

H pylori Ag Test

Lateral flow test to detect helicobacter pylori antigen in stool sample

Catalog #: AN1010C

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 15 minutes

PRINCIPLE OF THE TEST

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.

REAGENTS

Materials provided with the kits:

1. Test Device 25pcs
2. Extraction Buffer, 15mL
3. Instruction for use

PRECAUTION FOR USERS

1. For in-vitro diagnostic use only.
2. Must not use kit beyond the expiration date.

3. Do not mix components from kits with different lot number.
4. Avoid microbial contamination of reagents.
5. Do not pipet reagent by mouth and no smoking or eating while performing assays.
6. Wear gloves during the whole process and avoid reagents or specimen spilling-out.
7. Wipe up the spills using 5% hypochlorite solution.
8. Decontaminate all liquids or solid wastes before depositing.

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 the HP 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 OF TEST KIT

The HP Ag Test can be stored at any temperature between 4-30°C. **Do not freeze.** The stability of the kit under these storage conditions is 24 months. Use up the reagents as soon as possible after the kit is unpacked within 3 months.

ASSAY PROCEDURES

1. Allow all reagents to reach room temperature before use.
2. Place 0.5-1ml of extraction buffer in a properly marked testing tube.
3. Add a sample portion of approximately 5-6 mm size (25-100mg), with a swab, a wooden applicator or a bacteriology loop. Press the applicator to the tube and rotating it at the same time. For liquid or semi-solid stools add 100 microliters of stool using an appropriate pipette.
4. Vortex or stir to release the virus into diluent.
5. Add 3-4 drops to the sample well of the test device.
6. Incubate the test at room temperature and read the test after **5-15 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

Reference Test (Lateral Flow)

Positive	Negative
63	4

Asia-lion HP Ag Test

Positive	Negative
63	3
4	210

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

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

Assay Specificity

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

Microorganism and virus tested

<i>Adenovirus type II</i>	<i>Campylobacter jejuni</i>
<i>Campylobacter coli</i>	<i>Campylobacter lari</i>
<i>Campylobacter fetus</i>	<i>Candida albicans</i>
<i>Citrobacter freundii</i>	<i>Clostridium difficile</i>
<i>Clostridium perfringens</i>	<i>Enterococcus faecalis</i>
<i>Enterobacter cloacae</i>	<i>Escherichia coli</i>
<i>Escherichia fergusonii</i>	<i>Escherichia hermanii</i>
<i>Helicobacter cinaedi</i>	<i>Helicobacter mustelae</i>
<i>Klebsiella pneumoniae</i>	<i>Mycobacterium smegmatis</i>

<i>Providencia stuartii</i>	<i>Nocardia asteroides</i>
<i>Proteus vulgaris</i>	<i>Pseudomonas aeruginosa</i>
<i>Pseudomonas fluorescens</i>	<i>Rotavirus</i>
<i>Salmonella</i> (Group B)	<i>Salmonella dublin</i>
<i>Salmonella hilversum</i> (Group N)	<i>Salmonella typhimurium</i>
<i>Salmonella minnesota</i>	<i>Shigella boydii</i>
<i>Shigella dysenteriae</i>	<i>Shigella sonnei</i>
<i>Shigella flexneri</i>	<i>Staphylococcus epidermidis</i>
<i>Serratia liquefaciens</i>	<i>Staphylococcus aureus</i>
<i>Staphylococcus aureus</i> (Cowan)	<i>Staphylococcus faecalis</i>
<i>Staphylococcus galactiae</i>	<i>Yersinia enterocolitica</i>

1. Marshall, B.J. and Warren, J.R. Unidentified curved bacilli in the stomach of patients with gastric and peptic ulceration. *Lancet* 1:1984: 1311-1314.
2. Graham K.S and Graham D.Y. 1999. Contemporary Diagnosis and Management of *H. pylori*-Associated Gastrointestinal Diseases, Handbooks in Health Care Co., Newtown, PA., 1999: 39-67.
3. Howden C.W. Clinical expressions of *Helicobacter pylori* infection. *Am J Med*; 1996;100:27S-33S.
4. El-Zimaity HM, Al-Assi MT, Genta RM, Graham DY. Confirmation of successful therapy of *Helicobacter pylori* infection: number and site of biopsies or a rapid urease test. *Am J Gastroenterol*. 1995;90:1962-1964.
5. Talley NJ, Newell DG, Ormand JE, et al. Serodiagnosis of *Helicobacter pylori*: Comparison of enzyme-linked immunosorbent assays. *J. Clin Microbiol*. 1991;29:1635-1639.
6. Cutler AF. Testing for *Helicobacter pylori* in clinical practice. *Am J. Med*. 1996;100:35S-41S.
7. Klein PD, Malaty HM, Martin RF, et al. Noninvasive detection of *Helicobacter pylori* infection in clinical practice: the 13C urea breath test. *Am J. Gastroenterol*. 1996;91:690-694.
8. National Committee for Clinical Laboratory Standards. Internal quality control: Principles and definitions; Approval Guideline, NCCLS document C24-A (NCCLS, 771 East Lancaster Ave, Vallanova, PA 19085, 1991).
9. Marshall BJ. *JAMA*. 1995;274:1064-1066
10. Breuer T, Malaty HM, Graham DY. The epidemiology of *H. pylori*-associated gastroduodenal diseases. In: Ernst PB, Michetti P, Smith PD, eds. The Immunobiology of *H. pylori*: From Pathogenesis to Prevention. Philadelphia. Lippincott-Raven, 1997:1-14.
11. Graham DY, Malaty HM, Evans DG, Evans, Jr. DJ, Klein PD, and Adam E. Epidemiology of *Helicobacter pylori* in a asymptomatic population in the United States. Effect of age, race, and socioeconomic status. *Gastroenterology*. 1991;100:1495-1501.
12. Anand BS, Raed AK, Malaty HM, et al. Low point prevalence of peptic ulcer in normal individual with *Helicobacter pylori* infection. *Am J Gastroenterol*. 1996;91:1112-1115.
13. Tytgat GNJ, Noach LA, Rauws EAJ. *Helicobacter pylori* infection and duodenal ulcer disease. *Gastroenterol Clin North Am*. 1993;22:27-139.

Revision: AB200804

Manufacturer:

Biocare Diagnostics Ltd.
6F, Building B, 108 Xinghua Road
Xiangzhou, Zhuhai, China 519000
Tel: +86-756-8238560
E-mail: info@ivdbiocare.com
Website: www.ivdbiocare.com

April 10, 2008 Revision: 03
