Evaluation of a rapid, new method for detecting serum IgG antibodies to *Helicobacter pylori*

Tarun K. Sharma, Eugene L. Young, Sherry Miller, and Alan F. Cutler*

There is an increased need for rapid, inexpensive tests to diagnose *Helicobacter pylori* infection. Our objective was to determine the performance characteristics of an immunochromatographic test (ICT) for detection of anti-*H. pylori* IgG antibodies. A commercially available ICT kit (FlexSure® HP) was tested with a well-characterized cohort of banked sera as well as with fresh serum from randomly selected symptomatic patients. The ICT was evaluated with 107 stored sera and 96 prospective patients. The test correctly identified 65 of 68 *H. pylori*-infected and 37 of 39 noninfected stored sera and 54 of 57 infected and 30 of 39 noninfected patients. Sensitivity, specificity, and positive and negative predictive values were 96%, 95%, 97%, and 93% in stored serum and 95%, 77%, 86%, and 91% in fresh serum, respectively. We concluded that the ICT, reported at 4 min, is highly sensitive for detecting anti-*H. pylori* IgG antibodies in human serum. With a high negative predictive value, the test may be used to exclude *H. pylori* infection in symptomatic patients.

**INDEXING TERMS:** peptic ulcer • *Helicobacter* infections/diagnosis • immunoassay

Multiple invasive and noninvasive methods are available for the detection of *Helicobacter pylori* [1]. Invasive methods necessitate endoscopy and require gastric tissue. They include tests for urease activity, histologic evaluation, and culture of the bacterium. They are all limited by the high cost of endoscopy and sampling errors due to nonuniform distribution of *H. pylori* in the stomach [2–4].

Noninvasive techniques to detect bacterial infection include urea breath tests (UBT) and anti-*H. pylori* antibody detection by serologic methods.1 The presence of antibodies to *H. pylori* strongly correlates with histologic evidence of infection in untreated patients [5, 6]. Serologic detection of *H. pylori* antibodies with current methodologies is expensive and time consuming, and requires transport of the specimen to a reference laboratory. An office-based test to screen patients with upper gastrointestinal symptoms for *H. pylori* would expedite diagnosis and treatment of the infection. Such a test should be inexpensive, easy to perform, accurate, and produce results rapidly, i.e., during the encounter with the patient.

The immunochromatographic test (ICT) method of anti-*H. pylori* antibody detection is based on the principle of reverse-flow immunochromatography. The FlexSure® HP kit (SmithKline Diagnostics, San Jose, CA) includes the high-molecular-mass cell-associated protein (HM-CAP), an antigen highly specific to *H. pylori* [7], as the target antigen to detect *H. pylori*-specific IgG antibodies in human serum. The aim of our study was to determine the sensitivity, specificity, and positive and negative predictive values of this ICT for the detection of IgG antibodies to *H. pylori* in human serum.

**Material and Methods**

The study was conducted in two phases. Initially the ICT was used to detect anti-*H. pylori* IgG in banked serum from symptomatic patients with known *H. pylori* status (phase I). In the second phase the ICT was performed with fresh serum from symptomatic patients undergoing outpatient endoscopy who met entry criteria (phase II). The first cohort represents the “best-case scenario”, i.e., stored sera selected for complete concordance on all *H. pylori* tests, and the second cohort is the “average-case scenario.” The study was approved by the institutional human research review committee of Sinai Hospital.

**PHASE I: EVALUATION IN SELECT COHORT**

**Patient population.** Patients presenting to a gastroenterologist at a large clinic in Detroit for esophagogastroduo-
denoscopy from 1990 to 1993 underwent multiple tests to define their *H. pylori* infection status. Serum samples collected as part of the evaluation had been numbered and stored in a −70 °C freezer. Patients had given prior consent for future investigations involving stored sera.

Establishment of *H. pylori* status. Each patient’s infection status had previously been determined by using a panel of six tests: (a) rapid urease test (CLO® test only), (b) antral histology with hematoxylin and eosin stain, (c) antral histology with silver stain, (d) ELISA for IgG antibody, (e) ELISA for IgA antibody, and (f) \( ^{13}\text{C}\)UBT. From a large bank of sera, serum samples from donor patients that were either positive or negative by all six tests were selected. These banked serum samples were then used to evaluate the ICT by a single investigator (E.L.Y.) blinded to the results of all tests used to establish *H. pylori* status.

PHASE II: EVALUATION IN PROSPECTIVE COHORT

Patient population. Adult (≥18 years) patients who met entry criteria and were scheduled for esophagogastroduodenoscopy between August 1994 and March 1996 for the evaluation of upper gastrointestinal symptoms were offered participation. Patients with exposure to antibiotics, proton pump inhibitors, or bismuth compounds in the previous 4 weeks were excluded. Patients with previous treatment for *H. pylori* were excluded. Informed consent was obtained before endoscopy.

Establishment of *H. pylori* status. Infection status was determined in all patients by using three tests: (a) rapid urease tests (CLO test or PyloriTek® test); (b) antral histology with hematoxylin and eosin stain; silver stain was used at the pathologist’s discretion, when typical organisms were not seen upon histology with hematoxylin and eosin stain when chronic active inflammation was present.

In phase I, both *H. pylori* infection required both negative antral histology with silver stain (Warthin–Starry stain) was used in all tissue specimens to identify *H. pylori* organisms in phase I. In phase II, a silver stain (Steiner’s stain) was done, upon the pathologist’s discretion, when typical organisms were not seen upon hematoxylin and eosin stain when chronic active inflammation was present.

Noninvasive tests.

1) \( ^{13}\text{C}\)UBT: After an overnight fast, every patient underwent a 30- or 60-min breath test. The increase in mole fraction of isotope tracer \( ^{13}\text{CO}_2 \) compared with baseline was expressed as atom percentage excess units \( \frac{([^{13}\text{CO}_2]/[^{12}\text{CO}_2]) + ^{13}\text{CO}_2} \times 100 \). A UBT result of ≥6 atom percentage excess was considered positive. The sensitivity and specificity ranges of this cutoff value were 95–99% and 94–99%, respectively [9–11].

In phase II, \( ^{13}\text{C}\)UBT was performed with the following technical differences: (a) Posturea ingestion breath sample was collected only at 30 min; (b) an atom percentage excess value of ≥5 was considered positive and ≤5 was negative. With these cutoff values, the \( ^{13}\text{C}\)UBT is highly accurate, reliable, and reproducible. Sensitivity ranges were 95–98% [12, 13]. In both phases the UBT was performed after endoscopy.

2) ELISA: ELISAs (PyloriStat; BioWhittaker, Walkersville, MD) to detect the IgG and IgA antibodies to *H. pylori* were performed in phase I of the study by using sonicates of pooled *H. pylori* strains, as previously described [5]. Absorbance of ≥1.0 was considered seropositive and a value of <1.0 was considered seronegative. Sensitivity and specificity ranges of these assays (range) were 92–96% [6, 9, 12, 14]. ELISAs were not used in phase II of the study.

3) ICT: A commercially available ICT kit (FlexSure HP) was tested against characterized sera in both phases of the study. The kit contained a test card with a “pink pad” containing colored particles bound to anti-human IgG, a “blue pad” containing a colored dye, a “test line” containing purified *H. pylori* antigen (HM-CAP), and a “control line” containing conjugate-specific antibodies. In phase I, banked serum (stored at −70 °C in sealed containers) was thawed to room temperature and tested in the ICT kit. In phase II, patients undergoing endoscopy had ~5 mL of venous blood collected aseptically. Serum was separated
and utilized in the ICT kit. No serum samples were frozen before testing in phase II. The FlexSure HP test was performed as follows. Two drops of a buffer solution (PBS with 0.25 g/L sodium azide) were added to the pink pad to reconstitute the conjugate. One drop of undiluted serum from either the banked sample in phase I or the fresh sample in phase II was then added to the pad with the blue dye. The blue dye-colored serum migrated up the chromatographic test strip through a band of immobilized _H. pylori_ antigen (HM-CAP) in the test line. If antibodies specific to _H. pylori_ were present in the serum, they bound the antigen on the test line. The card was then folded, transferring the conjugate from the pink pad and initiating reverse-flow chromatography. The conjugate then migrated down the test strip, passing through the test line and control line. If antibodies to _H. pylori_ had been captured on the test line, the conjugate bound to both the test and control lines, producing two visible lines (a positive test). If antibodies to _H. pylori_ were absent from the serum being tested, the conjugate bound only to the control line (a negative test). Results were read visually at 4 min. The control line provided assurance that the test was functioning properly.

The FlexSure HP product insert reports 99% reproducibility of results in a blinded single-site study (5 serum samples, tested 10 times each on two lots of product by three technicians working independently) and 99% reproducibility of results in a multisite study (5 serum samples, tested 10 times each on two lots of product by individual technicians working in three geographically separated locations). In phase I of our study, banked serum samples with inaccurate FlexSure results were retested with the kit to check reproducibility. No serum samples from phase II of the study were retested.

**Statistics**

Sensitivity, specificity, and positive and negative predictive values were calculated in the traditional method [15]. All ages are reported as mean ± SD.

**Results**

**Phase I**

The ICT was performed on 107 select banked serum samples. The sera were obtained from a symptomatic cohort of 53 men and 54 women with a mean age of 53.7 ± 16.5 years. There were 54 Caucasian, 49 African-American, and 4 Asian-American patients.

Of 107 sera, 68 were _H. pylori_ positive (prevalence 63.5%) as established by the six tests. The remaining 39 (36.5%) sera were _H. pylori_ negative on all six tests.

Complete agreement between the ICT and _H. pylori_ status occurred in 102 of 107 serum samples (overall accuracy 95.3%). There were 65 true-positive, 37 true-negative, 2 false-positive, and 3 false-negative results. The ICT had sensitivity, specificity, and positive and negative predictive values of 96% [95% confidence interval (CI) 88–99%], 95% (83–99%), 97% (90–100%), and 93% (80–98%), respectively, in well-characterized banked sera with a _H. pylori_ prevalence rate of 63.5%.

The five banked serum samples with inaccurate _H. pylori_ results were retested with the FlexSure test kit. Results were fully reproducible, and no change in determined antibody status was found.

**Phase II**

Participating were 96 patients (45 men, 51 women) with mean age 51.1 ± 14.2 years. There were 50 Caucasians, 42 African-Americans, and 4 patients of Middle Eastern descent.

Of 96 patients, 57 (59.4%) were infected with _H. pylori_ as characterized by visualization of _H. pylori_ on antral biopsies or by the combination of a positive UBT and positive rapid urease test. The remaining 39 (40.6%) patients were negative on all tests and were deemed free of infection. The ICT correctly identified the _H. pylori_ status of 84 of 96 patients (overall accuracy 87.5%). There were 54 true-positive, 30 true-negative, 9 false-positive, and 3 false-negative results. Sensitivity, specificity, and positive and negative predictive values of the ICT in this population were 95% (95% CI 85–99%), 77% (61–89%), 86% (75–93%), and 91% (76–98%), respectively.

**Discussion**

Humans harboring _H. pylori_ in their gastric mucosa develop serum antibodies to the organism. Currently, these antibodies can be detected by several methods, including ELISA [16, 17]. This antibody detection method requires special equipment and skilled personnel and does not fulfill the need for a reliable, rapid, easy-to-use, in-office test for detection of anti- _H. pylori_ antibodies. In the present study, we evaluated the accuracy of an immunochromatographic method to detect anti- _H. pylori_ IgG in human serum. The ICT had a high sensitivity and specificity for detection of _H. pylori_ IgG antibody in banked sera (phase I). In sera from randomly selected patients (phase II), the test retained its high sensitivity but demonstrated only moderate specificity. Results similar to our analysis were obtained in another study [18].

Many factors affect the performance of a test used to screen a population. These include the prevalence rate of the disease, accuracy of the “gold standard” test(s) used to establish presence or absence of the disease, and the operational characteristics inherent to the methodology used to screen the population. There are several possible explanations for the reduction in specificity in phase II of the study.

Antibiotics are frequently prescribed for common infections (upper respiratory infections, urinary tract infections, etc.). Monotherapy with commonly used antibiotics, such as metronidazole or clarithromycin, can achieve _H. pylori_ eradication rates in up to 17–20% of patients [19, 20]. Serum antibody tests remain positive for a significant period, or perhaps indefinitely, after _H. pylori_ eradication.
Remote ingestion of certain antibiotics in potential study candidates could therefore result in eradication of \textit{H. pylori}. Such patients would have negative tests for urease activity (UBT and rapid urease test) and (or) nonvisualisation of bacteria on histology in the presence of detectable serum antibodies (a positive ICT). Such a situation would result in “false-positive” antibody test results and could contribute to a lower specificity of the ICT. Other conditions that could potentially lower the specificity of antibody-based testing include recent ingestion of antibiotics, bismuth compounds, and proton pump inhibitors. These agents alter gastric mucosal inflammation, bacterial distribution, and urease activity \cite{18,19,20}, making histologic detection of \textit{H. pylori} more difficult and thereby increasing the chances for false-negative \textit{H. pylori} infection status. The use of such products was an exclusion criterion for our study.

Various antigen preparations have been used to develop tests for detection of antibodies to \textit{H. pylori}. The ICT in this study has the HM-CAP antigen to detect anti-\textit{H. pylori} \(\text{IgG}\) antibody. The HM-CAP antigen is highly specific, yet cross-reactivity with other bacteria is always possible and could partly explain the observed decrease in specificity in phase II.

There is no single agreed-upon “gold standard” test for the establishment of \textit{H. pylori} infection. As such, research results can be affected by the manner in which bacterial infection is established. In our study, we used a combination of tests to eliminate biases inherent in individual test methodologies. Given our test requirements to determine \textit{H. pylori} infection, it is unlikely that the lowered ICT specificity in phase II was due to missed bacterial infection. Nevertheless, in phase I, only sera with agreement among all six tests, including \textit{H. pylori} \(\text{IgG}\) and \(\text{IgA}\) antibodies, was used. In phase II, infection status was determined by a three-test panel without regard to antibody status. Some spectrum bias between the two phases of the study is possible, in that \textit{H. pylori} infection status was more clearly established in phase I and less clearly delineated for phase II sera.

Identification of \textit{H. pylori} infection has become critical in the management of patients with gastroduodenal ulcer disease. The pathogenesis of \textit{H. pylori}-related gastroduodenal diseases (gastritis, ulcers, neoplasia) is not clearly understood, but eradication of this infection favorably alters the natural history of gastroduodenal ulcer disease. Currently, serology plays an important role in two situations, i.e., screening of various populations to understand the epidemiology of \textit{H. pylori} infection, and to identify those patients with gastroduodenal ulcer disease who are infected. Infected patients with active or quiescent gastroduodenal disease require antibiotic therapy \cite{18}. Absence of \textit{H. pylori} infection, as manifested by negative serological tests, in patients who are not exposed to nonsteroidal anti-inflammatory drugs almost completely excludes the presence of gastroduodenal ulcer disease as well as the precursor lesions of gastric carcinoma.

New whole-blood test technology has recently been approved for commercial distribution. However, whether this new methodology will be as accurate as the serum-based tests is unclear at present \cite{21}. Further clinical application of the whole-blood tests is required.

The ICT method used in this study is commercially available (FlexSure HP), inexpensive ($10 to $12 per kit), and easy to perform. Positive and negative controls are built into each test card. The positive predictive value of 86\% implies that the test could be used to identify \textit{H. pylori} infection in patients with upper gastrointestinal symptoms, though a small number of false-positive tests would result in some overtreatment. The negative predictive value of 91\% suggests that negative results could be used to rule out infection. Because results are available in 4 min, clinical decisions regarding patient care could be made during the visit. Antibody testing is expected to continue to improve and play an ever increasing role in the primary clinician’s diagnostic evaluation.

We thank SmithKline Diagnostics for supplying the FlexSure HP test kits.

\textbf{References}


