Helena ColoScreen® iFOBT

INTENDED USE
The Helena ColoScreen Immunochemical Fecal Occult Blood Test (iFOBT) is an immunochemical device intended for the qualitative detection of Fecal Occult Blood by laboratories or physicians offices. It is useful in determining gastrointestinal (GI) bleeding found in a number of gastrointestinal disorders, such as: diverticulitis, colitis, polyps, and colorectal cancer. This test is recommended for use in: 1) routine physical examinations or when hospital patients are first admitted, 2) hospital monitoring for GI bleeding in patients, and 3) screening for colorectal cancer or gastrointestinal bleeding.

SUMMARY AND EXPLANATION
The American Cancer Society and Centers for Disease Control recommend an occult blood feces test annually after age 50 to aid in the early detection of colorectal cancer.[1] Three types of assays for FOB testing are commercially available: 1) Guaiac Dye; 2) Hemoporphyrin; and, 3) Immunochemical.

The Guaiac test is widely available but lacks high accuracy. Guaiac is a naturally occurring phenolic compound that can be oxidized to quinone by hydrogen peroxidases with a detectable color change. The sensitivity and specificity of Guaiac tests are much lower than those of Hemoporphyrin tests and Immunochemical assays. The low accuracy of the Guaiac Dye method is related to dietary peroxidases, including hemoglobin and myoglobin from meat and uncooked fruits and vegetables. Non-cancerous gastrointestinal tract bleeding and iron intake may also cause false positive results from Guaiac test.[2]

The Hemoporphyrin test is not affected by dietary peroxidases, but false-positive results can occur in patients with upper gastrointestinal bleeding disorders such as gastric or duodenal ulcers because porphyrins are not broken down by stomach acids.[2]

The Helena immunochemical FOB rapid test is much more sensitive and has been designed to specifically detect low levels of human fecal occult blood. It is highly accurate for human hemoglobin (hHb) compared to the Guaiac and Hemoporphyrin methods. The results of immunochemical FOB rapid tests are not affected by dietary peroxidases, animal blood and ascorbic acid. A Japanese study demonstrated using immunochemical FOB tests reduced mortality by 60%.[3]

PRINCIPLE OF THE PROCEDURE
This assay is a one-step lateral flow chromatographic immunoassay. The test strip consists of: 1) a burgundy colored conjugate pad containing mouse anti-hHb antibodies conjugated with colloidal gold and 2) a nitrocellulose membrane strip containing a Test line (T-line) and a Control line (C-line). The T-line is coated with anti-hHb antibodies, and the C-line is coated with goat anti-mouse IgG antibodies.

When an adequate volume of test specimen is dispensed into the sample well of the device, the test specimen migrates by capillary action across the test strip. If the concentration of hHb in the specimen is at or above 50 ng/ml, the T-line appears as a visible burgundy line. If the concentration of hHb in the specimen is below the detectable level, no T-line develops.

The C-line is coated with goat anti-mouse antibody, which binds to the conjugated monoclonal antibody, regardless of the presence of hHb in the sample.

REAGENTS AND MATERIALS SUPPLIED
ColoScreen iFOBT Collection Tubes Cat. No. 5061
30 fecal collection tubes, each with 2mL FOB buffer (1x PBS with 0.02% sodium azide)
ColoScreen iFOBT Test Cassettes Cat. No. 5062
30 test devices (cassettes), each sealed in a foil pouch
ColoScreen iFOBT Mailers Cat. No. 5063
30 mailers, each with specimen pouch, collection paper, and Patient Instructions
ColoScreen iFOBT Office Pack Cat. No. 5064
30 fecal collection tubes, each with 2mL FOB buffer (1x PBS with 0.02% sodium azide)
30 test devices (cassettes), each sealed in a foil pouch
30 mailers, each with specimen pouch, collection paper, and Patient Instructions

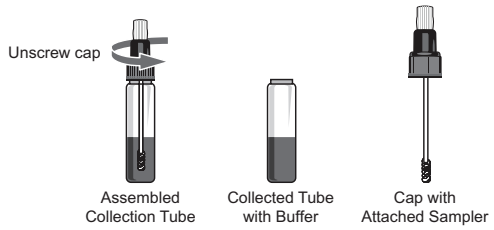
MATERIALS REQUIRED BUT NOT PROVIDED
1. Timer
2. An absorbent cloth or tissue (preferably disposable) or a clean disposable cup

PRECAUTION
1. This kit is for *in-vitro* diagnostic use only.
2. Do not use expired kit components.
3. Treat all specimens and used assay materials as if they are infectious.
4. Dispose of all used test components in a biohazard container, per clinical lab procedures.

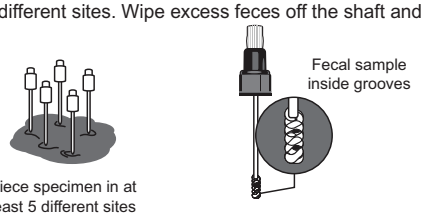
STORAGE
The test device is stable when stored in a controlled environment at 15-30°C (59-86°F) for up to 2 years or until the expiration date printed on the label, whichever comes first. Do not expose the kit components to temperatures over 30°C (86°F).

PATIENT LIMITATIONS
1. A specimen should not be collected from a patient with the following conditions that may interfere with the test results:
   - Menstrual bleeding
   - Bleeding hemorrhoids
   - Constipation bleeding
   - Urinary bleeding
2. Alcohol and certain medications such as aspirin, indomethacin, reserpine, phenylbutazone, corticosteroids and nonsteroidal anti-inflammatory drugs may cause gastrointestinal irritation and subsequent bleeding in some patients.

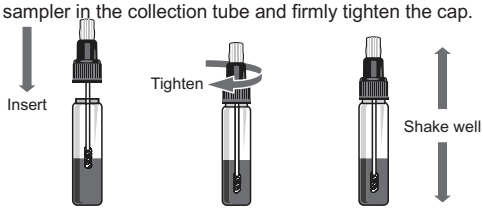
SPECIMEN COLLECTION
1. The specimen used in this assay is feces. It may be collected from toilet paper or caught in a clean cup. Avoid contact with toilet water.
2. Unscrew the cap (with the attached sampler) of the collection tube.



3. Randomly pierce the fecal specimen with the threaded end of the sampler in at least five (5) different sites. Wipe excess feces off the shaft and outer grooves.

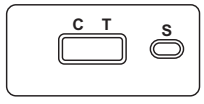


4. Insert sampler in the collection tube and firmly tighten the cap.



5. Shake the tube well to mix the specimen and the FOB buffer.
NOTE: Samples collected may be stored at least eight (8) days at ambient temperatures below 35°C (95°F), six (6) months at 2-8°C (36-46°F) and two (2) years at ≤ -20°C (≤ -4°F).

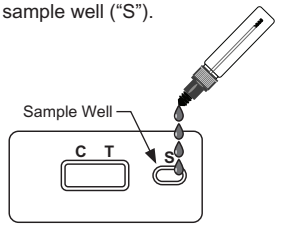
ASSAY PROCEDURE
1. Refrigerated specimens or other materials, including the test cassette, **must be equilibrated to room temperature before testing.**
2. Remove the test cassette from its pouch and place it on a flat surface. Label the device with appropriate identification.



3. Holding the collection tube upright, unscrew the clear tip cover.

ASSAY PROCEDURE, CONTINUED

4. Squeezing the collection tube, dispense **four drops** of the FOB buffer in the collection tube into the sample well ("S").

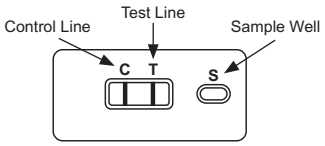


5. Read the result within **5-10 minutes** after adding the FOB buffer.
   IMPORTANT: Do not read the test results after ten (10) minutes.

INTERPRETATION

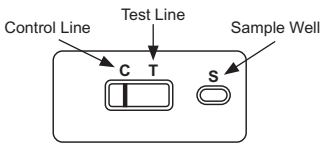
POSITIVE:

If both C-line and T-line are present, the result is positive. A positive result indicates the level of hHb in the specimen is over 50 ng hHb/mL FOB buffer or 50 µg hHb/g feces.



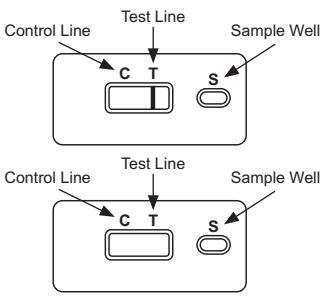
NEGATIVE:

If only the C-line develops in the control region of the test strip, the result is negative. A negative result indicates the hHb in the specimen is below 50 ng/mL.



INVALID:

If no C-line appears within 5 minutes, the result is invalid and the assay should be repeated with a new device. **NOTE: The test line may or may not be present. However, the absence of a control line indicates an invalid test.**



QUALITY CONTROL

- **Internal Quality Control**
  This device contains a built-in control feature, the Control line (C-line). The presence of this C-line indicates that an adequate sample volume was used and that the reagents migrated properly. If a C-line does not form, the test is considered invalid. In this case, review the entire procedure and repeat the testing with a new device.
- **External Quality Control**
  Operators should always follow the appropriate federal, state, and local guidelines concerning the running of external quality controls, including positive and negative, to assure the proper performance of the device.

LIMITATIONS OF THE PROCEDURE

1. Results cannot be considered conclusive evidence of the presence or absence of gastrointestinal bleeding or pathology. A positive result should be followed up with additional diagnostic procedures to determine the exact cause and source of the occult blood in the feces.
2. A negative result can be obtained even when a gastrointestinal disorder is present. For example, some polyps and colorectal cancers may bleed intermittently or not at all during certain stages of the disease.
3. False negative results may occur when occult blood is not uniformly distributed throughout the bowel movement and the formation of a fecal sample. Repeat testing is recommended if a pathological condition is suspected.

PERFORMANCE CHARACTERISTICS

1. Sensitivity

The sensitivity of the test is 50 ng hHb/mL buffer or 50 µg hHb/g feces.

2. Accuracy

• *Reference laboratory and Physicians Office Laboratory (POL) Studies*

One hundred (100) hHb-free feces extraction specimens collected in-house were divided into 5 groups of 20 each. The five groups of extraction samples were spiked with hHb for five different concentrations, respectively: 0, 37.5 ng hHb/mL, 50 ng hHb/mL, 62.5 ng hHb/mL, and 2000 ng hHb/mL. Those specimens were blind labeled and tested with the Helena Fecal Occult Blood Rapid Test at three (3) Physicians Office Laboratories and a Reference Laboratory.

The results obtained from the three POL sites by persons with diverse education background and work experiences agreed 97.7% (average) with the expected results. The results obtained from the Reference Laboratory agreed 99% with that expected. Overall, the accuracy of the Helena Fecal Occult Blood Rapid Test is 98%.

• *Comparison studies*

Those 100 specimens were also tested in house with the Helena Fecal Occult Blood Rapid Test and a predicate device. The correlation between the Helena Fecal Occult Blood Test and the predicate device was over 95%.

3. Specificity

The Helena Fecal Occult Blood Rapid Test is specific to human hemoglobin. The following substances, when spiked in both positive and negative specimens, did not interfere the test results.

Substance	Concentration (µg/mL)
Beef Hemoglobin	2,000
Chicken Hemoglobin	500
Fish Hemoglobin (meat extract)	100
Horse Hemoglobin	500
Goat Hemoglobin	500
Pig Hemoglobin	500
Rabbit Hemoglobin	500
Sheep Hemoglobin (meal extract)	100
Horseradish Peroxidase	20,000
Red radish	Aqueous extract
Raw turnip	Aqueous extract
Cauliflower	Aqueous extract
Broccoli	Aqueous extract
Parsnip	Aqueous extract
Cantaloupe	Aqueous extract
Vitamin C (ascorbic acid)	Dietary supplement
Iron	Dietary supplement

REFERENCES

1. American Cancer Society, Inc. Cancer Reference Information: Can Colon and Rectum Cancer Be Found Early? [Online] Available: http://www.cancer.org
2. Allison JB, Takawa IS, Ransom LJ, Adrian AL. A comparison of fecal occult blood tests for colorectal-cancer screening, N Engl J Med 1996; 334: 155-159.
3. Saito H. Screening for colorectal cancer by immunochemical fecal occult blood testing (Review). Jpn J Cancer Res 1996; 87: 1011-1024.

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