**H. pylori Ag Card test**

**LATERAL FLOW TEST FOR THE DETECTION OF HELICOBACTER PYLORI ANTIGENS IN STOOL SAMPLE.**

| REF | 88 88 97 | 20 tests |

**INTENDED USE**
The H. pylori Antigen Test is an *in vitro* qualitative immunochromatographic assay for the rapid detection of *Helicobacter pylori* antigens in human stool specimen. The test results are intended to aid in the diagnosis of *H. pylori* infection, to monitor the effectiveness of therapeutic treatment and to confirm the eradication of *H. pylori* in peptic ulcer patients.

**INTRODUCTION**
*Helicobacter pylori* is a corkscrew-shaped, gram-negative rod that lives in the mucous layer of the stomach. *H. pylori* infection is now accepted as the most common cause of gastritis, and is etiologically involved in gastric ulcer, duodenal ulcer, gastric adenocarcinoma and primary gastric B-cell lymphoma.\(^1,2\)
The organism is very common, infected at least half of the world’s population. *H. pylori* infection is typically acquired in childhood. Once acquired, infection persists chronically, probably continuing in the stomach throughout life. The damage to gastric structure and function of stomach is constant and direct. Approximately one in six of *H. pylori* infection develops peptic ulcer disease and a small portion of *H. pylori* infection leads to gastric cancer.\(^3\) The diagnostic tests for *H. pylori* can be classified into two categories: Invasive and Noninvasive tests. Direct detection by invasive test procedures requires an endoscopy and biopsy specimens from antrum and stomach body.\(^4\) The presence of *H. pylori* is then confirmed by direct culture, histological examination or rapid urease test. The endoscopy and biopsy specimens offer direct detection of active *H. pylori* infections. Although the procedure is highly specific and high positive predictive value, the cost and discomfort to the patients are very high. The most widely available noninvasive test is probably the serological based test. The serology test detects *H. pylori* specific IgG antibody in patient serum with current or prior infection.\(^5,6\)
Serology test is a simple, convenient test with relative high sensitivity. The main limitation of serology test is the inability to distinguish current and past infections. Antibody may be present in the patient’s serum long after eradication of the organism.\(^6\)
The urease breath test (UBT) with 14C or 13C labeled urea, is a noninvasive test based on the urease activity of the organism. UBT detects active *H. pylori* infection and is highly sensitive and specific. The UBT requires a high density and active bacteria and should not be performed until 4 weeks after therapy to allow residual bacterial to increase to a sufficient number for detection.\(^7\)

*H. pylori* Antigen Test is an immunochromatographic assay that uses antibody-coated colloidal gold to detect the presence of *H. pylori* antigens in stool specimens. The test detects directly antigens in specimens for an active infection.
The test is simple and easy to perform and the test results can be visually interpreted within 10 minutes.

**PRINCIPLE OF THE TEST**
AXIOM *H. pylori* Antigen Test is a sandwich solid phase immunochromatographic assay.
To perform the test, an aliquot of diluted stool sample is added to the sample well of the test cassette. The sample flows through a label pad containing *H. pylori* antibody coupled to red-colored colloidal gold. If the sample contains *H. pylori* antigens, the antigen will bind to the antibody coated on the colloidal gold particles to form antigen-antibody-gold complexes. These complexes move on the nitrocellulose membrane by capillary action toward the test line region on which *H. pylori* specific antibodies are immobilized. As the complexes reach the test line, they will bind to the antibody on the membrane in the form of a line. A second red control line will always appear in the result window to indicate that the test has been correctly performed and the test device functions properly. If *H. pylori* antigen is not present or lower than the detection limit of the test, only the control line will be visible. If the control line dose not developed, the test is invalid.
COMPONENTS

Materials provided
- Test Device 20pcs
- 3in1 vial, 20 pcs. Each vial contains 1.5ml Extraction Buffer.
- Instruction for use

Materials required but not provided
- Timer
- Disposable cloves

PRECAUTIONS
1. For professional in vitro diagnostic use only.
2. Do not use after expiration date.
3. Do not use test if the aluminum pouch has been damaged during storage.
4. All the specimens should be considered potentially hazardous and handled in the same manner as an infectious agent.
5. The test should be discarded in a proper biohazard container after testing.
6. Wipe up the spills using 5% hypochlorite solution.
7. Avoid microbial contamination of reagents.

SPECIMEN COLLECTION
Stool specimens should be collected in containers that do not contain media, preservatives, animal serum or detergents as any of these additives may interfere with H. pylori Ag test. Specimens may be stored at 2-8°C for 3 days without interfering with the assay performance. For long-term storage of specimens, -20°C or colder is recommended. Repeated freezing and thawing of specimens is not recommended and may cause erroneous results. Do not store specimens in self-defrosting freezers.

STORAGE AND STABILITY
Store as packaged in the sealed pouch at 4-30°C. The test is stable through the expiration date printed on the sealed pouch. The test must remain in the sealed pouch until use. Do not freeze.

ASSAY PROCEDURE
Step 1  Allow all reagents to reach room temperature before use.
Step 2  Remove the test card from the sealed foil pouch.
Step 3  Unscrew the cap of the 3in1 vial and remove the applicator stick. Insert the stick into the fecal specimen at several different sites.
Step 4  Insert the sample applicator back into the vial and tighten the cap securely. Shake the vial 5 seconds to release and disperse the stool sample into the extraction buffer.
Step 5  Hold the vial upright with tip pointes toward the direction away from the test performer. Snap off the tip.
Step 6  Hold the vial in a vertical position over the sample well and add 3-4 drops of the sample into the sample well.
Step 7  Incubate the test at room temperature and read the test after 5-10 minutes.

INTERPRETATION OF RESULTS

Negative:
One pink line appears in control line, showing the test has been carried out correctly.
There will be no line in test region

Positive:
In addition to a pink colored control line, a distinct pink colored band will also appear in the test region.

Invalid:
A total absence of color in both regions is an indication of procedure error and/or that the test reagent has deteriorated. The test should be repeated using a new strip.
**PERFORMANCE CHARACTERISTICS**

AXIOM H. pylori Antigen Test was evaluated on 280 adults. The test results were compared to diagnosis of *H. pylori* infection by reference tests, urease breath test and histology tests.

Patients were considered positive if both rapid urease and histology tests were positive. Patients with both negative urease breath test and histology tests were considered negative. Among 67 positive samples and 213 negative samples, AXIOM H. pylori Antigen Test showed:

<table>
<thead>
<tr>
<th>AXIOM H. pylori Ag test</th>
<th>Reference Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Positive</td>
<td>63</td>
</tr>
<tr>
<td>negative</td>
<td>4</td>
</tr>
</tbody>
</table>

Specificity: 210/(210+4) = **98%**

Sensitivity: 63/(63+3) = **95%**

**QUALITY CONTROL**

1. The control band is an internal reagent and procedural control. It will appear if the test has been performed correctly and the reagents are reactive.

2. Good Laboratory Practice recommends the daily use of control materials to validate the reliability of the device. Control materials which is not provided with this test kit may be commercially available.

**ASSAY SPECIFICITY**

Following bacterial and viral strains were used to test the specificity of AXIOM H. pylori Ag test. Positive and negative stools were spiked with >1x10⁸ organism/ml and tested by Axiom H. pylori Ag test. *H. pylori* positive stool remained positive with the spiked organisms. Negative stool remained negative with the spiked organisms.

Microorganism and virus tested:

- **Adenovirus type II**
- **Campylobacter jejuni**
- **Campylobacter fetus**
- **Clostridium difficile**
- **Enterobacter cloacae**
- **Escherichia coli**
- **Helicobacter mustelae**
- **Klebsiella pneumoniae**
- **Providencia stuartii**
- **Nocardia asteroides**
- **Pseudomonas aeruginosa**
- **Pseudomonas fluorescens**
- **Salmonella dublin**
- **Salmonella minnesota**
- **Shigella sonnei**
- **Staphylococcus epidermidis**
- **Serratia liquefaciens**
- **Staphylococcus aureus**
- **Staphylococcus faecalis**
- **Yersinia enterocolitica**

**LITERATURE**

8. National Committee for Clinical Laboratory Standards. Internal quality control: Principles and definitions; Approval Guideline,


**Helicobacter pylori Antigen Rapid test**

1. Unscrew the cap of the collection tube and remove the applicator stick.

2. Insert the stick into the fecal specimen at several different sites.

3. Insert the sample applicator back to the tube and tighten the cap securely.

4. Shake the tubes vigorously for about 5 seconds to release and disperse the stool sample into the collection buffer.

5. Hold the tube upright with tip pointed toward the direction away from the test performer.

6. Snap off the tip.

7. Hold the tube in a vertical position over the sample well of the test card and deliver 3 drops (120-150 μl) of the sample into the sample well markes as „S“ on the cassette.

8. Read the result within 10-15 minutes.