VITASSAY

FOB 50+200

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

IUE-7455002 Ed01 September 2023

CE IVD EN

Vitassay

EN

For professional *in vitro* diagnostic use only.

INTENDED USE

Vitassay FOB 50+200 is a rapid immunochromatographic, one step assay for the simultaneous qualitative detection of human hemoglobin in human stool samples.

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

INTRODUCTION

The majority of the colorectal cancers are developed through adenomatous polyps. Although the presence of polyps is frequent in the population, just a little percentage will become cancer. The average time needed to complete this progression is long, probably 10 years or more, and this fact allows early preventing or detecting the colorectal cancer and ameliorating the diagnosis.

Colorectal cancer (CRC) is the third most common cancer and the second most common cause of cancer deaths worldwide. It is one of the main cancers that is preventable with screening. Although colonoscopy is regarded as the most effective screening tool, it can be invasive and labour-intense. Faecal occult blood test (FOBT) is non-invasive and easily performed at home or in clinics; it is more suitable for population-based screening with FOBT has been demonstrated to reduce CRC incidence by 20% and mortality by 33%.

Like other cancers, the survival of patients with CRC is closely related to the stage at diagnosis. Early detection of CRC is not only associated with improvements outcomes, but also significantly reduces the cost of treatment. Current screening tests for CRC involve the detection of blood in stool samples and the visualization of gross abnormalities by colonoscopy. Although colonoscopy is still the gold standard method for CRC screening, diagnosis and treatment, it is invasive and associated with poor patient acceptability and high cost. In contrast, stool tests are non-invasive, do not require bowel preparation, may represent the entire colon, and are easy to transport.

PRINCIPLE

Vitassay FOB 50+200 is a qualitative immunochromatographic assay for the detection of human hemoglobin in human stool samples.

Strip A: The test line zone of the nitrocellulose membrane is pre-coated with monoclonal antibodies against human hemoglobin (≥50ng/mL).

Strip B: The test line zone of the nitrocellulose membrane is pre-coated with monoclonal antibodies against human hemoglobin (≥200ng/mL).

During the process, the sample reacts with the antibodies against human hemoglobin, forming conjugates. The mixture moves upward on the membrane by capillary action. If the sample is human hemoglobin positive (concentration ≥ 50ng/mL), 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 hemoglobin positive (concentration ≥ 200ng/mL), 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 50+200** Do not use any other commercial kit component or components from other batches.

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- 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.

MATERIALS

MATERIAL PROVIDED	MATERIAL REQUIRED BUT NOT PROVIDED
• **25** tests/kit **Vitassay FOB 50+200**. • 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)

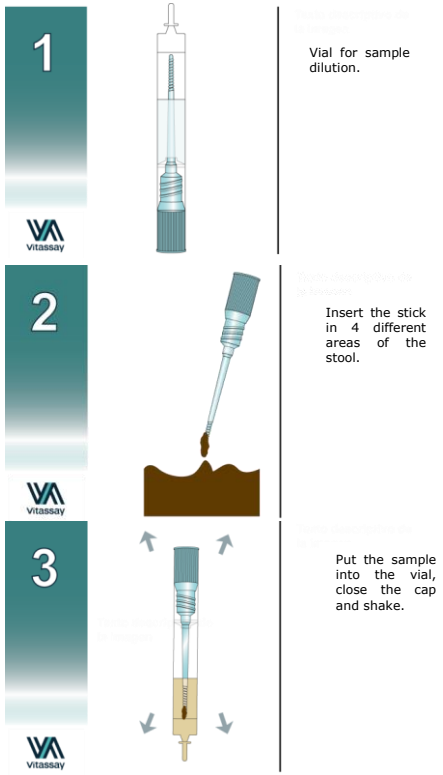
SPECIMEN COLLECTION

Collect sufficient quantity of 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 (2-8ºC) 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.

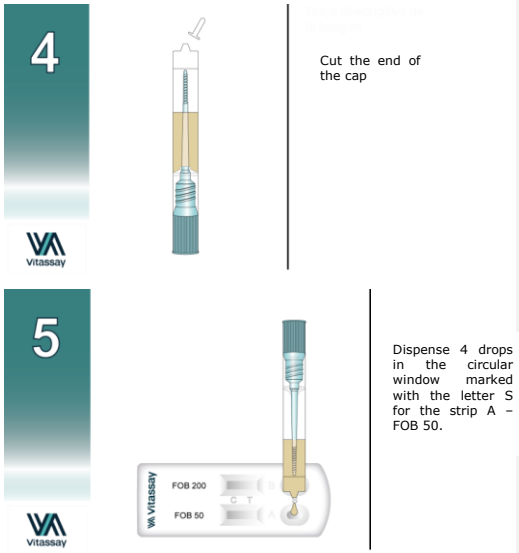


PROCEDURE

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 50+200** 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 50 (figure 5), and 4 drops, using the same vial in the circular window marked with the letter B-FOB 200 (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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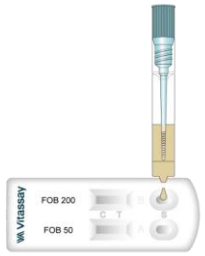

Dispense 4 drops in the circular window marked with the letter S for the strip B – FOB 200.

INTERPRETATION OF THE RESULTS

RESULTS	Strip A FOB 50 (≥50ng/mL)	Strip B FOB 200 (≥200ng/mL)	INTERPRETATION
	**Negative**	**Negative**	There is no human hemoglobin presence. Hemoglobin marker is not present in patient sample (<50ng/mL), which might mean no fecal occult blood and no gastrointestinal bleeding.
	GREEN	GREEN	
	**Positive**	**Positive**	High hemoglobin concentration (≥200ng/mL) is present in patient sample, which might mean some gastrointestinal bleeding problem (e.g. caused by colorectal cancer, bacterial infections, inflammatory bowel disease, etc.). Additional confirmatory diagnostic (endoscopy) should be followed up to determine the exact cause and source of the blood.
	GREEN-RED	GREEN-RED	
	**Positive**	**Negative**	Hemoglobin marker is present in patient sample (≥50ng/mL and <200ng/mL), which might mean small amounts of blood loss near the detection limit of the combo test. If the problem still persists, physician should evaluate all clinical and laboratory findings.
	GREEN-RED	GREEN	
	**Negative**	**Positive**	Invalid result. If B is positive, A has to be also positive.
	GREEN	GREEN-RED	
Any other result			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 50+200**. 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.
- The use of other samples different from human samples has not been established.
- The quality of **Vitassay FOB 50+200** depends on the quality of the sample; Proper fecal specimens must be obtained.
- Faecal samples whose antigen concentration is close to the cut-off of the Vitassay FOB 50+200 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 2-8ºC 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 hemoglobin in fecal 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 hemoglobin 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** samples may cause non-specific reactions in the test. Every mucous stool sample yielding a positive should be followed up with other techniques of diagnosis to confirm the result.

EXPECTED VALUES

Colorectal cancer (CRC) is the third leading cause of cancer mortality in US men and women, with nearly 50000 deaths estimated in 2011.

It is also the second leading cause of cancer death in Europe.

Colorectal cancer is the most frequent in Spain if both sexes are included. It is estimated an annual incidence of 35000 persons asociated with a mortality of 50%.

PERFORMANCE CHARACTERISTICS

Cut-off value

Cut-off value **Vitassay FOB 50+200**:

- Strip A: 50ng/mL (5.1µg hHb/g faeces)
- Strip B: 200ng/mL (20µg hHb/g faeces)

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Clinical sensitivity and specificity

An evaluation was performed comparing **Vitassay FOB 50+200** and another commercial test (ACTIM FECAL BLOOD, Medix Biochemica).

Results were as follows:

		ACTIM FECAL BLOOD		
		Positive	Negative	Total
Vitassay FOB 50+200	Positive	6	0	6
	Negative	0	121	121
	Total	6	121	127

Table 1. Results of **Vitassay FOB 50+200** compared to a commercial kit (ACTIM FECAL BLOOD).

Vitassay FOB 50+200 vs ACTIM FECAL BLOOD			
Sensitivity	Specificity	PPV	NPV
>99%	>99%	>99%	>99%

Table 2. Sensitivity, specificity, positive predictive values, and negative predictive values of the **Vitassay FOB 50+200** compared to a commercial kit.

In addition, **Vitassay FOB 50+200** (strip B) was evaluated using a commercial immunochromatographic kit. Discrepant results were analyzed by ELISA.

The results are shown below:

		Reference method		
		Positive	Negative	Total
Vitassay FOB (FOB 200)	Positive	29	0	29
	Negative	2	101	103
	Total	31	101	132

Table 3. Results of Vitassay FOB 50+200 (strip B) compared to the reference method, after assessing the discrepant results.

Vitassay FOB 50+200 vs Reference method			
Sensitivity	Specificity	PPV	NPV
93.54% (78.6-99.2)	100 (96.4–100)	100% (88.1–100)	98.1% (93.2–99.8)

Table 8. Sensitivity, specificity, positive predictive values and negative predictive values of the **Vitassay FOB 50+200 (strip B)** compared the reference method (CI=95%).

The results showed that **Vitassay FOB 50+200** has a high sensitivity and specificity for detecting human haemoglobin.

Cross reactivity

No cross reactivity was detected against other microorganisms, substances and faecal markers occasionally present in faeces:

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

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

REFERENCES

1. GRAU; A. CASTELLS. "Programas de detección precoz del cáncer colorrectal: un reto de integración y calidad asistencial": Revista de Calidad Asistencial. 2014; 29(4):185-187.

2. H. W. HIRAI; K.K. F. TSOI; J.Y.C. CHAN; S.H. WONG; J.Y.L. CHING; M.C.S. WONG; J.C.Y. WU; F.K.L. CHAN; J.J.Y. SUNG; S.C. NG. "Systematic review with meta-analysis; fecal occult blood test show lower colorectal cancer detection rates in the proximal colon in colonoscopy-verified diagnostic studies". Alimentary Pharmacology and Therapeutics, 2016; 43: 755-764.

3. BYUNG CHANG KIM; JUNGNAM JOO; HEE JIN CHANG; HYUN YANG YEO; BYONG CHUL YOO; BORAM PARK; JI WON PARK; DAE KYUNG SOHN; CHANG WON HONG; KYUNG SU HAN. "A predictive model combining fecal calgranulin B and Fecal Occult Blood tests can improve the diagnosis of Colorectal Cancer". PLoS ONE 9(9): e106182.

4. PRITI BANDI, MS; VILMA COKKINIDES; ROBERT A. SMITH; AHMEDIN JEMAL. "Trends in colorectal cancer screening with home-based fecal occult blood tests in adults ages 50 to 64 years, 2000-2008". Cancer 2012 Oct 15; 118(20):5092-9.

5. TAM CAM HA; SOOK KWIN YONG; KHENG-WEI YEOH; KAY KAMBERAKIS; RICHARD MING CHERT YEO; GERALD CHOON-HUAT KOH. "The effect of test kit provision, and individual and family education on the uptake rates of fecal occult blood test in an Asian population; a randomized controlled trial". Cancer Causes Control (2014) 25:1473-1488.

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-7455002 Ed00 January 2017	Original version.	01/2017
IUE-7455002 Ed01 September 2023	The format has been updated. New evaluation included. Limitations section has been updated and new cross-reactions have been added. Grammatical and editorial changes have been made to Precautions, Limitations, Sample Collection, Storage and Stability. Materials required, but not included updated with minor changes. A transcription error in the interpretation section has been corrected.	21/09/2023

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