Instant-View® Fecal Occult Blood (FOB) II Test *(Cassette)*

ONE STEP ASSAY
RAPID VISUAL RESULTS
FOR QUALITATIVE IN VITRO DIAGNOSTIC USE

INTENDED USE

The *Instant-View* Fecal Occult Blood (FOB) II Rapid Test is an immunochemical device intended for the qualitative detection of Fecal Occult Blood by laboratories or physicians offices. It is useful to determining gastrointestinal (GI) bleeding found in a number of gastrointestinal disorders, e.g., diverticulitis, colitis, polyps, and colorectal cancer. This test is recommended for use in 1) routine physical examinations, when hospital patients are first admitted, 2) hospital monitoring for GI bleeding in patients, and 3) screening for colorectal cancer or gastrointestinal bleeding from any source.

SUMMARY AND EXPLANATION

The American Cancer Society and Centers for Disease Control recommend an occult blood stool test annually after age 50 for the early detection for 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]

This immunological 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 compared to the Guaiac and Hemoporphyrin methods. The results of immunological 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 T line and a C line. The T line is coated with anti-hHb antibodies, and the C line is coated with Goat anti-mouse IgG antibody.

When an adequate amount of specimen is applied into the sample well of the device, the specimen migrates by capillary action through the test strip. If the concentration of hHb in the specimen is at or above the cutoff (50ng/ml) level, the T line appears as a visible burgundy line. If the concentration of hHb in the specimen is below the cutoff 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

1. 20 fecal collection tubes, each filled with extraction buffer (PBS buffer) This sample collection tube allows semi-quantitative collection of a fresh fecal sample. It is also leak-proof and mail transportable.
2. 20 test devices (cassettes), each sealed in a foil pouch
3. One insert (Instructions for Use)

MATERIALS REQUIRED BUT NOT PROVIDED

1. Timer
2. Clean disposable cup
3. An absorbent cloth or tissue (preferably disposable)

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

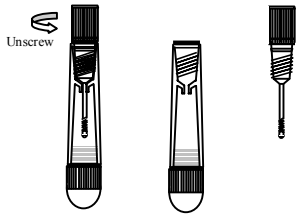
The test device is stable when stored in a controlled environment at 2-30°C (35.6-86°F) for up to 2 years or until the expiration date printed on the label, whichever comes first. Do not expose the kit components to temperatures over 30°C (86°F).

PATIENT LIMITATIONS

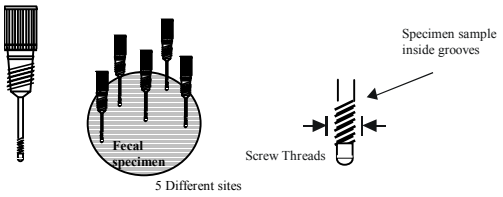
1. A specimen should not be collected while the patient is in the following conditions that may interfere with the results:
   - during menstrual bleeding
   - bleeding hemorrhoids
   - constipation bleeding
   - urinary bleeding
2. Alcohol and certain medications such as aspirin, indomethacin, reserpine, phenylbutazone, corticosteroids and nonsteroidal anti-inflammatory drugs may cause gastrointestinal irritation and subsequent bleeding in some patients.

SPECIMEN COLLECTION

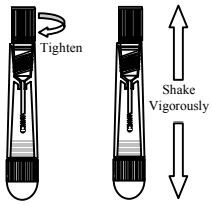
1. The specimen is stool. It may be collected from the toilet paper or caught in a clean cup. Contamination from toilet water should be avoided.
2. Unscrew the sampler of the collection tube.



3. Randomly insert the threaded end of the sampling stick into the fecal specimen in at least 5 different sites.



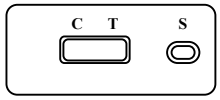
4. Insert sampler in the collection tube and firmly tighten it.



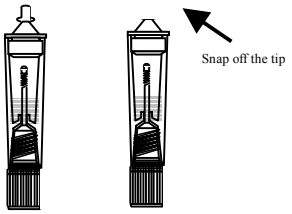
5. Shake the tube vigorously to mix the specimen and the extraction buffer.
6. Samples collected may be stored at least eight (8) days at temperature below 35°C (95°F), six (6) months at 4°C (39.2°F) and two (2) years at - 20°C (-4°F).

ASSAY PROCEDURE

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



3. Holding the collection tube upright, remove the screw cap, then snap off the tip.



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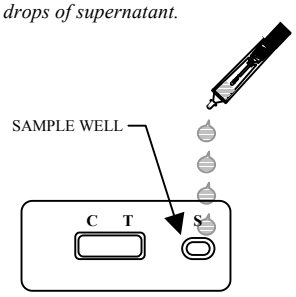
ASSAY PROCEDURE, CONTINUED

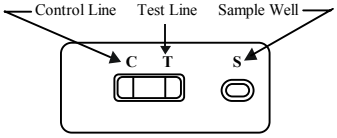
4. Squeezing the collection tube, dispense **four drops** of the supernatant in the collection tube into the sample well ("S").
   Note: If migration is not observed in 30 seconds in the results window, add one or two extra drops of supernatant.



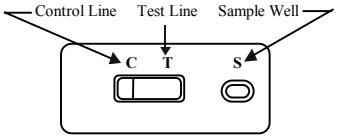
5. Read the result in **5-10 minutes** after adding the specimen.
   IMPORTANT: Do not read the test results after ten (10) minutes.

INTERPRETATION

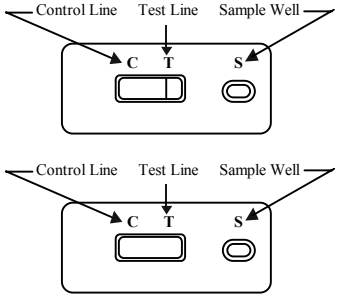
1. **POSITIVE:** If both **C line and T line** are present, the result is positive. A positive result indicates the level of hHb in the specimen is over 50ng hHb/ml extraction buffer or 50µg hHb/g feces.



2. **NEGATIVE:** If only the **C line** develops on the test strip, the result is negative. A negative result indicates the hHb in the specimen is below 50 ng/ml.



3. **INVALID:** If no C line appears within 5 minutes, the result is invalid. The assay should be repeated with a new device.



QUALITY CONTROL

- **Internal Quality Control**
  This device contains a built-in control feature, the Control (C) line. The presence of this Control 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 whole 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 by physicians to determine the exact cause and source of the occult blood in the stool. 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.
2. 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 50ng hHb/ml buffer of 50µg hHb/g feces.

2. Accuracy

- Reference Laboratory and Physicians Office Laboratory (POL) Studies
  One hundred (100) hHb-free stool extraction specimens collected in-house were divided into 5 groups of 20 each. The 5 groups of extraction samples were spiked with hHb for five different concentrations, respectively: 0, 37.5ng hHb/ml, 50ng hHb/ml, 62.5ng hHb/ml, and 2000ng hHb/ml. Those specimens were blind labeled and tested with the *Instant-View* 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 *Instant-View* Fecal Occult Blood Rapid Test is 98%.
- Comparison studies
  Those 100 specimens were also tested in house with the *Instant-View* Fecal Occult Blood Rapid Test and a predicate device. The correlation between the *Instant-View* Fecal Occult Blood Test and the predicate device was over 95%.

3. Specificity

The *Instant-View* 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 (meat 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/docroot/CRI/content/CRI_2_4_3x_Can_colon_and_rectum_cancer_be_found_early.asp? sitearea= (2002, February 22).
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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