Hb FECALE

For in *Vitro* diagnostic use only

A visual one-step immunoassay for the qualitative detection of human blood haemoglobin in human fecal samples

I. INTENDED USE

Hb Fecale is a rapid, visual immuno-chromatographic test for the qualitative detection of human blood haemoglobin in fecal samples. This test is intended as an aid in the diagnosis of lower gastrointestinal (g.i.) disorders. The principal use of the test is to screen for lower g.i. pathologies, such as colorectal cancers and large adenomas that bleed. Colorectal cancer is one of the most commonly diagnosed cancers and a leading cause of cancer death in the United States (Lieberman, 1994; MMWP, 1995). Screening for colorectal cancer probably increases the cancer detection at an early stage, therefore reduces the mortality (Dam et. al., 1995; Miller, 1995; and Lang, 1996).Earlier commercially available FOB tests utilized the guaiac test, which requires special dietary restriction to minimize false positive and false negative results. Hb Fecale is specially designed to detect human haemoglobin in fecal samples using Immunochemical methods, which improved specificity for the detection of lower g.i. disorders, including colorectal cancers and adenomas (Frommer et. al., 1988; St. John et. al., 1993).

II. PRINCIPLE

Hb Fecale has been designed to detect human haemoglobin in fecal samples through visual interpretation of color development in the test device. The test device contains a membrane strip, which is pre-coated with anti-human haemoglobin antibody on the test line region (T) and goat anti-mouse antibody on the control line region (C).An anti-human haemoglobin antibody-colloidal gold conjugate pad is placed at the end of the membrane. When human haemoglobin is present in the patient fecal sample dissolved in buffered saline, the mixture of colloidal gold conjugate and extracted sample moves along the membrane chromatographically by capillary action. This mixture then migrates to the test region (T) and forms a visible line as the antibodies complex with the human haemoglobin.When human haemoglobin is absent in the extracted sample, no visible color band will form on the test region (T). Therefore, the presence of a color band in the test region (T) indicates a positive result.A colored band will always appear at the control region (C) to serve as a procedural indicator for the proper performance of the test and the device.

III. STORAGE AND STABILITY

The test kit is to be stored at refrigerated (2-8℃) or at RT (up to 30℃) in the sealed pouch for the duration of the shelf-life.

IV. PRECAUTIONS

- For in-vitro diagnostic use only and for professional use only.
- Do not use test kit beyond expiration date.
- Do not mix sample collection tubes from different lots.
- Do not open the test cassette foil pouch until you are ready to perform the test.
- The control of human origin is obtained using only blood donors tested negative by tests approved by the FDA for the detection of HBsAg, HCV, and anti-HIV 1 and 2. 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.
- All patient samples should be treated as if capable of transmitting disease.
- Buffered saline contains sodium azide which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of buffered saline or extracted samples, always flush with copious quantities of water to prevent azide build up.
- Patients should closely follow the specimen collection procedures.
- Patients should not collect samples during their menstrual period, if they have bleeding hemorrhoids, blood in the urine, or if they have strained during bowel movement.

V. REAGENTS AND MATERIALS SUPPLIED

- ***Test Membrane - Hb Fecal****:* Individually wrapped test devices ***(CASSETTE)***. Each test device ***(CASSETTE)*** contains one test strip with anti-human haemoglobin monoclonal antibody coated membrane and colored anti-human haemoglobin monoclonal antibody pad.
- ***Extraction Liquid Tubes - FOB Diluent Buffer:*** Sample collection tubes. Each contains 2 mL of 0.1 M Tris-HCl buffered saline, with Bovine Albumin (BSA) and 0.05 % sodium azide.
- **Positive Control:** H. haemoglobin (Sigma Ref. H7379), for Ref. VT81520 and UD80010.To be dispensed directly into the well S.
- **Negative Control:** (only for Ref. UD80010). Solution containing biological additives and bacteriostatic agents. To be dispensed directly into the well S.
- **Instruction for use.**

MATERIAL REQUIRED BUT NOT PROVIDED

- A clean dry container or receptacle for the collection of fecal sample.
- A piece of tissue paper to prevent solution from splashing.

VII. SPECIMEN COLLECTION AND PREPARATION

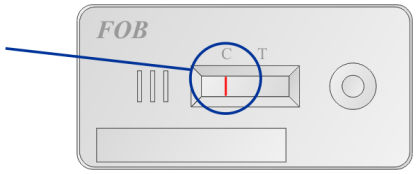
1. Collect a random sample of faeces in a clean dry container or receptacle.
2. Unscrew and remove the collection tube applicator stick. Be careful not to spill or spatter solution from container.
3. Collect random sample by using the applicator stick. Take sample from various surfaces of the faeces specimen
4. Re-insert the applicator stick into the tube and screw the cap tightly. Be careful not to break the tip of the sample collection tube.
5. The specimen is now ready to be stored at 2-30℃, transported or tested. Faecal samples in the buffered saline are stable for up to 15 days at room temperature (up to 30℃).

For liquid or semi-solid stools using a separate pipette, draw stool of the sample itself. Dispense 150 µL of each stool into a extraction tube. Mix carefully, then vortex 15 seconds.Then proceed as above from the point forward 4.

VIII. TEST PROCEDURE

Quality Control / Internal Procedural Control

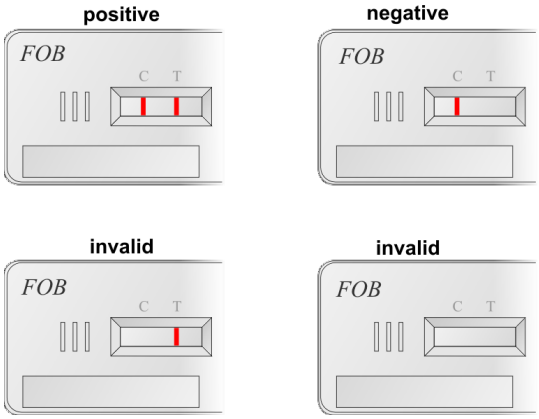
A procedural control is included in the test. A coloured band appearing on the control region (C) of the membrane indicates proper performance of the test and the device.
A clear background in the observation window is considered an internal negative control. However, when the faecal samples are tested, the background may appear slightly yellowish due to the original colour of the faecal samples. This is acceptable as long as it does not interfere with the interpretation of test result. The test is invalid if the background fails to clear and obscures the reading of the result.



IX. ASSAY PROCEDURE

1. Test device, patient's samples, (extracted sample) should be brought to room temperature (20°C to 30°C) prior to testing.
2. Remove the test device from its pouch when ready to perform the test. Bring the device to room temperature to avoid condensation of moisture in the membrane. Label the device with patient or control identification.
3. Shake the collection tube thoroughly to ensure proper mixing of the faecal sample with the extraction solution.
4. Using a piece of tissue paper, break the tip of the collection tube using a twisting motion. Hold the collection tube vertically and dispense 3-4 drops (app. 120 µL) of solution into the sample well of the test device.
5. For testing control, dispense 3-4 drops directly into the well S.
6. Observe the result in 5 minutes. Strong positive results may be observed sooner. Do not interpret results after 8 minutes.

X. INTERPRETATION OF RESULTS



Positive
Two pink-red colored bands appear. One in the control region (C) and one in the test region (T). When testing with strong positive samples, the intensity of the control band may be lighter than expected. Comparison of the line intensities is not recommended.

Negative
Only one pink-red colored band appears in the control region (C). No apparent faint pink to red colored band in the test region (T).

Invalid
A total absence of pink colored bands in both regions is an indication of procedural error or that test reagents may have deteriorated. Repeat the test with a new test device and if condition persists, contact the manufacturer for technical assistance.
The test lines may get darker after some time. This does not have any affect on the result.

XI. PERFORMANCE CHARACTERISTICS

A. Analytical Sensitivity

A sample containing human haemoglobin at concentration equal to or higher than 40 ng/mL produces a positive result.
Prozone effect: sample containing as high as 0.5 mg/ml haemoglobin can still test positive.

B. Test Specificity

Hb Fecale is specific for human haemoglobin and does not show any cross-reaction with the haemoglobin from bovine, pig, rabbit, horse,chiken and sheep.
Hb Fecale also does not show any cross reaction with bilirubin, vitamin C, ampicillin, caffeine, atropine, glucose, human albumin, urea, uric acid and horse radish peroxidase.

Attached HOW TO TAKE A FAECAL SAMPLE, to photocopy and distribute.

XII. BIBLIOGRAPHY

1. Dam, J.V., et al.; Fecal Blood Screening for Colorectal Cancer; Archive of Internal Medicine; (1995) 155: 2389-2402
2. Frommer, D.J. et. al.; Improved Screening for Colorectal Cancer by Immunological Detection of Occult Blood; British Medical Journal; (1988) 296: 1092-1094
3. Lieberman, D.; Screening/Early Detection Model for Colorectal Cancer, Why Screen? Cancer Supplement; (1994) 74 (7): 2023-2027
4. Miller, A.B.; An Epidemiological Perspective on Cancer Screening; Clinical Biochemistry (1995) 28 (1): 41-48
5. Ransohoff, D.F. and Lang, C.A.; Improving the Fecal Occult-Blood Test; The New England Journal of Medicine; (1996) 334 (3): 189-190
6. Screening for Colorectal Cancer-United States, 1992-1993, and New Guidelines; Mobility and Mortality Weekly Report; (1995) 45 (5): 107-110
7. St. John, D.J.B., et al.; Evaluation of New Occult Blood Test for Detection of Colorectal Neoplasia; Gastroenterology; (1993) 104: 1661-1668

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

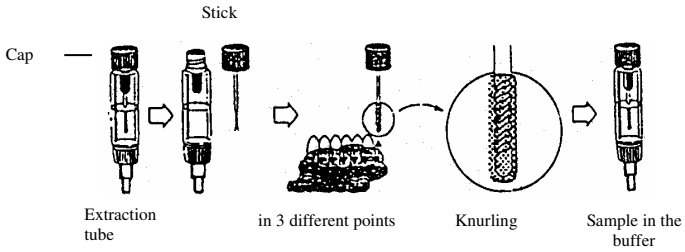
CONTENT	**VT81500A 50 cards**	**VT81500B 50 tubes**	**VT81510 50 tests**	**VT81520 100 tests+control**	**UD80010 Controls**
Test Membrane – Hb Fecale (cassette)	50 items		50 items	100 items	
Extraction Liquid Tubes–FOB Diluent Buffer		50 items	50 items	100 items	
Positive Control				1 x 1 ml	1 x 1 ml
Negative Control					1 x 1 ml
Instruction for use	1 item	1 item	1 item	1 item	1 item

EDMA Code 13 01 70 01 00

CE IVD

ANNEX TO THE INSTRUCTION FOR USE
HOW TO TAKE A FAECAL SAMPLE

1. Take faeces in a plane dry container.
2. Open the extraction buffer tube and take out the stick attached at the cap.
3. Dip in **3 different points** of faeces the stick (**only the knurling end**).
4. Take out the stick from the faeces.
5. Dip the stick in the extraction buffer tube.
6. Screw the cap, close and shake gently.



CE IVD