

PYLORITOP Ag

Rapid test for the detection of *Helicobacter pylori* antigen in human stool sample



INTENDED USE

The PYLORITOP® Ag Test is an immunochromatographic rapid test for the qualitative detection of *Helicobacter pylori* antigen in human faecal specimens. This kit is intended for use as an aid in the diagnosis of an *H. pylori* infection.

SUMMARY

Helicobacter pylori (also known as *Campylobacter pylori*) is a spiral-shaped gram negative bacteria which infects the gastric mucosa. *H. pylori* 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.

Many *H. pylori* strains have been isolated. Among them, the strain expressing CagA antigen is strongly immunogenic and is of utmost clinical importance. Literature articles report that in infected patients producing antibodies against CagA, the risk of gastric cancer is up to five times higher than reference groups infected with CagA negative bacteria. Other associated antigens such as CagII and CagC seems to act as starting agents of sudden inflammatory responses which can provoke ulceration (peptic ulcer), allergic episodes, and decrease of therapy efficacy.

At present several invasive and non-invasive approaches are available to detect this infection state. Invasive methodologies require endoscopy of the gastric mucosa with histologic, cultural and urease investigation, which are expensive and require some time for diagnosis. The PYLORITOP® Ag cassette is a non-invasive, qualitative, lateral flow immunoassay for the detection of *H. pylori* in human faeces specimen. It is precise, easy to perform, and rapid, generating the test result within several minutes.

PRINCIPLE

The PYLORITOP® Ag cassette is an immunochromatographic rapid test designed to detect *H. pylori* antigens in faecal specimens. The presence of *H. pylori* is indicated by a specific colour development for visual interpretation. Anti-human *H. pylori* antibodies are immobilized on the test line region of the membrane. During testing, the antigens extracted from the faecal specimen are captured by specific antibodies, which are adhered to pointer particles. The mixture migrates along the membrane and the antigen-antibody-particle complex binds to the specific antibody in the test line area. The agglomeration of complexes creates a colour line in the test line area. The appearance of the colour line in the test (T) line area indicates a positive result, while its absence indicates a negative result. A red line should always appear in the control (C) line area. It serves as a procedural control, confirming that sufficient specimen volume was used and indicates an adequate membrane wicking and proper procedural technique.

REAGENTS

The test devices include anti-*H. pylori* antibody coated pointer particles and *H. pylori* antibodies coated on the membrane.

PRECAUTIONS

- For professional in vitro diagnostic use only. Do not use after expiration date.
- The test should remain in the sealed pouch until use. Do not use test if the pouch is damaged.
- Do not eat, drink or smoke in the area where the specimens or kits are handled.
- Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout all procedures and follow the standard procedures for proper disposal of specimens.
- Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.
- The used test should be discarded according to local regulations.
- Humidity and temperature can adversely affect results.
- Do not mix reagents (e. g. test devices and diluent tubes) from different lots

STORAGE AND STABILITY

The kit should be stored at 2-30°C. The test is stable through 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 components in this kit from contamination. Do not use if there is evidence of microbial contamination or precipitation. Biological

contamination of dispensing equipment, containers or reagents can lead to false results.

MATERIALS

Materials Provided

- PYLORITOP® Ag cassettes, individually pouched
- Collection sets including diluent vial, zip bag and stool collection sheet with instructions for specimen collection.
- Package insert

Materials Required But Not Provided

- Centrifuge for treatment of specimens in special case
- Timer
- Pipettes to collect liquid specimens

SPECIMEN COLLECTION AND STORAGE

Specimen collection:

1. Use the specimen collection sheet provided in the kit for specimen collection. In order to facilitate the stool specimen collection the kit contains stool collection sheets (folded paper sheet). Follow the instructions on the specimen collection sheet. Other clean, dry containers may also be used for specimen collection. Collect sufficient quantity of feces (1-2 mL or 1-2 g) in a clean, dry specimen collection container to obtain maximum antigens (if present).
2. Note: Please ensure that the stool sample has no direct contact with the water of the toilet bowl to avoid a dilution or a contamination with detergents.
3. Unscrew the cap of the specimen collection tube to access to the applicator stick. Be careful not to spill or spatter solution from the tube.
4. **For solid specimen:** Collect specimens by inserting the applicator stick into at least 3 different sites of the faeces to collect approximately 50 mg of faeces. (Equivalent to 1/4 of a pea).

For liquid specimen: Hold the pipette vertically, aspirate fecal specimens, and then transfer 2 drops (approximately 80 µL) into the specimen collection tube containing the extraction buffer.

5. Replace the applicator back into the tube and screw the cap tightly.
6. Vigorously shake the diluent tube and make sure to mix thoroughly the specimen and diluent. Leave the tube alone for 2 minutes. Wrap the sample in a plastic bag and store it in a cool place. The specimen is now ready and should be tested as soon as possible.

Storage information

1. Perform testing immediately after specimen collection. Best results will be obtained if the assay is performed within 6 hours at room temperature after specimen collection.
2. Do not leave specimens at room temperature for prolonged periods. Collected specimens may be stored at 2-8°C up to 72 hours if not tested within 6 hours.
3. Prepared specimens in the specimen collection tube may be stored at room temperature (15-30°C) up to 72 hours if not tested within 1 hour after preparation.
4. For long term storage, raw specimens (not diluted in buffer) should be kept below -20°C.
5. If specimens are to be shipped, pack them in compliance with all applicable regulations for transportation of etiological agents.

DIRECTIONS FOR USE

Bring tests, reagents, stool specimens, and/or external controls to room temperature (15-30°C) before testing.

1. Remove the test from its sealed pouch, and use it as soon as possible. For best results, the assay should be performed within one hour after opening the sealed pouch. Best results are obtained if test is performed immediately after opening the pouch. Mark the device with patient or control reference.
2. Hold the specimen collection tube upright and open the cap onto the specimen collection tube to access to the dropper. Invert the specimen collection tube and transfer 2 full drops of the diluted specimen (approximately 80 µL) to the specimen well (S) of the test cassette, then start the timer. Avoid trapping air bubbles in the specimen well (S) and do not add any liquid to the reaction area. Start the timer as the test starts to run. As the test begins to run you will observe a coloured liquid migrate along the membrane of the reaction area.



3. Read results at 10 minutes after dispensing the specimen. Do not read results after 20 minutes.

Note: If the specimen does not migrate due to the presence of particles, centrifuge the extracted specimens contained in the diluent vial. Collect 80 µL of supernatant, dispense into the specimen well (S) of a new test device and start again, following the instructions described above.

INTERPRETATION OF RESULTS



POSITIVE: 2 lines appear. One line appears in the control line area (C) and one line in the test line area (T). A positive result indicates that H. Pylori antigen have been detected.



NOTE: The intensity of colour in the test area (T) may vary depending on the antigen concentration present in the specimen. Therefore, any shade of colour in the test area (T) should be considered positive.



NEGATIVE: One line appears in the control line area (C). No line appears in the test line area (T). A negative result indicates that no H. Pylori antigens are present in the specimen or that the antigens concentration is below the detection level of the test device.



INVALID: Control line fails to appear. Insufficient specimen volume, expired test components or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

If the presence of visible particles inhibited the migration they might be removed by centrifugation or sedimentation. For this, please refer to the related note in the test procedure chapter.

Note: When faecal samples are tested, the background may appear slightly yellowish due to the colour of the faecal samples. This is acceptable as long as it does not interfere with the interpretation of test result. The test is invalid if the background fails to clear and obscures the reading of the result.

QUALITY CONTROL

Internal Quality Control

An internal procedural control is included in the test. A red line appearing in the control area (C) is an internal positive procedural control. It confirms that sufficient specimen volume was used, and indicates an adequate membrane wicking and a proper procedural technique.

External Quality Control

It is recommended to perform a positive and negative external control for every kit, and as deemed necessary by internal laboratory procedures. External positive and negative controls are not supplied with the kit.

LIMITATIONS

1. The PYLORITOP® Ag is for in vitro diagnostic use only. The test should be used for the qualitative detection of H. pylori antigens in feces specimens only. Neither the quantitative value nor the rate of increase in H. pylori antigens concentration can be determined by this qualitative test.
2. The PYLORITOP® Ag will only indicate the presence of H.pylori in the specimen and should not be used as the sole criteria for H. pylori to be etiological agent for peptic or duodenal ulcer.
3. As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.
4. If the test result is negative and clinical symptoms persist, additional testing using other clinical methods is recommended. A negative result does not at any time preclude the possibility of H. pylori infection.
5. 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.

PERFORMANCE CHARACTERISTICS

Diagnostic Sensitivity and Specificity

The PYLORITOP® Ag has been evaluated with specimens obtained from a population of symptomatic and asymptomatic individuals. The result shows that the sensitivity of the PYLORITOP® Ag is >99.9% and the specificity is 98.1% relative to Endoscope-based methods.

Method	Endoscope based methods		
	Results	Positive	Negative
PYLORITOP®Ag	Positive	78	2
	Negative	0	101
Total Result		78	103
			181

Relative sensitivity: $78/78 = >99.9\%$ (95%CI*: 96.2%~100.0%);

Relative specificity: $101/103 = 98.1\%$ (95%CI*: 93.2%~99.8%);

Accuracy: $(78+101)/(78+103) = 98.9\%$ (95%CI*: 96.1%~99.9%).

*Confidence Intervals

Specificity

Cross reactivity with following organisms has been studied at 1.0E9 organisms/ml. The following organisms were found negative when tested with the PYLORITOP® Ag:

Acinetobacter calcoaceticus	Acinetobacter spp	Branhamella catarrhalis
Candida albicans	Chlamydia trachomatis	Enterococcus faecium
E.coli	Enterococcus faecalis	Gardnerella vaginalis
Group A Streptococcus	Group B Streptococcus	Group C Streptococcus
Hemophilus influenza	Klebsiella pneumonia	Neisseria gonorrhoea
Neisseria meningitides	Proteus mirabilis	Proteus vulgaris
Pseudomonas aeruginosa	Rotavirus	Salmonella choleraesuis
Staphylococcus aureus	Adenovirus	

Precision

Intra-Assay: Within-run precision has been determined by using 15 replicates of four specimens: Negative, low titer positive, middle titer positive and high titer positive specimens. The Specimens were correctly identified >99% of the time.

Inter-Assay: Between-run precision has been determined by 15 independent assays on the same four specimens: negative, low titer positive, middle titer positive and high titer positive specimens. Three different lots of the PYLORITOP® Ag have been tested using these specimens. The specimens were correctly identified >99% of the time.

REFERENCES

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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.

SYMBOLS

	Attention, see instructions for use		Lot number
	For in vitro diagnostic use only		Manufacturer
	Store between 2-30°C		Do not reuse
	Tests per kit		Catalog number
	Expiry		dilution buffer

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