

**Gut Check #3 – Diagnostic Testing for Helicobacter pylori**

*Helicobacter pylori* is widely recognized as the major cause of peptic ulcer disease, and as a probable risk factor in the development of gastric adenocarcinoma and a subtype of gastric lymphoma (marginal zone lymphoma or MALToma). The seroprevalence of *H. pylori* infection is between 25-30% in developed countries (ranging from 5-27% in early childhood to > 50% in adults over 50 years old), while the prevalence of peptic ulcer disease in the United States is 2.5%. This article will review laboratory tests available for the diagnosis and treatment of *H. pylori* infection.

### Pathology of *H. pylori* infection

Acute infection with *H. pylori* produces a nonspecific acute gastritis characterized by neutrophils along the surface mucosa and superficial gastric pits. After 1-4 weeks, the histologic appearance changes to the characteristic (though nonspecific) “chronic active gastritis” pattern where mononuclear inflammatory cells (lymphocytes and monocytes) mix with neutrophils in the lamina propria. Lymphoid hyperplasia (germinal center formation) is common. With time (or treatment), the histology may evolve to a chronic “inactive” gastritis, characterized by the loss of neutrophils. Finally, the inflammatory component may recede almost entirely leaving an “atrophic gastritis” pattern, often with associated intestinal metaplasia. Recognition of the spiral or gull-shaped organisms is facilitated by the use of special stains (Giemsa or silver stains), immunostains or fluorescence in situ hybridization. The distribution of the gastritis and prevalence of the bacteria is variable. Over 90% of duodenal ulcers are associated with *H. pylori* infection, with an increasing prevalence of bacteria as one progresses from the corpus to the gastric antrum, and an antral-dominant chronic active gastritis histology. A gastric antral (cf. duodenal) biopsy is necessary to visualize the offending microorganism. *H pylori* may also produce gastric ulcers. Biopsy of the ulcer base is recommended to exclude a neoplastic process, while biopsy of the adjacent mucosa is necessary to identify the bacteria and look for gastritis, atrophy, metaplasia or dysplasia. Atrophic gastritis may be multifocal or corpus dominant; bacteria are often absent or difficult to recognize. Patients with antral dominant chronic active gastritis have an increased risk of duodenal ulcer, but no increased risk of gastric adenocarcinoma, while patients with gastric ulcers have an increased risk of gastric adenocarcinoma. Patients seropositive for *H pylori* have a 6-fold increased risk for gastric MALT lymphoma. MALToma generally arises as a low grade B-cell lymphoma, but may evolve into a more aggressive diffuse large B-cell lymphoma. *H pylori* organisms are frequently visualized in biopsies of low grade gastric MALTomas, but are rarely observed in either high grade lymphomas or gastric adenocarcinomas. Finally, administration of antibiotics or proton pump inhibitors (e.g. esomeprazole or lansoprazole) may alter the histologic nature of the gastritis (e.g. chronic inactive gastritis instead of chronic active gastritis), the morphology of the bacteria, and reduce the prevalence of bacteria – leading to an erroneous “negative” interpretation.

### Diagnostic Tests for *H pylori*

There are 5 laboratory tests commonly employed to evaluate *H. pylori* infection. Two of these (gastric urease, gastric biopsy) are “invasive”, requiring gastroscopy to obtain tissue for testing, while the other three (serology, urea breath test, stool antigen test) are “noninvasive”. All have acceptable performance characteristics, but differ somewhat in costs, limitations and benefits. All (except for serology) can also be used to test for eradication of infection in those patients where this is important (complicated ulcer disease, low grade MALToma, gastric dysplasia or “early gastric adenocarcinoma”). If using these tests to confirm eradication of the bacteria, a waiting period of 6 weeks is recommended (to avoid false (+) results).
Serology: The overwhelming majority of patients with *H. pylori* infection have IgG antibodies directed against the bacteria. A smaller subset has IgA antibodies against the bacteria. Seroconversion occurs between 22 – 33 days after infection.¹ Serology can be very useful as an initial screening test in patients presenting with dyspepsia. A negative result effectively rules out *H. pylori* infection. A positive result does not discriminate between an active infection producing symptoms or past infection. Nevertheless, for uncomplicated chronic dyspepsia, a positive serologic test is sufficient evidence for initiation of eradication therapy.³ The sensitivities and specificities quoted in the table below are for the IgG assay used at Rex. Rapid whole blood tests have been developed, but have decreased sensitivity and specificity. The test is less sensitive in pediatric patients (especially less than 5 years old) and in immunocompromised patients (e.g. HIV infection). For these patients either stool antigen or urea breath testing is recommended. Serologic tests may revert to negative 6-12 months after adequate therapy.

Stool Antigen Test: A low cost, relatively convenient test (that for some reason has just not “caught on”). We may discontinue the test in the future if business does not pick up. A false (+) result may occur in the setting of other urease-producing bacteria (e.g. *H. heilmannii*) or achlorhydria. Bismuth, proton pump inhibitors or antimicrobials may produce false negatives (and should be discontinued at least 2 weeks prior to testing).

Urea Breath Test: We use the Diasorin ¹³C urea breath test designed to exploit the urease enzyme possessed by *H. pylori*. After obtaining a baseline breath specimen, the patient is given a cocktail of ¹³C urea. After 15 minutes, a second breath specimen is obtained. Urease-containing *H. pylori* will metabolize the ¹³C urea into ¹³CO₂ and NH₄. An increase in the ratio of ¹³CO₂/¹²CO₂ from the second (test) specimen over the baseline indicates the presence of urease-containing bacteria in the stomach. A false (+) result may occur in the setting of other urease bacteria (e.g. *H. heilmannii*) or achlorhydria. Bismuth, proton pump inhibitors or antimicrobials may produce false negatives (and should be discontinued at least 2 weeks prior to testing). ¹³C is a stable nonradioactive isotope and there is no radiation risk to the patient. The specimens are collected at Rex (Mon. – Fri. only) and forwarded to Mayo Medical Laboratories for analysis.

Gastric Urease: Gastric mucosal biopsies are placed in agar gel with pH indicators. Urease-producing bacteria metabolize urea into NH₄ and carbamate. The NH₄ increases the pH and results in a color change as early as 3 hours after incubation, but a 24-hour incubation maximizes sensitivity and specificity. In patients with duodenal ulcers, one gastric antral specimen is generally sufficient. For patients with gastric ulcers, sensitivity improves if both antral and corpus are sampled. Gastric urease is less sensitive in pediatric patients.¹

Gastric Biopsy: Patients with the following characteristics should be considered for upper endoscopy: “alarm factors” (unexplained weight loss, bleeding/anemia, dysphagia, protracted vomiting), change in chronic symptoms, fear of cancer or other organic disease, or age > 45 years.³ Histologic evaluation permits accurate verification of *H. pylori* infection, in addition to evaluating the type of inflammatory response and the presence of complicating conditions (atrophy, intestinal metaplasia, dysplasia, or neoplasia). Antral and corpus sampling is recommended for maximum sensitivity in confirming the presence of *H. pylori*. In our laboratory, a rapid Giemsa stain has performed quite well in facilitating recognition of the bacteria in biopsy specimens. Lymphocyte marker immunostains can be performed on formalin-fixed paraffin embedded tissue if a suspicious lymphocytic infiltrate is identified.

The table below summarizes the performance characteristics of the tests described above and gives the current Rex Outreach charges. The cost for the invasive tests does NOT include the fees associated with the endoscopic procedure itself, only the laboratory tests. The gastric biopsy fee includes the technical and professional component, as well as the Giemsa stain charge. The performance data is an amalgam of data from the literature¹ and package inserts from the assays used at Rex.

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**Laboratory Tests for *H. pylori***

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<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Cost</th>
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<tbody>
<tr>
<td>Serology</td>
<td>97%</td>
<td>99%</td>
<td>$41.00</td>
</tr>
<tr>
<td>Stool Antigen Test</td>
<td>96%</td>
<td>96%</td>
<td>$47.00</td>
</tr>
<tr>
<td>Urea Breath Test</td>
<td>&gt; 95%</td>
<td>&gt; 95%</td>
<td>$150.00</td>
</tr>
<tr>
<td>Gastric Urease</td>
<td>93-100%</td>
<td>91-100%</td>
<td>$41.00</td>
</tr>
<tr>
<td>Gastric Bx. (Pathology)</td>
<td>&gt; 95%</td>
<td>&gt; 95%</td>
<td>$217.00</td>
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References:


**BROOM VERSUS BRUSH/SPATULA COLLECTION DEVICES FOR SUREPATH™ SPECIMENS: A Validation Study**

Introduction: SurePath™ thin layer Pap specimens, according to current FDA criteria, must be collected with a broom device. The Rover’s Cervex-Brush® is preferred because it has a detachable head. The Rover’s broom device has been shown to be highly effective in obtaining adequate cervicovaginal samples and, because of its unique design, is felt to provide a more representative sample of the transition zone when compared to endocervical brush/spatula collection devices. This is mainly due to the tendency to overreach the transition zone and inadvertently sample the high endocervical canal or lower uterine segment when using an endocervical brush. It has also been noted that there can be a prolonged learning curve for clinicians to adjust to the proper use of broom devices. Many clinicians prefer to adjunctively add an endocervical brush collection device to the broom device collection to insure adequate endocervical sampling, especially in patients with a small or stenotic cervical os. Other clinicians prefer an endocervical brush with spatula for collection of Pap specimens. We recently performed a study comparing the endocervical brush with spatula with the standard broom device for collection of SurePath™ specimens.

Material and Methods: A five-clinician practice was provided with 100 kits each consisting of two SurePath™ collection vials, a Rover’s Cervex-Brush® broom, a Surgipath® snap-off endocervical brush and a Rover’s Detachable Spatula. The clinicians were instructed to collect a specimen with the broom device and first, then collect a second specimen using the brush and spatula. In each instance the head of the device was detached and placed in the collection vial. All specimens were processed using the standard SurePath™ technique. One senior cytotechnologist and one cytopathologist reviewed all slides. Overall cellularity of the squamous component, adequacy of the endocervical component (ECC) and diagnostic efficacy were evaluated.

Results: 88 of the 100 kits were returned. The diagnosis of “Negative for Epithelial Abnormality” was rendered in 79 of 88 cases (89.9%) An interpretation of “Atypical Squamous Cells of Uncertain Significance” (ASCUS) or more significant epithelial abnormality was provided in 9/88 cases (10.1%). There was complete diagnostic concordance in 87 of 88 cases (99%) including one case of squamous cell carcinoma easily recognized on both preparations. The one discordant case consisted of a case of ASCUS in which the atypical cells were only noted on the brush/spatula specimen. All broom samples were judged to have adequate cellularity, although one (1.3%) brush/spatula sample was deemed to have inadequate cellularity for evaluation. In one case (1.3%) both samples lacked an endocervical cell component (ECC), but in 14/88 (17.7%) the broom sample had no ECC and in 2/88 (2.5%) the brush/spatula had no ECC. In many cases the broom device, which was collected first, had a more abundant squamous component.
Conclusions: Both the standard Rovers’ broom device and the endocervical brush with spatula function satisfactorily in the collection of adequate SurePath™ specimens. The broom device consistently collected a more cellular squamous component. This is in part due to the unavoidable bias encountered by having the broom specimen collected first in each case. Nevertheless, the cutting action of the broom device’s bristles appears to maximize collection of squamous epithelial cells when compared to the smooth edge of a spatula. In several cases the endocervical brush collected an adequate endocervical component when the broom device did not. Based on the results of this study we feel confident that a clinician may use either the standard Rover’s broom device or a combination of an endocervical brush with an ectocervical spatula to collect an adequate SurePath™ specimen. However, we recommend the use of the Rover’s broom device with an adjunctive endocervical brush in order to maximize both ectocervical and endocervical sampling.

Keith V. Nance, MD


Laboratory Notes

- A Client Response Center is now available to assist you with a variety of inquiries: test results, patient preparation, specimen requirements, add-on testing or other customer service needs. The Center is open 0830 –1730, Monday through Friday. The phone number is 784-6000. The fax number is 784-6299.

- Effective immediately, the methodology for hemoglobin A1c has changed to an automated method based on a turbidometric inhibition immunoassay. The method has been certified by the National Glycohemoglobin Standardization Program and is quite similar to the HbA1c method offered at Mayo Medical Laboratories. It measures any glycated hemoglobin variants that are glycated at the beta-chain N-terminus of the hemoglobin molecule that have epitope sequences identical to HbA1c (amino acid sequence: VAL-HIS-LEU-THR). This includes HbS, HbC, HbG, HbE, etc. There is no interference from the labile fraction of glycohemoglobin, nor interference or cross reactivity with HbA0, HbA1a, HbA1d, acetylated Hb, carbamylated Hb, or glycated albumin. The reference range remains the same (4.8 – 6.0%). The American Diabetes Association recommends that a primary goal of therapy should be a HbA1c ≤ 7%. Physicians should reevaluate treatment regimens in patients with HbA1c levels consistently > 8%.

- Effective June 2, 2003 serologic tests for HIV and hepatitis C antibodies will be referred to Mayo Medical Laboratories. Effective immediately, most “special coagulation tests” not performed at Rex will be referred to Mayo Medical Laboratories.