INSTRUCTIONS FOR USE

HB FECALE PLUS

RAPID IMMUNOCHROMATOGRAPHIC TEST FOR QUALITATIVE DETECTION OF HUMAN HAEMOGLOBIN IN HUMAN FAECAL SAMPLES

1 – INTRODUCTION AND INTENDED USE

For ***in Vitro*** diagnostic use only

Colorectal cancer is the third most common cancer in the world. The appearance of occult blood in human faecal specimen is often associated with gastrointestinal diseases which might cause colorectal cancer if not treated promptly and properly. The traditional guaiac-based method lacks sensitivity and specificity, and has diet restrictions prior to the testing.
Hb FECALE PLUS test uses the technology of immunochromatographic sandwich assay. The test is more sensitive and more specific than the traditional guaiac assay. It is easier to interpret the result. In addition, unlike the guaiac assays, the accuracy of the test is not affected by the diet of the patients.
Hb FECALE PLUS is a qualitative test that detects human haemoglobin in human faecal specimens. The test is a visual one step, in-vitro assay. It is intended for professional use to help diagnose gastrointestinal bleeding. The kit include a positive control.

2 - PRINCIPLE OF THE METHOD

Hb FECALE PLUS is composed of two units, a faecal collection tube and a test device that contains a membrane strip. A faecal specimen is collected in the collection tube containing sample extraction buffer, and then added to the test device. When sample is added to sample pad, it moves through the conjugate pad and mobilizes the gold anti-h haemoglobin antibody conjugate that is coated on the conjugate pad. The mixture moves along the membrane by capillary action and reacts with anti-h haemoglobin antibody that is coated on the test region. If h haemoglobin is present at levels of 40 ng/mL or greater, the result is the formation of a coloured band in the test region. If there is no h haemoglobin in the sample, the area will remain colourless. The sample continues to move to the control area where goat anti-mouse IgG antibody will capture gold-antibody conjugate to form a pink to purple colour, indicating the test is working and the result is valid.

3 - MATERIALS PROVIDED – PACKAGING

Product	Type	REF	Pack
Hb FECALE PLUS	Immunochromatographic test	**VT81520** (100 tests)	100 sealed in foil pouch containing the test device, with dessicant. 100 FOB Collection Tube: sample collection tubes that contains 2 mL of buffer. Use as negative control. Positive control: 1 vial of positive control, solution of h. haemoglobin (Sigma Ref. H7379) ready to use (1x0,5 mL). Secondary packaging: cardboard box.

4 - MATERIALS REQUIRED BUT NOT PROVIDED

Disposable gloves, Timer.

5 - PRECAUTIONS AND WARNINGS

- All operations linked to the use of the test must be performed in accordance with Good Laboratory Practices.
- Hb FECALE PLUS is a qualitative *in vitro* diagnostic, for professional use only; it is to be used by qualified laboratory personnel also minimally skilled.
- This product is not classified as dangerous according to current European legislation.
- Read the package insert instruction before use the kit.
- All the specimens should be considered potentially hazardous and handled in the same manner as an infectious agent.
- Each test device is for single use only.
- Never use reagents from another lot.
- The test device should remain in the sealed pouch until use.
- Do not use the test if pouch is damaged.
- The control of human origin is obtained using a raw material tested. Plasma from each donor has been tested and found negative for antibody to HIV-1/HIV-2, Antibody to HCV, for HBSAG and for Syphilis. However, since no test is able to ensure that products derived from human blood will not pose a risk of transmitting infectious agents, you should consider the product still potentially at risk and therefore handled with the same precautions that are used for the samples taken by patients.
- Patients should closely follow the specimen collection procedures.
- Wear gloves when handling the sample.
- Disposable gloves, buffer solutions, plastic tubes and used devices in a propre biohazard container.
- Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
- The reagents' quality cannot be guaranteed beyond their shelf-life date or if the reagents are stored under inappropriate conditions.
- The Certificates of Analysis and the Safety Data Sheet of the product are available on the website www.masciabrunelli.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.
- Notify Mascia Brunelli Spa and the Relevant Authorities of any serious incidents occurring in connection with the in vitro diagnostic device. complaint@masciabrunelli.it
- As for all diagnostic procedures, a diagnosis should not be based on a single test result but, should only be made by a physician after pooling the laboratory results, clinical investigations and patient's history.

6 - STORAGE CONDITIONS AND STABILITY

Store as packaged in the sealed pouch at 4-30°C. Do not expose the kit over 30°C. Do not freeze the kit. If stored at 4°C-8°C, ensure that the test device is brought to room temperature before opening. The test device is stable through the expiration date printed on the sealed pouch.

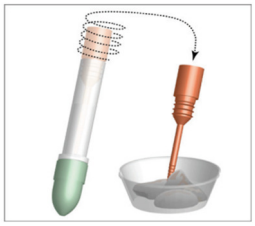
7 – Specimen collection and Preparation

PATIENT PREPARATION

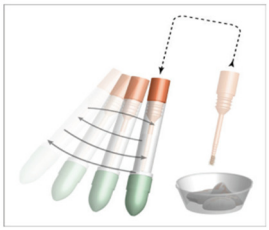
1. Specimen should not be collected during or within three days of a menstrual period, or if the patient suffers from bleeding hemorrhoids or blood in the urine.
2. Alcohol, aspirin and other medications, taken in excess, may cause gastrointestinal irritation resulting in occult bleeding. Such substances should be discontinued at least 48 hours prior to testing.
3. Dietary restrictions are not necessary.

SPECIMEN COLLECTION WITH SAMPLE TUBE SUPPLIED

1. Stool specimens can be collected at any time of the day.
2. Collect random sample of faeces in a clean, dry receptacle.
3. Unscrew the bottom cap (red end) of the collection tube and remove the applicator stick.
4. Insert the stick into the faecal specimen at several different sites.
5. Insert the sampled applicator back to the tube and tighten the bottom (red end) securely. The hold that only allows the stick goes through will prevent the access sample from getting into the tube.
6. Shake the tubes with bottom cap (red end) vigorously for about 5 seconds to release and disperse the stool sample into the collection buffer.



Specimen collection Steps 3 and 4



Specimen collection Steps 5 and 6

8 - Quality control

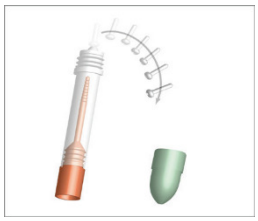
The control band is an internal reagent and procedural control, it is included in each test device. It will appear if the test has been performed correctly and the reagents are reactive.

9- Test procedure

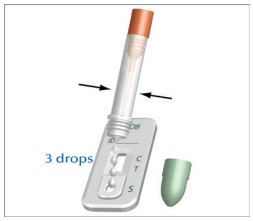
Allow the tests, samples and reagent to reach to room temperature (15-30ºC/59-86ºF) prior to testing. Do not open pouches until ready to perform the assay.

1. Remove an Hb FECALE device card from its foil pouch.
2. Remove the tip protection cap (green). Holding the tube upright with tip pointed toward the direction away from the test performer, Snap off the tip.
3. Hold the tube in a vertical position over the sample well of the test card and deliver 3 drops (120-150 µL) of the sample into the sample well marked as "S" on the cassette.
4. Read the test results at 5 minutes.

Note: Results read after 10 minutes may not be accurate.



Test Procedure Step 3



Test Procedure Step 4

TEST PROCEDURE for POSITIVE/NEGATIVE CONTROL

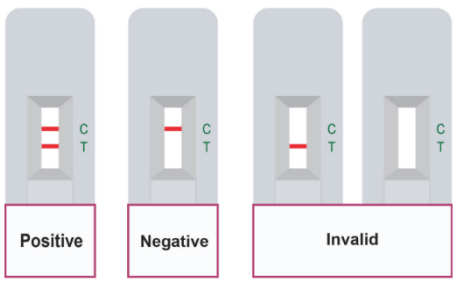
1. Dispense 3-4 drops directly into the well "S".

10 – Reading and interpretation of results

POSITIVE: Two lines appear within 5 minutes, the test result is positive and valid. One coloured line should be in the control line region "C" and another apparent coloured line should be in the test line region "T".
Note: Specimens containing very low levels of h Haemoglobin may develop two coloured bands over 10 minutes.

NEGATIVE: One coloured line appears in the control line region "C", the result is negative and valid. No line appears in the test line region "T".

INVALID: Control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test. If the problem persists, discontinue using the test kit immediately and contact your local distributor.



11 - Performances characteristics

A. Sensitivity

The analytical sensitivity of the test I 40 ng/mL h haemoglobin.

B. Specificity

The test is specific to human haemoglobin. Samples containing the following substances were tested on both positive and negative controls with no effect on test results.

Substance	Concentration	Substance	Concentration
Cattle haemoglobin	1 mg/mL	Pig haemoglobin	1 mg/mL
Chicken haemoglobin	1 mg/mL	Rabbit haemoglobin	1 mg/mL
Goat haemoglobin	1 mg/mL	Horseredish peroxidase	1 mg/mL
Horse haemoglobin	1 mg/mL		

C. Accuracy

Two hundred and fourteen (214) confirmed samples were used in the clinical evaluation of the Hb FECALE PLUS test. The clinical sensitivity, specificity and accuracy are respectively 100% (62/62), 99.3% (151/152) and 99.5% (213/214).

D. Interference testing

The following substances were added to h haemoglobin free and 40 ng/mL controls. No interference was found with any of the substances at the following concentrations:

Acetaminophen 20 mg/dL	Acetylsalicyclic acid 20 mg/dL	Ampicillin 40 mg/dL	Ascorbic acid 40 mg/dL
Atropine 40 mg/dl	Bilirubin 20 mg/dL	Caffeine 40 mg/dL	Gentisic acid 40 mg/dL
Glucose 2000 mg/dL	Human albumin 2000 mg/dL	Urea 4000 mg/dL	Uric acid 10 mg/dL

12 - LIMITATIONS OF THE TEST

1. Hb FECALE PLUS is for in vitro diagnostic use only. This test should be used for the detection of Human haemoglobin from human faecal specimens. Neither the quantitative value nor the rate of increase in haemoglobin concentration can be determined by this qualitative test.
2. A number of conditions, as mentioned in "Patient Preparation", can cause false positive results.
3. The results obtained with this kit must be interpreted together with other clinical information and laboratory findings available to the physician.

ATTACHED HOW TO TAKE A FAECAL SAMPLE, TO PHOTOCOPY AND DISTRIBUTE.

13 - REFERENCES

1. Simon J.B. "Occult blood screening for colorectal carcinoma: a critical review", Gastroenterology, Vol. 88 820, 1985.
2. Woo. H. and McDonald C. "Detection of faecal occult blood using monoclonal antibodies", Gastroenterology society of Australia, Annual general Meeting. Melbourne, Victoria, Australia, May 1986.
3. Adams, E.C. and Layman, K.M. "Immunochemical confirmation of gastrointestinal bleeding" , Ann. Elin. Lab. Sci., Vol. 4 343, (1974).
4. Ribet, A., et al. "Occult-blood test and colorectal tumors", Lancet, Vol. 1, 417, (1980).
5. Taranen, M.J., et al. "Immunological detection of faecal occult blood in colorectal cancer", Br. J. Cancer, Vol. 49 141, (1984).

TABLE OF APPLICABLE SYMBOLS

IVD	In Vitro Diagnostic Medical Device		Temperature limitation	LOT	Batch code (EXXX)		Manufacturer		Keep dry	UDI	Unique device identifier
	Consult Instructions for use		Use by (year/month)	REF	Catalogue number		Do not reuse		Fragile, handle with care		Keep away from heat

REVISION HISTORY

Version	Description of changes	Date
Instructions for Use (IFU) - Revision 5	Updated layout and content.	2025/09

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.