VITASSAY

FOB

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

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CE IVD EN

Vitassay

For professional *in vitro* diagnostic use only.

INTENDED USE

Vitassay FOB is a rapid, immunochromatographic, one step assay for the qualitative detection of human hemoglobin 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. 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.

PRINCIPLE

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

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

During the process, the sample reacts with the antibodies against hemoglobin, 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.
- 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. Single use device.
- 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**. 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 (2-30ºC/35.6-86ºF).

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
▪ **25** tests/kit **Vitassay FOB** ▪ Instructions for use. ▪ **25** vials with diluent for the sample dilution.	▪ Specimen collection container. ▪ Disposable gloves. ▪ Timer.

SPECIMEN COLLECTION

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

Samples can be stored in the refrigerator (2-8ºC/35.6-46.4ºF) for 7 days prior to testing.For longer storage, maximum 6 months, the specimen must be kept frozen at -20ºC (-4ºF). 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 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.

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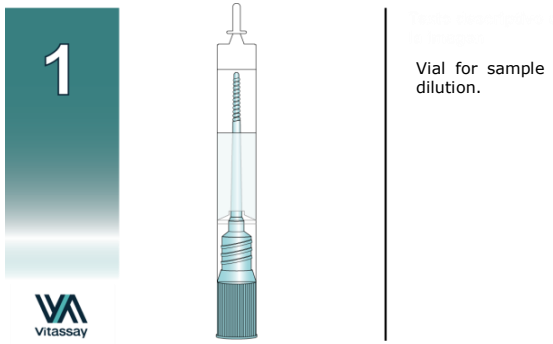
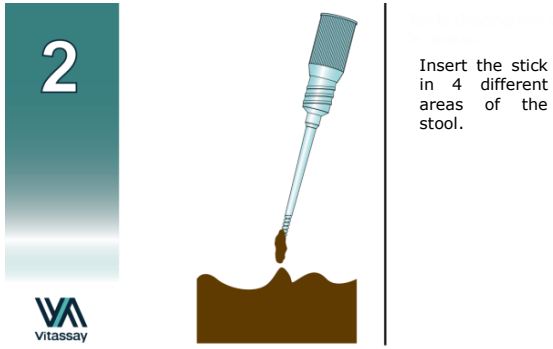
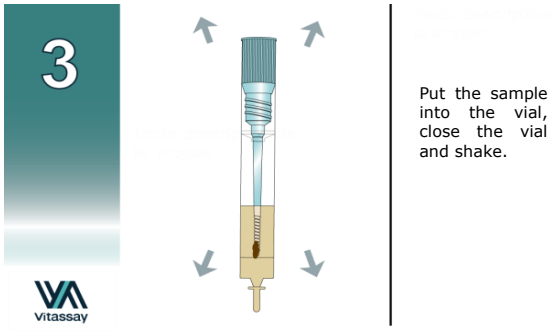
3. Close the tube with the diluent and stool sample. Shake vigorously the vial in order to assure good sample dispersion (figure 3). The vial with diluted sample can be stored for 7 days in the refrigerator (2-8ºC /36-46.4ºF) prior to testing.





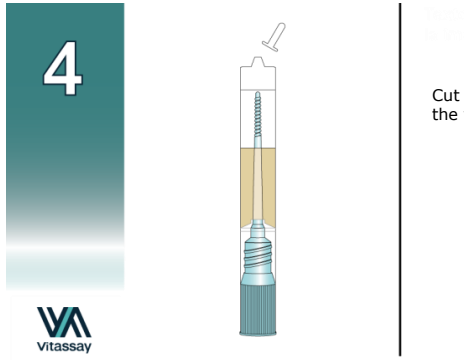


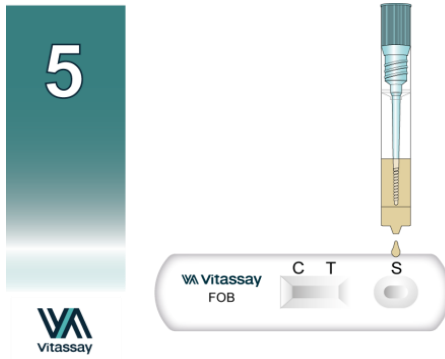
PROCEDURE

Allow the test, stool sample, controls and diluent to reach room temperature (15-30ºC / 59-86ºF) 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.





INTERPRETATION OF THE RESULTS

C T	**NEGATIVE** Only one green line in the control zone **(C)**.	There is no human hemoglobin presence which might mean no faecal occult blood and no gastrointestinal bleeding.
C T	**POSITIVE** In addition to the green line (**control line C**), a red line appears (**test line T**).	There is presence of human hemoglobin, which might mean gastrointestinal bleeding caused by ulcers, a colonic neoplasm, such as early-stage cancer and large adenomatous polyps.
ANY OTHER RESULTS		Invalid result, we recommend repeating the assay using the sample with another test. **Note:** Wrong procedural techniques 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 human hemoglobin 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

- **Vitassay FOB** 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 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 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

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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 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 hemoglobin.

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 FECAL BLOOD		
		Positive	Negative	Total
Vitassay FOB	Positive	6	0	6
	Negative	0	121	121
	Total	6	121	127

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

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

Cross reactivity

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

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

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

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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

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