VITASSAY

FOB + Transferrin

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

IUE-7455003 Ed01 September 2023

CE IVD EN

Vitassay

EN

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 haemoglobin and human transferrin in human stool samples.

Simple, non-invasive and highly sensitivity immunoassay for the detection of human haemoglobin 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. Faeca 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. Faeca 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 faeces or contents in the stomach indicates bleeding in gastrointestinal tract. Unlike haemoglobin, transferrin is resistant to degradation by digestive enzymes and bacteria. Thus, compared to haemoglobin, transferrin is more stable in faeces. It has been reported that faecal 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 haemoglobin 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 haemoglobin.

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 haemoglobin (strip A) and/or transferrin (strip B), forming conjugates. The mixture moves upward on the membrane by capillary action. If the sample is human haemoglobin 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.
- 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.
- Components provided in the kit are approved for use with the **Vitassay FOB+Transferrin.** Do not use any other commercial kit component or components from other batches.
- 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

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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.

MATERIALS

MATERIAL PROVIDED	MATERIAL REQUIRED BUT NOT PROVIDED
▪ **25** tests/kit **Vitassay FOB + Transferrin** ▪ 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)

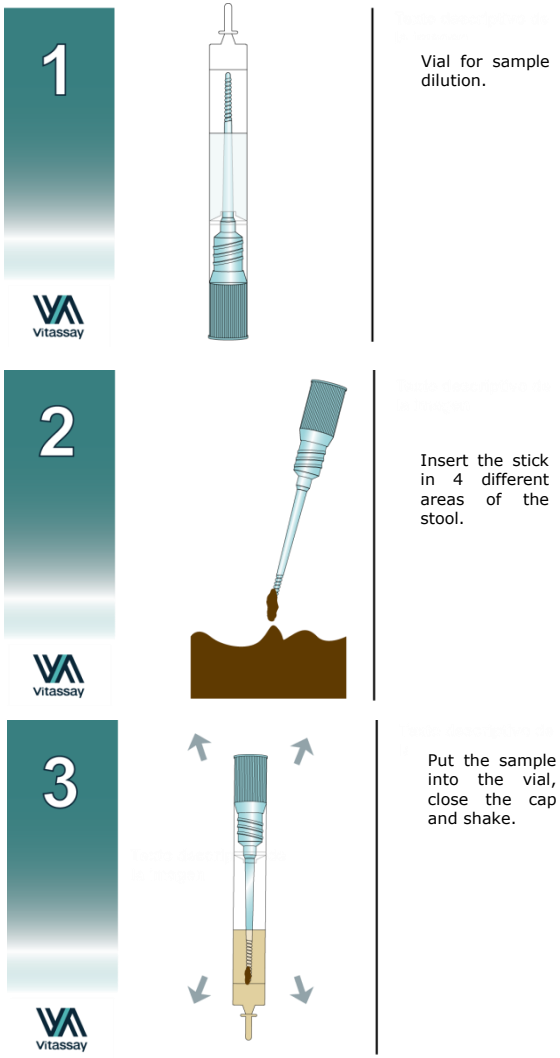
SPECIMEN COLLECTION

Collect sufficient quantity of faeces: 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 (2-8ºC) for 7 days prior to testing. For longer storage, maximum 6 months, the specimen must be kept frozen at -20ºC. 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 (2-8ºC) prior to testing.



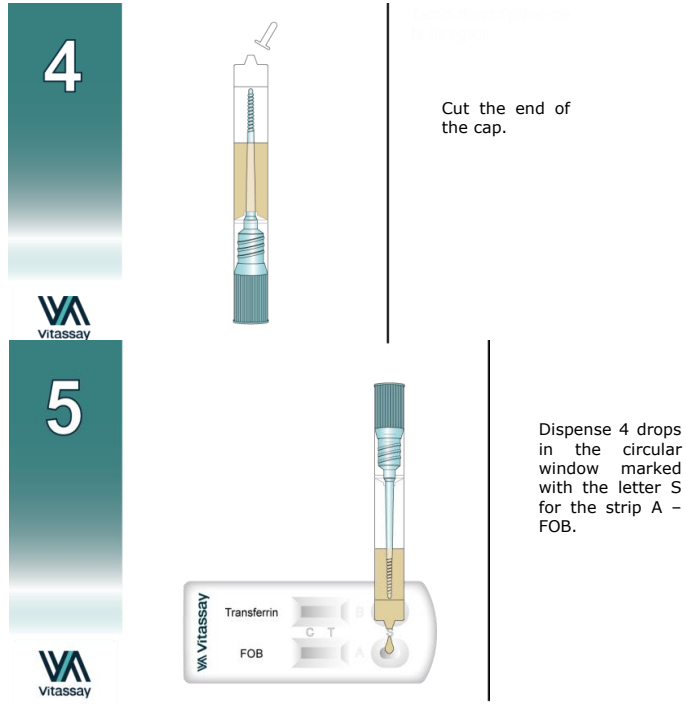
Precautions: Patients should not collect samples during their menstrual period, if they have bleeding hemorrhoids, blood in urine or if they have strained during bowel movement.

PROCEDURE

Allow the test, stool sample, controls and diluent to reach room temperature (15-30ºC) 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 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.



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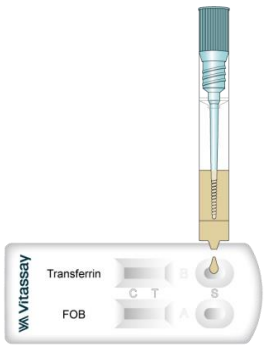
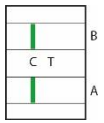
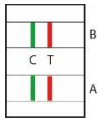
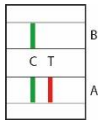
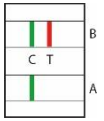


Dispense 4 drops in the circular window marked with the letter S for the strip B – Transferrin.

INTERPRETATION OF THE RESULTS

RESULTS	Strip A FOB	Strip B Transferrin	INTERPRETACIÓN
	**Negative**	**Negative**	There is no human haemoglobin and/or human transferrin presence in the sample. Haemoglobin and transferrin markers are not present (<50ng/mL for haemoglobin and <4ng/mL for transferrin), which mght mean no faecal occult blood and no gastrointestinal bleeding.
	GREEN	GREEN	
	**Positive**	**Positive**	There is human haemoglobin and human transferrin presence. It might mean a lower gastrointestinal bleeding disease (colorectal cancer). However, an upper gastrointestinal disease should not be discarded.
	GREEN-RED	GREEN-RED	
	**Positive**	**Negative**	There is human haemoglobin presence. It might mean a lower gastrointestinal bleeding disease (human transferrin concentration in blood is 100 times less than human haemoglobin, therefore, a FOB positive result means not much blood present in faeces).
	GREEN-RED	GREEN	
	**Negative**	**Positive**	There is human transferrin presence. It might mean an upper gastrointestinal bleeding disease (human haemoglobin was probably degraded in the gastrointestinal tract).
	GREEN	GREEN-RED	
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.

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

- 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 haemoglobin 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 faecal specimens must be obtained.
- Fecal samples whose antigen concentration is close to the cut-off of the Vitassay FOB +Transferrin test may be lost if the sample has been diluted and stored for up to 2 days at room temperature or up to 5 days at 2-8°C until tested. For faecal samples close to the detection limit value it is best to dilute the sample and test immediately.
- Positive results determine the presence of human haemoglobin and/or transferrin in faecal 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 haemoglobin 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.
- **Mucous stool** specimens may cause nonspecific reactions in the test. Any mucous stool specimen with a positive result should be followed up with other diagnostic techniques to confirm the result.

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.

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PERFORMANCE CHARACTERISTICS

Cut-off value

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

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

Clinical sensitivity and specificity

Vitassay FOB + Transferrin was evaluated in two studies, comparing the results obtained with the Vitassay method and other commercial reference kits.

- Study 1. Strip A, FOB and Strip B, Transferrin

The results of FOB and Tramsferrin detection were compared with a commercial immunochromatographic test. The results are shown below.

		IC Test: ImmunTech OcculTech		
		Positive	**Negative**	**Total**
IC test: Vitassay FOB + Transferrin (FOB)	**Positive**	10	0	10
	Negative	0	10	10
	Total	10	10	20

Table 1: Results obtained with **Vitassay FOB + Transferrin** (FOB strip) when compared to the reference method

Vitassay FOB + Transferrin (haemoglobin) **vs ImmunTech OccultTech**			
Sensitivity	**Specificity**	**PPV**	**NPV**
>99%	>99%	>99%	>99%

Table 2: Sensitivity, Specificity, Positive Predictive Values and Negative Predicted values for **Vitassay FOB + Transferrin** (FOB strip) when compared to the reference method.

- Study 2. Strip B, Transferrin

Vitassay FOB + Transferrin was compared with a commercial ELISA kit for the detection of Human Transferrin, taking into account a cut-off of 0.4 µg hTf/g:

		Reference method		
		Positive	**Negative**	**Total**
IC test: Vitassay FOB + Transferrin (Transferrin)	**Positive**	30	1	31
	Negative	2	99	101
	Total	32	100	132

Table 3: Results obtained with **Vitassay FOB + Transferrin** (Transferrin strip) when compared to the reference method.

Vitassay FOB + Transferrin (Transferrina) **vs Reference method**			
Sensitivity	**Specificity**	**PPV**	**NPV**
93.8% (79.2-99.2)	99.0% (94.6–100)	96.8% (83.3–99.9)	98.0% (93.0–99.8)

Table 4: Sensitivity, Specificity, Positive Predictive Values and Negative Predicted values for **Vitassay FOB + Transferrin** (Transferrin strip) when compared to the reference method.

The results showed that **Vitassay FOB + Transferrin** has a high sensitivity and specificity for detecting human haemoglobin and human transferrin.

Cross-reactivity

No cross-reactions were detected with the following faecal markers and micro-organisms occasionally present in faeces.

For strip A: FOB

Adenovirus	*Clostridium perfringens*	Human lactoferrin	*Salmonella typhi*
Astrovirus	*Cryptosporidium*	Human Transferrin	*Salmonella typhimurium*
Bovine Haemoglobin	*Entamoeba dispar*	*Legionella pneumophila*	*Shigella boydii*
Bovine Transferrin	*Entamoeba histolytica*	*Listeria monocytogenes*	*Shigella dysenteriae*
Bovine Lactoferrin	*Escherichia coli O111*	Norovirus GI	*Shigella flexneri*
Campylobacter coli	*Escherichia coli O26*	Norovirus GII	*Shigella sonnei*
Campylobacter jejuni	*Escherichia coli O157*	Porcine Haemoglobin	*Streptococcus pneumococcal*
C. difficile antigen GDH	*Giardia*	Rotavirus	*Streptococcus pyogenes*
C. difficile Toxin A	*Helicobacter pylori*	*Salmonella enteritidis*	*Yersinia enterocolitica O:3*
C. difficile Toxin B	Human Calprotectin	*Salmonella paratyphi A*	*Yersinia enterocolitica O:9*

For strip B: Transferrin

Adenovirus	*Clostridium perfringens*	Human transferrin	*Salmonella typhimurium*
Astrovirus	*Cryptosporidium*	*Legionella pneumophila*	*Shigella boydii*
Bovine haemoglobin	*Entamoeba dispar*	*Listeria monocytogenes*	*Shigella dysenteriae*
Bovine transferrin	*Entamoeba histolytica*	Norovirus GI	*Shigella flexneri*
Campylobacter coli	*Escherichia coli O111*	Norovirus GII	*Shigella sonnei*
Campylobacter jejuni	*Escherichia coli O26*	Porcine Haemoglobin	*Streptococcus pneumococcal*
C. difficile antigen GDH	*Escherichia coli O157*	Rotavirus	*Streptococcus pyogenes*
C. difficile Toxin A	*Giardia*	*Salmonella enteritidis*	*Yersinia enterocolitica O:3*
C. difficile Toxin B	*Helicobacter pylori*	*Salmonella paratyphi A*	*Yersinia enterocolitica O:9*
Human calprotectin	Human lactoferrin	*Salmonella typhi*	

There is no interference with any food (vitamin C, broccoli, carrots...). No special diet is recommended before the test.

REFERENCES

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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
CE	CE Marking		

Changes control		
Nº Version	**changes**	**date**
IUE-7455003 Ed00 November 2013	Original version	11/2016
IUE-7455003 Ed01 September 2023	The format has been updated. New cross-reactions and a new clinical evaluation for transferrin have been added and an old one deleted. Grammatical and editorial changes have been made to Precautions, Limitations, Sample Collection, Storage and Stability. Material required but not included updated with minor changes.	21/09/2023

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