Ref: 1110002

HEMOTRUST®

Rapid test for the detection of hemoglobin in stool samples.

CE

INTENDED USE

The HEMOTRUST® rapid test is a lateral flow chromatographic immunoassay for the qualitative detection of hemoglobin in stool samples. This kit is intended to be used as a screening test and as an aid in the diagnosis of low gastrointestinal diseases such as colorectal cancer and precancerous lesions.

SUMMARY

Colorectal cancer is one of the most commonly diagnosed cancers and a leading cause of cancer-related death (Lieberman 1994, MMWP 1995). Early detection of colorectal cancer improves the chances of survival and thus reduces mortality (Dam et al 1995, Miller 1995, and Lang 1996).

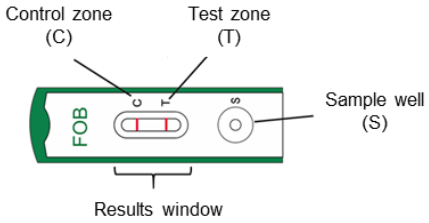
The first FOB (Fecal occult blood) tests commercially available used a gaiac method which required appropriate dietary restrictions to minimize false positive and false negative results. The HEMOTRUST® rapid test uses a sandwich immunoassay technique for the detection of hemoglobin in stool samples without such dietary restrictions (Frommer et. al. 1988; St. John et. al., 1993).

TEST PRINCIPLE

The HEMOTRUST® rapid test is a membrane-based immunoassay for the qualitative detection of hemoglobin in stools. In healthy subjects, trace amounts of blood in stools can be detected in the absence of cancer or other severe pathology. This is called physiological leakage. The threshold of the HEMOTRUST® rapid test was fixed above the threshold corresponding to physiological leak for these traces not to be detected.

A pair of anti-human hemoglobin antibodies is used for the qualitative detection of hemoglobin in stools. A first antibody is coated in the test region T of the nitrocellulose membrane and serves as capture antibody. The second antibody is conjugated to colored particles and serves as detection antibody. During migration, the diluted stool sample reacts with the detection antibodies to form an antigen-antibody-particles complex. The mixture migrates by capillarity along the membrane and the antigen-antibody-particles complex is capture by the antibody coated in the test region T. Agglomeration of these complexes creates a visible purple band in the test region T.

The appearance of a colored band in the test region T thus indicates a positive result, while its absence reveals a negative result. A purple band should always appear in the control region C, as it serves as a control of the procedure validating that a sufficient sample volume has been used and that the test has proceeded correctly.



PRECAUTIONS

- For professional *in vitro* diagnostic use only.
- The test is intended for single use only. Do not reuse.
- Do not freeze any item of the kit.
- Do not use after the expiration date printed on the pouch and on the box label
- Do no use the test if the sealed pouch is damaged
- Do not eat, drink or smoke in the area where the samples are handled
- All samples should be considered as potentially infectious
- Respect the precautions in force regarding microbiological hazard for all procedures as well as standard guidelines for proper sample disposal
- Wear personal protective equipment (lab coats, disposable gloves and safety goggles) when handling the samples
- The used test components should be discarded according to local regulations in force
- Humidity and high temperature may affects the results in a negative manner
- Allow all reagents to reach room temperature (15-30°C) before performing the test
- Do not drop or flip the sample in the reading window
- Do not touch the reading window to avoid any contamination
- The test cassette must remain in its sealed pouch until use
- Interprete the results after 5 minutes
- The kit can be stored and transported between 2 and 30°C
- Do not mix reagents (i.e., test cassettes and extraction buffer bottles) from different batches
- Avoid samples cross contamination by using a new extraction buffer bottle for each sample

STORAGE AND STABILITY

The kit should be stored between 2-30 ° C. The test is stable until the expiration date printed on the sealed pouch. The test must remain in the sealed pouch until use. Do not freeze.

Make sure to protect the components of this kit from contamination. Do not use the extraction buffer bottle if there is suspicion of microbial contamination (presence of a cloudiness or precipitate). Biological contamination of the extraction buffer may lead to a false result.

MATERIAL

Material provided

- Test cassettes HEMOTRUST® in individual pouches
- Dropper bottle (2 mL) of extraction buffer with applicator
- Instruction for use

Material required but not provided

- Timer
- Lab pipette (for liquid stool collection)

SAMPLE COLLECTION AND PREPARATION

- The HEMOTRUST® test is intended for the analysis of stool samples only.
- Patients should not collect samples during their menstrual cycle and in case of bleeding hemorrhoids, blood in the urine, occlusion or constipation that may cause injury.
- Alcohol, aspirin, non-steroidal anti-inflammatory drugs, or other irritating molecules can cause gastrointestinal irritation and unseen bleeding. The consumption of these substances must be stopped at least 48 hours before the test.
- No dietary restriction is required before the test.
- Aucun régime alimentaire particulier n'est requis avant la réalisation du test.
- If samples are to be shipped, package them in accordance with all applicable regulations for the transportation of biological agents.

Sample collection

1) Use a clean, dry container for stool sample collection.
2) Unscrew the extraction buffer bottle and remove the applicator. Do not splash or spill the solution. Collect stool samples by inserting the applicator at 3 different sites of the stool.
   Note : if liquid stools are used, take a 50 µL-sample using a lab pipette and introduce the sample directly into the extraction buffer bottle.
3) Replace the applicator in the tube and screw the cap. Shake the tube vigorously and make sure the sample and extraction buffer are thoroughly mixed. The diluted sample is now ready to be stored, transported or tested.
4) The diluted sample should be tested as soon as possible.

SAMPLE STORAGE

Raw stools: The best results will be obtained if the test is performed within 6 hours after sample collection at room temperature (15-30 ° C). The collected samples can be stored for 3 days between 2-8 ° C if not tested within 6 hours. For longer storage, raw stools can be stored at -20 ° C for 6 months

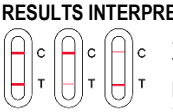
Sample diluted in extraction buffer : Samples can be stored up to 3 days à 15-30°C or 5 months at 2-8°C once diluted in the extraction buffer. For longer storage, samples can be stored at -20°C for 6 months.

PROCEDURE

Bring all test devices, reagents, stool samples and/or external controls to room temperature (15-30°C) before performing the test.

1. Remove the test cassette from its sealed pouch and use it immediately.
2. Label the test cassette with the patient ID or control number.
3. Unscrew the red cap of the extraction buffer bottle to access the dropper. Hold the bottle vertically and add 2 drops (about 80 µL) of solution in the sample well (s) of the cassette. **Avoid capturing air bubbles in the sample well (S) and flipping liquid in the results window**.
4. Start the timer when the migration begins.
5. When the test begins, a colored front will move along the membrane.
6. Read the results after 5 minutes. Do not interpret after 10 minutes.

RESULTS INTERPRETATION



POSITIVE: 2 colored bands appear. One colored band appears in the controle zone (C) and one band appears in the test zone (T). A positive result indicates that blood has been detected in the sample at a level above the threshold of the test.

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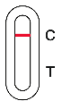
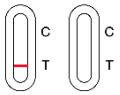
NOTE: The intensity of the colored band in the test zone (T) may vary depending on the concentration of hemoglobin in the sample. Any coloration in the test zone (T), whatever its intensity, should be considered as positive.
Please note that this test is only qualitative and cannot be used for the quantification of hemoglobin concentration.



NEGATIVE: One band appears in the control zone (C). No band appears in the test zone (T). A negative result indicates that no trace of blood has been detected in the sample or at a concertation below the threshold of the test. Une ligne apparait au niveau de la ligne contrôle (C).



INVALID : No band appears in the control zone (C). Insufficient sample volume, deficient test or improper procedure technique are the most likely causes of control failure. Review the procedure and repeat the test with a new cassette. If the problem

QUALITY CONTROL

Internal control of the procedure
An internal control of the procedure is included in the test. The appearance of a purple band in the control zone (C) is considered as a migration internal control. It confirms that a sufficient sample volume has been used, the membrane is intact, and that the technical procedure is correct. .

External quality control
External controls are not provided in this kit. However, it is recommended to test positive and negative controls according to Good Laboratory Practices to confirm the test procedure and to check the test performances.

LIMITATIONS

1. The HEMOTRUST® rapid test is intended for professional in vitro diagnostic use only. The test should be used for the qualitative detection of blood in stools.
2. As with all diagnostic tests, a definitive clinical diagnosis should not be based only on the results of a rapid test, but should be performed by a doctor after all clinical and laboratory results have been evaluated.
3. Do not read the test after 10 minutes.
4. Negative results do not exclude the presence of cancerous or precancerous lesions. Indeed, polyps or cancers of the colorectal areas may not bleed or intermittently. In addition, blood may not be evenly distributed in the stool samples. Colorectal polyps at an early stage may not bleed.
5. Contamination of the stool sample with urine or toilet water may affect the test results.
6. Colorectal bleeding is not necessarily due to precancerous or cancerous polyps. Note that the presence of blood in the stool samples may be related to causes other than colorectal bleeding, for example hemorrhoids, blood in the urine or irritation of the stomach.

The following risk-factors for cancer may cause blood to appear in stools:

1) Drugs
Aspirin (acetylsalicylic acid) or non-steroidal anti-inflammatory drugs may be responsible for bleeding and lead to a false positive result.

3) Hemorrhoids
Hemorrhoids can bleed. Stool can thus be contaminated with blood that is not related to cancer.

4) Menstruations
Small amounts of blood related to women's menstrual cycle can contaminate stool samples. Such blood is not related to cancer.

5) Samples contaminated with urine
Different pathologies can cause the appearance of blood in the urine. To avoid detection of urine-related blood, stool specimens should not be in contact with urine.

PERFORMANCE CHARACTERISTICS

Accuracy
The HEMOTRUST® test was compared to another commercial rapid test using clinical samples.

Method		Rapid test		Total
	Results	Positive	Negative	
HEMOTRUST®	Positive	210	6	216
	Negative	12	850	862
Total		**222**	**856**	**1078**

Relative sensitivity: 94.6% (95%CI*: 90.7%~97.2%)
Relative specificity: 99.3% (95%CI*: 98.5%~99.7%)
Accuracy: 98.3% (95%CI*: 97.4%~99.0%)
*Confidence intervals

Sensitivity
The HEMOTRUST® cassette detects blood in stools at a threshold of 6 µg/g stool (= 50 ng hemoglobins/mL extraction buffer).

Hook effect
A hook effect has been demonstrated for human hemoglobin concentrations greater than 10 µg/mL.
Note : if a negative result is obtained from a sample showing clear macroscopic evidence of the presence of blood, re-test the sample after carrying out a 1/10 dilution of the sample in extraction buffer.

Intra-assay accuracy
The intra-assay variability was determined using a series of 10 replicates of 3 samples containing different hemoglobin concentrations: negative, 50 ng/mL, and 10 µg/mL. Samples were correctly identified in more than 99% of cases.

Inter-assay accuracy
The inter-assay variability was determined using 10 independent tests on the same 3 samples containing different hemoglobin concentrations: negative, 50 ng/mL, and 10 µg/mL. 3 different batches of HEMOTRUST® test cassettes were used. Samples were correctly identified in more than 99% of cases.

Cross-reactivity
The HEMOTRUST® is specific for human hemoglobin. Samples containing the substances listed below were diluted in the extraction buffer solution at a concentration of 1.0 mg/mL and tested with negative and positive controls without affecting the results: cow hemoglobin, chicken hemoglobin, pig hemoglobin, goat hemoglobin, horse hemoglobin, rabbit hemoglobin and turkey hemoglobin.

Interfering substances
The following potentially interfering substances were added to the positive and negative hemoglobin samples.

Ascorbic acid 20mg/dL,	Oxalic acid 60mg/dL,
Bilirubin 100mg/dL,	Uric acid 60mg/dL,
Aspirin 20mg/dL,	Urea 2000mg/dL,
Glucose 2000mg/dL,	Cafeine 40mg/dL,
Albumin 2000mg/dL	

None of these substances showed interference when tested at the mentioned concentration.

REFERENCES

1. Simon JB. Occult Blood Screening for Colorectal Carcinoma: A Critical Review Gastroenterology, 1985; 88: 820.
2. Blebea J, Mcpherson RA. False-Positive Guaiac Testing With Iodine, Arch PatholLab Med, 1985;109:437-40.

SYMBOLES

	Attention, see instructions for use	LOT	Lot number
IVD	For *in vitro* diagnostic use only		Manufacturer
2°C 30°C	Store between 2-30°C		Do not reuse
Σ	Tests per kit	REF	Catalog number
	Expiration date	DIL	Diluent/ extraction buffer

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