**ONE STEP ASSAY**

**RAPID VISUAL RESULTS**

**FOR QUALITATIVE IN-VITRO DIAGNOSTIC USE**

**INTENDED USE**

The INSTANT-VIEW® Fecal Occult Blood (FOB) Rapid Test is an immunochromatographic 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. Three types of assays for FOB testing are commercially available: 1) Guaiac Dye; 2) Hemoporphyrin; and, 3) Immunochromatographic.

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 and Immunochromatographic 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.

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 INSTANT-VIEW® immunochromatographic 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 immunochromatographic FOB rapid tests are not affected by dietary peroxidases, animal blood and ascorbic acid. A Japanese study demonstrated using immunochromatographic FOB tests reduced mortality by 60%.

**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 test device, the test specimen migrates by capillary action across the test strip. If the concentration of hHb in the specimen is below the detectable level, no T-line develops. 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**

1. 20 fecal collection tubes, each with 2 ml FOB buffer (1x PBS with 0.02% sodium azide)
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. 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).

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**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).
**INSTANT-VIEW® Fecal Occult Blood Test (Cassette)**

**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 **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.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef Hemoglobin</td>
<td>2,000</td>
</tr>
<tr>
<td>Chicken Hemoglobin</td>
<td>500</td>
</tr>
<tr>
<td>Fish Hemoglobin (meat extract)</td>
<td>100</td>
</tr>
<tr>
<td>Horse Hemoglobin</td>
<td>500</td>
</tr>
<tr>
<td>Goat Hemoglobin</td>
<td>500</td>
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<tr>
<td>Pig Hemoglobin</td>
<td>500</td>
</tr>
<tr>
<td>Rabbit Hemoglobin</td>
<td>500</td>
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<tr>
<td>Sheep Hemoglobin (meat extract)</td>
<td>100</td>
</tr>
<tr>
<td>Horse radish Peroxidase</td>
<td>20,000</td>
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<tr>
<td>Red turnip</td>
<td>Aqueous extract</td>
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<tr>
<td>Vitamin C (ascorbic acid)</td>
<td>Dietary supplement</td>
</tr>
<tr>
<td>Iron</td>
<td>Dietary supplement</td>
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</tbody>
</table>

**REFERENCES**