VITASSAY

H. pylori + Transferrin

Rapid test for the qualitative detection of H. pylori and human transferrin in human stool samples.

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CE IVD EN

Vitassay

For professional *in vitro* diagnostic use only.

INTENDED USE

Vitassay H. pylori + Transferrin is a rapid immunochromatographic, one step assay for the simultaneous qualitative detection of Helicobacter pylori (H. pylori) and human transferrin in human stool samples.

Simple, non-invasive and highly sensitivity immunoassay for the detection of H. pylori that causes gastric and duodenal ulcers and the presence of gastrointestinal bleeding and moreover, to monitor patients treatment (H. pylori eradication).

INTRODUCTION

Most cases of colorectal cancer arise from premalignant adenomatous polyps which release small amounts of blood into the stool.

H pylori infection is a common chronic infection that is associated with upper gastrointestinal diseases, including chronic gastritis, peptic ulcers, and gastric cancer.

Transferrin, which is mainly responsible for the transport of iron in the blood, has also been identified as a potential biomarker for colorectal cancer. Bleeding into the gastrointestinal tract releases both transferrin and hemoglobin into feces where it can be measured by immunoassay-based tests. Furthermore, it has been reported from in vitro studies, and implied from studies done on colorectal cancer screening populations, that hemoglobin is more sensitive to degradation by enterobacteria and digestive enzymes than transferrin.

When gastrointestinal bleeding occurs, serum transferrin enters the gastrointestinal tract and is excreted in feces. Previous proteomic studies identified transferrin as a potential protein marker for a number of epithelial cancers, including colorectal cancer.

PRINCIPLE

Vitassay H. pylori + Transferrin is a qualitative immunochromatographic assay for the detection of H. pylori and human transferrin in human stool samples.

Strip A: The test line zone of the nitrocellulose membrane is pre-coated with monoclonal antibodies against H. pylori.

Strip B: The test line zone of the nitrocellulose membrane is pre-coated with monoclonal antibodies against human transferrin.

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

The presence of these green lines (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.
- Do not use the test if its pouch is damaged.
- Specimens should be considered as potentially hazardous and handle in the same manner as an infectious agent. A new test must be used for each sample to avoid contaminations errors. Single use device.
- Tests 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 + Transferrin**. Do not use any other commercial kit component.
- Follow Good Laboratory Practices, wear protective clothing, use disposal gloves, goggles and mask. Do not eat, drink or smoke in the working area.
- The presence of yellow lines in the result window (control line zone and test line zone), before using the test, is completely normal and does not imply failure of the test functionality.

STORAGE AND STABILITY

Store as packaged in the sealed pouch either at refrigerated or room temperature (2-30ºC/35.6-86ºF).

The test is stable until the expiration date printed on the sealed pouch.

The test must remain in the sealed pouch until use.

Do not freeze.

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MATERIALS

MATERIAL PROVIDED	MATERIAL REQUIRED BUT NOT PROVIDED
▪ **25** tests/kit **Vitassay H. pylori + Transferrin** ▪ Instructions for use. ▪ **25** H. pylori vials with diluent for the sample dilution. ▪ **25** Transferrin vials with diluent for the sample dilution.	▪ Specimen collection container. ▪ Disposable gloves. ▪ Timer.

SPECIMEN COLLECTION

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

Samples (for H pylori detection) can be stored in the refrigerator (2-8ºC/36-46.4ºF) for 1-2 days prior to testing. For longer storage, maximum 1 year, the specimen must be kept frozen at -20ºC (-4ºF).

Samples (for Transferrin detection) can be stored in the refrigerator (2-8ºC/36-46.4ºF) for 7 days prior to testing. For longer storage, maximum 6 months, the specimen must be kept frozen at -20ºC (-4ºF).

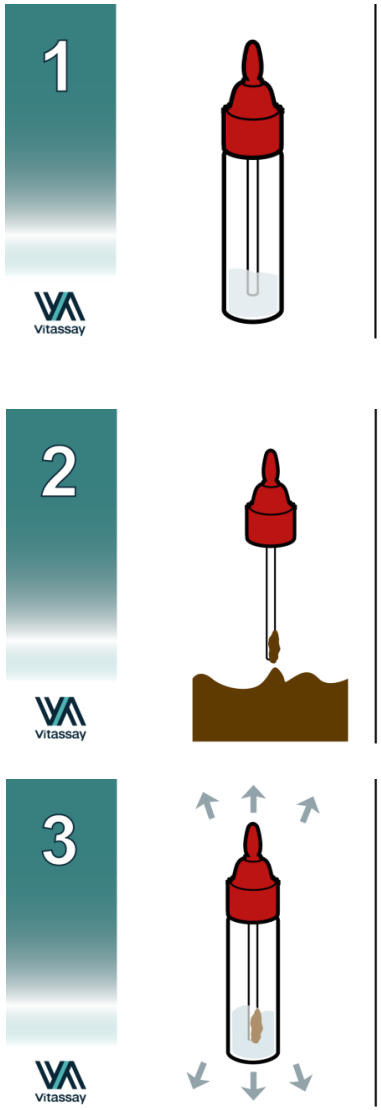
Samples must be brought to room temperature before testing.

SPECIMEN PREPARATION

Please take note that there are two different vials with diluent for sample dilution. One for H pylori strip and the other one for Transferrin strip.

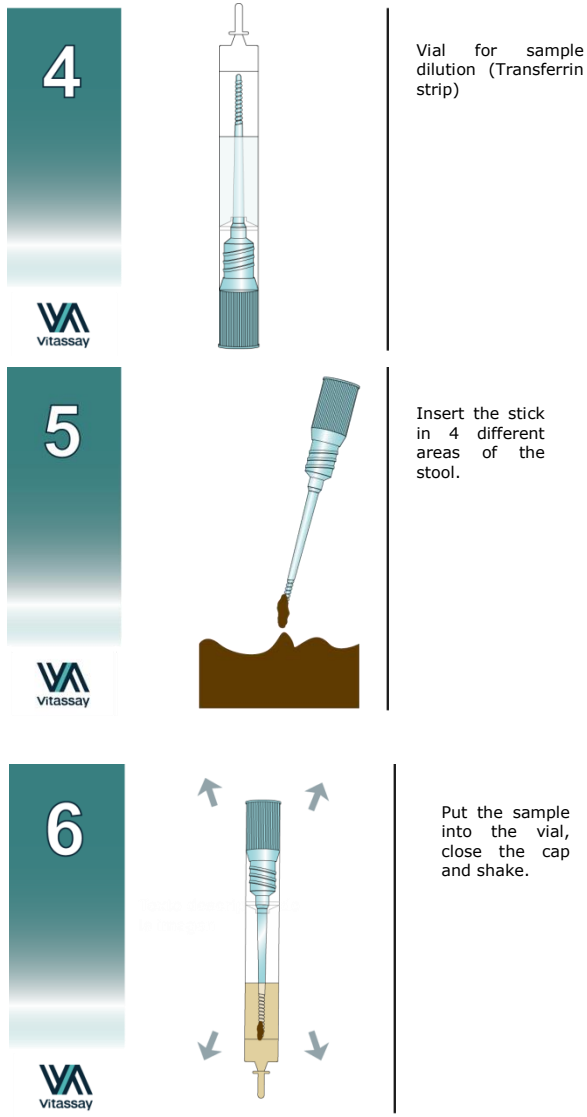
H. pylori procedure (specimen preparation)

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. 50mg, (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 assure good sample dispersion (figure 3).



Transferrin procedure (specimen preparation)

1. Take out the cap of the vial with diluent for the sample dilution (figure 4).
2. Use the stick to collect sufficient sample quantity. For solid stool, insert the stick in 4 different areas of the stool sample (figure 5), and add it into the vial with diluent for the sample dilution. For liquid stool, take 15 µL of the sample using a micropipette and transfer it into the vial with diluent for the sample dilution.
3. Close the tube with the diluent and stool sample. Shake vigorously the vial in order to assure good sample dilution (figure 6). The stool collection vial with diluted sample can be stored at room temperature until 2 days or until 5 days in the refrigerator (2-8ºC /35.6-46.4ºF) prior to testing.



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Precautions: Patients should not collect samples during their menstrual period, if they have bleeding hemorrhoids, blood in urine or if they have strained during bowel movement.

PROCEDURE

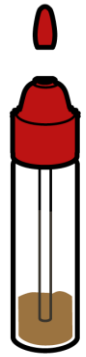
Allow the test, stool sample and controls to reach room temperature (15-30ºC / 59-86ºF) prior to testing.

Do not open pouches until the performance of the assay.

1. Shake the stool collection tube vigorously to obtain a good sample dilution.
2. Remove the **Vitassay H. pylori + Transferrin** from its sealed bag just before using it.
3. Take the H. pylori stool collection tube containing the diluted sample, cut the end of the cap (figure 7) and dispense 3 drops in the circular window marked with the letter S for the strip A–H. pylori (figure 8).
4. Take the Transferrin stool collection tube containing the diluted sample, cut the end of the cap (figure 9) and dispense 3 drops in the circular window marked with the letter S for the strip B–Transferrin (figure 10).
5. Read the results at 10 minutes. Do not read the results later than 10 minutes.

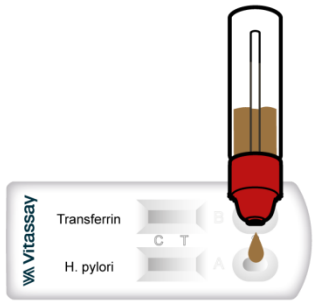
If the test does not run due to solid particles, stir the sample added in the sample window with the stick. If it does not work, dispense a drop of diluent until seeing the liquid running through the reaction zone.

7



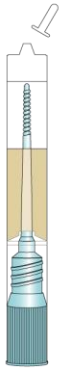
Cut the end of the cap.

8



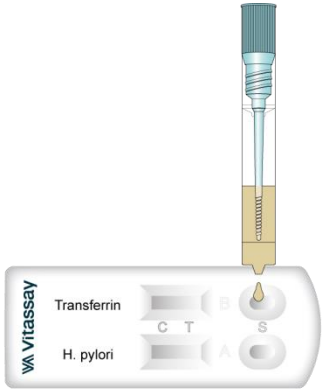
Dispense 3 drops in the circular window marked with the letter S for the strip A – H. pylori.

9



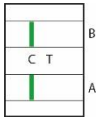
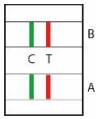
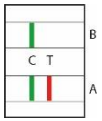
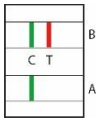
Cut the end of the cap.

10



Dispense 3 drops in the circular window marked with the letter S for the strip B – Transferrin.

INTERPRETATION OF THE RESULTS

RESULTS	Strip A H. pylori	Strip B Transferrin	INTERPRETATION
	**Negative**	**Negative**	No infection caused by H. pylori and no gastrointestinal bleeding problem.
	GREEN	GREEN	
	**Positive**	**Positive**	Infection caused by H. pylori, with gastrointestinal bleeding, which might mean gastrointestinal diseases (stomach and duodenum) like gastrtitis, peptic ulcer disease and gastric carcinona.
	GREEN-RED	GREEN-RED	
	**Positive**	**Negative**	Infection caused by H. pylori, without gastrointestinal bleeding.
	GREEN-RED	GREEN	
	**Negative**	**Positive**	No infection caused by H. pylori but gastrointestinal bleeding problem.
	GREEN	GREEN-RED	
Any other results			Invalid result, either A or B, it is recommended to repeat the assay using the sample with another test. **Note:** Wrong procedural techniques or deterioration of the reagents are mostly the main reasons for control line failure. If the symptoms or situation still persist, discontinue using the test kit and contact your local distributor.

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.

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QUALITY CONTROL

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

LIMITATIONS

- **Vitassay H. pylori + Transferrin** must be carried out within 2 hours of opening the sealed bag.
- 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 H. pylori and/or human transferrin.
- The use of other samples different from human samples has not been established.
- The quality of **Vitassay H. pylori+Transferrin** depends on the quality of the sample; Proper fecal specimens must be obtained.
- Positive results determine the presence of H. pylori and/or human transferrin in fecal samples; 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. Transferrin positive result can be also due to several causes besides colorectal bleeding, such as hemorrhoids, blood in urine or stomach irritations.
- A negative result is not meaningful because of it is possible the H. pylori and/or human transferrin concentration in the stool sample is lower than the detection limit or cut-off value. If the symptoms or situation still persist, H. pylori determination should be carried out on a sample from an enrichment culture or other any technique. Transferrin negative results do not exclude bleeding, as some polyps and colorectal cancers may bleed intermittently or not during certain stages of the disease. Moreover, blood may not be uniformly distributed in stool samples.
- To detect transferrin in strip B (to avoid false positive results) patients should not collect samples during their menstrual period, if they have bleeding hemorrhoids, blood in urine or if they have strained during bowel movement.
- Mucous stool samples can be 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.
- Faecal samples whose antigen concentration is near to the cut-off value of the Vitassay **H. pylori+Transferrin** (only for strip B: Transferrin) could be lost if the sample has been diluted and and preserved ultil 2 days at room temperature or until 5 days at 2-8ºC until to be tested. For faecal samples near to the cut-off value is better to dilute the sample and make immediately the test.

EXPECTED VALUES

Colorectal cancer (CRC) is the second leading cause of cancer in Canadian men, and the fourth leading cause of cancer death in the world.

The prevalence of H. pylori is estimated at ≈30% in the adult US population, based on data from the National Health and Nutrition Examination Survey, with higher rates with increasing age. Indviduals infected with H. pylori have a 15% (vs 4% among noninfected individuals) risk of developing peptic ulcer disease, and are at tenfold-higher risk of gastric adenocarcinoma. A large majority (70%-90%) of patients with peptic ulcer disease are infected with H. pylori, and its eradication is an important component of treatment for preventing ulcer recurrence.

PERFORMANCE CHARACTERISTICS

Analytical sensitivity (detection limit)

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

Cut-off value of Vitassay H. pylori+Transferrin (Transferrin strip) is 4 ng/mL (0.4µg hTf/g of faeces) for human transferrin.

Clinical sensitivity and specificity

An evaluation, with faecal samples, was performed comparing **Vitassay H. pylori + Transferrin**, and qPCR technique VIASURE Helicobacter pylori Real Time PCR Detection kit, CerTest), and another commercial immunochromatographic assay (Human Hexagon, OBTI). The samples were taken from patients with the same as *Helicobacter pylori* infection symptoms arrived in Gastroenterology area in a Spanish hospital.

Results were as follows:

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

Vitassay H. pylori+Transferrin vs VIASURE Helicobacter pylori Real Time PCR Detection Kit		
	Mean Value	95% 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%

		Human Hexagon		
		Positive	Negative	Total
Vitassay H. pylori + Transferrin Transferrin	Positive	10	0	10
	Negative	0	14	14
	Total	10	14	24

Vitassay H. pylori+Transferrin vs Human Hexagon		
	Mean Value	95% confidence interval
Sensitivity	100.0%	69.2-100.0%
Specificity	100.0%	76.8-100.0%
PPV	100.0%	69.2-100.0%
NPV	100.0%	76.8-100.0%

The results showed that **Vitassay H. pylori + Transferrin** has a high sensitivity and specificity to detect *H. pylori* and human transferrin.

Cross reactivity

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

Calprotectin	*Pig Haempglobin*	*Shigella boydii*
Campylobacter coli	*Lactoferrin*	*Shigella dysenteriae*
Campylobacter jejuni	*Listeria monocytognes*	*Shigella flexneri*
Clostridium difficile	*Salmonella enteritidis*	*Shigella sonnei*
Escherichia coli	*Salmonella paratyphi*	*Staphylococcus aureus*
Human hemoglobin	*Salmonella typhi*	*Yersinia enterocolitica*
Bovine hemoglobin	*Salmonella typhimurium*	

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3. PENG JIN; ZI-TAO WU; MING-MING MENG; XIN WANG; XIAO-WEI WANG; LI-JUAN GONG; DONG-LIANG YU; HUI XIE, AI-QIN LI; SHI-RONG LI; LAWRENCE YEN; JIANYU RAO; JIAN-QIU SHENG. "Combined Fecal Transferrin Test and Immuno Fecal Occult Blood Test for Detetcting Colorectal Cancer and Advanced Adenoma in Asymptomatic and Symptomatic Populations". Cancer Science and Therapy, 2012, volume 4(8) 243-248.

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

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