H. pylori Ag Rapid Test
(Faeces)

REF IVD

CATALOGUE NUMBER
D-HPAGD20

INTENDED USE
The H. pylori Ag Rapid Test is a rapid 15-minute, lateral flow immunoassay for the qualitative detection of *H. pylori* antigen in human faecal 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 Rapid Test must be confirmed with alternative testing method(s) and clinical findings.

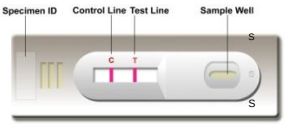
SUMMARY AND EXPLANATION OF THE TEST
Helicobacter pylori (*H. pylori*) a gram-negative, helical, rod-shaped bacterium, colonizes the gastric mucosa of approximately one-half of the world population[1]. *H. pylori* infection is a risk factor for a variety of gastrointestinal diseases including non-ulcer dyspepsia, duodenal and gastric ulcers and active, chronic gastritis[2-6]. Therefore elimination of *H. pylori* may be the most promising strategy to reduce the incidence of gastric cancer[7].

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 has a high accuracy but requires expensive lab equipment and use of a radioactive reagent[8]. Serologic antibody tests detect IgG specific to *H. pylori*, and cannot distinguish between current active infections and past infections. 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, and is not affected by the use of Proton Pump Inhibitors (PPI)[9].

The H. pylori Ag Rapid Test detects *H. pylori* antigen present in the fecal specimen by using specific antibodies. The test can be performed within 10 minutes by minimally skilled personal without the use of laboratory equipment.

TEST PRINCIPLE
The *OnSite* H. pylori Ag Rapid Test is lateral flow chromatographic immunoassay. The test strip in the card consists of: 1) a burgundy colored conjugate pad containing anti-*H.pylori* specific antibody conjugated with colloidal gold (anti-*H. pylori* conjugate) 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 anti-*H. pylori* antibody, and the C line is pre-coated with a control line antibody.



When an adequate volume of extracted fecal specimen is dispensed into the sample well of the cassette, the specimen migrates by capillary action across the card. The *H. pylori* antigen, if present in the specimen, will bind to the anti-*H. pylori* conjugate. The immunocomplex is then captured on the membrane by the pre-coated antibody forming a burgundy colored T line, indicating an H. pylori Ag Rapid Test positive result.

Absence of the T line suggests an H. pylori Ag Rapid Test negative result. The test contains an internal control (C line) which should exhibit a burgundy colored line of the immunocomplex of the control antibodies regardless of the color development on the T line. If no control line (C line) develops, the test result is invalid and the specimen must be retested with another card.

REAGENTS AND MATERIALS PROVIDED
1. Test Cards Individually Foil Pouched with a Desiccant
2. Collection Devices
3. Droppers
4. ID Labels
5. Package Insert

MATERIALS MAY BE REQUIRED AND NOT PROVIDED
1. Positive Control
2. Negative Control

MATERIALS REQUIRED BUT NOT PROVIDED
1. Clock or timer
2. A container to hold fecal specimen

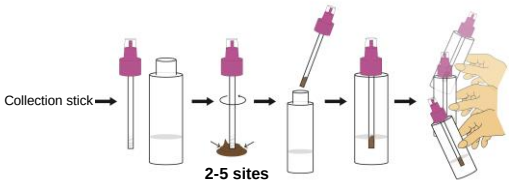
WARNINGS AND PRECAUTIONS
For *in Vitro* Diagnostic Use
1. This package insert must be read completely before performing the test. Failure to follow the insert causes inaccurate test results.
2. Do not open the sealed pouch, unless ready to conduct the assay.
3. Do not use any kit components beyond their 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 (15-30°C) before use.
6. ***Do not scoop stool sample as this may lead to excess fecal specimen that tends to clog 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 bio-safety.
9. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
10. Avoid extraction buffer contact with skin or eyes. Do not ingest.
11. Dispose of all specimens and materials used to perform the test as bio-hazardous waste.
12. The test results should be read 10-15 minutes after a specimen is applied to the sample well of the device. Any results interpreted outside of the 10-15 minute window should be considered invalid and must be repeated.
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 cards at 2-30°C. If stored at 2-8°C, ensure that the test card is brought to room temperature before opening. The test card 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 bio-safety procedures.

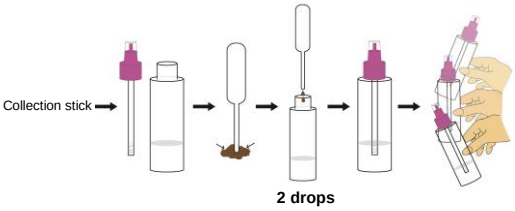
To prepare specimens using solid faeces samples follow Procedure A below. To prepare specimens using watery faeces samples follow Procedure B below.

Procedure A: Solid faeces samples
Step 1: Collect a random fecal specimen in a clean, dry receptacle.
Step 2: Label the collection device with the specimen's ID number (ID labels). Open the collection device by unscrewing the top and use the collection stick to randomly pierce in 2-5 different sites, twisting the collection stick into the fecal specimens to help collection if necessary. ***Do not scoop fecal specimen as this may lead to an invalid test result.***
Step 3: Ensure that all inner grooves of the collection stick are filled with fecal specimen. However, excess fecal specimen on the outside of grooves should be scraped off. ***Incorrect sampling may lead to an erroneous test result.***
Step 4: Replace the collection stick and tighten securely to close the stool collection device.
Step 5: **Shake the collection device vigorously.**



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

Procedure B: Watery faeces samples
Step 1: Collect a random fecal specimen in a clean, dry receptacle.
Step 2: Label the collection device with the specimen's ID number (ID labels). Open the collection device by unscrewing the top.
Step 3: Fill the dropper with the specimen; dispense 2 drops (70-85 µL) into the collection device.
Step 4: Replace the collection stick and tighten securely to close the collection device.
0Step 5: **Shake the collection device vigorously.**



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

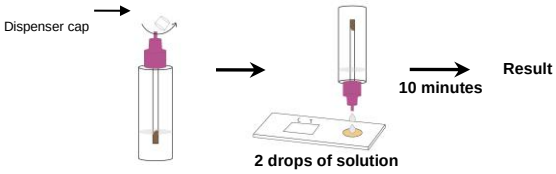
Note: The extracted specimens may be stored at 2-8°C or at room temperature up to 37°C for 10 days. For longer storage, the extracted specimen may be frozen at -20°C. Avoid multiple freeze-thaw cycles.

TEST PROCEDURE
Step 1: Bring the specimen and test components to room temperature if refrigerated or frozen. Once the specimen is thawed, mix well prior to performing the assay.

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

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

Step 4: Hold the collection device vertically. Twist off the cap. Dispense 2 drops (70- 90 µL) of the solution into the sample well of the card. Do not overload the solution.



Step 5: Set up the timer.

Step 6: Results can be read at 10 minutes. Positive results can be visible in as short as 1 minute. Negative results must be confirmed at the end of the 15 minutes only. ***However, any results interpreted outside of the 10-15 minute window should be considered invalid and must be repeated. Discard used device after interpreting the result following local requirements governing the disposal of device.***

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 card.

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 2-30°C.
   e. The temperature of the test area falls outside of 15-30°C.
   f. To verify a higher than expected frequency of positive or negative results.
   g. To investigate the cause of repeated invalid results.

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INTERPRETATION OF ASSAY RESULT

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

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

Fecal specimens with positive results should be interpreted in conjunction with other testing procedures and clinical findings before a diagnosis is made.

3. **INVALID:** If no C line develops, the assay is invalid regardless of any color development on the T line as indicated below. Repeat the assay with a new test card. **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**

A total of 157 fecal specimens were collected from symptomatic patients and healthy individuals. Specimens were tested by the H. pylori Ag Rapid Test. The urea breath test (UBT) gold standard is used as the reference test method. Comparison for all subjects is shown in the following table:

	H. Pylori Ag Rapid Test		
UBT reference	**Positive**	**Negative**	**Total**
Positive	58	2	60
Negative	6	91	97
Total	64	93	157

Relative Sensitivity: 96.7%, Relative Specificity: 93.8%, Overall agreement: 94.9%

2. **Analytic Sensitivity**

Six groups of fecal specimen extracts from 20 healthy individuals were spiked with *H. pylori* lysate antigen (Strain 43504) at concentrations of 0, 0.25, 0.5, 0.75, 1, and 2 ng/mL, respectively, and tested with the H. Pylori Ag Rapid Test. The results were showed in the follow table. The detection limit of the H. pylori Ag Rapid Test as defined as the level of ≥95% positive detection is 1 ng/mL of *H. pylori* lysate antigen.

	H. pylori Lysate Antigen (ng/mL)					
	0	**0.25**	**0.5**	**0.75**	**1**	**2**
Number of positive	0	0	0	9	20	20
Number of negative	20	20	20	11	0	0
Detection rate %	0%	0%	0%	45%	100%	100%

n=20, relative sensitivity at 1 ng/mL is 100%

3. **Cross-Reactivity**

The organisms listed below were tested for cross-reactivity with the H. Pylori Ag Rapid Test. No cross-reactivity was observed on the organisms at ≥ 1 x 10^8 org/mL.

Acinetobacter calcoaceticus
Adenovirus
Enterococcus faecalis
Escherichia coli
Gardnerella vaginalis
Geotrichum candidum
Haemophilus influenza
α-haemolytics streptococcus
B-haemolytic streptococcus
Klebsiella pneumonia
Moraxella catarrhalis
Neisseria gonorrheae
Neisseria meningitides
Proteus mirabilis
Proteus vulgaris Hauser
Pseudomonas aeruginosa
Rotavirus
Salmonella Paratyphi A
Salmonella Paratyphi B
Salmonella Paratyphi C
Salmonella typhi

4. **Interference**

The following common and potentially interfering substances may affect the performance of the H. Pylori Ag Rapid Test. This was studied by spiking these substances into negative and positive fecal specimens, respectively. The results demonstrate, at the concentrations tested, the substances studied do not affect the performance of the H. Pylori Ag Rapid Test.

List of potentially inferring substances and concentrations tested:

Tums® Antacid	5 mg/mL	Pepto-Bismol® Antacid	1:20
Tagamet® Antacid	5 mg/mL	Barium sulfate	5%
Prilosec® Antacid	5 mg/mL	Hemoglobin (tarry stool)	12.5%
Mylanta® Antacid	1:20		

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 and Handling procedure, may lead to inaccurate results.
2. The H. pylori Ag Rapid Test is limited to the qualitative detection of *H. pylori* antigen in 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 or non-reactive result 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 or non-reactive 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 specimen collected.
5. It is reported that the seroprevalence of *H. pylori* in specimens with positive fecal occult blood (FOB) test results is approximately 39.3%[10]. Therefore a specimen that tests positive with an FOB test may also be tested positive with the H. pylori Ag Rapid Test.
6. If symptoms persist and the result from the H. pylori Ag Rapid Test is negative or non-reactive, it is recommended to test with alternative test methods.
7. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

REFERENCES

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Index of Symbols

	Consult instructions for use	IVD	For *in vitro* diagnostic use only		Use by
REF	Catalog Number	LOT	Lot Number	Σ N	Tests per kit
2°C 30°C	Store between 2-30°C		Do not re-use		
	Manufacturer		Date of manufacture		

Manufactured by: Rapid Labs Ltd

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