INSTRUCTIONS FOR USE

HEPY STOOL CARD PLUS

RAPID TEST IN CARD FORMAT FOR DETECTION *HELICOBACTER PYLORI* ANTIGEN IN HUMAN STOOL SPECIMEN

1 – INTRODUCTION AND INTENDED USE

For ***in Vitro*** diagnostic use only

Hepy Stool Card Plus is a manual rapid chromatographic immunoassay for the qualitative detection of H. pylori antigens in human faeces specimens. The test offers a simple, highly sensitive and non-invasive screening assay to make a presumptive 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.

2 - PRINCIPLE OF THE METHOD

Hepy Stool Card Plus 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.

3 - MATERIALS PROVIDED – PACKAGING

Product	Type	REF	Pack
Hepy Stool Card Plus CND: W0105090102 EDMA: 15.01.04.01; RDM: 1435008/R	Immunochromatographic test	**VT82001P** (50 tests)	50 sealed in foil pouch containing the device, with dessicant. 50 plastic tubes with dropper tip containing the extraction buffer (solution hypotonic); to use also like negative control (50 x 1 mL) 1 glass dropper bottle containing Positive Control: mixture with non infectious components and NaN_3 as preservative (0.5 mL) 6 plastic pipettes. Secondary packaging: cardboard box.
Hepy Stool Card Plus CND: W0105090102 EDMA: 15.01.04.01; RDM: 1672148/R	Immunochromatographic test	**VT82003P** (20 tests)	20 sealed in foil pouch containing the device, with dessicant. 20 plastic tubes with dropper tip containing the extraction buffer (solution hypotonic); to use also like negative control (20 x 1 mL) 1 glass dropper bottle containing Positive Control: mixture with non infectious components and NaN_3 as preservative (0.5 mL) 3 plastic pipettes. Secondary packaging: cardboard box.

4 - MATERIALS REQUIRED BUT NOT PROVIDED

Specimen collection container, Disposable gloves, Timer, Tubes for test.

5 - PRECAUTIONS AND WARNINGS

- All operations linked to the use of the test must be performed in accordance with Good Laboratory Practices.
- Hepy Stool Card Plus is a qualitative *in vitro* diagnostic, for professional use only; it is to be used by adequately trained and qualified laboratory personnel.
- This product is not classified as dangerous according to current European legislation.
- 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.
- Each test device and each extraction buffer vial are for single use only.
- Never use reagents from another lot.
- The test should remain in the sealed pouch until use.
- The test must be carried out within 2 hours of opening the sealed bag.
- Do not use the test if pouch is damaged.
- The presence of yellow lines in the results window (control and test line zone) that are visible before using the test are completely normal. That not means failure on test functionality.
- Wear gloves when handling the sample.
- Disposable gloves, extraction buffer, test tubes, and used devices in a propre biohazard container.
- The reagents' quality cannot be guaranteed beyond their shelf-life date or if the reagents are stored under inappropriate conditions.
- The Certificates of Analysis and the Safety Data Sheet of the product are available on the website www.masciabrunelli.it.
- This device contains raw materials of animal origin. The raw material involved is derived from animals that have been slaughtered in an authorized slaughterhouse and, following an antemortem inspection, which have not shown any sign of disease transmissible to humans or animals. In any case is recommended that the kit be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal

consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

- Notify Mascia Brunelli Spa and the Relevant Authorities of any serious incidents occurring in connection with the in vitro diagnostic device.

6 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store the kit in their original pack at refrigerated or room temperature (2-30°C/36-89°F). If properly stored, the kit may be used up to the expiration date. The device test must remain in the sealed pouch until use. Do not use the device test after 2 hours of opening sealed-bag. Use the extraction buffer vial immediately before to perform the test. Do not freeze.

7 – SPECIMENS 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 *48* 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, *maximum 1 year. In this case, the sample will be totally thawed and brought to room temperature before testing.*

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 (about 125 µL) 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.

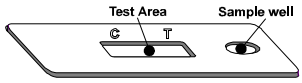
Formed / Solid Stools

Unscrew the top of the extraction tube. Collect the stool sample with the tip of the collection device by dipping in *four* different places of the same stool specimen.. Verify to transfer a small portion (approximately 50 mg) 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.

8 - TEST 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 card from the protective pouch. Identify the plastic cassette with the patients data.
2. Gently shake the test tube containing the sample under investigation.
3. Brake the tip of the test tube and squeeze 2-3 drops of the extracted mixture into the sample well "S" of the card.
4. Read the result at 10 minutes after dispensing the sample. Do not exceeded *10* minutes.

If the test does not run due to solid particles, stir the sample added in the sample window (S) with the stick. If it doesn´t work, dispense a drop of diluent until seeing the liquid running through the reaction zone.

PROCEDURE FOR CONTROLS

Remove the test card from the protective pouch (allow the device to reach to room temperature prior to open the pouch in order to avoid condensation on the membrane).

Open the vial of control (positive or negative) and dispense 2-3 drops in sample well S of the device.

Read the result at 10 minutes after dispensing the control. Do not exceeded *10* minutes.

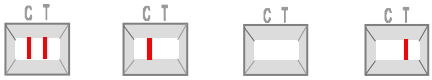
9 - READING AND INTERPRETATION

Interpret the results as follow:

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.

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.

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.



Positive Negative Invalid

10 - 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.

11 – EXPECTED VALUES

Studies have found that more than 90% of patients with duodenal ulcer and 80% of patients with gastric ulcer are infected with Helicobacter pylori. Hepy Stool Card Plus has been compared with different methods: cultures, Urea Breath Test and Urease Test, demostraiting an overall accuracy of >92%.

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12 - PERFORMANCES CHARACTERISTICS

A. Analytical Sensitivity – 0.78-0.09 ng/mL of *H. pylori* recombinant outer membrane protein.

B. Sensitivity and Specificity (correlation) - Accuracy

It was performed an evaluation using **HEPY STOOL CARD PLUS** with specimens obtained from patients with the same as *H. pylori* infection symptoms and from asymptomatic individuals. The **HEPY STOOL CARD PLUS** was valuated compared with a commercial qPCR kit CE mark kit. The result summarized in the following table:

	HEPY STOOL CARD PLUS vs VIASURE *Helicobacter pylori* Real Time Detection Kit	
		95% CI (Confidence interval)
Sensitivity	98.2%	90.3%-100.0%
Specificity	98.4%	91.2%-100.0%
PPV	98.2%	90.3%-100.0%
NPV	98.4%	91.2%-100.0%

C. 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: *Escherichia coli O157:H7, Campylobacter coli/jejuni, Salmonella enteritidis/paratyphi/typhi/typhimurium, C. difficile, Y. Enterocolitica, Shigella boydii/dysenteriae/flexneri/sonnei, Listeria monocytogenes, Staphylococcus aureus.*

13 - LIMITATIONS OF THE METHOD

- An excess of sample could cause wrong results (brown bands appear). Dilute the sample with the extraction liquid and repeat the test.
- The test is for the qualitative detection of Helicobacter pylori antigen in human fecal samples. The use of other samples has not been established. The quality of the test depends on the quality of the sample; proper faecal specimens must be obtained.
- A positive result suggests the presence of Helicobacter pylori antigens in the sample; nevertheless, a positive result should be followed up with additional invasive techniques (endoscopy) to confirm the results. A confirmed infection should only be made by a physician after all clinical and laboratory findings have been evaluated and must be based in the correlation of the results with further clinical observations.
- Neither the quantitative value nor the rate of increase in H. pylori antigens concentration can be determined by this test.
- Antimicrobials, proton pump inhibitors and bismuth preparations are known to suppress H.pylori, and ingestion of these prior to H.pylori testing (culture, histology, rapid urease, UBT and fecal antigen) may cause false-negative results. If a patient has ingested these compounds within two weeks prior to performing the antigen test, a false-negative result may occur. In such cases, the test should be repeated on a new specimen obtained two weeks after discontinuing treatment. A positive result for a patient ingesting these compounds within two weeks prior to performing the test, should be considered accurate. In case of a specific eradication therapy, a test for the therapy follow-up should be performed at least after 4 weeks from the end of the medication.
- An equivocal result should be checked with a new card and a new sample.
- If the test result is negative and clinical symptoms persist, additional testing using other clinical methods is recommended or on a sample from an enrichment culture. A negative result does not at any time preclude the possibility of H. pylori infection; could be that the antigens concentration in the stool sample is lower than the detection limit value.
- Mucous and/or bloody stool samples could cause non-specific reactions in the test. Mucous and/or bloody stool samples whose result is positive should be followed up with other techniques to confirm the result.

14 - REFERENCES

1. Bruce E. Dunn, Hartley Cohen & Martin J. Blaser. Helicobacter pylori. Clin. Microbiol. Rev. 10 (4), 720-741, Oct. (1997).
2. John L. Telford, Antonello Covacci, Rino Rappuoli & Paolo Ghiara. Immunobiology of Helicobacter pylori infections. Current Opinion in Immunology, 9; 498-503 (1997)
3. Martin J. Blaser. Helicobacter pylori and gastric diseases. BMJ; 316: 1507-1510 (1998).
4. 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)

TABLE OF APPLICABLE SYMBOLS

IVD	In Vitro Diagnostic Medical Device		Temperature limitation	LOT	Batch code (DXXX)		Manufacturer		Keep dry
	Consult Instructions for use		Use by (year/month)	REF	Catalogue number		Keep away from heat		Fragile, handle with care

REVISION HISTORY

Version	Description of changes	Date
Instructions for Use (IFU) - Revision 8	Updated layout and content; alignment to the Italian version revision index	2022/09

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.