

INTENDED USE

ForSure One Step Immunological Fecal Occult Blood (iFOB) Test is a rapid, qualitative, sandwich immunoassay for the determination of fecal occult blood in human stool sample by laboratories or physician offices. It is useful to determine gastrointestinal (GI) bleeding found in a number of gastrointestinal (GI) disorders, e.g., diverticulities, colitis, polyps, and colorectal cancer. This test is recommended for use in 1) routine physical examinations, when hospital patients are first admitted, 2) hospitals monitoring for bleeding in patients, 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 recommend an occult blood feces test annually after age 50 to aid in the early detection of colorectal cancer. Three types of assays for fecal occult blood testing are commercially available: 1) Gum Guaiac method, 2) Hemoporphyrin and 3) Sandwich Immunochemical method.

The gum Guaiac method 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 methods 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 Methods.

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.

The immunochemical 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 tests are not affected by dietary peroxidases, animal blood and ascorbic acid.

PRINCIPLE OF THE PROCEDURE

ForSure One Step iFOB Test is a qualitative, sandwich colloidal gold conjugate immunoassay for the determination of human hemoglobin in feces. The method employs a unique combination of monoclonal and polyclonal antibodies to selectively identify hemoglobin in test sample with a high degree of sensitivity. In less than 5 minutes, elevated levels of human hemoglobin as low as 50 ng/ml can be detected, and positive results for high levels of hemoglobin can be seen in the test as early as two to three minutes.

As the test sample flows through the absorbent device, the Colloidal Gold labeled antibody- conjugate binds to the hemoglobin in the specimen forming an antibody-antigen complex. This complex binds to the Antihemoglobin antibody in the positive reaction zone and produces a pink – rose color band when hemoglobin concentration is greater than the 50 ng/ml. In the absence of hemoglobin, there is no line in the positive reaction zone. The reaction mixture continues flowing through the absorbent device past the positive reaction zone and negative control zone. Unbind conjugate binds to the reagents in the negative control zone, producing a pink rose color band, demonstrating that the reagents and device are functioning correctly. A **NEGATIVE** specimen produces one distinct color bands in control area. A **POSITIVE** specimen produces two color bands, one in the control and one in the test area. There is no meaning attributed to color or its intensity for either line.

REAGENTS

ForSure One Step iFOB Test consists of a chromatographic absorbent device in which the hemoglobin in the stool sample binds with an antibody immobilized on a porous membrane. The method employs unique monoclonal (mouse) antibodies to selectively identify Fecal Occult Blood hemoglobin in test samples at the cutoff level of 50ng/ml hHb/ml buffer. The control zone of membrane of the test device is coated with goat anti-rabbit antibody. The sample pad contains a colloidal gold-labeled mouse monoclonal anti- Fecal Occult Blood hemoglobin antibody and colloidal gold labeled rabbit IgG. A **POSITIVE** specimen produces two distinct color bands in both the test area and control area. A **NEGATIVE** specimen produces only one color band in the

control area. There is no meaning attributed to color or its intensity for either line.

MATERIALS PROVIDED

1. Test device in foil wrapper (**Do not** open before use and if foil package is damaged do not use the device. (Call Syntron or its distributor). REF 10210.
2. Instructions for testing. REF PI-10210-D
3. Specimen Collection Tube (2.5 ml): Contains 2.5 ml of hemoglobin extraction buffer from stool sample, pH 7.2, stabilizers, and 0.1 % sodium azide as preservative. REF 10006T

MATERIALS REQUIRED BUT NOT PROVIDED

1. Specimen collection containers
2. Clock or Timer
3. Latex gloves

WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic use only.
2. For professional use only.
3. Do not use the test cassette beyond the expiration date imprinted on the outside of the foil pouch.
4. Use a new specimen collection tube for each test to avoid cross contamination of fecal samples.
5. Visually inspect the foil package to ensure it is intact. If the package is not intact discard the device.

STORAGE AND STABILITY

The ForSure One Step test cassette and Sample Collection Tube are stable when stored in a controlled environment at 2°- 30°C (35.6° - 86° F) until the expiration date printed on the label. Do not expose the test device to temperature over 30°C (86° F). However, the sample collected may be stored up to eighteen (18) days from 9° - 37°C (48.2°F - 98.6° F), six (6) months at refrigerated temperature 2° - 8°C (35.6° - 46.4° F) and two years (2) at less than or equal to -20° C (at less than or equal to -4° 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 non-steroidal anti-inflammatory drugs may cause gastrointestinal irritation and subsequent bleeding in some patients. Such substances should be discontinued at least 48 hours prior to testing.

SAMPLE COLLECTION AND STORAGE

1. Peel off the liner covering the adhesive tape on each end of the collection paper.



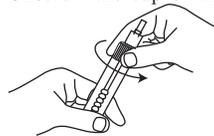
2. Lift toilet seat. Unfold collection paper to sides of the toilet rim.



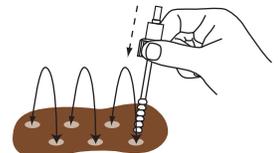
3. Make bowel movement onto collection paper.



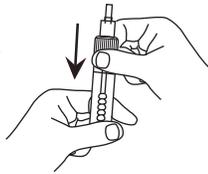
4. Unscrew the cap of the collection tube and remove the applicator stick.



5. Insert the stick into stool at 6 different sites. Use only enough fecal material to cover the tip of the applicator stick. Remove excess feces from the stick by gently wiping with an absorbent tissue.



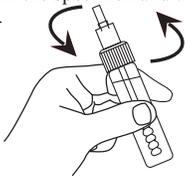
- Screw the applicator stick into the tube and secure tightly.



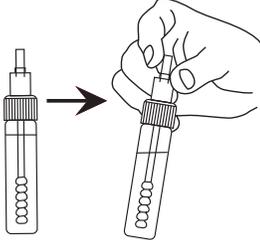
- The specimen is now ready for testing, transportation or storage.
Procedure Note: Do not use stool sample as test specimen if the stool hits the toilet water.

ASSAY PROCEDURE

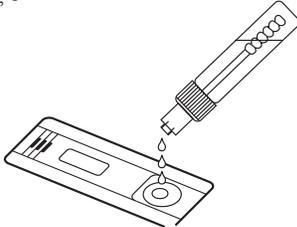
- Remove the cassette from the pouch.
- Shake the tube well to mix the specimen and the extraction buffer before you begin to conduct the test.



- Holding the tube upright, unscrew and remove cap



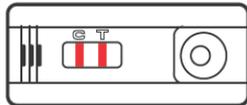
- Dispense four drops of the extraction solution from the collection tube into the sample well "S."



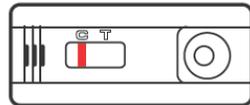
- Read the results at 5 minutes after adding the extraction solution.
Important: Do not read the result after 5 minutes.

Reading the Test Results

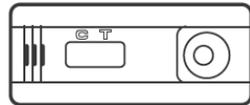
1. **Positive:** In addition to the control (C) region, a magenta band also appears on the test (T) region. If this is the case, the ForSure One Step iFOB Test level is above the detection cutoff level of 50 ng/ml extraction buffer or 50 µg hHB/g of feces.



2. **Negative:** One magenta band appears on the control (C) region with no visible band on the test region. This is an indication that the sample tested is below the ForSure One Step iFOB Test detection sensitivity level of 50 ng/ml extraction buffer or 50 µg hHB/g of feces.



3. **Invalid:** If there are no distinct color bands in both the control (C) region and test (T) region, then the test results are invalid. It is recommended that the specimen is retested. If, after performing the test no line is visible in the control (C) region of the cassette, then the results are invalid. It is recommended that the specimen is retested.



QUALITY CONTROL

- Internal Quality Control**
Each reaction cassette has its own built-in quality control indicator, the Control (C) line. If, after performing the test, no line is visible on the cassette, the device may have been over or under loaded with specimen or the test cassette may have deteriorated. The assay will have to be repeated using a new ForSure One Step iFOB Test cassette. Re-read the instructions carefully or call Syntron Bioresearch Co. or their local distributor for assistance.
- External Quality Control**
Good laboratory practice recommends the use of control material to test each product batch or whenever it is necessary to validate reagent performance and reliability.

AFTER TESTING

Stool specimens may be infectious. Properly handle and dispose all used reaction devices into an approved biohazard container. Residual specimens should be disposed in a medically approved manner after completion of all testing including the confirmatory testing. Sample disposal may be controlled by legal chain of custody considerations.

PERFORMANCE CHARACTERISTICS

1. Analytical Sensitivity:

ForSure One Step iFOB Test has been designed for the detection of Fecal Occult Blood test in feces at the detection cutoff of 50 ng hHb/ml buffer or 50 µg hHb/g feces.

2. Interfering Substances and Cross Reactivity:

A study was conducted with ForSure One Step iFOB Test to determine the cross-reactivity of non human hemoglobin, dietary peroxidases and other interfering substances. The following substances, when spiked in both positive and negative specimens. No false results were obtained.

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	500
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



1. Reproducibility:

The reproducibility of ForSure One Step iFOB Test was determined using replicate assays of samples from human hemoglobin free fecal extraction buffer known to be negative which were spiked with human hemoglobin concentration following levels: 0, 25, 50, 75, 200 and 2000 ng/ml, with kits from three different production lots. Each sample was run through fifteen parallel assays. The data showed 100% precision for the duplicates of each sample and 100% precision from different lots.

2. Method Comparison:

• Study I

The study on the interpretation of lay-users (N=180) from various educational backgrounds and the range of ages were 21 to 72 years old. A total of 180 samples were blind labeled and tested with ForSure One Step iFOB Test by 180 participants at the three geographical sites. The samples of hHb values ranged from 0 to 2000 ng/ml.

When compared to professional readings the positive percent agreement (PPA) and the negative agreement (NPA) were 96.7% (95% CI: 91.7% - 99.1%) and 100% (95% CI: 94% -100%), respectively. The overall percent agreement was 97.8% (95% CI: 94.4% - 99.4%).

• Study II

The study on 180 spiked samples were blind labeled and tested with the ForSure One Step iFOB Test and the predicate device at each hospital laboratory site. Three different people in each laboratory carried out the analysis. The results (N=3 x 180 samples) were obtained from three different hospital laboratory sites. Stool samples were spiked with hHb at 0, 37.5, 50, 62.5, 200 and 2000 ng hHb/ml.

When compared to expected result (ELISA) the positive percent agreement (PPA) and the negative agreement (NPA) were 99.2% (95% CI: 97.6% - 99.8%) and 96.7 % (95% CI: 92.9% - 98.8%), respectively. The overall percent agreement was 98.3% (95% CI: 96.9% - 99.2%).

When compared to predicate device result the positive percent agreement (PPA) and the negative agreement (NPA) were 98.3% (95% CI: 96.4% - 99.4%) and 94 % (95% CI: 89.4% - 96.9%), respectively. The overall percent agreement was 96.8% (95% CI: 95%-98.2%).

BIBLIOGRAPHY

1. Allison JB, Takawa IS, Ransom L J, Adrian AL. A comparison of fecal occult blood tests for colorectal-cancer screening. N. Engl J Med 1996; 334: 155-159.
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LIMITATIONS OF THE TEST

1. This product is designed to be used for the detection of human hemoglobin from human feces only.
2. Results can not be considered conclusive evidences 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.
3. 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.
4. 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.
5. The American Cancer Society recommends collection of two samples from three consecutive bowel movements.
6. Urine and excessive dilution of samples with water from the toilet bowl may cause erroneous test results. For best results, use the collection paper in the collection kit.
7. The ForSure One Step iFOBT test is not for use in testing urine, gastric specimens or other body fluids

