Helicobacter pylori Ag

Product-#: D-HP-F23I-002

Rapid test for the detection of H. pylori antigen in human stool samples

INTENDED USE

The DIMA® *H. pylori* Ag Test Device is an immunochromatographic screening assay for the qualitative detection of *Helicobacter pylori* antigen in stool samples. In contrast to serum assays that are based on the detection of antibodies and remain positive for a relatively long time even if the infection has subsided, the *H. pylori* Rapid Test indicates an active infection via the detection of antigen. Thus the test can be used for the determination of a suspected infection or re-infection with *H. pylori* or to monitor the success of an eradication therapy.

INTRODUCTION

Helicobacter pylori (also formerly known as *Campylobacter pylori*) is a spiral-shaped, Gram-negative bacterium with typical flagella. It is capable of infecting the gastric mucosa. It causes several gastro-enteric diseases such as non-ulcerous dyspepsia, gastric and duodenal ulcer, active gastritis and might even increase the risk of stomach adenocarcinoma, so that it has been classified as carcinogenic agent type I.

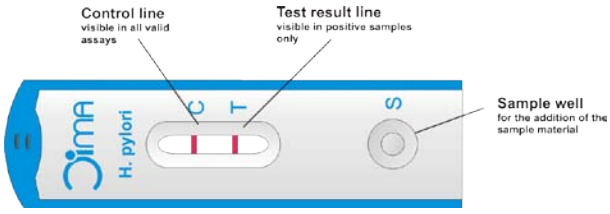
Various *H. pylori* strains have been isolated that differ in their virulence. Strains exhibiting a high virulence are generally characterized by the possession of the vacuolating cytotoxin (Vac A) and the so called cytotoxin associated genes cag pathogenicity island. These factors seem to be necessary for an effective infiltration of the gastric mucosa and seem to be associated with the persistence of the infection. They also contribute to sudden inflammatory responses, ulceration (gastric and duodenal ulcer), allergic episodes, and decrease of therapy efficacy.
Especially the CagA protein that is strongly immunogenic and is secreted into the gastric cells by a special mechanism is of special clinical importance. It has been widely reported in many literature articles that infected patients showing antibodies against the CagA gene product have a five times increased risk of developing gastric cancer if compared to a reference group infected with a CagA negative bacterial strain.

At present several invasive and non-invasive approaches are available to detect the infection state.
Invasive methodologies require endoscopy of the gastric mucosa with a histological, cultural and urease investigation. These examinations are expensive and time-consuming.
Alternatively, non-invasive methods are available such as Breath Tests with isotope labelled urea, which are complicated and cost-intensive, or classical ELISA or immunoblotting assays.

The DIMA® *H. pylori* Ag Test Device is an immunological rapid assay that takes advantage of a highly specific antibody/antigen reaction to detect bacterial protein of *H. pylori* (antigen) in stool samples.

PRINCIPLE OF THE TEST

The DIMA® *H. pylori* Ag Test Device is a non-invasive, qualitative, lateral flow immunoassay for the detection of *H. pylori* in human feces specimen. It is precise, easy to perform, and rapid, generating the test result within several minutes.
In this assay *H. pylori* is detected with the aid of specific antibodies against *H. pylori*. After the addition of the sample (feces diluted in buffer) color-labelled antibodies specifically bind to the bacteria if they are present in the sample. When these complexes migrate upward on the membrane by capillary action, they are captured with the aid of another specific antibody at the test result line region of the test. A red test result line is generated. If no bacteria are present, the color labelled antibody cannot bind at the test result line region. No red test result line is formed. So the presence of a colored test result line indicates a positive result, while its absence indicates a negative result.
To serve as a procedural control, a colored line will always appear in the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred.



REAGENTS AND MATERIALS SUPPLIED

- Individually pouched test cassettes
- Specimen collection tubes with dilution buffer for sample collection and dilution
- patient information regarding stool sampling
- Stool collection paper
- plastic bags
- Package insert

MATERIALS REQUIRED BUT NOT PROVIDED

Doctor's office or lab:
- Tissue paper for breaking the tip of the buffer tube.
- Timer

STORAGE AND STABILITY

Store kit as packaged either at room temperature or refrigerated (2-30°C). Under these conditions the test and the buffer is stable through the expiration date. The test must remain in the sealed pouch containing a desiccant until use. **DO NOT FREEZE.** Do not use beyond the expiration date.

PRECAUTIONS

- For in vitro diagnostic use by professionals only.
- For single use. Do not reuse tests
- Do not interchange or mix reagents from different lots.
- Do not use test if its foil pouch has been damaged. Do not use after expiration date.
- Handle all specimens as if they contain infectious agents. Do not eat, drink or smoke in the area where the specimens or kits are handled. Protective clothing such as laboratory coats, disposable gloves and eye protection are recommended. Observe established precautions against microbiological hazards throughout testing and follow standard procedures for proper disposal of specimens in accordance with local regulations.
- The dilution buffer contains low amounts of sodium azide.
- Humidity and temperature can adversely affect results.
- The components of the test (e.g. antibodies/chemicals) do not cause any danger if the test is used according to the instructions.
- Please follow the instructions for use carefully. Please inform your patients how to collect and dilute the stool sample.

SPECIMEN COLLECTION AND PREPARATION

Specimen collection and preparation by the patient

For collecting the stool sample the patient is given one of the specimen collection tubes of the kit and a stool collection paper. Please advise the patient to collect the stool sample in the following way:

- Read the instruction at the stool collection paper and follow them closely. So that you ensure that you collect a stool sample in a way that it has no contact with water in the toilet bowl to avoid a dilution of the sample or a contamination with detergents.
- Transfer a small amount of the stool into the specimen collection tube in the following way:
  - Hold the collection tube vertically. Unscrew the cap of the tube, and take out the specimen collection applicator.
  - Stab the spiral rod into the fecal specimen in at least 3 different sites. The amount of feces that sticks to the rod is sufficient. Do not try to scoop additional feces material into the tube.
  - Place the applicator back into the tube and screw the cap tightly. Shake the specimen collection tube to mix the specimen and the dilution buffer. Be careful not to break the tip of the collection tube.
  - Wrap the sample in a plastic bag and store it in a cool place. Return the sample to the doctor's office within the next 24-48 hours.

Note
If the patient feels uncomfortable in diluting the stool specimen into the collection tube himself, he might also return a container with the unprocessed stool sample to the doctor's office. The transfer of the specimen into the buffer of the tube can then be carried out as described above by the personnel of the doctor's office or lab.

Please advise your patients that it is necessary to follow the instructions precisely.

Sample preparation and storage doctor's office

On return of the sample, please ensure that the stool collection tube has been labelled with the patient's name for identification purposes. Samples should be used within the next 24 hours. Store samples refrigerated (2-8 °C/36-46 °F) until use. Short time exposure to temperatures up to 30 °C/86 °F e.g. during

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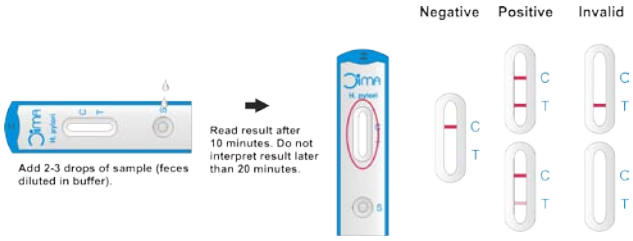
transportation do normally not affect the specimen. However, exposure times to high temperatures should be kept as short as possible.
Directly before the sample is used for the assay it must be brought to room temperature.

TEST PROCEDURE

1) Allow the test device and the diluted stool sample to reach room temperature (15-30°C) prior to testing.
2) Remove the test device from its pouch when ready to perform the test. The device must have room temperature to avoid condensation of moisture on the membrane. Label the device with patient or control identification.
3) Shake the collection tube thoroughly to ensure proper mixing of the fecal sample with the extraction solution.
4) Using a piece of tissue paper, break the tip of the collection tube with a twisting motion. Hold the collection tube vertically and dispense 2-3 drops of solution into the round sample well of the test device by applying a gentle pressure to the walls of the tube. Avoid air bubbles in the sample well or splashes of liquid into the oval result window.
5) Start the timer. As the test begins to work, you will see a reddish colored liquid front moving across the membrane.
6) Wait for the colored lines to appear. The result should be read after 10 minutes. Strong positive results may be observed sooner. Do not interpret the result after 20 minutes.



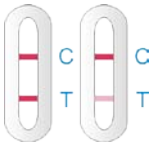
INTERPRETATION OF RESULTS

For reading the test result, the colored lines that have appeared in the test result window are interpreted.

Negative result

Only one colored line appears in the control line region (C). No line appears in the test line region (T). The absence of the test result line (T) indicates that no *H. pylori* antigen has been detected by the assay.

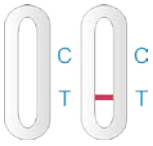
Positive Result



Two distinct colored lines appear on the membrane. One colored line forms in the control line region (C) and another apparent colored line appears in the test result line region (T). This result indicates that *H. pylori* antigen has been detected by the assay.

NOTE: The intensity of the color in the test result line region (T) will vary depending on the concentration of *H. pylori* antigen present in the specimen. Therefore, any shade of color in the test line region (T) indicates that *H. pylori* antigen has been detected. Do not try to quantify the amount of antigen with this qualitative assay.

Invalid Result



INVALID: The control line (C) fails to appear. Results from any test that has not produced a control line at the specified reading time should be discarded. Insufficient specimen volume, insufficient specimen migration, or incorrect procedural techniques are the most likely reasons for control line failure.

Review the procedure and repeat the test with a new test device. If the presence of visible particles inhibited the migration they might be removed by centrifugation or sedimentation. Transfer a part of the sample into a tube, sediment the particles by a brief centrifugation and pipette approximately 80-120 µl of the supernatant into the sample well of a new test device. Alternatively allow the particles to settle in the upright tube and use 80-120 µl from the topside of the liquid. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

Note

When fecal samples are tested, the background may appear slightly yellowish due to the color of the fecal 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

An internal procedural control is included in the test. A colored line appearing in the control line region (C) is an internal positive procedural control. It confirms sufficient specimen volume, adequate membrane wicking and correct procedural technique.
Control standards are not supplied with this kit; however, it is recommended that positive and negative controls are tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

LIMITATIONS

- As with all diagnostic rapid tests a definitive clinical diagnosis should not be solely based on the result of the test. All clinical and laboratory findings should be considered by the physician and test results should be confirmed by further examinations if indicated.
- Antibiotics, proton pump inhibitors and bismuth preparations inhibit *H. pylori.* Negative test results obtained during or shortly after a therapy might be not reliable because the amount of bacteria might be too reduced for detection with the assay. In this case it is useful to repeat the *H. pylori* test 2 weeks after the end of the therapy.

EXPECTED VALUES

Epidemiological studies show that infections with *H. pylori* are of worldwide occurrence. The exact route of infection between individuals is presently unknown. The values indicate that the incidence of infection rises about 1-2% with each year of life so that infection rates of 50% or higher are not uncommon for elderly people being 60 years or older. A high percentage of people colonized with *H. pylori* do not develop any clinical symptoms.

TEST PERFORMANCE

The sensitivity of the assay is 50-70 ng/ml *H. pylori* protein. No High Dose Hook (Prozone) Effect was observed up to a concentration of 2.5 mg/ml.

In a clinical study the DIMA® *H. pylori* Ag Test Device was tested against a commercially available ELISA and showed the following performance characteristics:

Diagnostic Sensitivity:	82.1 %
Diagnostic Specificity:	96.9 %
Positive Predictive Value:	91.4 %
Negative Predictive Value:	93.1 %
Reproducibility:	92.6 %

REFERENCES

1. Graham, David Y. (Editor), (**2007**) Journal compilation© 2007 Blackwell Publishing Ltd., Helicobacter 12 (Suppl.1): 1-56.
2. Kist, M.; Glocker, E.; Suerbaum S. (**2005**): Pathogenese, Diagnostik und Therapie der *Helicobacter pylori*-Infektion, Bundesgesundheitsbl – Gesundheitsforsch – Gesundheitssschutz 2005, 48: 669-678, Springer Verlag 2005.
3. Meurer, Linda N.; Meurer, M.D., M.P.H., Bower, Douglas J.,M.D. (**2002**): Management of *Helicobacter pylori* infection, American Family Physician Vol.65, No.7, 1. April 2002.
4. Weiss, Judith; Mecca, James; da Silva, Elvira; Gassner, Dieter (**1994**): Comparison of PCR and other Diagnostic Techniques for Detection of *Helicobacter pylori* Infection in Dyspeptic Patients, Journal of Clinical Microbiology Vol.32, No.7, p.1663-1668, (1994).

EXPLANATION OF SYMBOLS

IVD	For in-vitro diagnostic purposes only		For single use only
▼	Content		Expiration date
LOT	Charge number		Storage temperature
	Observe instructions for use		Manufacturer

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