

COMBI Hb-TRANSFERRIN FECAL

For *in Vitro* diagnostic use only

Immunochromatographic rapid test for the qualitative detection of human haemoglobin (hHb) and transferrin (hTf) in human faeces

I. INTRODUCTION AND INTENDED USE

The COMBI Hb-TRANSFERRIN FECAL is a rapid chromatographic immunoassay (non-invasive assay) for the qualitative detection of human haemoglobin and transferrin in human faeces specimens, which might be useful for the diagnosis of bleeding gastrointestinal disorders.

Colorectal cancer is cancer that occurs in the colon or rectum, and affects both men and women of all racial and ethnic groups, and is most often found in people aged 50 years or older. For men, colorectal cancer is the third most common cancer after prostate and lung cancers. For women, colorectal cancer is the third most common cancer after breast and lung cancers.

Fecal occult blood should be an important indicator in the diagnostic evaluation of patients with suspected gastrointestinal bleeding of any etiology, not just as an indication of colorectal cancer. The presence of human haemoglobin in faeces is inadequate as a screening test for stomach cancer (upper gastrointestinal disorders), because of human haemoglobin derived from the upper digestive tract is broken down in the intestinal tract (the antigenicity is lost).

Detection of fecal transferrin, which is more stable in stool than haemoglobin, provides an alternative way of diagnosing the disease in the upper digestive tract.

Blood in the stool may be the only symptom of cancer, but not all blood in the stool is caused by cancer. Other conditions that can cause blood in the stool include: Haemorrhoids, Anal fissures, Colon polyps, Peptic ulcers, Ulcerative colitis. Gastroesophageal reflux disease (GERD). Crohn's disease, use of non-steroidal anti-inflammatory drugs (NSAIDs).

II. PRINCIPLE OF THE TEST

The COMBI Hb-TRANSFERRIN FECAL is a qualitative immunoassay for the detection of human haemoglobin and human transferrin in faeces samples. The membrane is pre-coated with monoclonal antibodies against human haemoglobin and human transferrin on the test lines region. During testing, the sample reacts with the particles coated with anti-human haemoglobin and anti-human transferrin antibodies which were pre-dried on the test strip. The mixture moves upward on the membrane by capillary action. In the case of a positive result the specific antibodies present on the membrane will react with the mixture conjugate and generate coloured lines. A green coloured band always appears in the control line and serves as verification that sufficient volume was added, that proper flow was obtained and as an internal control for the reagents.

III. REAGENTS AND MATERIALS

Each kit contains:

1. COMBI Hb-TRANSFERRIN FECAL (25 card)
2. Extraction tubes with buffer (1mL x 25 tubes)
3. Instruction for use (1)

Required materials (not supplied)

Specimen collection container, disposable gloves, plastic pipette and timer.

IV. SPECIAL PRECAUTIONS

- For professional *in vitro* diagnostic use only.
- Do not use after expiration date.
- The test should remain in the sealed pouch until use.
- Do not use the test if pouch is damaged.
- Follow Good Laboratory Practices, wear protective clothing, use disposal gloves, do not eat, drink or smoke in the area.
- All the specimens should be considered potentially hazardous and handled in the same manner as an infectious agent.
- The test should be discarded in a proper biohazard container after testing.
- The test must be carried out within 2 hours of opening the sealed bag.

V. STORAGE AND STABILITY

Store as packaged in the sealed pouch either at refrigerated or room temperature (2-30°C/36-86°F). The test is stable through the expiration date printed on the sealed pouch. The test must remain in the sealed pouch until use. Do not freeze.

VI. SPECIMENS COLLECTION FOR STOOL SAMPLES

Collect sufficient quantity of faeces (1-2 g or mL for liquid sample). Stool samples should be collected in clean and dry containers (no preservatives or transport media). The samples can be stored in the refrigerator (2-4°C/36-40°F) for 1-2 days prior to testing. For longer storage the specimen must be kept frozen at -20°C/4°F. In this case, the sample will be totally thawed, and brought to room temperature before testing.

VII. PROCEDURE FOR STOOL SAMPLES

To process the collected stool samples

Use a separate tube with extraction buffer for each sample. Introduce the stick two times into the faecal specimen to pick up a little sample (150mg) and put into the testing tube with buffer. Shake the testing tube in order to assure good sample dispersion. For liquid stool samples, aspirate the faecal specimen with a dropper and add 150µL into the testing tube with buffer.

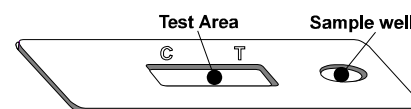
Test Procedure

Allow the tests, stool samples and buffer to reach to room temperature (15-30°C/59-86°F) prior to testing. Do not open pouches until ready to perform the assay.

1. Remove the card from its sealed pouch and use it as soon as possible.
2. Use a separate device for each sample.
3. Extract some liquid from the topside with a dropper. Dispense 4 drops or 100µL into the specimen well. Start the timer.
4. Read the result at **10 minutes** after dispensing the sample.

VIII. INTERPRETING THE RESULTS

Tf positive: Two lines appears across the central window, in the result line region (red test line marked with the letter T) and in the control line region (green control line marked with the letter C). A Transferrin (Tf) positive result could be indicative of upper gastrointestinal bleeding.

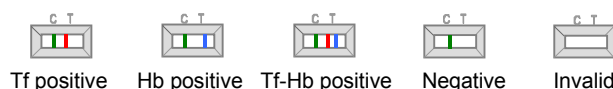


Hb positive: Two lines appears across the central window, in the result line region (blue test line marked with the letter T) and in the control line region (green control line marked with the letter C). The result Haemoglobin (Hb) positive is indicative of little blood in faeces; transferrin exists in only trace amounts in blood and could be not detected.

Tf-Hb positive: Three lines appears across the central window, in the result line region two lines (red test line and blue test line marked with the letter T) and in the control line region (green control line marked with the letter C). Transferrin (Tf) and Haemoglobin (Hb) positive result could be indicative of lower gastrointestinal bleeding disorders.

NEGATIVE: Only one GREEN control band appears across the central window in the site marked with the letter C (control line). Not occult blood in faeces.

INVALID: A total absence of the green control coloured band regardless the appearance or not of the red test line. Note: Insufficient specimen volume, incorrect procedural techniques or deterioration of the reagents 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 and contact your local distributor.



The intensity of the blue or red coloured band in the result line region (T) will vary depending on the concentration of human haemoglobin or human transferrin in the specimen. However, neither the quantitative value, nor the rate of increase in haemoglobin/transferrin can be determined by this qualitative test.

IX. INTERNAL QUALITY CONTROL

Internal procedural controls are included in the test. A GREEN line appearing in the control region (C) is an internal control. It confirms sufficient specimen volume and correct procedural technique.

X. PERFORMANCE

A. Expected Values

Common causes of Upper GI bleeding: duodenal ulcer (20-30%), gastric or duodenal erosions (20-30%), varices (15-20%), gastric ulcer (10-20%), erosive esophagitis (5-10%), angioma (5-10%), arteriovenous malformation (<5%), gastrointestinal stromal tumours.

Common causes of Lower GI bleeding (percentages vary with the age group sampled): anal fissures, angiodysplasia (vascular ectasia), colitis (radiation, ischemic, infectious), colonic carcinoma, colonic polyps, diverticular disease, inflammatory bowel disease: ulcerative, proctitis/colitis, Crohn's disease, internal haemorrhoids.

B. Sensitivity and Specificity

The detection limit of COMBI Hb-TRANSFERRIN FECAL is 5.1ug/g faeces for human haemoglobin and 0.4ug/g faeces for human transferrin.

COMBI Hb-TRANSFERRIN FECAL was highly specific (>99%) and also highly sensitive (>99%) compared with the results of that guaiac assay.

The detection of hHb with COMBI Hb-TRANSFERRIN FECAL showed >99% of sensitivity and >99% of specificity compared with others commercial rapid tests (ImmunoTech OcculTech and Human Hexagon OBTI).

C. Cross-Reactivity and interferences

It was performed an evaluation to determine the cross reactivity and interferences of COMBI Hb-TRANSFERRIN FECAL. There is not cross reactivity with common gastrointestinal pathogens, other organisms and substances occasionally present in feces.

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|--------------|--------------------------|---------------------------|--------------|
| - Rotavirus | - <i>Campylobacter</i> | - <i>Escherichia coli</i> | - Adenovirus |
| - Astrovirus | - <i>Giardia lamblia</i> | - Lactoferrin | - |

No special diet is recommended prior to testing. There are not interferences with any foods (vitamin C, broccoli, carrots...) and supplements (iron).

XI. LIMITS OF THE KIT

- COMBI Hb-TRANSFERRIN FECAL will only indicate the presence of human haemoglobin or/and transferrin in the specimen (qualitative detection) and should be used for the detection of haemoglobin and transferrin in faeces specimens only. Neither the quantitative value nor the rate of increase in haemoglobin or transferrin concentration can be determined by this test.
- An excess of sample could cause wrong results (brown bands appear). Dilute the sample with the buffer and repeat the test.
- Some stool samples can decrease the intensity of the control line.
- In the case of patients have bleeding haemorrhoids, blood in urine, strained during bowel movement or women during their menstrual period, should be not collect samples.
- Positive results confirm the presence of occult blood in faecal samples; nevertheless, it can be due to several causes, besides colorectal bleeding, such as haemorrhoids, anal fissures, colon polyps, peptic ulcers, ulcerative colitis, gastroesophageal reflux disease (GERD), Crohn's disease. Positive results should be followed up with additional diagnostic procedures by a physician to determine the exact cause and source of the blood in the stool. Endoscopy is the method of choice in diagnosing the cause of upper and lower gastrointestinal bleeding.
- Negative results do not exclude bleeding since some polyps and colorectal cancers may bleed intermittently or not at all during certain stages of the disease. Additionally, blood may not be uniformly distributed in stool samples

XII. REFERENCES (See Italian version)

IVD	In Vitro Diagnostic Medical Device	Temperature limitation	LOT	Batch code (EXXX)	Manufacturer	Keep dry	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 (25 tests)

COMBI Hb-TRANSFERRIN FECAL
Extraction tubes with Buffer
Instruction for use

Ref. VT81650

25 Device (Card)
25 tubes x 1 mL
1 item

EDMA Code 12 70 03 90 00

