OnSite™ FOB-Hi Rapid Test

REF R2011C CE

INTENDED USE

The *OnSite* FOB-Hi Rapid Test is a lateral flow chromatographic immunoassay for the qualitative detection of fecal occult blood in human fecal specimens in laboratories or physician offices. It is intended to be used by professionals as a screening test and provides a preliminary test result to aid in the detection of bleeding caused by a number of gastrointestinal disorders, e.g. diverticulitis, colitis, polyps, and colorectal cancer.

Any use or interpretation of this preliminary test result must also rely on other clinical findings and the professional judgment of health care providers. Alternative test method(s) should be considered to confirm the test result obtained by this device.

SUMMARY AND EXPLANATION OF THE TEST

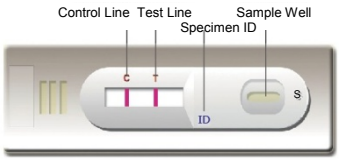
The American Cancer Society and Centers for Disease Control recommend an occult blood feces test annually after age 50 to aid in the early detection of colorectal cancer[1].Two types of FOB tests are commercially available: guaiac dye tests and immunochemical tests (iFOBT).

The guaiac tests are widely used but lack accuracy. The guaiac dye is a naturally occurring phenolic compound that can be oxidized to quinone by hydrogen peroxidase activity of human hemoglobin (hHb) resulting in a detectable color change. The sensitivity and specificity of guaiac tests are much lower than those of immunochemical assays. The low accuracy of the guaiac tests 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 with guaiac tests[2].

Immunochemical tests are highly accurate for the detection of hHb compared to the guaiac method. The results of immunochemical FOB tests (iFOBT) are not affected by dietary peroxidases, animal blood or ascorbic acid. A Japanese study demonstrated that iFOB screening tests reduced mortality of colorectal cancer by 60%[3].

The *OnSite* FOB-Hi Rapid Test is an iFOBT designed to specifically detect low levels of human fecal occult blood. It can be performed within 10 minutes by minimally skilled personnel without the use of laboratory equipment.

TEST PRINCIPLE



The *OnSite* FOB-Hi Rapid Test is a lateral flow chromatographic immunoassay. The test strip in the 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 line (T line) and a control line (C line). The T line is pre-coated with another monoclonal anti-hHb antibody, and the C line is pre-coated with a control line 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 by the pre-coated reagent forming a burgundy colored T line, indicating a FOB positive test result.

Absence of the T line suggests a negative result. Each test contains an internal control (C line) which should exhibit a burgundy colored line of the immunocomplex of the control line antibodies regardless of the color development on the T line. If the C line does not develop, the test result is invalid, and the specimen must be retested with another device.

REAGENTS AND MATERIALS PROVIDED

1. Individually sealed foil pouches containing:
   a. One cassette test device
   b. One desiccant
2. Stool collection devices, each containing 2 mL of sample extraction buffer (REF SB-R2011)
3. Patient ID stickers
4. One package insert (instruction for use)

MATERIALS MAY BE REQUIRED AND AVAILABLE FOR PURCHASE

1. *Positivia* FOB Rapid Test Control Kit (Cat # C2011) contains positive control and negative control.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Clock or timer
2. A container to hold fecal specimen

WARNINGS AND PRECAUTIONS

For *in Vitro* Diagnostic Use

1. This package insert must be read completely before performing the test. Failure to follow the instructions may give inaccurate test results.
2. Do not open the sealed pouch unless ready to conduct the assay.
3. Do not use any kit components beyond their stated expiration date.
4. Do not use the components in any other type of test kit as a substitute for the components in this kit.
5. Bring all reagents to room temperature (15-30°C) before use.
6. **Do not scoop fecal specimen as this may lead to excess fecal specimen that may block the sample well and result in an invalid test result.**
7. **Do not use specimens for testing if blood is visible.**
8. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
9. Users of this test should follow the US CDC Universal Precautions for bio-safety.
10. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
11. Dispose of all specimens and materials used to perform the test as bio-hazardous waste.
12. The testing results should be read 10 minutes after a specimen is applied to the sample well of the device. Any results interpreted outside 10 minutes should be considered invalid and must be repeated.
13. Do not perform the test in a room with strong air flow, i.e. 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 2-30°C. The positive and negative controls should be kept at 2-8°C. If stored at 2-8°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 to temperature above 30°C.

PATIENT PREPARATION

1. Specimens should not be collected from patients with the following conditions which may interfere with the test results:
   - Menstrual bleeding
   - Bleeding hemorrhoids
   - Constipating bleeding
   - Urinary bleeding
2. Dietary restrictions are not necessary.
3. Alcohol and certain medications such as aspirin, indomethacin, phenylbutazone, reserpine, corticosteroids, and nonsteroidal anti-inflammatory drugs may cause gastrointestinal irritation and subsequent bleeding, and produce positive reactions. On the advice of a physician, these medicines may be temporarily discontinued for 7 days prior to and during the test period.

SPECIMEN COLLECTION AND HANDLING

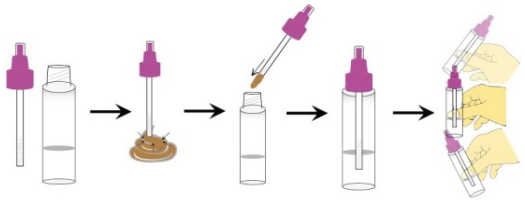
Consider any materials of human origin as infectious and handle them using standard bio-safety procedures.

Step 1: Collect a random sample of feces in a clean, dry receptacle.

Step 2: Label the stool collection device with the specimen's ID number (patient ID sticker). Open the stool collection device by unscrewing the top and use the collection stick to randomly pierce the stool specimen in at least five different sites. ***Do not scoop stool specimen. Ensure that stool specimen is only in the grooves of the collection stick. Excess stool specimen may lead to an invalid test result.***

Step 3: Replace the collection stick in the tube and tighten securely to close the sample extraction tube.

Step 4: Shake the stool collection device vigorously to extract the hHb in the specimen.



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

Note: It is recommended to test the specimen immediately after extraction. If not tested immediately, the extracted specimen may be stored at room temperature (20-37°C) for up to 10 days or at 2-8°C for up to 21 days. For longer storage, the extracted specimen may be frozen at -20°C. Avoid multiple freeze-thaw cycles.

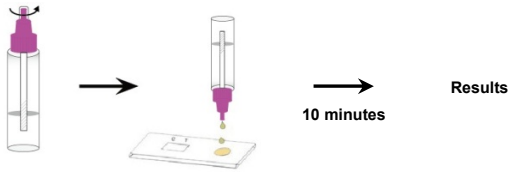
TEST PROCEDURE

Step 1: Bring the specimen and test components to room temperature if refrigerated or frozen. Once the specimen is thawed, mix well prior to performing the assay.

Step 2: When ready to test, open the pouch at the notch and remove device. Place the test device on a clean, flat surface.

Step 3: Shake the stool collection device vigorously to ensure an homogenous liquid suspension.

Step 4: Hold the stool collection device vertically. Twist off the tip. Dispense 2 drops (70-90 µL) of the solution into the sample well of the cassette. Do not overload samples.



Step 5: Set up timer.

Step 6: Results can be read at 10 minutes. Positive results can be visible in as short as 1 minute. Negative results must be confirmed at the end of the 10 minutes only. ***However, any results interpreted outside 10 minutes should be considered invalid and must be repeated. Discard used device after interpreting the result following local laws governing the disposal of device.***

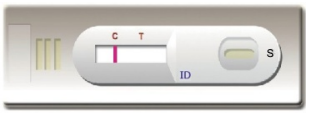
QUALITY CONTROL

1. **Internal Control:** This test contains a built-in control feature, the C line. The C line develops after adding sample. If the C line does not develop, review the whole procedure and repeat test with a new device.

2. **External Control:** Good Laboratory Practice recommends using external controls, positive and negative, to assure the proper performance of the assay, particularly under the following circumstances:
   a. A new operator uses the test kit, prior to performing testing of specimens.
   b. A new lot of test kits is used.
   c. A new shipment of test kits is used.
   d. The temperature during storage of the kit falls outside of 2-30°C.
   e. The temperature of the test area falls outside of 15-30°C.
   f. To verify a higher than expected frequency of positive or negative results.
   g. To investigate the cause of repeated invalid results.

INTERPRETATION OF ASSAY RESULTS

1. **NEGATIVE RESULT:** If only the C line develops, the test indicates that the concentration of hHb in the sample is below 25 ng/mL in buffer. The result is negative or non-reactive.



2. **POSITIVE RESULT:** In addition to the presence of the C line, if the T line develops, the test indicates that the concentration of hHb in the sample is equal to or higher than 25 ng/mL in buffer. The result is FOB-Hi positive or reactive.

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

3. **INVALID:** If no C line develops, the assay is invalid regardless of any color development on the T line as indicated below. Repeat the assay with a new device. ***If caused by an excess amount of fecal specimen collected, collect a new specimen and retest.***

PERFORMANCE CHARACTERISTICS

1. **Sensitivity**

The analytical sensitivity of the test is 25 ng/mL hHb in buffer or 3.5 µg/g hHb in feces.

2. **Specificity**

The *OnSite* 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.

1. Chicken Hemoglobin	2 mg/mL	6. Horse Hemoglobin	2 mg/mL
2. Turkey Hemoglobin	2 mg/mL	7. Sheep Hemoglobin	2 mg/mL
3. Pig Hemoglobin	2 mg/mL	8. Fish Hemoglobin	2 mg/mL
4. Beef Hemoglobin	2 mg/mL	9. Rabbit Hemoglobin	2 mg/mL
5. Goat Hemoglobin	2 mg/mL		

3. **Dose Hook Effect**

The *OnSite* FOB-Hi Rapid Test cassettes do not show any hook effect or prozone effect up to the concentration of 4 mg/mL hHb in buffer.

4. **Reproducibility**

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

5. **Clinical Performance**

A total of 135 specimens were collected and tested by the *OnSite* FOB-Hi Rapid Test and by a leading commercial FOB rapid test. Comparison for all specimens is shown in the following table:

	OnSite FOB-Hi Rapid Test		
Reference Test	**Positive**	**Negative**	**Total**
Positive	46	2	48
Negative	1	86	87
Total	47	88	135

Relative Sensitivity: 95.8%, Relative Specificity: 98.8%, Overall Agreement: 97.8%

6. **Interference**

Common substances (such as pain and fever medication, blood components) may affect the performance of the *OnSite* FOB-Hi Rapid Test. This was studied by spiking these substances into negative serum and negative serum samples spiked with two levels of FOB standard controls (negative and positive). The results demonstrate, at the concentrations tested, the substances studied do not affect the performance of the *OnSite* FOB-Hi Rapid Test.

List of potentially interfering substances and concentrations tested:

1. Ascorbic acid	20 mg/dL	4. Dietary iron (Fe^{2+}/Fe^{3+})	5 mg/dL
2. Bilirubin	100 mg/dL	5. Glucose	2,000 mg/dL
3. Caffeine	40 mg/dL	6. Horseradish Peroxidase	20 mg/mL

LIMITATIONS OF THE TEST

1. The Test Procedure and the Interpretation of Assay Result sections must be followed closely when testing for the presence of occult blood in feces. Failure to follow the procedure may give inaccurate results.
2. The *OnSite* 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.
3. A negative or non-reactive 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. A negative or non-reactive result can also be obtained if the quantity of occult blood present in the specimen is below the detection limit of the assay.
4. The *OnSite* FOB-Hi Rapid Test has not been validated for testing of patients with hemaglobinopathies.
5. Specimens containing visible blood may produce negative results due to the hook effect.
6. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

REFERENCES

1. America 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. Eng. 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.

Index of CE Symbols

	Consult instructions for use	IVD	For *in vitro* diagnostic use only		Use by
REF	Catalog #	LOT	Lot Number	Σ N	Tests per kit
2°C 30°C	Store between 2-30°C	EC REP	Authorized Representative		Do not reuse
	Manufacturer		Date of manufacture		

CTK Biotech, Inc.
10110 Mesa Rim Road
San Diego, CA 92121, USA
Tel: 858-457-8698
Fax: 858-535-1739
E-mail: info@ctkbiotech.com

EC REP **MDSS GmbH**
Schiffgraben 41
30175 Hannover, Germany

PI-R2011C Rev. E
Date released: 2016-03-14
English Version

For Export Only, Not For Re-sale In the USA