was positive or organisms with characteristic morphology were seen on histological examination (6). Cultures from adult patients were done on selective *H. pylori* agars (Brucella agar with 10% bovine blood, 1% IsoVitaleX, and 20 mg of nalidixic acid, 6 mg of vancomycin, 8 mg of amphotericin B, and 100 mg of cycloheximide per liter) and incubated in CampyPak jars. Pediatric biopsies were cultured on Columbia agar containing 5% sheep blood. Antral biopsies were stained with a modified Giemsa technique or Brown-Hopps (6). For each group, all slides were examined by the same pathologist who was unaware of the results of culture or ELISA.

ELISA used an outer membrane preparation of *H. pylori* as previously described and validated by Czinn et al. (1, 2). In brief, we inoculated polystyrene microtiter plates with 2  $\mu$ g of protein per well of antigen and stored them at 4°C overnight. Nonspecific binding sites were blocked with 1% bovine serum albumin (BSA) in phosphate-buffered saline PBS for 90 min at room temperature, and then the plates were washed with 0.1% BSA in PBS. One hundred microliters of each serum sample, diluted 1:1,024, was added per well. After incubation at room temperature for 90 min, the plates were washed three times with 0.1% BSA in PBS, and 100  $\mu$ l of a 1:1,000 dilution of goat antibody to human immunoglobulin G-alkaline phosphatase conjugate was added to each well for 90 min. After washing the plates, we developed them with 100  $\mu$ l per well of a 1-mg/ml solution of *p*-nitrophenyl phosphate in glycine buffer for 1 h. The  $A_{410}$  was measured. On the basis of a previous study (1), we defined a positive result as an optical density of  $\geq 2$  standard deviations above the mean value for patients who were negative for *H. pylori* infection.

We performed the Pyloriset testing according to the manufacturer's directions. Serum from each patient was diluted 1:2 and mixed with the latex reagent. At 3 min, a positive or negative agglutination reaction was read by comparing the sample with the positive and negative controls included in each test kit. All readings were done by two independent observers without knowledge of the results of culture, histological examination, or ELISA. Statistical analysis of data

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TABLE 1. Results of Culture, Histology, ELISA, and Pyloriset testing with 100 adult patients

Test	No. of results <sup>a</sup>				Sensitivity (%)	Specificity (%)	PPV (%) <sup>b</sup>	NPV (%) <sup>c</sup>
	True positive	False positive	True negative	False negative				
Culture	50	0	35	15	77	100	100	70
Histology	56	0	35	9	86	100	100	80
ELISA	52	9	26	13	80	74	85	67
Pyloriset	60	12	23	5	92	66	83	82

<sup>a</sup> Infection defined as either positive culture or positive histological examination.

<sup>b</sup> PPV, positive predictive value.

<sup>c</sup> NPV, negative predictive value.

was performed by using Statgraphics 3.0 (Graphics Software Systems, Inc., Rockville, Md.).

### RESULTS

We compared results from Pyloriset testing with culture, histological examination, and ELISA results for 100 adult patients (Table 1). Sixty-five of the 100 patients had *H. pylori* infection, and the latex test correctly identified 60 of the 65. Five patients had false-negative latex test results. Twenty-three of 35 *H. pylori*-negative patients were correctly identified as negative by the test. There were 12 false-positive reactions seen. With this sample of patients, the Pyloriset test had a sensitivity of 92% and a specificity of 66%. The positive predictive value was 83% and the negative predictive value 82%. Pyloriset was more sensitive than culture (77%), histological examination (86%), and immunoglobulin G by ELISA (80%) but had a slightly lower specificity than the other tests.

With the pediatric patients, the test had a specificity of 85%, but its sensitivity dropped to 36% (Table 2). The test identified only 4 of 11 *H. pylori* infections. It correctly identified 33 of 39 *H. pylori*-negative patients but gave six false-positive results. The positive predictive value in this group of patients was only 40%.

### DISCUSSION

With the adult sample of patients, the Pyloriset test had a positive predictive value of 83% and a negative predictive value of 82%. These numbers are slightly lower than those reported by the manufacturer in its pilot study but still suggest that Pyloriset could become an important rapid test for *H. pylori* infection. The clinical implications are manifold. A rapid noninvasive test could lead to presumptive diagnosis of *H. pylori* infection as early as during the initial patient visit, without the need for more costly laboratory evaluations. That could lead to quicker decisions regarding

the patient's disposition and therapy as well as reduced costs. If it is used as a screening test, it is likely to identify patients who would best benefit from additional evaluation with endoscopy and, by that, increase the cost effectiveness of the procedure. In a study of 1,153 patients, Sobala et al. showed that such screening with an immunoglobulin G ELISA reduced endoscopy work load by 23.3% while a sensitivity of 97.4% for detecting peptic ulcer was retained (10). A simple latex agglutination test also can mean new possibilities for diagnosis in developing countries where sophisticated laboratory services are often lacking.

In light of its strong performance with adult patients, it was a surprise to find that Pyloriset was inadequate in diagnosing pediatric infections. We do not think that this result was due to technical problems. The kits used for pediatric patients were brand new and had gone through the manufacturer's regular quality control. We tested each serum twice with two different lots of tests, and the results were almost identical. One of the two testers had also participated in testing all the adult serum samples (T.U.W.) and served as a guarantor that identical criteria were used in the interpretation.

We think that the problems seen may relate directly to the patient population tested. Megraud et al. have recently reported preliminary data that support our findings (8). They tested Pyloriset with a group of adult and pediatric African patients. With 238 adults, the test had a sensitivity of 75%, but among 202 children the sensitivity dropped to 39%. When they tested three other *H. pylori* antibody kits with their pediatric serum samples, they observed a similar drop in sensitivity. This suggests that the phenomenon is real and not simply a technical problem with Pyloriset.

Previous studies have shown that there is a difference in cellular immune response between children and adults. Pediatric patients infected with *H. pylori* predominantly have a chronic inflammation in the gastric mucosa, while adult patients show a more neutrophilic response (4). Our

TABLE 2. Results of Culture, Histology, ELISA, and Pyloriset testing with 50 pediatric patients

Test	No. of results <sup>a</sup>				Sensitivity (%)	Specificity (%)	PPV (%) <sup>b</sup>	NPV (%) <sup>c</sup>
	True positive	False positive	True negative	False negative				
Culture	11	0	39	0	100	100	100	100
Histology	11	0	39	0	100	100	100	100
ELISA	9	0	39	2	82	100	100	95
Pyloriset	4	6	33	7	36	85	40	83

<sup>a</sup> Infection defined as either positive culture or positive histological examination.

<sup>b</sup> PPV, positive predictive value.

<sup>c</sup> NPV, negative predictive value.

current data suggest that there is also a difference in humoral response. There could be several explanations for such a difference. It is our experience that the mean titers among infected pediatric patients usually are lower than the mean titers in infected adult patients. The mean optical density ( $\pm$  standard deviation) for our positive pediatric serum samples was 0.62 ( $\pm$  0.28) compared with 0.97 ( $\pm$  0.30) for the positive adult serum samples. This difference was highly significant ( $P = 0.002$ ) and could explain some of the decreased sensitivity. There also could be a difference in the quality of antibody response between adults and children. We know that this is true for some infections such as *Haemophilus influenzae*, for which only children older than 6 years consistently show the same antibody response as adults (11). Our patients were older than that, but very little is known about *H. pylori* antigen recognition in different age groups. We have found considerable individual variation in antibody response to one of the three major outer membrane proteins of *H. pylori* (1). By Western blot (immunoblot) analysis, almost half of the serum samples from infected pediatric patients lacked antibody to a 32-kDa protein and only 1 of 15 recognized a fourth 14-kDa protein. We have also found a difference in antibody class response between adults and children. In contrast to adults, infected children fail to uniformly mount a significant systemic immune response by serologic immunoglobulin A (2). Since Pyloriset measures total antibody, such differences also could theoretically affect sensitivity.

We conclude that Pyloriset could become an important alternative to other more time-consuming diagnostic tests for *H. pylori* infection in adult patients. In contrast, we find that the test is inadequate for diagnosing *H. pylori* infection in pediatric patients. A modified test, possibly using different antigens or a different serum dilution, might be needed for the pediatric patient population.

#### ACKNOWLEDGMENTS

This work was supported in part by grant A1-25818 from the National Institute of Allergy and Infectious Diseases and by Orion Diagnostica.

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