VITASSAY

H. pylori
-Turbidimetric Assay-

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

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CE IVD EN

Vitassay

For professional *in vitro* diagnostic use only. Professional trained in Turbidimetric techniques.

INTENDED USE

Vitassay H. pylori –Turbidimetric Assay- is a rapid Turbidimetric assay for the quantitative detection of Helicobacter pylori (H. pylori) in human stool samples.

Simple, non-invasive and highly sensitive assay that helps in the diagnosis of Helicobacter pylori infection. This product has been optimized for several automated analyzer.

INTRODUCTION

Helicobacter pylori (H. pylori) is a spiral gram-negative, microaerobic human pathogen. H. pylori infection is strongly related with many gastroduodenal diseases, atrophic gastritis, mucosa associated lymphoid tissue (MALT) lymphoma and noncardia gastric cancer.

H. pylori colonize approximately 50% of world´s population, but the prevalence of H. pylori is of high quality in developing countries. Risk factors for H. pylori infection varies widely by geographic area, age, race, and socioeconomic status.

PRINCIPLE

Vitassay H. pylori –Turbidimetric Assay- is a quantitative turbidimetric assay for the detection of Helicobacter pylori in human solid stool samples.

H. pylori Turbidimetric Assay is based on antigen-antibody agglutination reactions between the antigen contained in the sample and the antibodies anti-antigen coated on polystyrene latex particles. Such agglutination is measured as an increase in absorbance proportional to the quantity of antigen contained in the sample.

The use of two external controls, Control 1 and Control 2, is used to verify that the test is working properly.

PRECAUTIONS

- For professional *in vitro* use only.
- A trained person in Turbidimetric technique and autoanalyzer use is required.
- Do not use after expiration date.
- Do not use the test if its primary containers are damaged.
- Specimens should be considered as potentially hazardous and handle in the same manner as an infectious agent. Avoid contamination errors, follow proper work procedure.
- The reagents after use should be discarded in a proper biohazard container after testing.
- Reagents contain preservatives. Avoid any contact with the skin or mucous membrane. Consult safety data sheet, available on request.
- Components provided in the kit are approved for use with the **Vitassay H. pylori -Turbidimetric Assay-**. Do not use any other commercial component.
- Follow Good Laboratory Practices, wear protective clothing, use disposal gloves, goggles and mask. Do not use any other commercial kit component.
- If measure range is exceeding, use the sample diluent to dilute the sample and repeat the assay again.
- Read and follow up the instructions for use provided in the kit.
- Prepare and adjust the analyzer before starting measurements.

STORAGE AND STABILITY

Store as packaged in the original primary container, the reagents should be preserved at refrigerated temperature (2-8ºC/35.6-46.4ºF), the sample diluent could be preserved refrigerated or at room temperature (2-30ºC/35.6-86ºF).

The product is stable until the expiration date printed on the label, if they have been preserved under the recommended conditions.

Do not freeze and keep away from the sunlight.

MATERIALS

MATERIAL PROVIDED	MATERIAL REQUIRED BUT NOT PROVIDED
▪ Reagent R1 (Enhancer buffer) ▪ Reagent R2 (Particles Latex) ▪ Calibrator 0 ▪ Calibrator 1 ▪ Calibrator 2 ▪ Calibrator 3 ▪ Calibrator 4 ▪ Calibrator 5 ▪ Control 1 ▪ Control 2 ▪ Vials H. pylori with diluent for the sample dilution. ▪ Instruction for use. ▪ Additional screw cap.	▪ Specimen collection container. ▪ Disposable gloves and laboratory equipment. ▪ Vortex. ▪ Automatic analyzer. ▪ Microcentrifugue (10000g).

SPECIMEN COLLECTION

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

Samples can be stored in the refrigerator (2-8ºC/36.5-46.4ºF) for 7 days prior to testing. For longer storage, maximum 6 months, the specimen must be kept frozen at -20ºC (-4ºF). Samples must be

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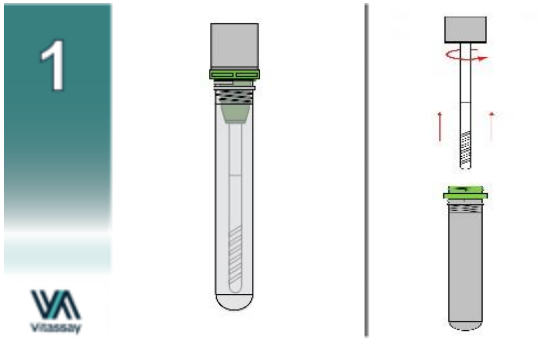
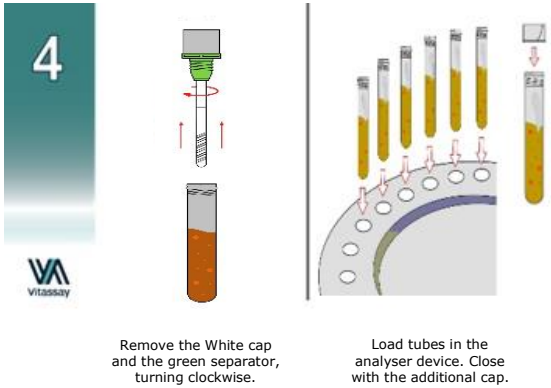
brought to room temperature before testing. Homogenize the sample as thoroughly as possible prior to preparation.

SPECIMEN PREPARATION

1. Label the vial with the patients´s identification.
2. Open the cap of the vial (anticlockwise) without removing the green separator and use the stick to pick up sufficient quantity of sample (figure 1).
3. Use the stick to collect sufficient sample quantity. Introduce the stick into 4 different zones and rotate the stick in order to make the samples as homogenous as possible and make sure all grooves are filled with faecal material (figure 2).
4. Remove the stool excess by rotating the stick on the internal wall of the sterile container.
5. Insert the stick with the sample into the vial through the green separator. Close the white cap by holding the green separator to prevent it from opening.
6. Shake each vial (30-60 seconds) by Vortex in order to assure good sample dispersion (figure 3). Proceed to step 7 if the grooves are visually empty of any stool material, otherwise repeat the shaking up to 120 seconds and proceed to step 7 regardless some residual stool sample in the grooves. The sample dilution vial with diluted sample can be stored for 7 days in the refrigerator (2-8ºC) prior to testing and bring the samples to room temperature.
7. Centrifuge the vial for 10 minutes at 1000-3000 x g in order to remove the possible residuals of faecal material. Alternatively, let the vial sit upright for a minimum of 15 minutes.
8. Remove the white screw cap and the green separator from vials by rotating the separator (clockwise). The faecal extract is now ready to be tested. Insert the opened device directly into the clinical chemistry analyser or transfer the supernatant to an adaptor cup (not provided) (figure 4).
9. Close the collection vial by the provided additional screw cap (figure 4).

Note: Do not use the sample vials directly in the analyzer.





PROCEDURE

Reagent R1 y Reagent R2 are ready to use.

Calibration curve

For calibration only use H. pylori Calibrator: Cal0, Cal1, Cal2, Cal3, Cal4 and Cal5. Contain Helicobacter pylori antigen at diferent concentrations indicated on the label of each of the vials.

	Reference	Calibrator 1	Calibrator 2	Calibrator 3	Calibrator 4	Calibrator 5
Con.	0 ng/mL	2.5 ng/mL	5 ng/mL	10 ng/mL	20 ng/mL	40 ng/mL
Vol.	1000 µL	1000 µL	1000 µL	1000 µL	1000 µL	1000 µL

Reagents are ready to use. The frequency in the realization of the calibration curve must be established by the end user in the function of the criteria established for the clinical laboratory.

Note: See section quality control.

Analytical procedure

Measure range: 1 – 40 ng/mL.

Procedure	Steps	
R1 addition	305 µL	0 s
Sample addition	15 µL	10 s
R2 addition	25 µL	300 s
Blank measure	450 nm – 800 nm	310 s
Mainly measure	450 nm – 800 nm	610 s

*Data obtained by the analyzer Biolis i24 (Tokio Boeki)

INTERPRETATION OF RESULTS

Negative results values: Lower to the limit of detection of 0.8 ng of H. pylori antigen/mL (*).

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Negative results determine the absence of H. pylori antigen in higher concentration than the detection limit.

Grey area results: Between 0.8 and 1 ng H. pylori antigen/mL. For these values near to the detection limit, a second sampling is recommended to clarify the diagnosis, in case, the second result also shows a value in this range, the recommendation is to follow up the patient some time later.

Positive results values: Higher concentrations of 1 ng H. pylori antigen/mL.

Positive results determine the presence of H. pylori antigen in human stool samples.

(*) All the previous data have been obtained by the analyser Biolis 24i (Tokyo Boeki). Detection limit value depends on the analyzer used so the value may vary from 0.5 to 1 ng H. pylori/mL depending on the equipment used.

QUALITY CONTROL

H. pylori C1 & C2 Controls are ready to use.

H. pylori Control 1: is liquid control at a certain concentration of Helicobacter pylori (lower than Control 2). Concentration is indicated on the label of the vial.

H. pylori Control 2: is liquid control at a certain concentration of Helicobacter pylori (higher than Control 1). Concentration is indicated on the label of the vial.

The use of controls at two different concentrations is recommended to verify test precision.

If the obtained results are out of the tolerance range, the equipment, the reagents or the technique must be reviewed. If the problems persists, stop using the reagents and contact your distribuitor.

LIMITATIONS

- **Vitassay H. pylori -Turbidimetric Assay** should be only used for the detection of Helicobacter pylori in human stool samples.
- The quality of **Vitassay H. pylori -Turbidimetric Assay-** depends on the quality of the sample; Proper fecal specimens must be obtained.
- Positive results determine the presence of Helicobacter pylori in fecal samples; a positive result should be followed up with additional invasive techniques (endoscopy) to confirm the results.
- Positive results can be obtained even when the symptoms had disappeared.
- 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 still persist, a Helicobacter pylori determination should be carried out, on a sample from an enrichment culture or using an invasive technique.

EXPECTED VALUES

H. pylori infection affects more than half of the adult population worldwide and is strongly related with many peptic ulcer diseases and gastric cancer. The prevalence of H. pylori infection varies widely by geographic area, age, race and socio-economics status. Usually, the prevalence of H. pylori increases with age in most countries, being able to cause more than 80% of peptic ulcer diseases and approximately 75% of gastric cancer. Peptic ulcer and gastric cancer together cause more than a million deaths per year in the world.

PERFORMANCE CHARACTERISTICS

Analytical sensitivity:

Detection limit: 0.8 ng/mL.

Prozone:

Lower concentrations of 0.2 mg/mL of stool do not show prozone effect and no false negative results have been observed.

Within-Run Precision:

	Low (1 ng/mL)	Media (10 ng/mL)	High (40 ng/mL)
N	20	20	20
Media (µg/g)	1.08	10.23	39.76
DS (µg/g)	0.12	0.79	2.01
CV (%)	11	8	5

*Data obtained by the analyzer Biolis i24 (Tokio Boeki)

Cross reactivity:

No cross reactivity was detected against other fecal markers that are occasionally present in feces:

Campylobacter Coli	*Salmonella enteritidis*	*Shigella boydii*
Campylobacter jejuni	*Salmonella paratyphi*	*Shigella dysenteriae*
C. difficile Toxin B	*Salmonella Typhi*	*Shigella flexneri*
Escherichia coli O157	*Salmonella typhimurium*	*Yersinia enterocolitica O:3*
Listeria monocytogenes	*Shigella sonnei*	*Yersinia enterocolitica O:9*

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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
	Keep out of the sunlight		

ADAPTED EQUIPMENT

- Biolis 24i/Biolis 50i
- BS200E (Mindray)
- ChemwellT (Awareness)
- TC220 (Tecom)

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