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HELICOBACTER PYLORI ANTIGEN TEST

CE REF R13-525 IVD

Immunochromatographic rapid assay for the Detection of Helicobacter pylori Antigens in Human Stool Specimens

INTENDED USE

Immunospec 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 ^{14}C or ^{13}C 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]

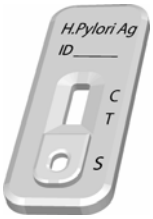
H. pylori Antigen Test is an immune-chromatographic assay that uses antibody- coated colloidal gold to detect the presence of *H. pylori* antigens in stool specimens. The test detects directly antigens in specimens for an active infection. The test is simple and easy to perform and the test results can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

PRINCIPLE OF THE TEST

Immunospec 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.

MATERIALS PROVIDED

1. H. Pylori Antigen test card
   Each cassette contains a test strip with H. pylori specific antibody on the test region of the membrane and colored H. pylori antibody-gold conjugate pad.



H. Pylori Antigen Test Card


Sample bottle

2. Sample bottle
   Each sample bottle contains 1 ml of stool specimen collection buffer. Store at 4-30°C

MATERIALS NOT PROVIDED

1. Specimen collection container
2. Timer.

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic use.
2. Wear protective glove while handling kit components and test specimens.
3. Patient specimens and inactivated Positive Control may contain infectious agents and should be handled and disposed of as potential biohazards.
4. Do not use kit components beyond expiration date.
5. Dispose all used materials in appropriate container. Treat as potential biohazard.

STORAGE INSTRUCTION

1. The expiration date is indicated on the package label.
2. Sample Collection Tubes without introducing the sample can be stored at 4-30^{o}C.
3. Test device can be stored at 4-30 $^{\circ}$C.

SPECIMEN COLLECTION AND STORAGE

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 the H. pylori Antigen Test.

Specimens may be stored at 2-8^{o}C for 3 days without interfering with the assay performance. For long-term storage of specimens, -20^{o}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.

REAGENT PREPARATION

Bring all reagents, including test device, to room temperature before use.

SPECIMEN PREPARATION

1. Unscrew the sample bottle, use the attached applicator stick attached on the cap to transfer small piece of stool (5-6 mm in diameter; approximately 100 mg – 200 mg/0.1-0.2 g) into the sample bottle containing specimen preparation buffer.
2. Replace the stick in the bottle and tighten securely. Mix stool sample with the buffer thoroughly by shaking the bottle for a few seconds.

   Note: Watery or diarrhea specimens are inappropriate for testing.

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PROCEDURE

1. Bring all materials and specimens to room temperature.
2. Remove the test card from the sealed foil pouch.
3. Hold the sample bottle upright with the tip point toward the direction away from the test performer, snap off the tip.
4. Hold the bottle in a vertical position over the sample well of the test card, deliver 3 drops (120 -150 µL) of diluted stool sample to the sample well.
5. Read the result within 10 to15 minutes. A strong positive sample may show result earlier.

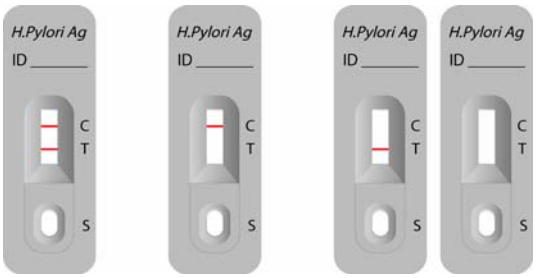
   Test results after 15 minutes may not be accurate.

INTERPRETATION OF RESULTS

Positive result: A distinct pink colored band appears on test line regions, in addition to a pink line on the control line region.

Negative result: No line appears in the test line region. A distinct pink line shows on the control line region.

Invalid: The control line next to the test line does not become visible within 10 minutes after the addition of the sample.



POSITIVE **NEGATIVE** **INVALID**

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.

LIMITATIONS

1. The test is for qualitative detection of H. pylori antigen in stool sample and dose not indicate the quantity of the antigens.
2. The test is for *in vitro* diagnostic use only.
3. The test result should be used only to evaluate with patient with signs and symptoms of gastrointestinal disease. A definitive clinical diagnosis should only be made by the physician after all clinical and laboratory finding have been evaluated.

EXPECTED VALUES

Helicobacter pylori infects more than half the people in the world.[9] The prevalence of the infection varies among countries and among different groups within the same country.[10] The prevalence rate in the United State suggests an incidence of infection of 2%. The lifetime prevalence of peptic ulcer disease is about 12% in men and 9% in women.[11] Studies have found that more than 90% of patients with duodenal ulcer and 80% of patients with gastric ulcer are infected with *H. pylori*.[12,13]

H. pylori Antigen Test detects the presence of *H. pylori* antigens in stool specimens. Expected values for any given population should be determined for each laboratory. The positivity rate of any given laboratory may vary depending on geographic location, ethnic group, and living environment.

PERFORMANCE CHARACTERISTICS

Sensitivity and Specificity

Helicobacter pylori Antigen Test was evaluated on 170 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 fifty (50) positive samples and one hundred and twenty (120) negative samples, H. pylori Antigen Test showed 94.0% clinical sensitivity and 96.7% specificity. The accuracy is 97.5%.

	Reference Test		
Immunospec H. Pylori Antigen Test		Positive	Negative
	Positive	47	4
	Negative	3	116

Sensitivity = 94.0% (47/50)
Specificity = 96.7% (116/120)
Positive Predictive Value = 92.2% (47/51)
Negative Predictive Value = 97.5% (116/119)
Accuracy = 95.9% (163/170)

Reproducibility

Reproducibility of H. pylori Rapid Antigen Test was determined using negative, low positive, and high positive samples along with negative and positive controls. These samples were tested in replicates of 8 in a blind study by 5 operators working independently in the same laboratory. The agreement of the expected result was 100%.

Assay Specificity

Following bacterial and viral strains were used to test the specificity of H. pylori Antigen Test. Positive and negative stools were spiked with $>1x10^8$ organism/ml and tested by H. pylori Antigen 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 coli
Campylobacter fetus
Campylobacter jejuni
Campylobacter lari
Candida albicans
Citrobacter freundii
Clostridium difficile
Clostridium perfringens
Enterococcus feacalis
Enterobacter cloacae
Escherichia coli
Escherichia fergusonii
Escherichia hermanii
Helicobacter cinaedi
Helicobacter mustelae
Klebsiella pnumoniae
Mycobacterium smegmatis
Providencia stuartii
Nocardia asteroids
Proteus vulgaris
Pseudomonas aeruginosa
Pseudomonas fluorescen
Rotavirus
Salmonella(Group B)
Salmonella dublin
Salmonella hilversum(Group N)
Salmonella typhimurium
Salmonella minnesota
Shigella boydii
Shigella dysenteriae
Shigella flexneri
Shigella sonnei
Serratia liquefaciens
Staphylococcus aureus
Staphylococcus aureus (Cowan)
Staphylococcus faecalis
Staphylococcus galactiae
Staphylococcus epidermidis
Yersinia enterocolitica

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H.pylori Ag

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