# H. pylori Ag Cassette Rapid Test - (Fecal Specimen)

BIOGENIX INC. PVT. LTD.

IVD **In Vitro Diagnostic**

### INTENDED USE

The H. pylori Ag Cassette Rapid Test is a lateral flow chromatographic immunoassay for the qualitative detection of H. pylori antigen in human fecal specimen. It is intended to be used by professionals as a screening test and as an aid in the diagnosis of infection with H. pylori. Any reactive specimen with the H. pylori Ag Cassette Rapid Test must be confirmed with alternative testing method(s) and clinical findings.

### SUMMARY AND EXPLANATION OF THE TEST

Helicobacter pylori is associated with a variety of gastrointestinal diseases including non -ulcer dyspepsia, duodenal and gastric ulcers and active, chronic gastritis[1,2]. The prevalence of H. pylori infection could exceed 90% in patients with signs and symptoms of gastrointestinal diseases. Recent studies indicate an association of H. pylori infection with stomach cancer[3].

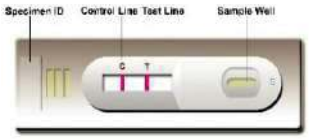
H. pylori can be transmitted through the ingestion of food or water that is tainted with fecal matter. Antibiotics in combination with bismuth compounds have been shown to be effective in treating active H. Pylori infection.

H. pylori infection is currently detected by invasive testing methods based on endoscopy and biopsy (i.e. histology, culture) or non-invasive testing methods such as urea breath test (UBT), serologic antibody test and stool antigen test. UBT requires expensive lab equipment and consumption of a radioactive reagent. Serologic antibody tests do not distinguish between currently active infections and past exposures or infections that have been cured. The stool antigen test detects antigen present in the feces, which indicates an active H. pylori infection. It can also be used to monitor the effectiveness of treatment and the recurrence of an infection.

The H. pylori Ag Cassette Rapid Test uses a colloidal gold conjugated monoclonal anti-H. pylori antibody and another monoclonal anti-H. pylori antibody to specifically detect H. pylori antigen present in the fecal specimen of an infected patient. The test is user friendly, accurate, and the result is available within 15 minutes.

### TEST PRINCIPLE

The H. pylori Ag Cassette Rapid Test is a sandwich lateral flow chromatographic immunoassay. The test strip consists of: 1) a burgundy colored conjugate pad containing monoclonal anti-H. pylori antibody conjugated with colloidal gold (anti-H.p. conjugates) and 2) a nitrocellulose membrane strip containing a test line (T line) and a control line (C line). The T line is pre-coated with another monoclonal anti-H. pylori antibody, and the C line is pre-coated with goat anti-mouse IgGantibody.




When an adequate volume of extracted fecal specimen is dispensed into the sample well of the test cassette, the specimen migrates by capillary action across the cassette. H. pylori antigens, if present in the specimen, will bind to the anti-H.p. conjugates. The immunocomplex is then captured on the membrane by the pre-coated antibody forming a burgundy colored T line, indicating an H. pylori positive test result. Absence of the T line suggests that the concentration of H. pylori antigens in the specimen is below the detectable level, indicating an H. pylori negative test result.

The test contains an internal control (C line) which should exhibit a burgundy colored line of the immunocomplex of goat anti-mouse IgG/mouse IgG-gold conjugate regardless of the color development on the T line. If the C line does not develop, the test result is invalid and the specimen must be retested with another device.

### REAGENTS AND MATERIALS PROVIDED

1. Individually sealed foil pouches containing:
   a. One cassette device
   b. One desiccant
2. Extractiontubes
3. Sample extration (1ml/ bottle)
4. One package insert (instruction for use)

### MATERIALS MAY BE REQUIRED AND NOT PROVIDED

1. qH. pylori Ag Rapid Test Control Kit (Cat # 5315R) contains one vial of positive control and one vial of negative control

### MATERIALS REQUIRED BUT NOT PROVIDED

1. Clock or Timer
2. A container to hold fecal specimen

### WARNINGS AND PRECAUTIONS

***For in Vitro Diagnostic Use***

1. This package insert must be read completely before performing the test. Failure to follow the insert causes inaccurate test results.
2. Do not open the sealed pouch, unless ready to conduct the assay.
3. Do not use any kit components beyond their stated expiration dates.
4. Do not use the components from any other type of test kit as a substitute for the components in this kit.
5. Bring all reagents to room temperature (15°C-30°C) before use.
6. Do not scoop stool sample as this may lead to excess fecal specimen that tends to clot the sample pad and interfere with sample migration.
7. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
8. Users of this test should follow the US CDC Universal Precautions for biosafety.
9. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
10. Extraction buffer contains 0.1% $NaN_3$. Avoid contact with skin or eyes. Do not ingest.
11. Dispose of all specimens and materials used to perform the test as biohazardous waste.
12. The test results should be read within 15 minutes after a specimen is applied to the sample well of the device. Reading results after 20 minutes may give erroneous results.
13. Do not perform the test in a room with strong air flow, i.e. electric fan or strong air-conditioning.

### REAGENT PREPARATION AND STORAGE INSTRUCTIONS

All reagents are ready to use as supplied. Store unopened test devices at 2°C-30°C. If stored at 2°C-8°C, ensure that the test device is brought to room temperature before opening. The test device is stable through the expiration date printed on the sealed pouch. Do not freeze the kit or expose the kit to temperatures above 30°C.

### SPECIMEN COLLECTION AND HANDLING

Consider any materials of human origin as infectious and handle them using standard biosafety procedures.

To prepare specimens using solid stool samples follow Procedure A below. To prepare specimens using watery stool samples follow Procedure B below.

**Procedure A: Solid stool samples**

**Step 1:**Collect a random stool sample in a clean, dry receptacle.

**Step2:** Open the stool collection device by unscrewing the top, and then use the collection stick to randomly pierce the stool sample in at least five different sites. Do not scoop stool sample as this may lead to an invalid test result.

**Step 3:** Ensure stool sample is only in the grooves of the collection stick. Excess stool sample may lead to an invalid test result.

**Step 4:** Replace the collection stick and tighten securely to close the stool collection device.

**Step 5:** Shake the stool collection device vigorously.


**≥5 sites**

The specimen is now ready for testing, transportation or storage.

**Procedure B: Watery stool samples**

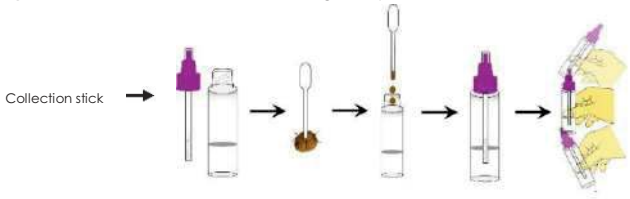
**Step 1:** Collect a random stool sample in a clean, dry receptacle.

**Step 2:** Open the stool collection device by unscrewing the top.

**Step 3:** Fill the plastic dropper with the sample; dispense 2 drops (70-85 µL) into the stool collection device.

**Step 4:** Replace the collection stick and tighten securely to close the stool collection device.

**Step 5:** Shake the stool collection device vigorously.




**2 drops**

The specimen is now ready for testing, transportation or storage.

**Note:** Specimens extracted may be stored at 2°C-8°C for up to 3 days. If longer storage is required, freezing at ≤-20°C is recommended.

### ASSAY PROCEDURE

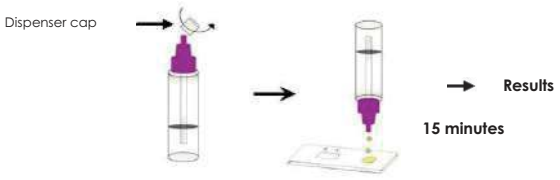
**Step 1:** Bring the specimen and test components to room temperature if they`re refrigerated or frozen.

**Step 2:** When ready to test, open the pouch at the notch and remove the test device. Place the test device on a clean, flat surface.

**Step 3:** Shake the stool collection device vigorously to ensure a homogenous liquid suspension.

**Step 4:** Position the stool collection device upright and twist off the dispenser cap. Holding the stool collection device vertically, dispense 2 drops of the solution into the sample well of the test device. Do not overload sample.

# H. pylori Ag Rapid Test - (Fecal Specimen)




**Step 5:** Set up the timer.
**Step 6:** Results can be read 15 minutes after adding the specimen. Positive results can be visible in a time period as short as 1 minute.
***Don't read result after 20minutes. To avoid confusion, discard the test device afteri nterpreting the result.***

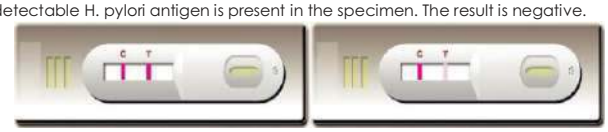
**QUALITY CONTROL**

**1. Internal Control:** This test contains a built-in control feature, the C line. The C line develops after adding specimen extract. If the C line does not develop, review the entire procedure and repeat the test with a new device.
**2. External Control:** Good Laboratory Practice recommends using external positive and negative controls to assure the proper performance of the assay, particularly under the following circumstances:

a. A new operator uses the kit, prior to performing testing of specimens.
b. A new lot of test kits is used.
c. A new shipment of test kits is used.
d. The temperature used during storage falls outside of 2°C-30°C.
e. The temperature of the test area falls outside of 15°C-30°C.
f. To verify a higher than expected frequency of positive or negative results.
g. To investigate the cause of repeated invalid results.

**INTERPRETATION OF ASSAY RESULT**

**1. NEGATIVE RESULT:** If only the C line is developed, the test indicates that no detectable H. pylori antigen is present in the specimen. The result is negative.



**2. POSITIVE RESULT:** If both C and T lines are developed, the test indicates the presence of H. pylori antigen in the specimen. The result is positive.

***Samples with positive results should be interpreted in conjunction with other testing procedures and clinical findings before a diagnostic decision is made.***

**3. INVALID:** If no C line is developed, the assay is invalid regardless of any color development on the T line as indicated below. Repeat the assay with a new test device.
**Excess fecal specimen can lead to invalid test results; if this is the cause, re-sample and re-test (see instructions for collection of specimen).**

**PERFORMANCE CHARACTERISTICS**

1. **Clinical Performance**

324 fecal samples collected from subjects with symptomatic gastrointestinal disorders and non-gastrointestinal symptoms were tested with the qH. pylori Ag Rapid Test and with the UBT as reference test. A comparison of the results for all subjects is shown in the following table:

| | H. pylori Ag Rapid Test | | |
|---|---|---|---|
| **UBT** | **Positive** | **Negative** | **Total** |
| **Positive** | 118 | 7 | 125 |
| **Negative** | 0 | 199 | 199 |
| **Total** | 118 | 206 | 324 |

Relative Sensitivity: 94.4% , Relative Specificity: 100.0%, Overall Agreement: 97.8%

2. **Analytical Sensitivity**

The detection limit for the H. pylori Ag Rapid Test is 5 ng/ml of *H. pylori* lysate. Fecal specimen extractions containing *H. pylori* lysate equal to or greater than 5 ng/ml routinely test positive. Specimens containing *H. pylori* lysate less than 5 ng/ml may also produce a very faint positive line, especially with an assay time extended beyond 15 minutes.

The following experiments were done to validate the sensitivity of the qH. pylori Ag Rapid Test:

Normal fecal specimen extractions were spiked with *H. pylori* lysate to concentrations of 0, 1.25, 2.5, 5, 10, 20 ng/ml. The specimens were run on the qH. pylori Ag Rapid Test. Results are shown in the table below.

| ***H. pylori* lysate ng/ml** | **0** | **1.25** | **2.5** | **5** | **10** | **20** |
|---|---|---|---|---|---|---|
| Number of positive | 0 | 0 | 12 | 20 | 20 | 20 |
| Number of negative | 20 | 20 | 8 | 0 | 0 | 0 |

n=20 Relative Sensitivity at 5 ng/ml = 20/20 x 100% = 100%

**LIMITATIONS OF TEST**

1. The Assay Procedure and the Interpretation of Assay Result sections must be followed closely when testing for the presence of H. pylori antigen in feces. Failure to follow the procedure, particularly the Specimen Collection procedure, may cause inaccurate results.
2. The CellexqH. pylori Ag Cassette Rapid Test is limited to the qualitative detection of H. pylori antigenin human fecal specimen. The intensity of the test line does not have a linear correlation with the antigen titer in the specimen.
3. A negative result for an individual subject indicates the absence of detectable H. pylori antigen. However, a negative test result does not preclude the possibility of infection with H. pylori.
4. A negative result can occur if the quantity of the H. pylori antigen present in the specimen is below the detection limits of the assay or if the antigens that are detected are not present in the fecal sample collected.
5. If symptoms persist and the result from the CellexqH. pylori Ag Cassette Rapid Test is negative or non-reactive, it is recommended to re-sample the patient a few days later or test with alternative test methods.
6. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

**WASTE MANAGEMENT OR DISPOSABLE:**

The contents of RDTs can be divided into :
**Infectious waste:**

- sharps (lancets, needles, scalpel blades)
- blood collection devices (tubes, straws, and loops); gloves; swabs; and cotton
- used cassettes.

**Non-infectious waste (Recyclable):**

- packaging materials, desiccant, buffer, and unused or unusable RDTs.

*****You must collect and dispose each type of waste in separate containers as per your waste management policies.***

**REFERENCES**

1. Marshall BJ, et al, pyloric camplylobacter infection and gastroduodenal disease. Med.J. Aust. 1985, 142: 439-444
2. Lambert IR, Lin SK, and Aranda-Michel. J, helocobacter pylori Scan. J. Gasteroenterol. 1995, 30 suppl 208: 33-46
3. Vans DJ, Evans DG, et at A sensitive and specific seriologic test for detection of campylobacter pylori infection. Gastroenerology. 1989, 96:1004
4. Shimoyama T, KatoC, et al. Applicability of a monoclonal antibody-based stool antigen test to evaluate the results of Helicobacter pylori eradication therapy. 2009, May 62(3): 225
5. Krausse R, Muller G, Doniec M. Evaluation of a rapid new stool antigen test for diagnosis of Helicobacter pylori infection in adult 1008, 46(6): 2062
6. Altman E, Fernanez H. et al Analysis of Helicobacter pylori isolates from Chile: Occurrence of selective type I Lewis b antigen expression in lipopolysaccharide. 2008, 57(pt 5): 585

## Index of CE Symbols

| | | |
|---|---|---|
| Consult instructions for use | For in vitro diagnostic use only | Use by |
| Catalog # | Lot Number | Tests per kit |
| Store between 2-30°C | AuthorizedRepresentative | Do not reuse |
| Manufacturer | Date of manufacture | |

**Mfg. By: BIOGENIX INC. PVT. LTD.**
**Factory:** B - 19/A, S.I.L Ancillary Estate, Amausi Industrial Area, Nadarganj, Kanpur Road,Lucknow - 226008 (U.P.), India
**Email:** biogenix2007@yahoo.com, info@biogenixinc.com
**Website:** www.biogenixinc.com
**Customer care no:**+919140971443
**Document No.** BIPL-IFU-080