HEPYLORI

For *in Vitro* diagnostic use only

Rapid test strip for detecting Helicobacter pylori antigen in human stool specimen

I. INTRODUCTION AND INTENDED USE

Hepylori is a rapid chromatographic immunoassay for the qualitative detection of H. pylori antigens in human faeces specimens to aid in the diagnosis of H. pylori infection.
Helicobacter pylori (also known as Campylobacter pylori) is a Gram negative bacteria, infecting gastric mucosa. H. pylori infection can cause chronic gastritis and can predispose to gastric and duodenal ulcer; can even increase the risk of stomach adenocarcinoma, so as to be classified as carcinogen agent type I.
Infection with Helicobacter pylori is very common and has been estimated to occur in 40-50% of the population in developed countries and 80-90% of the population in developing regions.

II. PRINCIPLE OF THE TEST

Hepylori is a non-invasive lateral flow assay, rapid, precise and easy to perform.
This test makes use of monoclonal specific antibody against H. pylori antigen adsorbed onto a reactive membrane. During testing, the sample reacts with the particle coated with anti-H. pylori antibodies which was pre-dried on the test strip. The mixture moves upward on the membrane by capillary action. If H. pylori is present in stool specimen, the specific antigen is bound by the antibody which is conjugated with latex and generate a coloured line. A generic antibody, fixed onto the reactive membrane, in shape of the band, is able to capture the conjugated antibody, assuring the correctness of the test performance.

III. REAGENTS AND MATERIALS

Each kit contains:
1. Hepylori dipstick – each strip are contained in a sealed bottle, with dessicant.
2. Extraction buffer – specimen collection with extraction buffer (solution hypotonic).
3. Instruction for use

Required materials (not supplied)
Specimen collection container, Disposable gloves, Timer, Tubes for test, droppers.

IV. PRECAUTIONS

- All operations linked to the use of the test must be performed in accordance with Good Laboratory Practices.
- Hepylori is for in vitro diagnosis only and professional use only.
- Avoid touching the nitrocellulose with your fingers.
- All the specimens should be considered potentially hazardous and handled in the same manner as an infectious agent.
- Disposable gloves, swabs, test tubes, and sensitized strips in accordance with GLP.
- Never use reagents from another lot.
- The strip should remain in the sealed bottle until use.
- The reagents' quality cannot be guaranteed beyond their shelf-life date or if the reagents are stored under inappropriate conditions.

V. STORAGE AND STABILITY

Store as packaged in the sealed bottle either at refrigerated or room temperature (2-30ºC/36-86ºF). The test is stable through the expiration date printed on the label. The test must remain in the sealed bottle until use. Do not freeze.

VI. SAMPLES AND PREPARATION

The specimen should be transported in an airtight container and stored at +2°C - +8°C until tested. The specimen should be tested as soon possible, but may be held up to 72 hours at +2°C - +8°C prior to testing. If testing cannot be performed within this time frame, specimens should be frozen immediately on receipt and stored frozen (≤ -20°C) until tested. Specimens may be frozen and thawed twice.

Note
Stool in transport media, on swabs, or mixed with preservatives is not appropriate for testing.
Mix stool as thoroughly as possible prior to pipetting.

Liquid or Semi-Solid Stools
Using a separate pipette (included with the kit) for each stool, draw stool of the sample itself. Dispense 6-7 drops of each stool into a separate extraction tube. Mix carefully, then vortex 15 seconds.
Care should be taken when pipetting semi-solid stool. The addition of less than indicated of stool may cause a false-negative test. The addition of more than indicated of stool may cause invalid results due to restricted sample flow.

Formed / Solid Stools
Unscrew the top of the extraction tube. Collect the stool sample with the tip of the collection device by dipping in three different places of the same stool specimen.. Verify to transfer a small portion (approximately 6 mm diameter) of stool. Put the collection device back into the plastic test tube. Shake the extraction tube in order to get an homogeneous solution. Wait at least 3 minutes. Repeat the operations just to obtain a dark yellow-brown solution, if necessary.
The transfer of too little stool, or failure to mix and suspend the stool in extraction tube completely may result in a false-negative test results. Care should be taken to transfer no less and no more than the amount indicated. The sample should be thoroughly mixed with a vortex before testing. The addition of excessive amount of stool may cause invalid results due to restricted sample flow.

VII. PROCEDURE

Allow the tests, stool samples and buffer to reach to room temperature (15-30ºC/59-86ºF) prior to testing. Do not open pouches until ready to perform the assay.

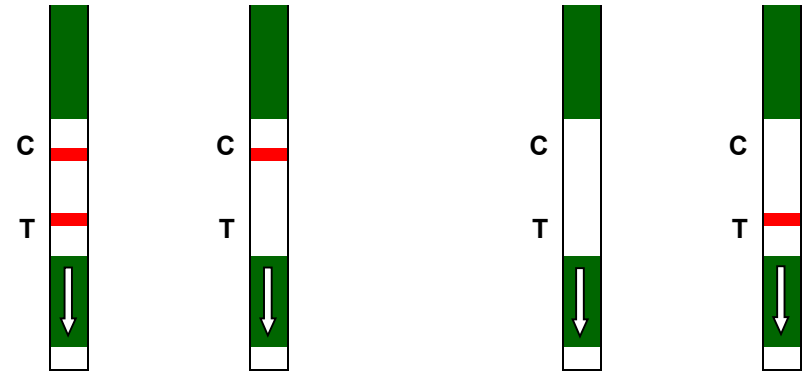
1. Remove the test strip and use it as soon as possible when opening the tube.
2. Gently shake the test tube containing the sample under investigation.
3. Brake the tip of the test tube and dispense 5-6 drops (200 µL) of the extracted mixture into the testing tube.
4. Leave the test strip to stand vertically taking care of not surpassing the limit of immersion indicated with the arrows.
5. Start the timer and read the result at 10 minutes after dispensing the sample (no read after 15 minutes).

VIII. INTERPRETING THE RESULTS

Interpret the results as follow:
Positive: two red lines (C) and (T) are visible in the control and test areas of the window. The intensity of the band colour in the test region is proportionally to the antigen concentration in the sample. However, neither the quantitative value, nor the rate of increase in antigens can be determined by this qualitative test.
Negative: In the reading window only 1 red band appears in the control region "C". This is the control line assuring the correctness of test performing.

Invalid: No band appears in the control region. A sample should never be identified as positive if you do not generate a control line. If the control line is not formed, the test is invalid and must be repeated.

Illustration 1



IX. INTERNAL QUALITY CONTROL

Internal procedural controls are included in the test. A line appearing in the control region (C) is an internal control. It confirms sufficient specimen volume and correct procedural technique.

X. CHARACTERISTICS

A. Analytical Sensitivity – 6.25 x 10^3 CFU/mL (0.78 ng/mL).

B. Sensitivity and Specificity (correlation) - Accuracy

The was validated comparing the results obtained with Hepylori versus those ones obtained with a commercial ELISA test (Premier Platinum HpSA EIA test) for the sensitivity and versus Amplified IDEIATM HpStAR™ for the specificity.

Sensitivity and specificity of kit have been determined on specimens obtained from patients with the same as H. pylori infection symptoms and from asymptomatic individuals. The results are:

sensitivity = > 94 % (35 samples confirmed positive versus 37 positive with ELISA test).
specificity = > 99 % (10 samples confirmed negative versus 10 negative with ELISA test).
PPV >99% NPV > 84%
Accuracy = > 92 % compared with other analysis methods (culture, Urea Breath Test and ureasi test).

C. Linearity : Signal visible up to concentration of 9 mg/mL.

C. Prozone effect (Kook effect): Prozone effect was not observed up to concentration of 9 mg/mL.

D. Cross-reactivity: No cross-reactions have been found with bacteria normally present in the gastro-intestinal tract and those ones generally infecting the same area such as Rotavirus, Adenovirus, Escherichia coli, Campylobacter, Giardia lamblia.

A second study performed on a series of potentially cross-reactive samples for Rotavirus, several protozoan or helminth enteric pathogens, contaminated with bacteria such as Salmonella and Campylobacter, has identified a specific value by about 100%, resulting negative.

All bacteria and viruses tested are negative: Rotavirus, Campylobacter jejunii at concentration of about 2 mg/g of stool; Campylobacter uppsaliensis (2 mg/g); Salmonella; C. difficile; Klebsiella; Y. Enterocolitica; Giardia lamblia; S. enteridis; Shigella; Proteus; P. mirabilis; Entamoeba hystolytica; Citrobacter; P. vulgaris; Strongyloides stercoralis; EPEC: E. coli entheropatogenich; ETEC: E. coli entherotoxic.

E. Interferences: the follow substances not interfere: human hemoglobin, bovine immunoglobulins (IgG) and HCG hormone. Diarrheal, blood samples do not interfere.

XI. LIMITS OF THE TEST

- An excess of sample could cause wrong results (brown bands appear). Dilute the sample with the buffer and repeat the test.
- The test is for the qualitative detection of Helicobacter pylori antigen in human fecal samples. A positive result suggests the presence of Helicobacter pylori antigens in the sample.
- An equivocal result should be checked with a new card and a new sample.
- As will 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

XII REFERENCES

1. 1997 – International update conference on H. pylori by the American Digestive Health Foundation's
2. 1998 – Guidelines for the management of H. pylori infection – Colin W. Howden, MD FACG and R. H. Hunt
3. 1999 – Detection of H. pylori CagA gene by PCR in fecal samples – Russo F., Leoci C., Notarnicola M., Di Matteo G., Caruso M. L., Pirelli M., Caradonna M., Morandi L., Di Leo A.
4. 1999 – Detection of H. pylori DNA in fecal samples from infected individuals – William A., Gramley A.
5. 1999 – Analysis of VacA, CagA, and IS605 genotypes and those determined by PCR amplification of DNA between repetitive sequences of H. pylori strains isolated from patients with nonulcer dyspepsia or mucosa-associated lymphoid tissue lymphoma – van Doorn N. E., Namavar F., van Doorn L. J., Durrani Z., Kuipers E. J., Vandenbroucke-Grauls C. M.; J. Clin. Microbiol.; 37 (7): 2348-9
6. 2008 – Helicobacter pylori: valutazione di un nuovo test diretto – Casella P., Straface M.C. SMeL, A.O. "Ospedale Civile di Vimercate" Presidio di Vimercate (MI)

IVD	In Vitro Diagnostic Medical Device		Temperature limitation	LOT	Batch code (EXXX)		Manufacturer		Keep dry	NON STERILE	Non-sterile
	Consult Instructions for use		Use by (year/month)	REF	Catalogue number		Do not reuse		Fragile, handle with care		Keep away from heat

CONTENT (50 tests)	**REF. VC1150**
Hepylori dipstick	50 items
Extraction liquid	50 x 1.0 mL
Instruction for use	1 item

EDMA Code 15010401 CE IVD

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