

VITASSAY

FOB

-Turbidimetric Assay-

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

IUE-7115001 Ed07 June 2022



For professional *in vitro* diagnostic use only. Professional trained in Turbidimetric techniques.

INTENDED USE

Vitassay FOB –Turbidimetric Assay- is a rapid Turbidimetric assay for the quantitative detection of human hemoglobin in human stool samples.

Simple, non-invasive and highly sensitivity assay for the detection of human hemoglobin in feces that helps in the search for gastrointestinal bleeding problems. This product has been optimized for several automated analyzer.

INTRODUCTION

The increased amount of hemoglobin (hHb) in feces is the main symptoms of illnesses accompanied by hemorrhagic lesions in the digestive tract, particularly in the lower digestive tract. Therefore, measuring the amount of hemoglobin in feces is an effective method for the early detection and treatment of colon cancer and other diseases of the lower digestive tract that are accompanied by hemorrhaging.

PRINCIPLE

Vitassay FOB –Turbidimetric Assay- is a quantitative turbidimetric assay for the detection of human hemoglobin in human solid stool samples.

FOB Turbidimetric Assay is based on antigen-antibody agglutination reactions between the antigen contained in the sample and the antibodies anti-antigen coated on polystyrene latex particles.

Such agglutination is measured as an increase in absorbance proportional to the quantity of antigen contained in the sample.

The use of two external controls, Control 1 and Control 2, is used to verify that the test is working properly.

PRECAUTIONS

- For professional *in vitro* use only.
- A trained person in Turbidimetric technique and autoanalyzer use is required.
- Do not use after expiration date.
- Do not use the test if its primary containers are damaged.
- Specimens should be considered as potentially hazardous and handle in the same manner as an infectious agent. Avoid contamination errors, follow proper work procedure.
- The reagents after use 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-Turbidimetric Assay-**. Do not use any other commercial component.
- Follow Good Laboratory Practices, wear protective clothing, use disposal gloves, goggles and mask. Do not use any other commercial kit component.
- If measure range is exceeding, use the sample diluent to dilute the sample and repeat the assay again.
- Read and follow up the instructions for use provided in the kit.
- Prepare and adjust the analyzer before starting measurements.

STORAGE AND STABILITY

Store as packaged in the original primary container, the reagents should be preserved at refrigerated temperature (2-8°C/35.6-46.4°F), the sample diluent could be preserved refrigerated or at room temperature (2-30°C/35.6-86°F).

The product is stable until the expiration date printed on the label, if they have been preserved under the recommended conditions.

Do not freeze and keep away from the sunlight.

MATERIALS

MATERIAL PROVIDED	MATERIAL REQUIRED BUT NOT PROVIDED
<ul style="list-style-type: none">▪ Reagent R1 (Enhancer buffer)▪ Reagent R2 (Latex particles)▪ Calibrator 0▪ Calibrator 1▪ Calibrator 2▪ Calibrator 3▪ Calibrator 4▪ Calibrator 5▪ Control 1▪ Control 2▪ Universal vials with diluent for the sample dilution.▪ Instruction for use.▪ Additional screw cap	<ul style="list-style-type: none">▪ Specimen collection container.▪ Disposable gloves and laboratory equipment.▪ Vortex▪ Automatic analyzer.▪ Microcentrifuge (10000g).

SPECIMEN COLLECTION

Collect sufficient quantity of faeces: 1-2 g or mL for liquid samples. Stool should be collected in clean and dry containers and must be kept away from the sunlight.

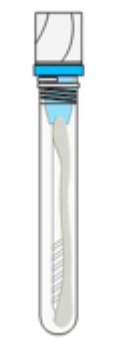
Samples can be stored in the refrigerator (2-8°C/35.6-46.4°F) for 3 days prior to testing. For longer storage, maximum 6 months, the specimen must be kept frozen at -20°C (-4°F). Samples must be

brought to room temperature before testing. Homogenize the sample as thoroughly as possible prior to preparation.

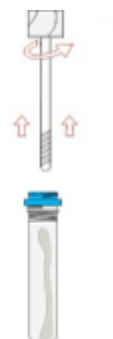
SPECIMEN PREPARATION

1. Label the vial with the patients 's identification.
2. Open the cap of the vial (anticlockwise) without removing the blue separator and use the stick to pick up sufficient quantity of sample (figure 1).
3. Use the stick to collect sufficient sample quantity. Introduce the stick into 4 different zones and rotate the stick in order to make the samples as homogenous as possible and make sure all grooves are filled with faecal material (figure 2).
4. Remove the stool excess by rotating the stick on the internal wall of the sterile container.
5. Insert the stick with the sample into the vial through the blue separator. Close the white cap by holding the blue separator to prevent it from opening.
6. Shake each vial (30-60 seconds) by Vortex in order to assure good sample dispersion (figure 3). Proceed to step 7 if the grooves are visually empty of any stool material, otherwise repeat the shaking up to 120 seconds and proceed to step 7 regardless some residual stool sample in the grooves. The sample dilution vial with diluted sample can be stored for 7 days in the refrigerator (2-8°C) prior to testing and bring the samples to room temperature.
7. Centrifuge the vial for 10 minutes at 1000-3000 x g in order to remove the possible residuals of faecal material. Alternatively, let the vial sit upright for a minimum of 15 minutes.
8. Remove the white screw cap and the blue separator from vials by rotating the separator (clockwise). The faecal extract is now ready to be tested. Insert the opened device directly into the clinical chemistry analyser or transfer the supernatant to an adaptor cup (not provided) (figure 4).
9. Close the collection vial by the provided additional screw cap (figure 4).

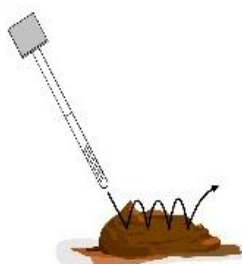
Note: Do not use the sample vials directly in the analyzer.



Sample dilution vial.



Pull out the shaped stick by turning the screw cap.



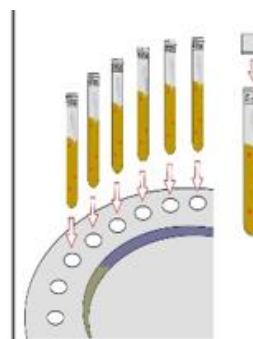
Introduce the stick into 4 different zones of the sample.



Shake the sample dilution vial with diluent+sample. Use a vortex. Centrifuge.



Remove the White cap and the blue separator, turning clockwise.



Load tubes in the analyser device. Close with the additional cap.

PROCEDURE

Allow reagents and solid stool to reach room temperature (15-30°C/59-86°F) prior the testing. Reagent R1 y Reagent R2 are ready to use.

For calibration only use FOB Calibrator: Cal0, Cal1, Cal2, Cal3, Cal4 and Cal5. Contain human hemoglobin at different concentrations indicated on the label of each of the vials.

	Reference	Calibrator 1	Calibrator 2	Calibrator 3	Calibrator 4	Calibrator 5
Con.	0 ng/mL	50 ng/mL	100 ng/mL	250 ng/mL	500 ng/mL	1000 ng/mL
Vol.	1000 µL	1000 µL	1000 µL	1000 µL	1000 µL	1000 µL

Reagents are ready to use. The frequency in the realization of the calibration curve must be established by the end user in the function of the criteria established for the clinical laboratory.

Note: See section quality control.

Analytical procedure

Measure range: 10 – 1000 ng hHb/mL.

Procedure	Steps	
R1 addition	200 µL	0 s
Sample addition	20 µL	10 s
R2 addition	55 µL	300 s
Blank measure	505 nm – 800 nm	310 s
Mainly measure	505 nm – 800 nm	610 s

*Data obtained by the analyzer Biolis i24 (Tokio Boeki)

INTERPRETATION OF RESULTS

Positive results: higher or equal than the cut-off fixed by the clinical laboratory.

Recommended cut-off values: 5 µg de hHb/g of stool (50 ng/mL) for diagnostics procedures and 20 µg de hHb/g of stool (200 ng/mL) for screening procedures.

Positive results determine the abnormal presence of human Haemoglobin (hHb) in stool samples.

Vitassay FOB Turbidimetric Assay Cut-off value:

Haemoglobin concentration values lower than 50 ng of hHb/mL of stool are considered normal values and that os not indicative of a bleeding in the gastrointestinal tract.

Haemoglobin concentration values between 50 and 200 ng hHb/mL of stool are considerer as an abnormal presence being indicative of slight bleeding, therefore monitoring and follow-up of the patient is recommended.

Haemoglobin concentration values higher than 200 ng of hHb/mL of stool are ndicative of a strong bleeding in the gastrointestinal tract.

Cut-off value of **Vitassay FOB-Turbidimetric Assay**:-: 50-200 ng hHb/mL (:10 dilution factor, 5-20 µg hHb/g of sample)

QUALITY CONTROL

FOB C1 & C2 Controls are ready to use.

FOB Control 1: is liquid control at a certain concentration of human hemoglobin (lower than Control 2). Concentration is indicated on the label of the vial.

FOB Control 2: is liquid control at a certain concentration of human hemoglobin (higher than Control 1). Concentration is indicated on the label of the vial.

The use of controls at two different concentrations is recommended to verify test precision.

If the obtain results are out of the tolerance range, the equipment, the reagents or the technique must be reviewed. If the problems persists, stop using the reagents and contact your distributor.

LIMITATIONS

- **Vitassay FOB -Turbidimetric Assay** should be only used in human solid stool samples.
- The quality of **Vitassay FOB -Turbidimetric Assay**- depends on the quality of the sample; Proper fecal specimens must be obtained.
- Positive results determine the abnormal levels of human hemoglobin in fecal samples; nevertheless, it can be due to several causes, besides colorectal bleeding, (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.
- If the test result is negative and the clinical symptoms or situation persist, it is recommended to perform another detection method. Negative results do not exclude hemorrhages caused by some

polyps or colorectal cancer, as they can cause bleeding intermittently or not cause them during any phase of the disease. In addition, blood may not have been distributed uniformly in the stool sample.

- 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.
- This test may be less sensitive for the detection of upper gastrointestinal bleeding.
- This test has not been validated in patients with hemoglobinopathies.
- All results must be interpreted along with the rest of the clinical information and other results obtained in the laboratory by a specialist doctor.

EXPECTED VALUES

The cut-off value commonly established is 5.1 µg of hemoglobin per g of feces. However, this value is not established in all studies. The Spanish CRC programs establish a value of 20 µg/g to study the convenience of performing colonoscopies. Therefore, those tests that are capable of quantifying in the range between 5.1 and 20 µg/g of feces are interesting. It should be noted that the conversion between µg/g to ng/mL is dependent on the sampling process of each product, in this case it must be multiplied by a factor of 10.

PERFORMANCE CHARACTERISTICS

Analytical sensitivity:

Detection limit: 8 ng hHb/mL.

Prozone:

Lower concentrations of 1 mg of hHb/mL of stool do not show prozone effect and no false negative results have been observed.

Within-Run Precision:

	Low (20 µg/g)	Media (80 µg/g)	High (250 µg/g)
N	20	20	20
Media (µg/g)	21.2	82.7	255.9
DS (µg/g)	1.3	4.9	9.1
CV (%)	6.1	5.9	3.5

*Data obtained by the analyzer Biolis i24 (Tokio Boeki)

Cross reactivity:












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

Human calprotectin	None	Bovine haemoglobin	Concentration lower than 0.2 µg/mL
Human lactoferrin	None	Pig haemoglobin	Concentration lower than 0.9 µg/mL
Human transferrin	None	Bovine Transferrin	Concentration lower than 250 µg/mL

REFERENCES

- 1.Vilkin A, Rozen P, Levi Z, Waked A, Maoz E, Birkenfeld S, Niv Y. Performance characteristics and evaluation of an automated-developed and quantitative, immunochemical, fecal occult blood screening test. Am. J. Gastroent. 2005 Nov; 100(11):2519-25.
- 2.Burt RW. Colon cancer screening continues as pivotal to cancer prevention. J Natl Compr Canc Netw. 2013 Dec 1;11(12):1457-8.
3. Fenocchi E, Martínez L, Tolve J, Montano D, Rondán M,Parra-Blanco A, Eishi Y. Screening for colorectal cancer in Uruguay with an immunochemical faecal occult blood test. Eur J Cancer Prev. 2006 Oct;15(5):384-90.

SYMBOLS FOR IVD COMPONENTS AND REAGENTS

	in vitro diagnostic device		Keep dry
	Consult instructions for use		Temperature limitation
	Use by		Manufacturer
	Batch code		Contains sufficient for <n> test
	Sample diluent		Catalogue number
	Keep out of the sunlight		

ADAPTED EQUIPMENT

- A15 (Biosystems)
- Alinity (Abbot)
- Architect 4000/8000 (Abbott)
- AU680 (Beckman Coulter)
- Biolis 24i/Biolis 50i (Tokio Boeki)
- BS200E (Mindray)

- CA800 (Furano)
- ChemwellIT (Awareness)
- Cobas c501 (Roche)
- Cobas c111 (Roche)
- Respons 910 (DiaSys)
- Vitros 5600 (Ortho)
- TC220 (Tecom)
- XL-180 & XL-200 (Erba)

