

**In vitro Diagnostic
Catalog Number R2011C**

INTENDED USE

The FOB Hi Rapid Test is an immunochemical device intended for the qualitative detection of fecal occult blood to be used in laboratories or physicians offices. It is a useful aid to detect bleeding caused by a number of gastrointestinal disorders, e.g., diverticulitis, colitis, polyps, and colorectal cancer. Fecal occult blood tests are recommended for use in 1) routine physical examinations, 2) routine hospital testing, 3) screening for colorectal cancer or gastrointestinal bleeding from any source.

SUMMARY AND EXPLANATION OF THE TEST

The American Cancer Society and Centers for Disease Control recommended an occult blood feces test annually after age 50 to aid in the early detection of colorectal cancer⁽¹⁾. Three types of FOB tests are commercially available: 1) Guaiac Dye; 2) Hemoporphyrin; and, 3) immunochemistry.

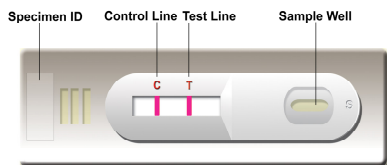
The Guaiac test is widely used but lacks high accuracy. The Guaiac dye is a naturally occurring phenolic compound that can be oxidized to quinone by hydrogen peroxidase activity of hHb with a detectable color change. The sensitivity and specificity of Guaiac tests are much lower than those of Hemoporphyrin test and immunochemical assays. The low accuracy of the Guaiac Dye test is related to dietary peroxidases, including hemoglobin 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 FOB Hi Rapid Test is 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 that immunochemical FOB screening test reduced mortality of colorectal cancer by 60%(3).

TEST PRINCIPLE

The FOB Hi Rapid Test is a sandwich lateral flow chromatographic immunoassay. The test cassette consists of: 1) a burgundy colored conjugate pad containing monoclonal anti-hHb antibody conjugated with colloid gold (anti-hHb conjugates) and 2) a nitrocellulose membrane strip containing a test band (T band) and a control band (C band). The T band is pre-coated with another monoclonal anti-hHb antibody, and the C band is pre-coated with goat anti-mouse IgG antibody.



When an adequate volume of test specimen is dispensed into the sample well of the test cassette, the specimen migrates by capillary action across the cassette. hHb if present in the specimen at or higher than 25 ng/mL will bind to the anti-hHb conjugates. The immunocomplex is then captured on the membrane by the pre-coated antibody, forming a burgundy colored T band, indicating a FOB positive test result. Absence of this band suggests that the concentration of hHb in the specimen is below the detectable level, indicating a FOB negative result.

Absence of the T band suggests a negative result. The test contains an internal control (C band) which should exhibit a burgundy colored band of the immunocomplex of goat anti-mouse IgG/mouse IgG-gold conjugate regardless of the color development on the T band. Otherwise, the test result is invalid and the specimen must be retested with another device.

REAGENTS AND MATERIALS PROVIDED

- Each package contains 25 test devices, each sealed in a foil pouch with two items inside:
 - One cassette test device.
 - One desiccant.
- 25 Sample extraction tubes, each containing 1 mL of extraction buffer.
- One package insert (instruction for use).

MATERIALS REQUIRED AND AVAILABLE FOR PURCHASE

- Positive Control (1 vial, red cap, 1 mL)
- Negative Control (1 vial, green cap, 1 mL)

MATERIALS REQUIRED BUT NOT PROVIDED

- Clock/watch or Timer
- A container to hold fecal specimen

WARNINGS AND PRECAUTIONS

For in Vitro Diagnostic Use

- This package insert must be read completely before performing the test. Failure to follow the insert gives inaccurate test results.
- Do not open the sealed pouch, unless ready to conduct the assay.
- Do not use any kit components beyond their stated expiration date.
- Do not use the components in any other type of test kit as a substitute for the components in this kit.
- Bring all reagents to room temperature (15°C-30°C) before use.
- Do not use specimen with visible blood for the testing.**
- Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
- Users of this test should follow the US CDC Universal Precautions for prevention of transmission of HIV, HBV and other blood-borne pathogens.
- Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
- Extraction buffer contains 0.1% NaN₃. Avoid contact with skin or eyes. Do not ingest.
- Dispose of all specimens and materials used to perform the test as biohazardous waste.
- The testing results should be read within 10 minutes after a specimen is applied to the sample well of the device. Read result after 10 minutes may give erroneous results.
- Do not perform the test in a room with strong air flow, ie. electric fan or strong air-conditioning.

REAGENT PREPARATION AND STORAGE INSTRUCTIONS

All reagents are ready to use as supplied. Store unused test devices unopened at 2°C-30°C. The positive and negative controls should be kept at 2°C-8°C. If stored at 2°C-8°C, ensure that the test device is brought to room temperature before opening. The test device is stable through the expiration date printed on the sealed pouch. Do not freeze the kit or expose the kit over 30°C.

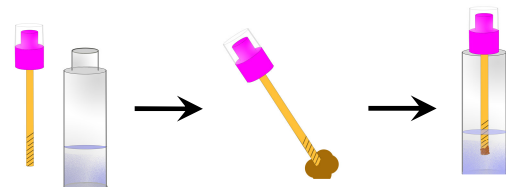
PATIENT PREPARATION

- A specimen should not be collected from a patient with following conditions that may interfere with the test results:
 - Menstrual bleeding
 - Bleeding hemorrhoids
 - Constipating bleeding
 - Urinary bleeding
- Dietary restrictions are not necessary.
- Alcohol and certain medications such as aspirin, indomethacin, phenylbutazone, reserpine, cortocosteroids, and nonsteroidal anti-inflammatory drugs may cause gastrointestinal irritation and subsequent bleeding, thus gives positive reactions. On the advice of the physician, these medicines might be temporarily discontinued for 7 days prior to and during the test period.

SPECIMEN COLLECTION AND HANDLING

Consider any materials of human origin as infectious and handle them using standard biosafety procedures.

- Collect a random sample of feces in a clean, dry receptacle.
- Unscrew the top of the collection tube and remove the applicator stick.
- Randomly pierce the fecal specimen in at least five (5) different sites.
- Remove excess sample off the shaft and outer grooves. Be sure sample remains on inside grooves.
- Replace the stick in the tube and tighten securely.



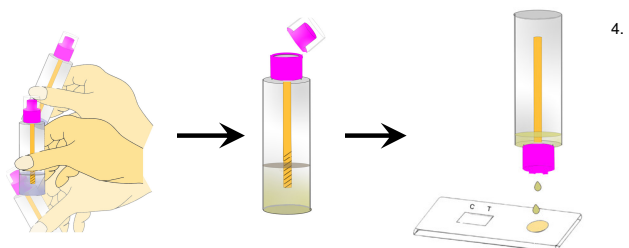
The specimen is now ready for testing, transportation or storage.

Note: Specimens collected may be stored at least eight (8) days at room temperature below 35°C, six (6) months at 2°C -8°C, and two (2) years at ≤20°C.

TEST PROCEDURE

- Step 1: Bring the specimen and test components to room temperature if refrigerated or frozen.
- Step 2: When ready to test, open the pouch at the notch and remove the test strip. Place the test strip on a clean, flat surface.
- Step 3: Shake the sample collection tube vigorously to ensure an effective liquid suspension.

Step 4: Hold the tube upright, snap off the tip. Dispense 2 drops of the solution into the sample pad (s) of the strip. Do not over load samples.



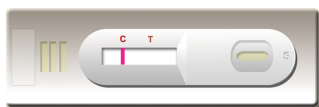
Step 5: Set up clock/watch or timer.

Step 6: Results can be read in 10 minutes after adding the specimen. Positive results can be visible in as short as 1 minute.

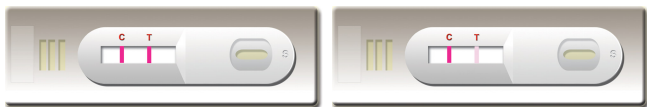
Don't read results after 10 minutes. To avoid confusion, discard the test device after interpreting the result.

INTERPRETATION OF ASSAY RESULT

1. **NEGATIVE RESULT:** If only the C band is developed, the test indicates that the hHb in the specimen is below 25 ng/mL FOB buffer. The result is negative.



2. **POSITIVE RESULT:** If both C and T bands are developed, the test indicates that the concentration of hHb in the specimen is equal or higher than 25 ng hHb/mL FOB buffer or 25 µg hHb/g feces. The result is positive.



Samples with positive results should be confirmed with alternative testing method(s) and clinical findings before a positive determination is made.

3. **INVALID:** If no C band is developed, the assay is invalid regardless of color development on the T band as indicated below. Repeat the assay with a new device.



PERFORMANCE CHARACTERISTICS

Sensitivity

The analytical sensitivity of the test is 25 ng hHb/mL buffer or 25 µg hHb/g feces.

Specificity

The FOB Hi Rapid Test is specific to human hemoglobin. The following substances, when spiked in both positive and negative specimens, did not interfere with the test results.

Substances	Concentration
Chicken Hemoglobin	100 µg/mL
Pork Hemoglobin	100 µg/mL
Beef Hemoglobin	100 µg/mL
Goat Hemoglobin	100 µg/mL
Horse Hemoglobin	100 µg/mL
Sheep Hemoglobin	100 µg/mL

Reproducibility

Known positive samples were tested in multiple assays and identically positive results were observed. Similarly, known negative samples produced negative results when tested in multiple assays.

LIMITATIONS OF THE TEST

- The Assay Procedure and the Test Result Interpretation must be followed closely when testing the presence of occult blood in feces. Failure to follow the procedure may give inaccurate results.
- The FOB Hi Rapid Test is to aid in diagnosis and is not intended to replace other diagnostic procedures such as G.I. fibroscope, endoscopy, colonoscopy, or X-ray analysis. Test results should not be deemed conclusive with respect to 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 for the occult blood in the feces.

- 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.
- If the symptom persists, while the result from FOB Hi Rapid Test is negative, it is recommended to re-test the specimen with an alternative test device.

REFERENCES

- America Cancer Society, Inc. Cancer Reference Information: Can Colon and Rectum Cancer be Found Early? (Online) Available: <http://www.cancer.org>
- Allison JB, Takawa IS, Ransom LJ, Adrian AL. A comparison of fecal occult blood tests for colorectal –cancer screening. N. Eng. J. Med. 1996; 334:155-159.
- 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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