Glenbio
Diagnostics & Research Laboratory Solutions

FOB LATEX REAGENT

KIT SPECIFICATIONS:

Cat. No.	Quantity	Reagent	Storage
GLFOB202	2 x 20 ml 1 x 11 ml 6 x 0.3 ml 2 x 0.5 ml 2 x 0.5 ml	FOB R1 FOB R2 Calibrators 0 – 5 Control Level 1 Control Level 2	2 – 8 ºC

INTENDED USE:

Glenbio FOB Latex Reagent is a latex turbidimetric assay used for the quantitative detection of haemoglobin (faecal occult blood) in human stool samples. The intended use of the test is to make a presumptive diagnosis of faecal occult blood (gastrointestinal bleeding).

This assay is simple and widely applicable and test results are obtained in a very short time. This product is optimised for several automated analysers.

SUMMARY AND EXPLANATION:

Colorectal cancer is the second leading cause of illness and death in Western world. The screening with faecal occult blood tests is based on the concept that important target colonic neoplasm, such as early-stage cancer and large adenomatous polyps. Colorectal cancer is also associated with local acute inflammatory reaction being visualized, in some cases, by white cell neutrophil scanning.
Haemoglobin is the iron-containing oxygen-transport protein in the red blood cells of all vertebrates that may be leaked into gastrointestinal tract and then discharged with the faeces in gastrointestinal bleeding diseases. When gastrointestinal blood is lost, the stool will contain a combination of intact or nearly intact haemoglobin, intact heme and heme-derived porphyrins in amounts that depend on the site and amount of bleeding and the transit time through the gut. The Glenbio FOB test detects intact or nearly intact human haemoglobin, being a very specific technique for detecting loss of blood from the lower intestine.

PRINCIPLE OF THE TEST:

FOB latex turbidimetric assay is based on agglutination reactions. These involve in vitro aggregation of microscopic latex particles. This aggregation consists in the specific reaction between antigen and antibodies, antigen contained in the sample and the antibodies anti-antigen coated on polystyrene latex particles. The sample is mixed with a suspension containing antibodies against the antigen bound to latex particles. If antigen is present in the sample it will react with the antibodies and form an aggregate. If no antigen is present in the sample the mixture will keep its appearance as a smooth suspension. Such turbidity is measured as an increase in absorbance at the determinate wave and is proportional to the quantity of antigen contained in the sample.

WARNINGS AND PRECAUTIONS:

For In Vitro Diagnostics Use Only – For Professional Use Only.
Carefully read instructions for use. Deviations from this procedure may alter performance of the assay.

- Read and follow the instructions for use provided with the kit.
- If the result exceeds the measurement range, use the sample diluent to dilute the sample and repeat the assay.
- Do not use after the expiration date.
- Do not use the reagents if pack is damaged or opened.
- Follow Good Laboratory Practices, wear protective clothing, use disposal gloves, goggles and mask.
- All the specimens should be considered potentially hazardous and handled the same way as an infectious agent.
- The solutions should be discarded in a proper container after testing following local regulations.

INSTRUMENTS:

Refer to relevant user's manual or Laboratory internal practice for routine maintenance procedures.

REAGENT PREPARATION AND STABILITY:

Kit components must be stored at a refrigerated temperature (2 - 8ºC). Do not freeze.

R1 and R2 are ready to use.

Reagents are stable up to the expiration date printed on the label, always considering that reagent containers must be properly closed to avoid any contamination, must be kept away from the sunlight and conserved at temperature indicated on the label of each reagent.

TYPE OF SPECIMEN:

Collect sufficient quantity of human stool samples. These samples should be collected in clean and dry containers (no preservatives or transport media). The samples can be stored completely dissolved in sample diluent in the refrigerator (2-8ºC) for 7 days prior to testing. Homogenise stool samples as thoroughly as possible prior to preparation.

TEST PROCEDURE:

Materials required but not supplied:

Description	*Cat No.*	*Description*	*Cat No.*
Specimen Collection Container	N/A	General Laboratory Equipment	N/A
FOB Sample Diluent	GL2122	Clinical Chemistry Automated Analyser	N/A
FOB Sample Dilution Vials	GL2123		

Assay procedure:
Refer to relevant user's manual for instructions on instrument start-up, loading components and samples, calibration, sample testing procedures, calculating and reporting results.

To process the collected stool sample:
Use a centrifugation tube for each sample to be tested. Label centrifugation tube with name or number of patient.
1. Homogenize the sample. Add 20mg of sample into centrifugation tube.
2. Add 2mL of sample diluent.
3. Shake vigorously the tube in order to assure good sample dispersion (vortex) until sample is completely dissolved.
4. Centrifuge for 15 minutes at 10000 g or 10 minutes at 15000 g.
5. Take the supernatant to automated analyser vial.

Calibration:
Using the recommended calibrator, calibrate the assay:
- Weekly (extremely recommended).
- When using a new reagent kit or changing lot number.
- Following preventive maintenance or replacement of a critical part.
- When Quality Controls are out of the assigned range.

Allow the calibrator to reach room temperature (15°C - 30°C) before testing. Concentration of each calibrator is indicated on the label of each vial. Calibrators are ready to use.

Quality Control:
All clinical laboratories should establish an Internal Quality Control program. Verify instrument and reagent performance with recommended controls or similar. The values obtained for QC should fall within manufacturer's acceptable ranges or should be established according to Laboratory's QC program.
Controls should be assayed:
- Each day before running patient faecal sample extract to validate the calibration curve.
- Prior to reporting patient results.
- Following any maintenance procedure.
- At intervals established by the Laboratory QC program.

Allow the controls to reach room temperature (15°C - 30°C) before testing. Concentration of each control is indicated on the label of each vial. The use of control materials at two different concentrations is recommended in order to verify test precision. Controls are ready to use.

RESULTS:

Positive results: higher or equal than the cut-off fixed by the clinical lab.
Recommended: 5 µg of hHb/g of stool for diagnostic procedures and 20 µg of hHb/g of stool for screening procedures.
Positive results determine the abnormal presence of human Haemoglobin (hHb) in stool samples.

EXPECTED VALUES:

Colorectal cancer affects both men and women of all racial and ethnic groups, being most often found in people aged 50 years or older. Regarding men, colorectal cancer is the third most common cancer after prostate and lung cancers. Regarding women, colorectal cancer is the third most common cancer after breast and lung cancers.
Some studies established equal or higher 5.1 µg hHb/g faeces as cut-off values to allow detect adult patients with gastrointestinal bleeding that will require to diagnosis additional diagnostic invasive procedures. In the Glenbio FOB test, this value is equivalent to 50 ng hHb/mL of diluent.

LIMITATIONS:

1. FOB latex reagent should only be used in human stool samples. The use of other samples has not been established. The quality of the test depends on the quality of the sample; proper faecal specimens must be obtained.
2. Positive results determine the presence of human haemoglobin in faecal samples. A positive result should be followed up with additional diagnostic invasive procedures to determine the exact cause and source of the blood in the stool.
3. If symptoms or situation still persist, haemoglobin determination should be carried out invasive techniques. 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.
4. This test may be less sensitive for detecting upper gastrointestinal bleeding because blood degrades as it passes through the gastrointestinal tract.
5. 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.

PERFORMANCE CHARACTERISTICS:

Performance results can vary with the instrument used. Data obtained in each individual laboratory may differ from these values.

Prozone:
Studies have been made up to a concentration of 10 µg of hHb/mL and no false negative results have been observed. Studies using higher concentrations have not been carried out.

Measuring Range:
20-1000 ng of hHb/mL of stool.
Samples higher concentrated than 1000 ng of hHb/mL of stool must be diluted (sample diluent) for proper quantification.

Interfering substances:
An evaluation was performed to determine the cross reactivity; no cross reactivity was founded against other faecal markers occasionally present in faeces, such as: bovine lactoferrin, human calprotectin, lactoferrin and transferrin. The cross reactions with bovine and pig haemoglobins are not present at concentrations below 1 µg/mL and 2.5 µg/mL respectively.

Analytical Sensitivity:

Limit of Detection (LOD): 15 ng hHb/mL of stool determined x20 replicates with sample diluent and reported as mean +2xSD.
FOB cut-off value:
Haemoglobin concentration values low than 50 ng of hHb/mL are considered normal values and that is not indicative of an inflammation of gastrointestinal tract. Haemoglobin concentration values higher than 200 ng of hHb/mL are indicative of a bleeding in the gastrointestinal tract.
FOB cut-off value: 50-200 ng hHb/mL (X100 dilution factor, 5-20 µg hHb/g of stool).

Precision:

Within Run Precision (N = 20)	Mean (ng/mL)	SD (ng/mL)	% CV
Level 1 (20 ng/mL)	21.2	1.3	6.1
Level 2 (80 ng/mL)	82.7	4.9	5.9
Level 3 (250 ng/mL)	255.9	9.1	3.5

BIBLIOGRAPHY:

1. Towler BP, Irwig L, Glasziou P, Weller D, Kewenter J. Screening for colorectal cancer using the faecal occult blood test, Hemoccult. Cochrane Database Syst Rev. 2000;(2): CD001216.
2. Ransohoff DF and Lang CA. Screening for colorectal cancer with the Fecal Occult Blood Test: a background paper. Ann Intern Med. 1997; 126: 811-822.
3. Ransohoff DF and Lang CA. Suggested technique for Faecal Occult Blood testing and interpretation in colorectal cancer screening. Ann Intern Med. 1997; 126: 808-810.

SYMBOLS:

The following symbols are used in the labelling of Glenbio systems:

IVD	In Vitro Diagnostics	REF	Catalogue No
LOT	Batch Code	CONT	Content
REAG	Reagent	CAL	Calibrator
CE	CE Mark - Device complies with the Directives 98/79/EC		
	Storage temperature	→	Reconstitute with
	Expiry Date (Last day of the month)		Manufactured By
	Biological risk		Consult Instruction for Use

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* For Reagent Instrument Application Settings please contact: **applications@glenbio.com**

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