

VITASSAY

FOB 50+200

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

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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-intensive. 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 ($\geq 50\text{ng/mL}$).

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

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 $\geq 50\text{ng/mL}$), 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 $\geq 200\text{ng/mL}$), 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.
- 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 50+200**. 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 50+200. Instructions for use. 25 vials with diluent for the sample dilution. 	<ul style="list-style-type: none"> Specimen collection container. Disposable gloves. Timer.

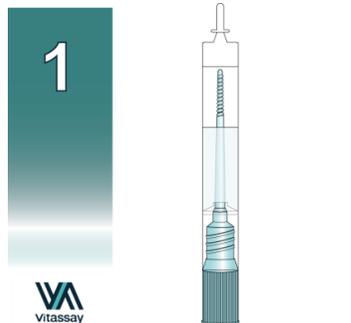
SPECIMEN COLLECTION

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

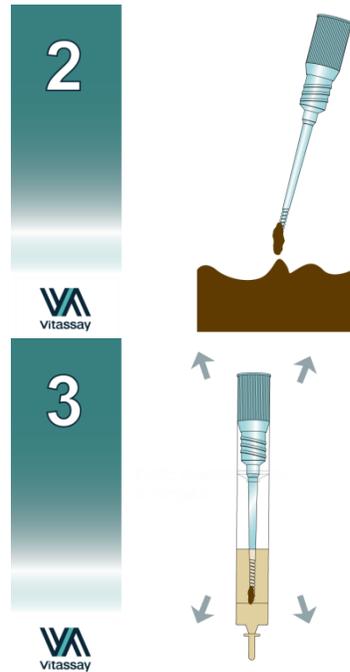
Samples can be stored in the refrigerator (2-8°C/36-46.4°F) for 7 days prior to testing. For longer storage, maximum 6 months, the specimen must be kept frozen at -20°C (-4°F). Samples must be brought to room temperature before testing.

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.

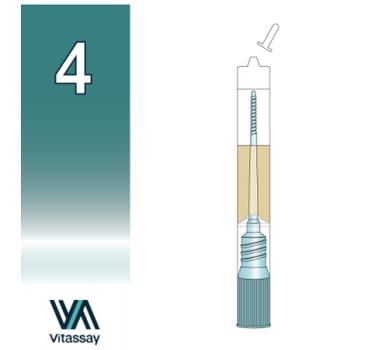
Put the sample into the vial, close the cap and shake.

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 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.



Cut the end of the cap

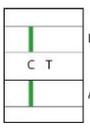
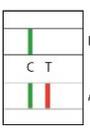
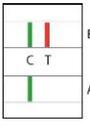


Dispense 4 drops in the circular window marked with the letter S for the strip A - FOB 50.



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 GREEN	Negative GREEN	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.
	Positive GREEN- RED	Positive GREEN- RED	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.
	Positive GREEN- RED	Negative GREEN	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.
	Negative GREEN	Positive GREEN- RED	Invalid result. If B is positive, A has to be also positive.

Any other result

Invalid result, we recommend repeating the assay using the sample with another test. **Note:** Wrong procedural techniques or deterioration of the reagents are the main reasons of 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.

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

- **Vitassay FOB 50+200** 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 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.
- 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.

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 associated 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 feces)
- Strip B: 200ng/mL (20µg hHb/g feces)

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

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

The results showed that **Vitassay FOB 50+200** has a high sensitivity and specificity to detect human hemoglobin.

Cross reactivity

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

Bovine and pig hemoglobine	Human calprotectin	Human transferrin
Bovine and pig transferrin	Human lactoferrin	

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

REFERENCES

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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.
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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.
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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

