

VITASSAY

FOB + Transferrin

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

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For professional *in vitro* diagnostic use only.

INTENDED USE

Vitassay FOB + Transferrin is a rapid immunochromatographic, one step assay for the simultaneous qualitative detection of human hemoglobin and human transferrin in human stool samples.

Simple, non-invasive and highly sensitivity immunoassay for the detection of human hemoglobin and human transferrin to make a presumptive diagnosis of gastrointestinal bleeding.

INTRODUCTION

Colorectal cancer screening is usually undertaken as a one-step or two-step process depending on whether colonoscopy is used as the only test or its use is preceded by a simpler test to determine who undergoes colonoscopy. Fecal occult blood test has been the traditional first-step test in the two-step process. Their value is proven in randomized controlled trials at the population level. Fecal occult bloods test meet World Health Organization requirements in that they are simple screening tests that serve to select those with a higher probability of having colorectal cancer.

Transferrin, which is present in plasma by the release of neutrophil-specific granules, is undetectable in normal human gastrointestinal tract. Detection of transferrin in feces or contents in the stomach indicates bleeding in gastrointestinal tract. Unlike hemoglobin, transferrin is resistant to degradation by digestive enzymes and bacteria. Thus, compared to hemoglobin, transferrin is more stable in feces. It has been reported that fecal transferrin is elevated in patients with colorectal tumor, compared to healthy individuals. Recently, a number of proteomic studies showed that transferrin could be used as a marker expressing in a number of cancers.

PRINCIPLE

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

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

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 hemoglobin (strip A) and/or transferrin (strip B), forming conjugates. The mixture moves upward on the membrane by capillary action. If the sample is human hemoglobin 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 human 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.
- 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 FOB + 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.

STORAGE AND STABILITY

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

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.

MATERIALS

MATERIAL PROVIDED	MATERIAL REQUIRED BUT NOT PROVIDED
<ul style="list-style-type: none">• 25 tests/kit Vitassay FOB + Transferrin• Instructions for use.• 25 vials with diluent for the sample dilution.	<ul style="list-style-type: none">• Specimen collection container.• Disposable gloves.• Timer.

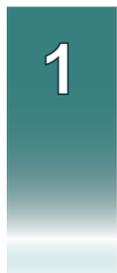
SPECIMEN COLLECTION

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 can be stored in the refrigerator (2-8°C/36-46.4°F) for 7 days prior to testing. For longer storage, maximum 6 months, the specimen must be kept frozen at -20°C (-4°F). Samples must be brought to room temperature before testing.

SPECIMEN PREPARATION

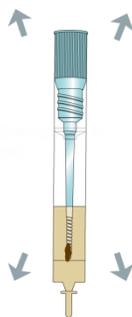
1. Take out the cap of the vial with diluent for the sample dilution (figure 1).
2. Use the stick to collect sufficient sample quantity. For solid stool, insert the stick in 4 different areas of the stool sample (figure 2), and add it into the vial with diluent for the sample dilution. For liquid stool, 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 3). The stool collection vial with diluted sample can be stored for 7 days in the refrigerator (2-8°C /35.6-46.4°F) prior to testing.



Vial for sample dilution.



Insert the stick in 4 different areas of the stool.



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

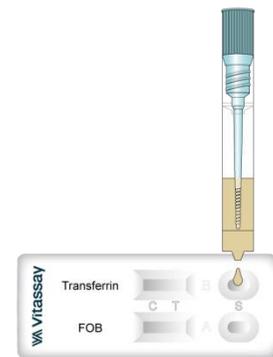
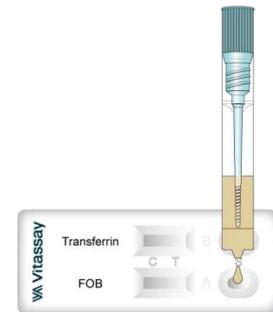
Allow the test, stool sample, controls and diluent to reach room temperature (15-30°C / 59-86°F) prior to testing. Do not open pouches until the performance of the assay.

1. Shake the vial with the sample to obtain a good sample dilution.
2. Remove the **Vitassay FOB+Transferrin** 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 4 drops in the circular window marked with the letter A-FOB (figure 5), and 4 drops, using the same vial in the circular window marked with the letter B-Transferrin (figure 6).
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 4 drops in the circular window marked with the letter S for the strip A - FOB.

Dispense 4 drops in the circular window marked with the letter S for the strip B - Transferrin.

INTERPRETATION OF THE RESULTS

RESULTS	Strip A FOB	Strip B Transferrin	INTERPRETACIÓN
	Negative	Negative	There is no human hemoglobin and/or human transferrin presence in the sample. Hemoglobin and transferrin markers are not present (<50ng/mL for hemoglobin and <4ng/mL for transferrin), which might mean no fecal occult blood and no gastrointestinal bleeding.
	GREEN	GREEN	



	Positive	Positive	<p>There is human hemoglobin and human transferrin presence. It might mean a lower gastrointestinal bleeding disease (colorectal cancer). However, an upper gastrointestinal disease should not be discarded.</p>
	GREEN-RED	GREEN-RED	
	Positive	Negative	<p>There is human hemoglobin presence. It might mean a lower gastrointestinal bleeding disease (human transferrin concentration in blood is 100 times less than human hemoglobin, therefore, a FOB positive result means not much blood present in feces).</p>
	GREEN-RED	GREEN	
	Negative	Positive	<p>There is human transferrin presence. It might mean an upper gastrointestinal bleeding disease (human hemoglobin was probably degraded in the gastrointestinal tract).</p>
	GREEN	GREEN-RED	
Any other results			<p>Invalid result, either A or B, it is recommended to repeat the assay using the sample with another test.</p> <p>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.</p>

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

LIMITATIONS

- **Vitassay FOB + 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 human hemoglobin and/or human transferrin.
- The use of other samples different from human samples has not been established.
- The quality of **Vitassay FOB+Transferrin** depends on the quality of the sample; proper fecal specimens must be obtained.
- Positive results determine the presence of human hemoglobin and/or transferrin in fecal samples; nevertheless, it can be due to several causes, besides colorectal bleeding, such as hemorrhoids, blood in urine or stomach irritations. A positive result should be followed up with additional diagnostic procedures to determine the exact cause and source of the blood in the stool.
- Negative results should not be considered as conclusive; it is possible that the concentration of human hemoglobin and/or human transferrin is lower than the cut-off value. 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.
- 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.

EXPECTED VALUES

Colorectal cancer is one of the most prevalent cancers worldwide and the lifetime risk is almost 6%.

The annual incidence rate is approximately one million of patients and 500 thousand of death. It is estimated that the absolute number of cases will improve in the next two decades because of the aging and the expansion of the population.

PERFORMANCE CHARACTERISTICS

Cut-off value

Cut-off value **Vitassay FOB+Transferrin**:

- Strip A: 50ng/mL (5.1µg hHb/g feces)
- Strip B: 4ng/mL (0.4µg hTf/g feces)

Clinical sensitivity and specificity

An evaluation was performed comparing **Vitassay FOB + Transferrin** and another commercial test (ImmunoTech OccultTech, YD Diagnostics, and Human Hexagon, OBTI).

The Results were as follows:

		IC Test: ImmunoTech OccultTech		
		Positive	Negative	Total
IC test: Vitassay FOB + Transferrin (hemoglobin)	Positive	10	0	10
	Negative	0	10	10
	Total	10	10	20

Vitassay FOB + Transferrin (hemoglobin) vs ImmunoTech OccultTech			
Sensitivity	Specificity	PPV	NPV
>99%	>99%	>99%	>99%

		IC Test: Human Hexagon		
		Positive	Negative	Total
IC test: Vitassay FOB + Transferrin (transferrin)	Positive	10	0	10
	Negative	0	14	14
	Total	10	14	24

Vitassay FOB + Transferrin (transferrin) vs Human Hexagon			
Sensitivity	Specificity	PPV	NPV
>99%	>99%	>99%	>99%

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

Cross reactivity

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

Bovine and pig hemoglobin	Human hemoglobin (Strip B)
Bovine and pig transferrin	Human lactoferrin
Human calprotectin	Human transferrin (Strip A)

REFERENCES

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2. JI-GUI CHEN; JUAN CAI; HUAN-LEI WU; HUA XU; YU-XING ZHANG, CHAO CHEN; QIAN WANG; JUN XU; XIANG-LIN YUAN. "Colorectal cancer screening: Comparison of transferrin and immune fecal occult blood test". World J Gastroenterology 2012 June 7; 18(21): 2682-2688.
3. JOHANN KARL; NORBERT WILD; MICHAEL TACKE; HERBERT ANDRES; URSULA GARCZAREK; WOLFGANG ROLLINGER; WERNER ZOLG. "Improved Diagnosis of Colorectal Cancer Using a Combination of Fecal Occult Blood and Novel Fecal Protein Markers". Clinical gastroenterology and hepatology, Vol. 6, 2008, pp. 1122-1128.
4. JUAN ALBERTO PEREZ CARRASCO; MARIO ÁLVAREZ MARCER; ENRIQUE ABRAHAM MARCEL; ISABEL GIRALDINO FALERO. "Detección de hemoglobina humana en heces". Rev Mex Patol Clin, Vol. 58, No. 3, 2011, pp. 144-150.

SYMBOLS FOR IVD COMPONENTS AND REAGENTS

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

