VITASSAY

H. pylori

Rapid test for the qualitative detection *of Helicobacter pylori* in human stool samples.

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Vitassay

EN

For professional *in vitro* diagnostic use only.

INTENDED USE

Vitassay H. pylori is a rapid one step immunochromatographic assay for the qualitative detection of *Helicobacter pylori* (*H. pylori*) in human stool samples.

Simple, non-invasive and a highly sensitive screening assay to make a presumptive diagnosis of *Helicobacter pylori* infection.

INTRODUCTION

Helicobacter pylori is a spiral shaped pathogenic bacterium found on the human gastric mucosa.

Infection is generally asymptomatic, with the majority of those persons infected not developing clinical disease. However, because *H. pylori* has been recognized as a major cause of gastritis and is associated with duodenal ulcer disease, gastric ulcer disease, gastric lymphoma, and gastric cancer in humans, it is a public health problem in both developed and developing countries.

PRINCIPLE

Vitassay H. pylori is a qualitative immunochromatographic assay to make a presumptive diagnosis of *Helicobacter pylori* infection in human stool samples.

The test line zone of the nitrocellulose membrane is pre-coated with monoclonal antibodies against *Helicobacter pylori.*

During the process, the sample reacts with the antibodies against *H. pylori*, forming conjugates. The mixture moves upward on the membrane by capillary action. If the sample is positive, antibodies present on the membrane (test line) capture the conjugate complex and a red line will be visible. Although the sample is positive or negative, the mixture continues to move across the membranes and the green control line always appears.

The presence of this green line (in the control zone (C)) indicates that sufficient volume is added; proper flow is obtained and serves as an internal control for the reagents.

PRECAUTIONS

- For professional *in vitro* use only.
- Do not use after expiration date.
- Read the instructions for use carefully before using the test.
- Do not use the kit if the label sealing the outer carton is torn or if the bags are open or damaged on arrival.
- Do not use the tests if the desiccant material is missing or broken inside the aluminium pouch.
- Tests should be discarded in a proper biohazard container after testing.
- Specimens should be considered potentially hazardous and should be handled in the same manner as an infectious agent, following local/national regulations. A new test should be used for each sample to avoid contamination errors.
- Material exposed to the samples should also be considered potentially hazardous and should be handled in the same manner as an infectious agent, following local/national regulations.
- Do not reuse. This is a single-use device.
- Used material should be disposed of in an appropriate biohazard container after testing.
- Reagents contain preservatives. Avoid any contact with the skin or mucous membrane. Consult safety data sheet, available on request.
- All reagents included in the kit are approved for use with **Vitassay H. pylori** only. Do not mix or use the components with other batches of Vitassay. Do not use with reagents from other kits or commercial assays.
- Follow Good Laboratory Practices. These practices should include, but are not limited to, personal protective equipment (PPE), such as lab coat, surgical or appropriate mask or face shield, disposable gloves and eye protection. Take the necessary precautions during sample collection, transport, storage, handling, and disposal. Each sample must be correctly and unequivocally identified to ensure proper traceability of samples.
- In case of spillage, clean thoroughly with a suitable disinfectant.
- Do not eat, drink or smoke in the workplace.
- The presence of yellow lines in the result window (control line area and test line area), before using the test, is completely normal and does not imply a failure in the functionality of the test.
- The visual interpretation of the results is done by coloured lines, the interpretation of the results should be done by a professional user without problems of visualisation and colour interpretation.
- A certificate of analysis can be provided on request (not included)

STORAGE AND STABILITY

The storage temperature of the kits should be 2-30ºC.

Do not freeze.

Under these conditions, they can be used until the expiry date indicated on the kit label.

All kit components are for single use only and must remain in their primary packaging until use. The test must remain in the sealed pouch until use.

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MATERIALS

MATERIAL PROVIDED	MATERIAL REQUIRED BUT NOT PROVIDED
▪ **25** tests/kit **Vitassay H. pylori** ▪ Instructions for use. ▪ **25** vials with diluent for the sample dilution.	• PPE, such as disposable gloves ▪ Specimen collection container ▪ Timer • Micropipette (in case of liquid stool)

SPECIMEN COLLECTION

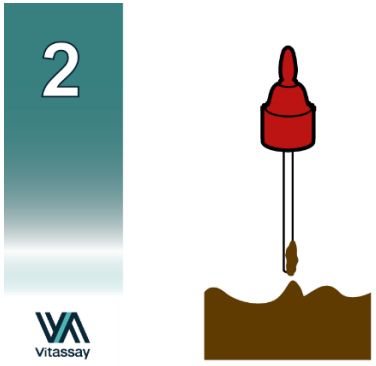
Collect enough quantity of faeces: 1-2g or mL for liquid samples. Stool specimens should be collected in clean and dry containers.

Samples can be kept refrigerated for 1-2 days before use. If the sample is to be stored for a prolonged period, maximum 1 year, it should be frozen at -20°C. The sample should be fully thawed to room temperature before use.

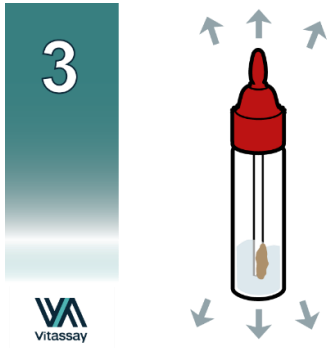
SPECIMEN PREPARATION

1. Remove the cap of the vial with diluent for the sample dilution (figure 1).
2. Use the stick to collect sufficient sample quantity. For solid stool, insert the stick in 4 different areas of the stool sample, taken approx. 50 mg, (figure 2), and add it into the vial with diluent for the sample dilution. Not to exceed the stick´s screw to avoid wrong results. For liquid stool, take 125µL of the sample using a micropipette and transfer it into the vial with diluent for the sample dilution.
3. Close the vial with the diluent and stool sample. Shake vigorously the vial in order to guarantee good sample dispersion (figure 3).

Vial for sample dilution.



Insert the stick in 4 different areas of the stool.



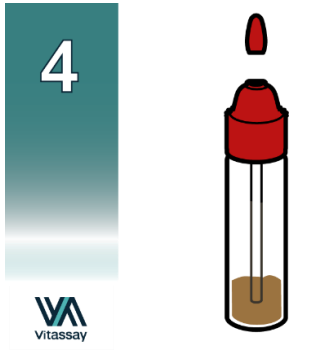
Put the sample into the vial, close the cap and shake.

PROCEDURE

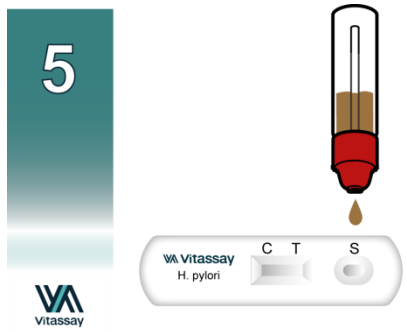
Allow the test, stool sample, controls (if any) and diluent to reach room temperature (15-30ºC) prior to testing. Do not open pouches until the performance of the assay.

1. Shake the vial with the sample to obtain a good sample dilution.
2. Remove the **Vitassay H. pylori** from its sealed bag just before using it.
3. Take the vial containing the diluted sample, cut the end of the cap (figure 4) and dispense 3 drops in the circular window marked with the letter S (figure 5).
4. Read the results at **10 minutes**. Do not read the results later than 10 minutes.

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



Cut the end of the cap.



Dispense 3 drops into the circular window, marked with the letter S

INTERPRETATION OF THE RESULTS

	**NEGATIVE** Only one **green** line in the control zone **(C).**	There is no *Helicobacter pylori* presence. No infection caused by *Helicobacter pylori*.
	**POSITIVE** In addition to the **green** line (**control line C**), a **red** line appears (**test line T**).	There is presence of Helicobacter pylori. Possible *Helicobacter pylori* infection, which might mean gastrointestinal diseases (stomach and duodenum) like gastritis, peptic ulcer or gastric carcinoma.
ANY OTHER RESULTS		Invalid result, we recommend repeating the assay using the sample with another test. **Note**: Wrong procedural techniques, insufficient sample volume or deterioration of the reagents are mostly the main reasons for control line failure. If the symptoms or situation persist, discontinue using the test kit and contact your local distributor.

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Notes: The intensity of the red colored test line in the result line zone (T) will vary depending on the concentration of antigens in the specimen.

QUALITY CONTROL

Internal procedural control is included in **Vitassay H. pylori**. Green line appearing in the results window is an internal control, which confirms sufficient specimen volume and correct procedural technique.

LIMITATIONS

- An excess of stool sample could cause wrong results (brown bands appear). Dilute the sample with the diluent and repeat the test.
- The intensity of test line may vary depending on the concentration of antigens.
- The use of other samples different from human samples has not been established.
- The quality of **Vitassay H. pylori** depends on the quality of the sample; proper faecal specimens must be obtained.
- Positive results determine the presence of *Helicobacter pylori* in faecal samples. 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.
- Negative results should not be considered as conclusive; it is possible that the concentration of antigens is lower than the detection limit. If symptoms or situation persist, a *Helicobacter pylori* determination should be carried out, on a sample from an enrichment culture or using an invasive technique.
- **Bloody stool samples** and/or **mucous** stool samples can cause non-specific reactions in the test. These types of samples whose result is positive should be followed up with other techniques of diagnosis to confirm the result.

EXPECTED VALUES

Helicobacter pylori is a common bacterium, and approximately 50 percent of the world's population has been estimated to be infected. Rates appear to be higher in developing than in developed countries, with most of the infections occurring during childhood.

The overall prevalence of *H. pylori* infection is strongly correlated with socioeconomic conditions. The prevalence among middle-aged adults is over 80 percent in many developing countries, as compared with 20 to 50 percent in industrialized countries.

PERFORMANCE CHARACTERISTICS

Clinical sensitivity and specificity

An evaluation with faecal samples was performed using **Vitassay H. pylori** and qPCR technique (VIASURE Helicobacter pylori Real Time PCR Detection kit, CerTest). The results were as follows:

Results were as follows:

		qPCR test: VIASURE Helicobacter pylori Real Time detection kit		
		Positive	Negative	Total
Vitassay H. pylori	Positive	54	1	55
	Negative	1	60	61
	Total	55	61	116

Table 1. Results obtained by immunochromatographic test of **Vitassay H. pylory** compared to a commercial kit qPCR (VIASURE Helicobacter pylori Real Time Detection kit, Certest).

Vitassay H. pylori vs VIASURE Helicobacter pylori Real Time PCR Detection Kit		
	Mean Value	95% confidence interval
Sensitivity	98.2%	90.3-100%
Specificity	98.4%	91.2-100%
PPV	98.2%	90.3-100%
NPV	98.4%	91.2-100%

Table 2. Sensitivity, specificity, positive predictive values, and negative predictive values of the **Vitassay H. pylori** compared to a commercial kit.

In addition**, Vitassay H. pylori** was evaluated in a Spanish clinical hospital, where 250 stool samples from patients with suspected *H. pylor*i infection were used. To determine the presence of *H. pylori*, the samples were evaluated with **Vitassay H. pylori** and a commercial immunoassay. Subsequently, a commercial ELISA test assay was performed on the discrepant samples.

The results are shown below:

		Reference method		
		Positive	Negative	Total
Vitassay H. pylori	Positive	95	1	96
	Negative	1	153	154
	Total	96	154	250

Tabla 3. Results obtained by comparing **Vitassay H.pylori** with a commercial immunochromatographic and enzyme-linked immunosorbent assay.

Vitassay H. pylori vs Reference method			
Sensitivity	Specificity	PPV	NPV
99% (94.3-100%)	99.4% (96.4-100%)	99% (94.3-100%)	99.4% (96.4-100%)

Table 4. Sensitivity, specificity, positive predictive values and negative predictive values of the **Vitassay H. pylori** kit compared to a commercial kit.

After pooled analysis of these two evaluations, the **Vitassay H. pylori** kit obtained overall clinical sensitivity and specificity values of 98.7% (95.3-99.8%) and 99.1% (96.7-99.9%), respectively (CI=95%).

The results showed that **Vitassay H. pylori** has a high sensitivity and specificity for detecting *Helicobacter pylori*.

Analytical sensitivity

Limit of Detection range is: 0.78 ng/mL-0.09 ng/mL of *Helicobacter pylori* recombinant outer membrane protein.

Cross reactivity

No cross reactivity was detected against gastrointestinal pathogens that are occasionally present in faeces:

Campylobacter coli	*Salmonella enteritidis*	*Shigella dysenteriae*
Campylobacter jejuni	*Salmonella paratyphi*	*Shigella flexneri*
Clostridium difficile	*Salmonella typhi*	*Shigella sonnei*
E. coli O157:H7	*Salmonella typhimurium*	*Staphylococcus aureus*
Listeria monocytogenes	*Shigella boydii*	*Yersinia enterocolitica*

No special diet is recommended prior to testing. There are not interferences with any foods (Vitamin C, broccoli, carrots).

REFERENCES

1. LINDA MORRIS BROWN. "Helicobacter pylori: Epidemiology and Routes of Transmission". Epidemiologic reviews, Vol. 22, No. 2, 2000, pp. 283-297.

2. SEBASTIAN SUERBAUM, M.D., and PIERRE MICHETTI, M.D. "Helicobacter pylori Infection". N Engl J Med, Vol. 347, No. 15, Oct. 2002, pp. 1175-1186.

SYMBOLS FOR IVD COMPONENTS AND REAGENTS

IVD	*in vitro* diagnostic device		Keep dry
	Consult instructions for use		Temperature limitation
	Use by		Manufacturer
LOT	Batch code	Σ n	Contains sufficient for <n> test
DIL	Sample diluent	REF	Catalogue number
CE	CE Marking		

Changes control		
Nº version	**Changes**	**Date**
IUE-7355020 Ed02 August 2023	Change of format. Addition of a new evaluation. Addition of Analytical sensitivity section. Format has been updated. Limitations sections has been updated. Transcription error in interpretation section has been corrected. Wording and grammatical changes have been implemented in Precautions, Limitations, Specimen collection, Storage and Stability. Material required but not included updated with minor changes.	25/08/2023

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