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TECHNICAL REPORT ON *H. pylori* on Stool

TECHNICAL REPORT ON
***H. pylori* on Stool**
Helicobacter pylori

Cod.C06/C07

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Rapid One Step Immunochromatographic Test
for the detection of *H. pylori* antigen in stool samples

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TECHNICAL REPORT ON *H. pylori* on Stool**1 PRODUCTS DESCRIPTION****1.1 DESCRIPTION OF THE PACKAGING**

Products Names	Packaging		References
	Immediate container	Outer container	
<i>H. pylori</i> Card	Aluminium pouches	20/50 Test	C06/C07

Table 1 Packaging

Immediate container is the packaging that protects the device from humidity, contamination, and/or damage.

H. pylori Test is temperature and humidity sensitive, proper packaging can control these variables. Dessicants are included in packages and in test strips.

Product	Label size	Label Reference	Description	Contents
Immediate container: <i>H. pylori</i> card	Printed message: <i>H. pylori</i> CE LOT: MED- <i>uuuuuu</i> EXP: <i>yyyy-mm</i> Manufactured: <i>yyyy-mm</i>		Laminated pouch	1 test/pouches + desiccant material
Sample diluent	1x(35x50)	C011	Colour white	Sample diluent (3 mL)

Table 2 Identification

2 BASICS

Helicobacter pylori (*H. pylori*) is a spiral-shaped bacterium that is found in the gastric mucous layer or adherent to the epithelial lining of the stomach. *H. pylori* causes more than 90% of duodenal ulcers and up to 80% of gastric ulcers.

The importance of *Helicobacter pylori* testing has increased greatly since the strong correlation between the presence of bacteria and confirmed gastrointestinal diseases (stomach and duodenum) like gastritis, peptic ulcer disease and gastric carcinoma.

2.1 INTENDED USE

H. pylori Test has been developed with the purpose of providing quick help in the diagnosis of *Helicobacter pylori* infection, by detection of *H. pylori* antigen in human stool, and to monitor response during and post-therapy in patients.

H. pylori Test is a direct non-invasive testing method, that not to produce risk and discomfort to the patient. Instrumentation are not required to interpret results

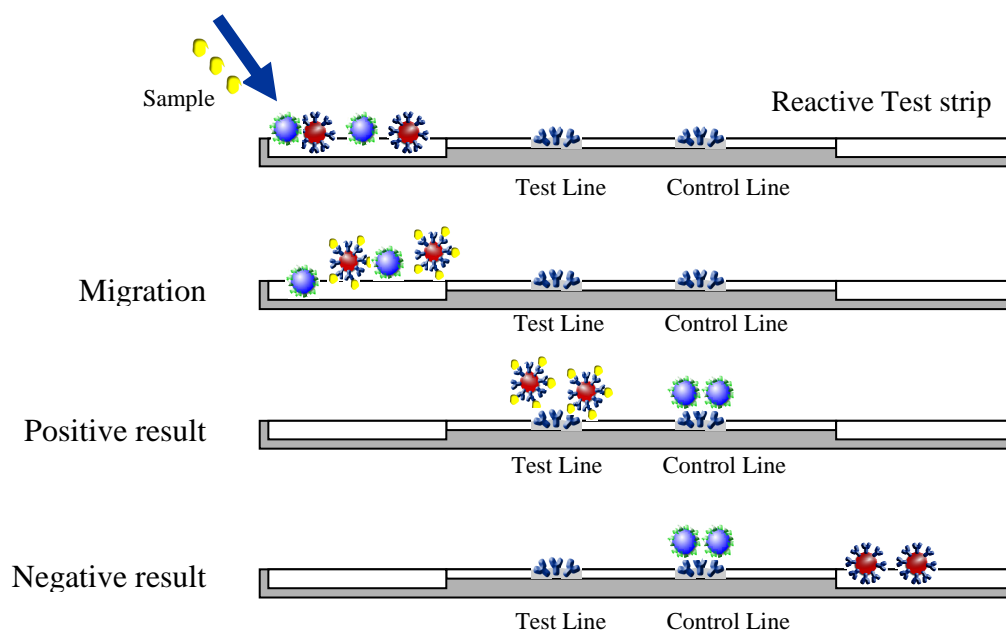
H. pylori Test is a one step coloured chromatographic immunoassay for the qualitative detection of *Helicobacter pylori* in faeces.

The device is for professional *in vitro* diagnostic use.

2.2 BASIC PRINCIPLES OF THE ASSAY

H. pylori Test is a qualitative immunochromatographic assay for the determination of *H. pylori* in fecal samples. The membrane is pre-coated with polyclonal antibodies, on the test band region, against viral antigens.

During testing, the sample is allowed to react with the coloured conjugate (anti-*H. pylori* polyclonal antibodies-red polystyrene microspheres), which was pre-dried on the test. The mixture then moves upward on the membrane by capillary action. As the sample flows through the test membrane, the coloured particles migrate. In the case of a positive result the specific antibodies present on the membrane will capture the coloured conjugate. The mixture continues to move across the membrane to the immobilized antibody placed in the control band region, a green coloured band always appears. The presence of this green band serves as 1) verification that sufficient volume is added, 2) that proper flow is obtained and 3) as an internal control for the reagents.

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2.3 REAGENTS AND KIT COMPONENTS

To perform the test are necessary the test device and a stool collection tube.

The sample buffer is a mix of biological buffers, salts, detergents and proteins. Also contains NaN_3 as preservative at a concentration less than 0.1%.

Stool samples must be observed as potentially infectious. Adopt adequate security practices.

2.4 STORAGE AND STABILITY

Store as packaged in the pouch at 2-30°C. The test is stable through the expiration date printed on each pouch. The test must remain in the closed pack until use. Keep it the remaining test in the sealed pouch with the date printed. Do not freeze.

2.5 RUNNING THE ASSAY

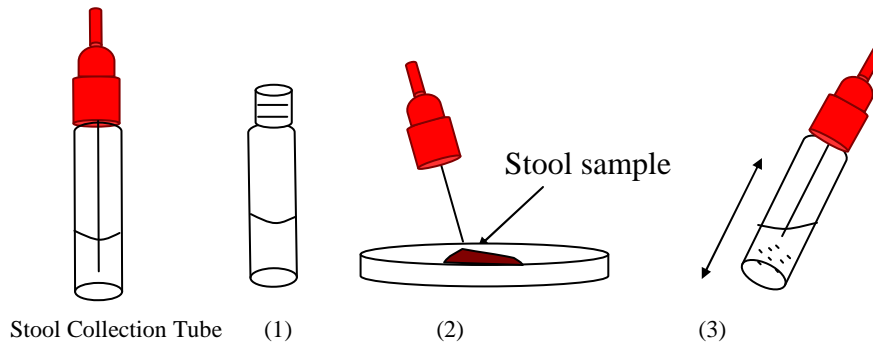
2.5.1 Samples

Liquid, semi-solid or solid (formed), can be used. Watery and diarrhoeal specimens are not appropriate for testing. Stool samples should be collected in clean containers and the assay should be done right after collection. The samples can be stored in the refrigerator (2-4 °C) for 1-2 days prior to testing. For longer storage, maximum 1 year, the specimen must be kept frozen at -20°C. In this case, the sample will be totally thawed, and brought to room temperature before testing.

Specimen preparation using stool collection tube:

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- (1) Take out the top of the stool collection tube.
- (2) Use the stick to pick up a little sample. Close the tube with the diluent and stool sample. (3) Shake the tube in order to assure good sample dispersion.



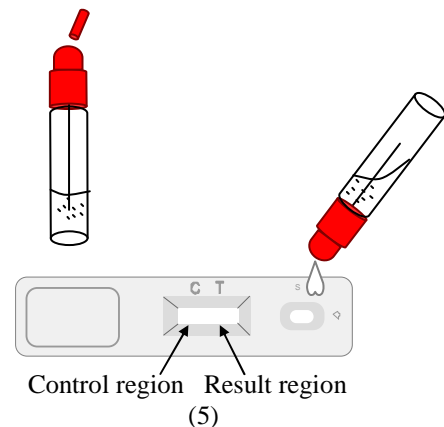
2.5.2 Card Test procedure with stool collection tube:

Allow the tests, stool samples and controls to reach to room temperature (15-30°C) prior to testing. Do not open the package until ready to perform the assay.

1. Proceed to shake the stool collection tube in order to assure good sample dispersion. Cut the end of the top (4).

2. Remove the *H. pylori* Card device from its sealed bag just before using.

3. Use a separate stool collection tube and device for each sample or control. Dispense exactly 4 drops or 150 µL into the circular window marked with an arrow, avoiding to add solid particles with the liquid (5).



In case the tests did not run due to solid particles fallen into the round window, stir the sample added or dispense a drop of extraction buffer until seeing the liquid running through the reaction zone.

4. - Read the result at **10 minutes** (the coloured bands appear).

3 REACTION DEVICE

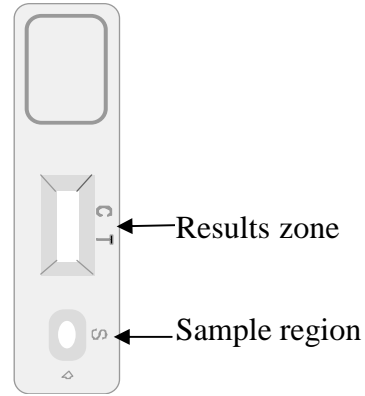
3.1 DESCRIPTION AND COMPOSITION OF TEST

The *H. pylori* Card is a plastic device containing a reaction strip. The inside reaction strip is made of different laminated materials.

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The device has two regions of interest:

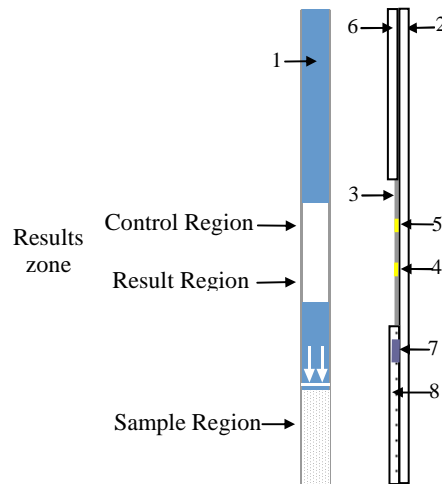
H. pylori Card



The strip has two defined areas:

1. Sample region: the white end of the strip is submerged into the sample or the sample is dispensed on it.
2. Results zone: the white intermediate zone where the coloured bands appear.

- 1) Plastic protective material
- 2) Backing
- 3) Nitrocellulose membrane
- 4) Immobilised antibodies
- 5) Control line
- 6) Final absorbent material
- 7) Coloured conjugate
- 8) Initial absorbent material



H. pylori Test

H. pylori Test consists in a strip with several layers: an absorbent material pre-dried with a coloured latex conjugate to polyclonal antibodies against *H. pylori* antigens, a nitro-cellulose membrane with coated antibodies against *H. pylori* antigens and a cellulose absorbent.

H. pylori sample diluent (DIL) contains biological buffer, NaCl, proteins, surfactants and 0.095% of sodium azide as preservative.

3.2 POUCHES PACKAGING:

Laminated aluminium pouches, containing a dessicant material, are the packaging that protects *H. pylori* Tests from humidity and damage.

4 INTERPRETATION OF RESULTS

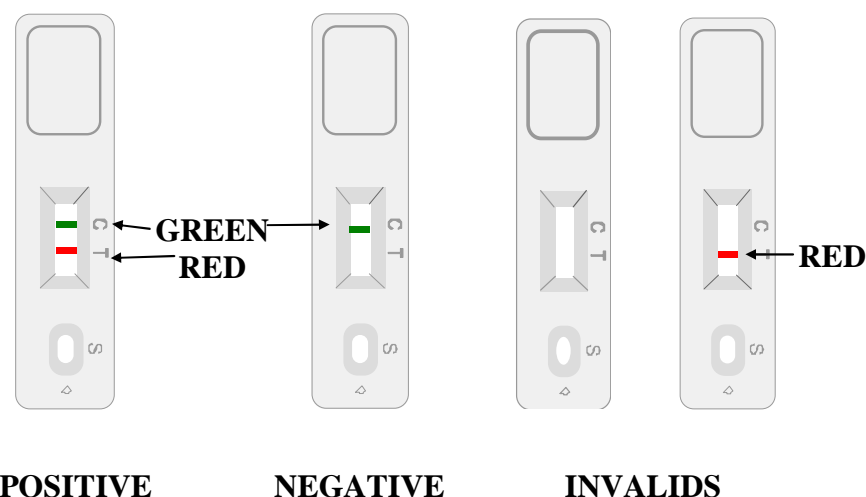
As soon as the re-suspended sample is poured into sample region or the strip is submerged into the sample, a chromatographic process moves the labelled latex particles towards the results zone.

If the sample is positive, *H. pylori* antigens react with the antibodies bound to the red colloidal latex particles. When this complex (particles/antibodies/ *H. pylori* antigens) reaches the first line result zone, it reacts again with the immobilised antibodies anti-*H. pylori*, producing a reddish band, which increases its colour intensity with the course of the chromatography and with *H. pylori* concentration.

If the sample is negative there is no *H. pylori* antigens to react with the antibodies bound to the colloidal red latex particles. When the red colloidal latex particles reach the results zone, there is no interaction with the anti-*H. pylori* antibodies immobilised there. The line remains invisible while the chromatographic process advances.

In both cases, the green particles -which have not been retained in the first line of results zone- continue their chromatographic advance to the second invisible line, where they react producing a green band (control band).

The interpretation of the results is as follows:



H. pylori Card Test

4.1 POSITIVE

Two bands or lines appear in the result zone: a reddish (positive) line and a green (control) line. The upper line, green control line, will always appear as a strong line.

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The colour intensity of the lower red line (results line) will depend on the *H. pylori* antigens concentration of the specimen.

This intensity may vary from very strong at high *H. pylori* antigens concentrations to faint when the *H. pylori* antigens concentration is close to the sensitivity limit of the test.

4.2 NEGATIVE

Only one green band appears on result zone (control line).

4.3 INVALID

No band appears at result region. Other invalid possibilities would be showing two bands of the same colour or a unique red band.

Invalid results may be caused by deterioration of the reagents or by improper handling. In this case the test should be repeated using a new test.

H. pylori Test is sensitive to humidity, close the container always tightly. If the container is stored in the refrigerator, do not open it until the room temperature has been reached in order to avoid water condensation inside the cool container.

Some stool samples can decrease the intensity of the control red line.

4.4 NOTES ON THE INTERPRETATION OF RESULTS

The intensity of the red coloured band in the test line region will vary depending on the concentration of *H. pylori* presents in the specimen. However, neither the quantitative value, nor the rate of increase in *H. pylori* can be determined by this qualitative test.

4.5 QUALITY CONTROL

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

5 TEST PERFORMANCE

5.1 SENSITIVITY

5.1.1 Detection Limit

A culture of *H. pylori* bacteria was sonicated, centrifuged and its protein concentration was determined. This reference antigen preparation of *H. pylori* was diluted in the PBS-BSA buffer and tested in accordance with the kit instructions.

ng/mL	256	128	64	32	16	8	4	2	0
Signal (10 ³)	+	+	+	+	+	±	±	-	-

Table 3 Detection limit

We found that, under such conditions, the detection limit using the reference antigen preparation of *Helicobacter pylori* is 4-8 ng/mL.

5.2 SPECIFICITY

The use of specific polyclonal antibodies in the elaboration of Medimar *H. pylori* Test assures its high degree of specificity for antigens of *Helicobacter pylori*.

Using stool samples from Europe and Italian Hospitals, the diagnosis was obtained by clinical evaluation. A first small evaluation of the test gave the following results:

<i>H. pylori</i> TEST	CLINICAL EVALUATION		
	+	-	Total
+	11	1	12
-	0	8	8
Total	11	9	20

Table 4 Results of *H. pylori* Test samples checked with clinical evaluation.

Sensitivity	>99%
Specificity	89%
Positive Predictive Value	92%
Negative Predictive Value	>99%
Correlation	95%

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These preliminary values have to be taken with precaution until more evaluation data will be available.

5.2.1 Comparison of *H. pylori* Test and another commercial rapid test for detection of *H. pylori* antigen

12 stool samples were tested in parallel with *H. pylori* Test and with another commercial rapid test, in accordance with the kit instructions. These patients showed gastrointestinal symptoms (like gastritis).

<i>H. pylori</i> Test	Clinical Evaluation			Other rapid Test	Clinical Evaluation		
	+	-	Total		+	-	Total
+	7	1	8	+	7	2	9
-	0	4	4	-	0	3	3
Total	7	5	12	Total	7	5	12

Table 5 *H. pylori* test and other rapid Test

	<i>H. pylori</i> Test	Other commercial rapid test (<i>H. pylori</i> antigen)
Sensitivity	100%(>99%)	100% (>99%)
Specificity	80%	60%
Positive Predictive Value	87.50%	77.78%
Negative Predictive Value	100%(>99%)	100%(>99%)
Correlation	91.67%	83.33%

Results show in both tables 100% sensitivity (>99%) to *H. pylori* Test and the other rapid test.

These results also show that the specificity, PPV and the correlation of *H. pylori* Test is better than the other commercial rapid test.

The detection of *Helicobacter pylori* showed 95% of concordance with the commercial assay.

The antibodies used to elaborate the *H. pylori* Test recognise epitopes present in the antigen found in stool of patients, as well as in preparations from the bacteria

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cultures in vitro. Sonicated *Helicobacter pylori* extract from different commercial samples reacts with *H. pylori* Test.

6 FINAL

H. pylori Test is a quick "one step" test for detection of *H. pylori* antigen in stool samples. It is presented in a "ready to use" format and the interpretation of results is very simple. In order to ensure the best results, it is necessary to carry out the procedure as described in the kit insert and to store it under the best conditions, preferably in cool room if the room temperature is close to 30 °C. The recommended store temperature is between 2 and 30°C.

Notwithstanding the high sensitivity of *H. pylori* Test, a *H. pylori* infection in a negative sample cannot be totally excluded. Furthermore, although specificity is high, it may show some occasional false positive reaction; consequently, as a general rule, all samples giving positive results should be re-tested and rechecked with another different method with the same or better sensitivity.

This test provides a presumptive diagnosis for *H. pylori* infections. A confirmed infection diagnosis should only be made by a physician after all clinical and laboratory findings have been evaluated.

7 BIBLIOGRAPHY/PUBLICATIONS











Bibliography

- 1.- Stephen L. W. On. *Identification Methods for Campylobacters, Helicobacters, and Related Organims*. Clin. Microbiol. Rev. **9** (6), 405-422, July (1996)
- 2.- Bruce E. Dunn, Hartley Cohen & Martin J. Blaser. *Helicobacter pylori*. Clin. Microbiol. Rev. **10** (4), 720-741, Oct. (1997)
- 3.- Martin J. Blaser. *Helicobacter pylori and gastric diseases*. BMJ; **316**: 1507-1510 (1998).
- 4.- John L. Telford, Antonello Covacci, Rino Rappuoli & Paolo Ghiara. *Immunobiology of Helicobacter pylori infections*. Current Opinion in Immunology, **9**; 498-503 (1997).

8 ANNEX

Annex I: Interpretation for the symbols used:

+	very clear band, positive result
±	low intensity band, positive result
-	no band, negative result

	<i>In vitro</i> diagnostic device		Batch code (Pxxx)
	Consult instructions for use		Catalogue number
	Keep dry		Contains sufficient for <n> tests
	Temperature limitation		Manufacturer
	Use by (yyyy-mm: year-month)		Do not use if package damaged