Novel diagnostic tests to detect *Helicobacter pylori* infection: A pediatric perspective

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JD Snyder, S Veldhuyzen van Zanten. Novel diagnostic tests to detect *Helicobacter pylori* infection: A pediatric perspective. Can J Gastroenterol 1999;13(7):585-589. Because of the widespread problem of *Helicobacter pylori* infections, there is an increased need for rapid, reliable and inexpensive diagnostic tests. Five recently developed tests that offer potential advantages because they are less invasive or permit easier acquisition of samples than available tests are assessed. The tests assessed are whole blood, saliva and urine assays that measure systemic antibody response to *H pylori*, stool tests that measure *H pylori* antigens and string tests that recover *H pylori* organisms.

Key Words: *Children; Enzyme-linked immunosorbant assay;* Helicobacter pylori*; Serological testing; Urease*

Nouveaux tests diagnostiques pour déceler une infection à *Helicobacter pylori* : une perspective pédiatrique

RÉSUMÉ : Étant donné l'importance du problème des infections à *Helicobacter pylori*, on a de plus en plus besoin de tests diagnostiques rapides, fiables et peu coûteux. On a évalué cinq tests récemment mis au point et qui présentent des avantages potentiels parce qu'ils sont moins effractifs ou qu'ils permettent de recueillir des échantillons plus facilement que les tests disponibles. Les tests évalués sont des dosages de sang entier, d'urine et de salive qui mesurent la réponse systémique des anticorps à *H. pylori*, des examens de selles qui mesurent les antigènes de *H. pylori* et des épreuves du fil qui recueillent *H. pylori*.

Helicobacter pylori is one of the most common bacterial infections in humans, and has been found in differing rates in persons of all age groups, nationalities and socioeconomic classes (1). Because the role of *H pylori* in human disease continues to be explored, there is an increased need for rapid, reliable and inexpensive diagnostic tests (2,3). The most commonly used standard tests are listed in Table 1. To date, the only test that can detect infection and disease activity is endoscopy with biopsy, which is invasive and expensive.

Five recently developed tests that offer potential advantages because they are less invasive or permit easier acquisition of samples are assessed. These tests are whole blood, saliva and urine assays that measure systemic antibody response to *H pylori*, stool tests that measure *H pylori* antigens and string tests that use methodology less invasive than upper gastrointestinal endoscopy to recover *H pylori* organisms.

The tests are compared with current standard tests and criteria for optimal tests. The criteria are non- or minimally invasive nature; high sensitivity, specificity, and positive and negative predictive values in diagnosing infection and assessing disease activity and effectiveness of therapy; convenience, including ease of acquisition of samples and rapid determination of results; and low cost.

WHOLE BLOOD ANTIBODY TESTS

Background: Whole blood antibody tests are based on the fact that systemic antibodies to *H pylori* are found in whole blood, making testing possible in a primary care setting (2-9). Whole blood tests have several important potential advantages compared with standard serological tests; the samples are easier to obtain, testing can be done in an office setting and the results can be available within 10 mins. In addition, the cost is projected to be less than that for the standard serological tests.

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TABLE 1
Comparison of tests used to diagnose *Helicobacter pylori infection*

Test	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Cost (US$)
Serology	90	95	95	95	50.00
Urea breath test	95	95	99	90	200.00
Rapid urease test*	90	98	95	94	10.00
Histology*	95	99	97	99	150.00
Culture*	80	100	90	90	150.00

**Includes endoscopy (about US$250)*

TABLE 2
Comparison of whole blood assays to diagnose *Helicobacter pylori* infection

Author, year (reference)	Tests	Gold standard	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Reilly et al, 1997 (4)	Helisal serum (Corex, London, United Kingdom) Helio-G (Shield Diagnostics, Dundee, United Kingdom)	Two or more tests	82	75	79	83
Faigel et al, 1998 ((5)	FlexSure HP (SmithKline Diagnostics, Philadelphia, Pennsylvania) QuikVue HP (Quidel, San Diego, California) Accustat (Boehringer, Mannheim, Germany)	Two or more tests	80	92	85	92
Sadowski et al, 1998 ((6)	OneStep (Cortex, San Leandro, California) Stat-Pak *H Pylori* (CQI-Biomed, Montreal, Quebec)	Breath test or histology	85	76	65	93
Chey et al, 1998 (7)	Flex-Pack AP (Abbott Diagnostics, Chicago, Illinois)	Histology	72	92	81	88
Liquornik et al, 1998 (8)	FlexSure HP	Two or more tests	50	100	—	—

Methodology: Whole blood samples are obtained by finger prick yielding one to two drops of blood that are placed directly onto a test slide. The most effective assays measure immunoglobulin (Ig) G antibodies to highly specific *H pylori* antigens using ELISA techniques (2,3). Colorimetric results can usually be read in less than 10 mins. Several commercial kits are available (Table 2).

Comparison with serological assays: Most of the studies evaluating whole blood antibody tests have been reported in abstract form and have used one standard test as the 'gold standard' to diagnose *H pylori* (4-9). Individual tests have not been validated formally by multiple investigators or evaluated in an office setting. Evaluation of these tests in pediatric populations is limited; preliminary data indicate that their performance is likely to be similar to that in adults (8). The sensitivity, specificity, and positive and negative predictive values of these tests are not as high as those of serological testing (Table 2).

Summary: Despite the potential advantages of whole blood antibody testing, available tests have several important limitations; the sensitivity, specificity, and positive and negative predictive values are lower than those of serological testing, and this greatly limits their usefulness in clinical situations. None of the tests available is sufficiently accurate to be recommended for general use. As with other antibody tests, an important limitation of these assays is that they cannot detect disease activity. In addition, because antibody levels may persist for up to a year after successful eradication therapy, they cannot be used to document cure of the infection (2,3).

SALIVA ANTIBODY TESTS

Background: Systemic antibodies to *H pylori* are found in saliva (2-4,10-15). Saliva samples are easier to obtain than serum samples, do not require separation steps and can be refrigerated for storage. These tests may be useful in populations with constraints for obtaining blood samples.

Methodology: Saliva samples are obtained using absorbent disks placed in the mouth for a few minutes; the collection of a sufficient quantity of saliva is indicated by a colour change in the disk. The disk is then placed into a separator tube containing buffer, and the extracted saliva can be stored at room temperature for up to one month (4). Several systems have been evaluated and all use ELISA tests to determine the presence of antibody (11-15) (Table 3). IgG antibody has been found to be a more reliable test of infection than the

TABLE 3
Comparison of saliva assays to diagnose *Helicobacter pylori infection*

Author, year (reference)	Tests	Gold standard	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Patel et al, 1994 (10)	Experimental ELISA	Two or more tests	85	85	82	90
Luzza et al, 1995 (11)	Experimental ELISA	Urease or histology	82	71	95	40
Christie et al, 1996 (12)	Experimental ELISA	Histology or urease or culture	88	71	65	90
Reilly et al, 1997 (14)	Helisal (Cortex, London, United Kingdom)	Two or more tests	88	39	59	76
Shaw et al, 1998 (13)	Experimental EIA	Urease or histology	88	81	83	87
Luzza et al, 1997 (14)	Experimental ELISA	Urease or histology	91	81	82	90
Ballam et al, 1998 (15)	Experimental Western blot	Serology	71	84	71	84

EIA Enzyme immunoassay

TABLE 4
Comparison of urine assays to diagnose *Helicobacter pylori* infection

Author, year (reference)	Tests	Gold standard	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Alemohammad et al, 1993 (16)	Experimental ELISA	Histology or culture	96	77	93	86
Weston et al, 1995 (17)	Commercial ELISA	Histology	70	85	81	76

IgA antibody (10,11,14). The testing requires laboratory conditions equivalent to those of standard ELISAs, and takes the same amount of time to perform.

Comparison with serological assays: As with whole blood tests, many of the reports of saliva antibody assays are available in abstract form only (10-15). The overall sensitivity, specificity, and positive and negative predictive values of these tests are not as high as those of serological testing, and preclude the current tests from being clinically relevant. Preliminary data in children indicate that the tests do not perform any better in pediatric than in adult populations (14,15).

Summary: If accurate, saliva antibody tests can offer advantages over standard serological tests. However, the lower overall sensitivity, specificity, and positive and negative predictive values of the antibody tests limit their clinical usefulness. There is no reduction in time to determine results compared with serological testing. Although not discussed in the initial studies, concentration effects can affect the accuracy of these tests. Even if the efficacy of the tests is improved, reliance on systemic antibody response limits their ability to detect disease severity or measure eradication effectively.

URINE ANTIBODY TESTS

Background: Systemic IgG and IgA antibodies to *H pylori* are found in urine. Accordingly, several assays have been developed for measurement (16,17). These tests have the potential advantages of being noninvasive, nonpainful and permitting easy specimen collection.

Methodology: Postfasting urine samples are collected and the presence of antibody is determined by IgG ELISA or Western blot analysis (16,17). Samples can be refrigerated and stored before analysis.

Comparison with serological assays: IgG antibodies more accurately identify infection than IgA antibodies (17). The water content of urine and the freezing of samples for storage negatively influence the reliability of urine antibody tests (16). Initial studies have reported sensitivity, specificity, and positive and negative predictive values too low to permit urine screening to be a clinically useful test (Table 4). Trials in children have yet to be undertaken.

Summary: The concentration of urine can affect results, and freezing samples also appears to affect accuracy. There is no reduction in time to determine results compared with serological testing. Urine antibody tests also share the limitations of measuring systemic antibody response to disease activity that limits their ability to detect disease severity or measure eradication effectively.

STOOL *H PYLORI* ANTIGEN

Background: *H pylori* has been detected in feces, and stool testing has the potential to be an effective, inexpensive and noninvasive method of determining the presence of the organism both before and after eradication therapy. The relatively expensive urea breath test is the standard noninvasive test for determining the presence or absence of active infection (2,3). Alternatively, endoscopy and biopsy for histology and culture can be used, but this method is invasive and more expensive. Several groups have tested a new commer-

TABLE 5
Comparison of stool antigen to diagnose *Helicobacter pylori* infection

Author, year (reference)	Tests	Gold standard	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Vaira et al, 1998 (19)	Commercial test, HpSA	Two or more tests	95	92	93	94
Veldhuyzen van Zanten et al, 1998 (20)	Commercial test, HpSA	Three or more tests	98	95	90	99
Casswall et al, 1999 (25)	Experimental polymerase chain reaction	Urea breath test	69	52	66	56

HpSA H pylori *stool antigens*

TABLE 6
Comparison of string tests to diagnose *Helicobacter pylori* infection

Author, year (reference)	Tests	Gold standard	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Perez-Trallaro 1995 (26)	Enterotest + culture	Histology	75	75	86	60
Lynch 1998 (27)	Enterotest + polymerase chain reaction	Histology or urease	80	100		

Positive predictive value: true positive/true positive + false positive; Negative predictive value: true negative/true negative + false positive

cially available antigen detection assay in adults (18-22), and others have tested isomagnetic separation polymerase chain reaction (PCR) detection of *H pylori*-specific DNA in feces, including one study involving children (23-25).

Methodology: For antigen detection, stool samples can be refrigerated if assayed within 48 h of passage, or the samples can be frozen indefinitely (22). The available commercial test uses polyclonal anti-H pylori IgG antibody adsorbed to microwells to detect stool antigen. If antigen is present, a colour change occurs that can be read visually or with a spectrophotometer. The immunomagnetic separation PCR technique is a research laboratory test at this time (25). Briefly, slurries of previously frozen fecal material are mixed with magnetic beads coated with polyclonal anti-*H pylori* rabbit antibody. After purification, the presence of *H pylori* is measured by PCR.

Comparison with urea breath test and serological testing: Results from several studies published in abstract form (Table 5) indicate that the sensitivity, specificity, and positive and negative predictive values of the commercial antigen detection test are comparable with those of serological testing and the urea breath test (19,20). The results of serial determinations indicate that the test can detect eradication as early as five days after the start of therapy (21,22). Data using this test are not yet available in children. The results of the immunomagnetic separation PCR test in children are not as encouraging (25).

Summary: Initial results indicate that stool antigen testing for *H pylori* may prove to be an effective, rapid and noninvasive method to evaluate both active disease and response to treatment (19-22). The test also has the potential to be less expensive than the urea breath test because it requires less specialized equipment and less costly reagents. Because patients may have difficulty obtaining and handling stool samples, compliance may be a problem with this test. Although the test has some important potential advantages, it shares the important limitation of all of the noninvasive tests of not being able to measure disease activity.

STRING TESTS

Background: String tests have the potential to provide a less invasive, less expensive method to recover *H pylori* organisms. Currently, the recovery of *H pylori* organisms requires invasive techniques, primarily endoscopy and biopsy. Enterical string tests have been used for many years to obtain upper gastrointestinal fluid to aid in the assessment of gastric pH, bile salts and duodenal parasitic infections (26,27). This methodology has also been used to recover viable *H pylori* from the gastric mucus layer (26,27). The recovery of organisms can provide important information about strain type and the presence of antibiotic resistance.

Methodology: One end of a highly adsorbent polymeric string is taped to the patient's cheek, and the remainder of the string is swallowed in a gelatin-coated capsule (26,27). The string remains in the stomach for 30 to 60 mins and is then removed manually. Gastric mucus is expressed from the string, and either cultured or tested by PCR for the presence of *H pylori*.

Comparison with endoscopic retrieval: The results from preliminary studies have not shown as high a rate of retrieval of organisms compared with that of endoscopy (Table 6). Data are not yet available in children.

Summary: This test offers a minimally invasive method for obtaining viable organisms. If the recovery of organisms is enhanced, it can offer an important, less costly alternative to endoscopy and biopsy.

CONCLUSIONS

The whole blood, saliva and urine tests of antibody response have thus far had lower accuracy than serological testing. In addition, saliva and urine tests appear to be affected by con-

centration effects. Because tests of antibody response cannot determine disease severity or assess eradication in a timely manner, these tests are not likely to have a major role in *H pylori* evaluation, even if they undergo further improvements. However, improved saliva tests may prove useful in pediatric populations with constraints to blood drawing.

The preliminary results of stool antigen determination are very encouraging for the detection of *H pylori* both before and after treatment. The initial findings indicate that this test has high sensitivity, specificity, and positive and negative predictive values, and may become less expensive than the urea breath test. This assay cannot measure disease severity, but it may have an important role in assessing the effectiveness of eradication therapy. It remains to be determined whether this test performs as well when used outside clinical trials.

The string test affords a potentially less invasive and less expensive method to recover *H pylori* organisms. The current test does not recover organisms as efficiently as endoscopy and biopsy, but it offers a less invasive and less expensive alternative methodology.

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