ROJAN®

H. pylori Antigen

Rapid Test Device (Feces)

REF R-2512	English

INTENDED USE

The *H. pylori* Antigen Rapid Test Device (Feces) is a rapid visual immunoassay for the qualitative presumptive detection of *Helicobacter pylori* antigens in human fecal specimens. This kit is intended to be used as an aid in the diagnosis of *H. pylori* infection.

INTRODUCTION

Helicobacter pylori (also known as Campylobacter pylori) is a spiral-shaped with a typical flagellum, Gram negative bacteria, infecting gastric mucosa. It causes several gastro-enteric diseases such as non-ulcerous dyspepsia, gastric and duodenal ulcer, active gastritis and can even increase the risk of stomach adenocarcinoma, so as to be classified as carcinogen agent type I.
Many *H. pylori* strains have been isolated: among them, the strain expressing CagA antigen is strongly immunogenic and, according to this, it is of utmost clinical importance because it is associated to the cytotoxic factor. It is widely reported in many literature articles that, in infected patients showing antibodies against CagA gene product, the risk of gastric cancer is up to five times higher than the reference group infected with a CagA negative bacterial strain.
The presence of the gene itself determines the persistence of the infection, the ulceration and the protein associated, VacA toxin is frequently the main cause of infiltrations in the gastric mucosa.
This antigen associated to others, such as CagII, CagC, seems to act as starting agent of a sudden inflammatory response which can provoke ulceration (peptic ulcer), allergic episodes, and a decrease of the therapy efficacy.
At present several invasive and non-invasive approaches are available to detect this infection state.
Invasive methodologies requires endoscopy of the gastric mucosa with a histologic, cultural and urease investigation, which are cost-effective and requiring long times to come to a correct final diagnosis.
Alternatively, non-invasive methods are available such as Breath Test, which is extremely complicated and not highly selective, or classical ELISA and immunoblotting assays.

PRINCIPLE

The *H. pylori* Antigen Rapid Test Device (Feces) has been designed to detect *Helicobacter pylori* through visual interpretation of color development in the internal strip. The membrane was immobilized with anti-*H. pylori* antibodies on the test region. During the test, the specimen is allowed to react with colored anti-*H. pylori* antibodies colloidal gold conjugates, which were precoated on the sample pad of the test. The mixture then moves on the membrane by a capillary action, and interact with reagents on the membrane. If there were enough *H. pylori* antigens in specimens, a colored band will form at the test region of the membrane. Presence of this colored band indicates a positive result, while its absence indicates a negative result. Appearance of a colored band at the control region serves as a procedural control. This indicates that proper volume of specimen has been added and membrane wicking has occurred.

MATERIALS

Materials Provided

- Test devices
- Specimen collection tubes with extraction buffer
- Package insert

Materials Required but Not provided

- Specimen collection container
- Timer

PRECAUTIONS

- For professional *in vitro* diagnostic use only.
- Do not use after the expiration date indicated on the package. Do not use the test if the foil pouch is damaged. Do not reuse tests.
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not completely guarantee the absence of transmissible pathogenic agents. It is therefore, recommended that these products be treated as potentially infectious, and handled by observing usual safety precautions (e.g., do not ingest or inhale).
- Avoid cross-contamination of specimens by using a new specimen collection container for each specimen obtained.
- Read the entire procedure carefully prior to testing.
- Do not eat, drink or smoke in the area where the specimens and kits are handled. Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout the procedure and follow standard procedures for the proper disposal of specimens. Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.
- Humidity and temperature can adversely affect results.
- Used testing materials should be discarded according to local regulations.

STORAGE AND STABILITY

- The kit should be stored at 2-30°C until the expiry date printed on the sealed pouch.
- The test must remain in the sealed pouch until use.
- **Do not freeze.**
- Care should be taken to protect the components of the kit from contamination. Do not use if there is evidence of microbial contamination or precipitation. Biological contamination of dispensing equipments, containers or reagents can lead to false results.

SPECIMEN COLLECTION AND STORAGE

- The *H. pylori* Antigen Rapid Test Device (Feces) is intended only for use with human fecal specimens.
- Perform the testing immediately after the specimen collection. Do not leave the specimens at room temperature for prolonged periods. Specimens may be stored at 2-8°C for up to 72 hours.
- Bring specimens to room temperature prior to testing.
- Pack the specimens in compliance with applicable regulations for transportation of etiological agents, in case they need to be shipped.

PROCEDURE

Allow test device, specimen collection tube, specimen, and/or controls to equilibrate to room temperature (15-30°C) prior to testing.

1. To collect fecal specimens:

   Collect feces in a clean, dry specimen collection container. Best results will be obtained if the assay is performed within 6 hours after collection. Specimen collected may be stored for 3 days at 2-8°C if not tested within 6 hours.

2. To process fecal specimens:

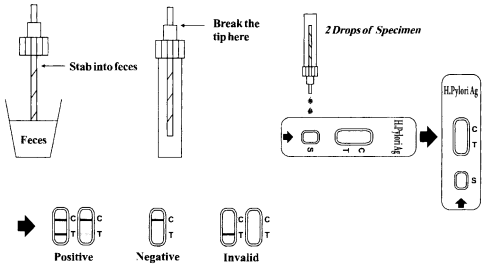
   Unscrew the cap of the specimen collection tube, then randomly **stab the specimen collection stick into the fecal specimen in at least 3 different sites**. Do not scoop the fecal specimen.

   Screw on and tighten the cap onto the specimen collection tube, then **shake the specimen collection tube vigorously** to mix the specimen and the extraction buffer. Specimens prepared in the specimen collection tube may be stored for 6 months at -20°C if not tested within 1 hour after preparation.

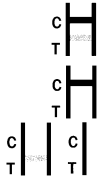
3. Remove the test device from the sealed pouch and use it as soon as possible.

4. Hold the specimen collection tube upright and **break off the tip** of the specimen collection tube. Invert the specimen collection tube and **transfer 2 full drops of the extracted specimen** (approx. 90 µL) to the specimen well (S) of the test device, then start the timer. Avoid trapping air bubbles in the specimen well (S). See illustration below.

5. Wait for the colored line(s) to appear. **Read results at 10 minutes.** Do not interpret the result after 20 minutes.



INTERPRETATION OF RESULTS



POSITIVE: Two colored bands appear on the membrane. One band appears in the control region (C) and another band appears in the test region (T).

NEGATIVE: Only one colored band appears, in the control region (C). No apparent colored band appears in the test region (T).

INVALID: Control band fails to appear. Results from any test which has not produced a control band at the specified read time must be discarded. Please review the procedure and repeat with a new test. If the problem persists, discontinue using the kit immediately and contact your local distributor.

NOTE:

1. The intensity of color in the test region (T) may vary depending on the concentration of analytes present in the specimen. Therefore, any shade of color in the test region should be considered positive. Note that this is a qualitative test only, and cannot determine the concentration of analytes in the specimen.
2. Insufficient specimen volume, incorrect operating procedure or expired tests are the most likely reasons for control band failure.

QUALITY CONTROL

- Internal procedural controls are included in the test. A colored band appearing in the control region (C) is considered an internal positive procedural control, confirming sufficient specimen volume and correct procedural technique.
- External controls are not supplied with this kit. It is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

LIMITATIONS OF THE TEST

1. The *H. pylori* Antigen Rapid Test Device (Feces) is for professional *in vitro* diagnostic use, and should be used for the qualitative detection of *Helicobacter pylori* only.
2. Following certain antibiotic treatments, the concentration of *H. pylori* antigens may decrease to the concentration below the minimum detection level of the test. Therefore, diagnosis should be made with caution during antibiotic treatment.
3. As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.

PERFORMANCE CHARACTERISTI

Table: *H. pylori* Antigen Rapid Test vs. Endoscope-based methods

Relative Sensitivity:
>99.9% (97.3%-100.0%)*
Relative Specificity:
>99.9% (97.6%-100.0%)*
Overall Agreement:
>99.9% (98.7%-98.8%)*
***95% Confidence Interval**

		H. pylori Antibody Rapid Test		
		+	-	Total
Biopsy/ Histology/ RUT	+	132	0	132
	-	0	154	154
		132	154	286

Specificity:
Cross reactivity with following organisms has been studied at 1.0 x 10^9 organisms/ml. The following organisms were found negative when tested with the One Step H. pylori Antigen Test Device (Feces).

Staphylococcus aureus	*Proteus mirabilis*	*Neisseria gonorrhea*
Pseudomonas aeruginosa	*Acinetobacter spp*	*Group B Streptococcus*
Enterococcus faecalis	*Salmonella choleraesius*	*Proteus vulgaris*
Group C Streptococcus	*Gardnerella vaginalis*	*Enterococcus faecium*
Klebsiella pneumoniae	*Acinetobacter calcoaceticus*	*Hemophilus influenzae*
Branhamella catarrhalis	*E.coli*	*Neisseria meningitidis*
Candida albicans	*Chlamydia trachomatis*	*Rotavirus*

LITERATURE REFERENCES

1. Marshall, BJ, McGechie, DB, Rogers, PAR and Glancy, RG. Pyloric Campylobacter infection and gastroduodenal disease. Med. J. Australia. (1985), 149: 439-44.
2. Soll, AH. Pathogenesis of peptic ulcer and implications for therapy. New England J. Med. (1990), 322: 909-16.
3. Hazell, SL, et al. Campylobacter pyloridis and gastritis I: Detection of urease as a marker of bacterial colonization and gastritis. Amer. J. Gastroenterology. (1987), 82(4): 292-96.
4. Cutler AF. Testing for Helicobacter pylori in clinical practice. Am j. Med. 1996; 100:35S-41S.
5. Anand BS, Raed AK, Malaty HM, et al. Loe point prevalence of peptic ulcer in normal individual with Helicobacter pylori infection. Am J Gastroenterol. 1996,91:1112-1115.

Index of Symbols

	Attention, see instructions for use		Tests per kit		Manufacturer
IVD	For *in vitro* diagnostic use only		Use by		Do not reuse
30°C 2°C	Store between 2-30°C	LOT	Lot Number		

ISO, GMP

Rojan Azma
Rojan Azma mfg. Co.
No. 41, Golestan 4, Baharestan Industrial Estate, 5th km Karaj-Qazvin Highway, Tehran-Iran
Tel: +98-261-47 60 610
www.rojanazma.com