BIOSYNEX
Easy Diagnostics for Life

Ref: 1110002

HEMOTRUST

Rapid test for the detection of human hemoglobin in faecal samples.

CE

INTENDED USE

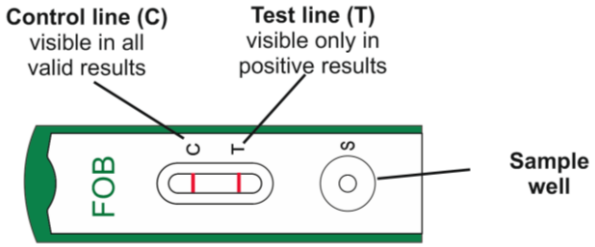
The HEMOTRUST cassette is an immunochromatographic rapid test for the qualitative detection of human haemoglobin in human faecal specimens. This kit is intended for use as an aid in the diagnosis of lower gastrointestinal pathologies such as colorectal cancer and its previous stages.

SUMMARY

Colorectal cancer is one of the most commonly diagnosed cancers and a leading cause of cancer-related death (Lieberman, 1994; MMWP, 1995). Screening for colorectal cancer is likely to improve the odds of detecting cancer at an early stage, reducing mortality (Dam et. al., 1995; Miller, 1995; und Lang, 1996).
Earlier commercially available FOB tests utilized a guaiac test, requiring special dietary restrictions to minimize false positive and false negative results. The highly specific HEMOTRUST cassette is designed especially to detect human haemoglobin in faecal specimens.
The test uses immunochemical methods, improving sensitivity and specificity for the detection of lower gastrointestinal disorders in comparison with the conventional guaiac test (Frommer et. al., 1988; St. John et. al., 1993).

PRINCIPLE

The HEMOTRUST cassette is an immunochromatographic rapid test designed to detect human haemoglobin in faecal specimens. The presence of haemoglobin is indicated by a specific colour development for visual interpretation. Anti-human haemoglobin antibodies are immobilized on the test line region of the membrane.
During testing, the antigens extracted from the faecal specimen are captured by specific antibodies, which are adhered to pointer particles. The mixture migrates along the membrane and the antigen-antibody-particle complex binds to the specific antibody in the test line area. The agglomeration of complexes creates a colour line in the test line area.
The appearance of the colour line in the test (T) line area indicates a positive result, while its absence indicates a negative result. A red line should always appear in the control (C) line area. It serves as a procedural control, confirming that sufficient specimen volume was used and indicates an adequate membrane wicking and proper procedural technique.



REAGENTS

The test devices include anti-haemoglobin antibody coated pointer particles and haemoglobin antibodies coated on the membrane.

PRECAUTIONS

- For professional in vitro diagnostic use only
- For single use only
- Do not freeze any components of the test kit
- Do not use components after stated expiration date (see pouch and box label)
- Do not use test if pouch is damaged
- Do not eat, drink or smoke in the area where the specimens or kits are handled
- Handle all specimens as if they contained infectious agents
- Observe established precautions for microbiological risks throughout all procedures and standard guidelines for appropriate disposal of specimens
- Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are being tested
- Used testing materials should be discarded according to local regulations
- Humidity and high temperature can adversely affect results
- Bring all reagents to room temperature (15-30°C) before use
- Do not spill the specimens into the reaction area
- Do not touch the reaction area of the device to avoid contamination
- The test device should remain in the sealed pouch until use
- Interpret results after 5 minutes
- Store and transport the test device always at 2-30°C
- Do not mix reagents (e. g. test devices and buffer tubes) from different lots
- Avoid cross-contamination of specimens by using a new buffer tube for each specimen
- The extraction buffer solution contains small amounts sodium azide which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of these solutions always flush with copious amounts of water to prevent azide build-up

STORAGE AND STABILITY

The kit should be stored at 2-30°C. The test is stable through the expiry date printed on the sealed pouch. The test must remain in the sealed pouch until use. Do not freeze.
Care should be taken to protect components in this kit from contamination. Do not use if there is evidence of microbial contamination or precipitation. Biological contamination of dispensing equipment, containers or reagents can lead to false results.

MATERIALS

Materials Provided

- HEMOTRUST Test Devices, individually pouched
- Patient sets including buffer vial (with approx. 2 ml extraction buffer, contains very small amounts of sodium azide below the declaration limit)
- Package insert

Materials Required But Not Provided

- Timer

SPECIMEN COLLECTION AND PREPARATION

- The HEMOTRUST cassette is intended for analysis of human faecal specimens only.
- Patients should not collect specimens during their menstrual period, if they have bleeding haemorrhoids, blood in the urine, or if they experience obstructions or hard stool that may cause injuries.
- 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.
- No dietary restrictions are necessary before testing.
- If specimens are to be shipped, pack them in compliance with all applicable regulations for transportation of etiological agents.

Specimen collection:

1) Use a clean, dry container for specimen collection.
2) Unscrew the buffer tube at the blue end and remove the buffer tube applicator. Be careful not to spill or splatter solution from the tube. Collect specimens by inserting the applicator stick into at least 3 different sites of the faeces
3) Replace the applicator back into the tube and screw the cap tightly. Vigorously shake the buffer tube and make sure to mix thoroughly the specimen and extraction buffer. The buffered specimen is now ready to be stored, transported or tested.
4) The buffered specimen should be tested as soon as possible.

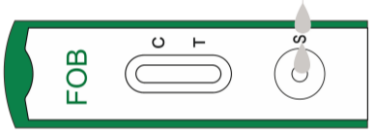
SPECIMEN STORAGE

Raw feces: Best results will be obtained if the assay is performed within 7 hours after collection at room temperature (15-30°C). Specimen collected may be stored for 4 days at 2-8°C if not tested within 7 hours.
Buffered specimen: Once prepared in the specimen collection tube, specimens may be stored for up to 4 days at 15-30°C or 6 months at -20°C if not tested within one hour.

DIRECTIONS FOR USE

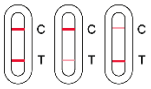
Bring tests, reagents, stool specimens, and/or external controls to room temperature (15-30°C) before testing.

1. Remove the test from its sealed pouch, and use it as soon as possible. For best results, the assay should be performed within one hour after opening the sealed pouch. Best results are obtained if test is performed immediately after opening the pouch. Mark the device with patient or control reference.
2. Unscrew the red cap of the buffer tube. Hold the tube vertically and dispense 2 drops (approx 80 µl) of solution into the specimen well of



the test device. **Avoid trapping air bubbles in the specimen well (S) and do not add any liquid to the reaction area.** Start the timer as the test starts to run.
3. As the test begins to run you will observe a coloured liquid migrate along the membrane of the reaction area.
4. Interpret results after 5 minutes. Do not interpret any results after more than 10 minutes.

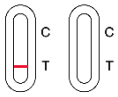
INTERPRETATION OF RESULTS



POSITIVE: 2 lines appear. One line appears in the control line area (C) and one line in the test line area (T). A positive result indicates that human haemoglobin has been detected.

NOTE: The intensity of colour in the test area (T) may vary depending on the concentration of haemoglobin present in the specimen. Therefore, any shade of colour in the test area (T) should be considered positive. Please note that this is a qualitative test only. The haemoglobin concentration cannot be determined with this test.

NEGATIVE: One line appears in the control line area (C). No line appears in the test line area (T). A negative result indicates that no haemoglobin is present in the specimen or that it is below the detection level of the test device.



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

QUALITY CONTROL

Internal Quality Control

An internal procedural control is included in the test. A red line appearing in the control area (C) is an internal positive procedural control. It confirms that sufficient specimen volume was used, and indicates an adequate membrane wicking and a proper procedural technique.

External Quality Control

It is recommended to perform a positive and negative external control for every kit, and as deemed necessary by internal laboratory procedures. External positive and negative controls are not supplied with the kit.

LIMITATIONS

1. The HEMOTRUST cassette is for professional in vitro diagnostic use only. The test should be used for the qualitative detection of human haemoglobin in faecal samples.
2. As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single rapid test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
3. Please note the reading time of max. 10 minutes, as a prolonged reading time may increase the test sensitivity.
4. Negative results do not exclude bleeding since some polyps and colorectal region cancers can bleed intermittently or not at all. Additionally, blood may not be uniformly distributed in faecal specimens. Colorectal polyps at an early stage may not bleed.
5. Urine and excessive dilution of specimens with toilet water may cause erroneous test results.
6. This test may be exhibit decreased sensitivity for upper gastrointestinal bleeding, as blood degrades as it passes through the gastrointestinal track.
7. Not all colorectal bleedings are due to precancerous or cancerous polyps. Please note that the presence of blood in stool specimens may be due to causes other than colorectal bleeding, such as haemorrhoids, blood in urine or stomach irritation.

The following non-cancer related factors may cause blood in faeces samples:

1) Iron:

Food supplementation with iron leads to increased release of blood in the colon. Iron itself is not cross-reacting with the test.[10]

2) Acetylsalicylic acid

ASA is main compound in a lot of drugs against headache (e.g. Aspirin® from Bayer), and is sometimes used to substitute Marcumar as a blood diluter. Almost always there are very small amounts of blood in faecal samples in case of healthy humans. This is far below the sensitivity of our test and has nothing to do with cancer or any other serious matter. However, if a patient takes blood diluters bleeding can be more intensive. Therefore the cut-off of HEMOTRUST cassette may be reached.[9]

3) Coumarin

Coumarin is used as a drug (e.g. Marcumar®) for prevention of heart attacks, against thrombosis and stroke. Similar to ASA, coumarin is a blood diluter. Almost always there are very small amounts of blood in faecal samples in case of healthy humans. This is far below the sensitivity of HEMOTRUST cassette and has nothing to do with cancer or any other serious matter. However, if a patient takes blood diluters, bleeding can be more intensive. Therefore the cut-off of HEMOTRUST cassette may be reached.

4) Haemorrhoids

Haemorrhoids may bleed. Therefore faecal sample may be contaminated with blood which is not associated with cancer.

5) Monthly period

Small amounts of blood released because of female´s period may contaminate the faecal sample. This is blood which is not associated with cancer.

6) Urine samples

Several diseases may cause blood in urine samples. To avoid detection of urine-related blood, stool sample should not get in contact with urine.

PERFORMANCE CHARACTERISTICS

Accuracy

The HEMOTRUST cassette has been compared to another leading commercially available rapid test using clinical specimens.

Method		**Other Rapid Test**		**Total**
Hemotrust	Results	Positive	Negative	
	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 human haemoglobin in faecal specimens as low as 6µg/g stool (=50 ng haemoglobin/mL buffer after extraction).

Intra-Assay-Precision

An Intra-Assay was performed using a series of 15 identical specimens each of 3 positive haemoglobin concentrations: 50ng/ml, 100ng/ml and 10µg/ml. The specimens were correctly identified in >99%.

Inter-Assay-Precision

An Inter-Assay was performed using a series of 15 identical specimens each of 3 positive haemoglobin concentrations: 50ng/ml, 100ng/ml and 10µg/ml. 3 different lots of HEMOTRUST cassettes were used. The specimens were correctly identified in >99%.

Cross-reactivity

The HEMOTRUST cassette is specific for human haemoglobin. The following compounds were diluted in extraction buffer at a concentration of 1.0 mg/ml and tested both with positive and negative controls without affecting the results: haemoglobin from cow, chicken, pork, goat, horse, rabbit and turkey.

LITERATURE

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.

SYMBOLS

	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
	Expiry	DIL	dilution buffer

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