FOB Rapid Test
(Fecal Specimen)

INTENDED USE
The First View FOB 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 cancer1 .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 FOB screening tests reduced mortality of colorectal cancer by 60%3. The First View FOB Rapid Test is an FOB 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 First View FOB Rapid Test is a lateral flow chromatographic immunoassay. The test strip in the cassette consists of: 1) a 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 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 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 MATEREIALS PROVIDED
FOB Test cards contain the following:
- Test Device with activated silica gel
- Extractions Tubes
- Package Insert (Instruction for use)

MATERIALS MAY BE REQUIRED AND NOT PROVIDED
- Positive Control
- Negative Control

MATERIALS REQUIRED BUT NOT PROVIDE
- Timer
- Digital Clock
- Specimen collection container Tube

WARNINGS AND PRECAUTIONS:
For in Vitro Diagnostic Use
- Read these Instructions for Use completely before performing the test. Failure to follow the instructions could lead to 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 (15-30°C) before use.
- **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.**
- **Do not use specimens for testing if blood is visible.**
- Users of this test should follow the US CDC Universal Precautions for bio-safety.
- Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.

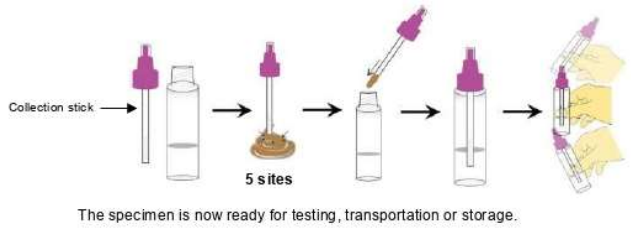
REAGENT PREPARATION AND STORAGE INSTRUCTIONS:
All reagents are ready to use as supplied. Store unused test devices unopened at 2-30°C. If stored at 2-8°C, ensure that the test device is brought to room temperature before opening. The test device is stable through the expiration date printed on the sealed 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.

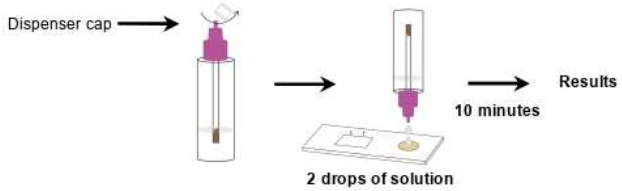
3. Alcohol and certain medications such as aspirin, indomethacin, phenylbutazone, reserpine, corticosteroids, and nonsteroidal anti-inflammatory drugs may cause gastrointestinal irritation and subsequent bleeding, and produce positive reactions. On the advice of a physician, these medicines may be temporarily discontinued for 7 days prior to and during the test period.

SPECIMEN COLLECTION, STORAGE AND HANDLING
Consider any materials of human origin as infectious and handle them using standard bio safety procedures.
Step 1: Collect a random stool sample in a clean, dry receptacle.
Step2: 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. Ensure stool sample is only in the grooves of the collection stick. Excess stool sample may lead to an invalid test result.***
Step 4: Replace the collection stick and tighten securely to close the stool collection device.
Step 5: Shake the stool collection device vigorously to extract the hHb in the specimen.



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.

DIRECTIONS FOR USE
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 a homogenous liquid suspension
Step 4: Hold the stool collection device vertically. Twist off the tip. Dispense 2 drops (70-80 µL) of the solution into the sample well of the cassette. Do not overload samples.



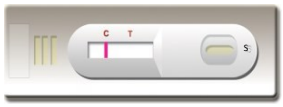
Step 5: Set up timer and wait for the colored line(s) to appear.
Step 6: Results can be read at 10 minutes. Positive results can be visible in as short as 1 minute. Negative results must be confirmed at the end of the 10 minutes only. ***However, any results interpreted outside 10 minutes should be considered 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 specimen extract. If the C line does not develop, review the entire procedure and repeat the test with a new device.

INTERPRETATION OF ASSAY RESULT

NEGATIVE RESULT: If only the control (C band is developed the test indicates that no detectable FOB antibodies are present in the specimen. The result is negative.



POSITIVE RESULT: If the both control C test bend and T are developed the test indicate for the presence of FOB positive results

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

INVALID: If no C line is developed 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. **Clinical Performance**

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

	First View FOB 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: 98.9%,
Overall Agreement: 97.8%

2. Interference

Common substances (such as pain and fever medication, blood components) may affect the performance of the First View FOB 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 First View FOB Rapid.

List of potentially interfering substances and concentrations tested:

- Ascorbic acid 20 mg/dL
- Dietary iron (Fe2+/Fe3+) 5 mg/dL
- Bilirubin 100 mg/dL
- Glucose 2,000 mg/dL
- Caffeine 40 mg/dL
- Horseradish Peroxidase 20 mg/mL

3. PRECISION

Intra-Assay

Within-run precision has been determined by using 15 replicates of three specimens: 50ng/ml, 100ng/ml and 10ug/ml positive specimens. The specimens were correctly identified >99% of the time.

Inter-Assay

Between-run precision has been determined by 15 independent assays on the same three specimens: 50ng/ml, 100ng/ml and 10µg/ml positive specimens. Three different lots of the FOB Rapid Test Cassette (Feces) have been tested using these specimens. The specimens were correctly identified >99% of the time.

LIMITATIONS OF 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 First View FOB Rapid Test is to aid in diagnosis and is not intended to replace other diagnostic procedures such as G.I. fibro scope, 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. 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 First View FOB Rapid Test has not been validated for testing of patients with hemoglobinopathies.
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. Simon JB. Occult Blood Screening for Colorectal Carcinoma: A Critical Review, Gastroenterology, 1985; 88: 820.

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.

4. Blebea J, Mcpherson RA. False-Positive Guaiac Testing With Iodine, Arch Pathol Lab Med, 1985;109:437-40.