FOB. Immunoturbidimetric Ref.:FOB-220
Immunoturbidimetric latex determination of human hemoglobin in feces
Only for *in vitro* use in clinical laboratory
Store at 2-8ºC 1x20 mL / 1x20mL

FOB

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PRINCIPLE OF THE METHOD
The FOB test is an immunodiagnostic kit developed for providing sensitive, accurate and reproducible measurements of human hemoglobin levels in feces specimens.
It is based on an antigen-antibody agglutination reaction between the human hemoglobin contained in the sample and the polyclonal antibodies anti-human hemoglobin coated on polystyrene particles. Such a agglutination is measured as an increase in absorbance at 570 nm and is proportional to the quantity of human hemoglobin contained in the sample.

CLINICAL SIGNIFICANCE
The FOB test allows the quantitative determination of human hemoglobin (Hb) in feces and, being easily applicable on clinical chemistry automated analyzers, it can be used for screening many lower gastrointestinal tract conditions associated with bleeding such as colorectal carcinoma, colon polyps, Crohn's disease and ulcerative colitis.
The method is specific for human hemoglobin and no restricted diet (meat-free or peroxidase-free diet) is required.

REAGENTS
- REAGENT 1
Tris buffer pH 8.5, methylisothiazolinone (MIT) ≤ 0.03%
- REAGENT 2
Suspension of latex particles coated with rabbit polyclonal antibodies anti-human hemoglobin, pH 7.3, sodium azide < 0.1%

PREPARATION
- **R 1 and R2**: Are ready to use.

STORAGE AND STABILITY
All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8ºC and contaminations are prevented during their use. Do not freeze the reagents.
Once opened, R1&R2 are stable 30 days at 2-8ºC and also 30 days on board the analyzer at 2-12°C.
Do not use reagents over the expiration date.

SAMPLES
1. Wear disposable gloves.
2. Verify that the FOB tube does not interfere with the sampling probe or the sample disk of the instrument; if necessary, transfer the sample from the tube to the instrument sample cup.
3. Remove the flat bottom colorless cap from the FOB tubes and place the tube on the sample disk of the instrument.
WARNING: do not unscrew the light violet cap or the green cap, in order to avoid liquid spreading.

ADDITIONAL EQUIPMENT
FOB Pack (500 pcs) – **REF:** FOP-561: the kit contains
- 1 instruction sheet for sample collection and 500 plastic bags for collection tube storage and transport.
- Spectrophotometer
- Saline solution (sodium chloride 9g/L)

CALIBRATION
For calibration, use only the following materials:
FOB Calibrator REF: FOCA-573: 2x2 mL
Lyophilized calibrator containing human hemoglobin at a concentration of about 1000 ng/mL. Concentration is reported on the label of the vial. For use, follow the instructions contained in the kit. Use the calibrator for preparing the calibration curve as in the following table.

PREPARATION OF THE CALIBRATION CURVE

Working calibrators	1	2	3	4	5	6
Calibrator Solution*	500µL	500µL	-	-	-	-
Normal saline	-	500µL	500µL	500µL	500µL	500µL
Working calibrator 2	-	-	500µL	-	-	-
Working calibrator 3	-	-	-	500µL	-	-
Working calibrator 4	-	-	-	-	500µL	-
Dilution Ratio	-	1:2	1:4	1:8	1:16	-
[hemoglobin] ng/mL	[C]	0.5x[C]	0.25x[C]	0.125x[C]	0.0625x[C]	0x[C]

[C] = concentration of hemoglobin in ng/mL indicated on the label of the vial of the kit FOB Calibrator. * = FOB Calibrator Solution

Calibration stability on an automated instrument is 2 weeks.

PROCEDURE

Wavelength:	Main = 570 nm / Reference= 800 nm*
Cuvette:	1 cm light path
Temperature:	37ºC
Sample/REAGENT 1/REAGENT 2:	1/10.4/10.4
Reaction:	Fixed Time (increase)

***= instead of the 800 nm as a reference wavelength, it can also be used the 700 nm if considering that the decrease in delta abs will be about 30%.**

Allow reagents to reach working temperature before using.
A proportional variation of the reaction volumes indicated in the analytical procedure does not change the result.

CALCULATIONS
1. Plot a calibration curve on a graph paper by tracing absorbance (y axis) according to corresponding ng/mL concentration (x axis) for each Calibrator.
2. Indicate on the calibration curve the absorbance value obtained for Samples and Controls.
3. Extrapolate the ng/mL value for Samples and Controls from the calibration curve.

Conversion factor: Hb [ng/mL]: 1000 = Hb [µg/mL]

QUALITY CONTROL
The use of following control materials at 2 different levels of analyte is recommended to verify test accuracy:
FOB Control REF: FOCO-570 2x (2x2 mL)
Lyophilized controls at 2 different levels of analyte. For use, follow the instructions contained in the kit.

SAMPLE - for collecting samples, use only following materials:
FOB Tube REF: FOTU-561 (100 tests)
FOB Tube NG REF: FOTU-561N (100 tests)

Feces collection tube containing a buffer solution for the extraction and storage of hemoglobin.
For the complete solubility of the feces sample in the extraction buffer, wait at least **60** minutes after having gently shaken the collection tube or wait until the sample has dissolved.
For use, follow the instructions contained in the kit.
The collected sample stored in the FOB tube is stable for 1 week at 2-8 °C, protected from direct light.

PERFORMANCE CHARACTERISTICS
Interferences: the test is not affected by the presence of bilirubin up to **250** µg/mL, ascorbic acid (vitamin C) up to **100** µg/mL, BSA up to **40** g/L, BaSO4 up to **80** µg/mL and iron up to **1000** µg/dL. Other hemoglobins from animal origin (bovine, pig, rabbit, horse, fish, sheep, chicken and goat) do not significantly interfere in the test up to **2500** ng/mL.

Cross reactivity: based on strong similarity to Hb beta-chain, human myoglobin showed a cross-reactivity of about **25**%.

Human Hemoglobin variants: the assay recognises the following human Hb variants: Hb S, Hb C, Hb A2 and Hb D.

Measuring Range: **15** ng/mL to the highest calibrator concentration. Measuring range depends on highest calibration point. Samples with concentration exceeding the Measuring Range must be diluted 1:10 with normal saline and result multiply by 10.

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Total Precision: it was determined for 20 days on 2 replicates per 2 runs/day on 3 different sample concentration levels (L1/L2/L3).
Results were in compliance with the following limits:

Concentration	Total imprecision
≤ 90 ng/mL	CV ≤ 7.0% or SD ≤ 5.0 ng/mL
≥ 90 ng/mL	CV ≤ 5.0%

Following table shows representative data obtained in defined conditions.
Results obtained in individual laboratories may vary:

	Mean	Repeatability		Run to run		Total	
	ng/mL	SD	CV%	SD	CV%	SD	CV%
L1	62.5	1.5	2.4	1.8	3.0	2.3	3.9
L2	93.4	2.2	2.5	0.7	0.8	2.7	3.0
L3	301.7	3.7	1.2	5.9	2.0	7.0	2.3

Limit of Quantitation (LOQ): **15** ng/mL. The LOQ is the analyte concentration at which imprecision ≤ 20% as CV%.

Prozone effect: no prozone effect up to **35000** ng/mL (35 µg/mL).
It is recommended to verify this value on each analyzer by setting the alarms necessary in case of high dose of analyte.

Methods comparison: this FOB NG test (y) was compared with a commercially available method. Results were as follows:
N = 131, r = 0.989, y = 0.94 x +13
Min tested value: **0** ng/mL - MAX tested value: **1456** ng/mL.

BIBLIOGRAPHY
1) M. Nagata, T. Tanaka: Journal of Immunological Methods, 153, 185-192, 1992.
2) Review Criteria for Assessment of Qualitative Fecal Occult Blood In Vitro Diagnostics Devices. FDA; Guidance for Industry and FDA Staff. August 8th 2007.

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