VITASSAY

FOB

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

IUE-7355001 Ed01 September 2023

CE IVD

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Vitassay

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For professional *in vitro* diagnostic use only.

INTENDED USE

Vitassay FOB is a rapid, immunochromatographic, one step assay for the qualitative detection of human haemoglobin in human stool samples.

Simple, non-invasive and highly sensitivity immunoassay 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. Faecal 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. Faecal 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.

PRINCIPLE

Vitassay FOB is a qualitative immunochromatographic assay to make a presumptive diagnosis of faecal occult blood in human stool samples (gastrointestinal bleeding).

The test line zone of the nitrocellulose membrane is pre-coated with monoclonal antibodies against human haemoglobin.

During the process, the sample reacts with the antibodies against haemoglobin, 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.
- 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 specimens 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 FOB** 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 FOB** ▪ 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: 1-2g or mL for liquid samples. Stool specimens should be collected in clean and dry containers.

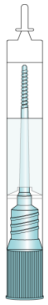
If processed the day after collection, samples can be stored at room temperature. However, we recommend testing immediately after sample collection.

Samples can be stored for up to 7 days at 2-8ºC, and frozen at -20°C for up to one year. Samples shall be brought to room temperature before testing.

Ensure that only the necessary quantity is thawed, as freezing freeze-thaw cycles are not recommended. Homogenise stool samples as thoroughly as possible prior to preparation.

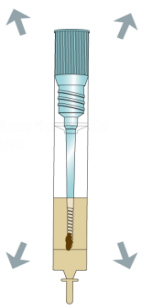
SPECIMEN PREPARATION

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 once 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 dispersion (figure 3). The vial with diluted sample can be stored for 7 days in the refrigerator (2-8ºC) prior to testing.



Vial for sample dilution.

Insert the stick in 4 different areas of the stool.



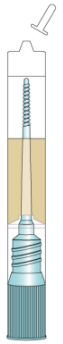
Put the sample into the vial, close the vial and shake.

PROCEDURE

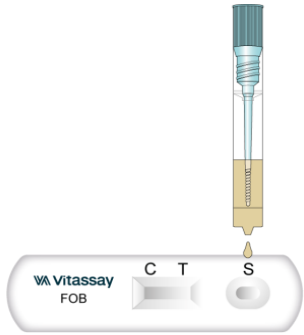
Allow the test, stool sample, controls and diluent to reach room temperature (15-30ºC) prior to testing. Do not open pouches until the performance of the assay.

1. Shake the vial with the sample to obtain a good sample dilution.
2. Remove the **Vitassay FOB** 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 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 vial.



Dispense 4 drops in 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 human haemoglobin presence which might mean no faecal occult blood and no gastrointestinal bleeding.
	**POSITIVE** In addition to the green line (**control line C**), a red line appears (**test line T**).	There is presence of human haemoglobin, which might mean gastrointestinal bleeding caused by ulcers, a colonic neoplasm, such as early-stage cancer and large adenomatous polyps.

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ANY OTHER RESULTS	Invalid result, we recommend repeating the assay using the sample with another test. **Note:** Improper procedure, deterioration of reagents, or insufficient sample volume could be the cause of the invalid result. If the symptoms or situation continues, stop using the kit and contact your distributor.

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

QUALITY CONTROL

Internal procedural control is included in **Vitassay FOB**. 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 FOB** depends on the quality of the sample; Proper faecal specimens must be obtained.
- Positive results determine the presence of human haemoglobin in faecal samples; nevertheless, it can be due to several causes, besides colorectal bleeding, such as haemorrhoids, 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 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.
- Mucuos stool samples can contain components that may cause non-specific reactions in the test. These type of specimens yielding positive results should be confirmed with other diagnostic techniques.
- Patients should not collect samples during their menstrual period, if they have bleeding haemorrhoids, 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** is 50ng/mL (5.1µg hHb/g feces) for human haemoglobin.

Clinical sensitivity and specificity

An evaluation was performed comparing **Vitassay FOB** and another commercial test (ACTIM FECAL BLOOD, Medix Biochemica).

Results were as follows:

		ACTIM FAECAL BLOOD		
		Positive	**Negative**	**Total**
Vitassay FOB	**Positive**	6	0	6
	Negative	0	121	121
	Total	6	121	127

Table 1. Results of **Vitassay FOB** compared to a commercial kit (ACTIM FACAL BLOOD).

Vitassay FOB vs ACTIM FAECAL BLOOD			
Sensitivity	**Specificity**	**PPV**	**NPV**
>99%	>99%	>99%	>99%

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

The results showed that **Vitassay FOB** has a high sensitivity and specificity to detect human haemoglobin.

Subsequently**, Vitassay FOB** was also compared to a commercial ELISA Kit in 132 remnants of frozen faecal samples from patients.

Results are shown below:

		ELISA test		
		Positive	**Negative**	**Total**
Vitassay FOB	**Positive**	38	4	42
	Negative	1	89	90
	Total	39	93	132

Table 3. Results of **Vitassay FOB** compared to a commercial kit based on ELISA technique for the detection of occult haemoglobin in faeces

Vitassay FOB vs ACTIM FAECAL BLOOD			
Sensitivity	**Specificity**	**PPV**	**NPV**
97.4% (86.5-99.9%)	95.7% (89.4–98.8%)	90.5% (77.4–97.3%)	98.9% (94.0–100.0%)

Table 4. Sensitivity, specificity, positive predictive values, and negative predictive values of the Vitassay FOB compared to a ELISA commercial kit for the occult haemoglobin in faeces (CI=95%).

In summary, **Vitassay FOB** shows high clinical **sensitivity** and **specifity** for the occult haemoglobin in faeces detection.

Cross reactivity

No cross reactivity was detected against other faecal markers and gastrointestinal pathogens that are occasionally present in faeces:

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

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

REFERENCES

1. ALICIA SMITH; GRAEME P. YOUNG, STEPHEN R. COLE, PETER BAMPTON. "Comparison of a Brush-Sampling Fecal Immunochemical Test for Hemoglobin with a Sensitive Guaiac-Based Fecal Occult Blood Test in Detection of Colorectal Neoplasia". American Cancer Society, 2006, pp. 2152-2159.

2. 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.

3. JUAN ALBERTO PEREZ CARRASCO; MARIO ÁLVAREZ MARCER; ENRIQUE ABRAHAM MARCEL; ISABEL GIRALDINO FALERO. "Detección de hemoglobin humana en heces". Rev Mex Patol Clin, Vol. 58, No. 3, 2011, pp. 144-150.

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**
IU-7355001 Ed00 January 2017	Original Version	01/2017
IU-7355001 Ed01 September 2023	Addition of a new evaluation. Cross-reactivity section has been updated with new compounds/microorganisms. 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.	21/09/2023

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