Second Generation FIT™
Fecal Immunochemical Test

Procedure Guide / Technical File

Rev 4.8.01
March 2015

Format: Cassette
Method: Color Indicator
Testing Procedure: Manual
Storage Temperature: 15-28 Degrees Centigrade
Sensitivity: 50 ng hHb/mL buffer or 5 ug hHb/g feces

Expected Values:
Negative: Pink band in the control region
Positive: Pink band in the control region and the test region
Invalid: total absence of band in the control region OR test band appears without control band

Test “shelf” life from date of manufacture:
24 months

Principle:
The Second Generation FIT™ Fecal Immunochemical Test employs a unique combination of polyclonal and monoclonal antibodies to selectively identify hemoglobin in test samples with a high degree of sensitivity. In less than five minutes, elevated levels of human hemoglobin as low as 50ng Hb/mL can be detected and positive results for higher levels of hemoglobin can be seen in the test as early as 2-3 minutes.

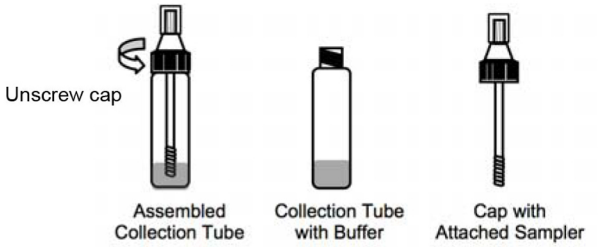
Intended Use:
For the determination of human hemoglobin in feces by professional laboratories or physician’s offices. Useful as an aid in the detection of lower GI bleeding , colorectal cancer, et. al.

PATIENT LIMITATIONS

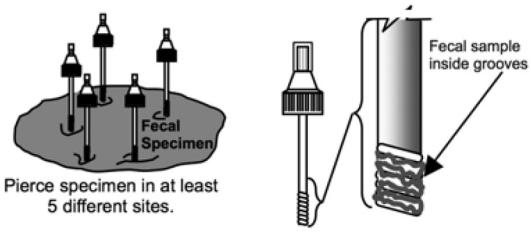
1. A specimen should not be collected from a patient with the following conditions that may interfere with the test results:
   - Menstrual bleeding
   - Bleeding hemorrhoids
   - Constipation bleeding
   - Urinary bleeding
2. Alcohol and certain medications such as aspirin, indomethacin, reserpine, phenylbutazone, corticosteroids and nonsteroidal anti-inflammatory drugs may cause gastrointestinal irritation and subsequent bleeding in some patients.

SPECIMEN COLLECTION

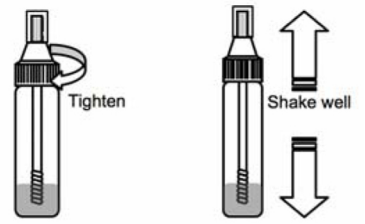
1. The specimen used in this assay is feces. It may be collected from toilet paper or caught in a clean cup. Avoid contact with toilet water.
2. Unscrew the cap (with the attached sampler) of the collection tube.



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.



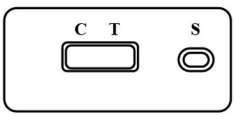
4. Insert sampler in the collection tube and firmly tighten the cap.



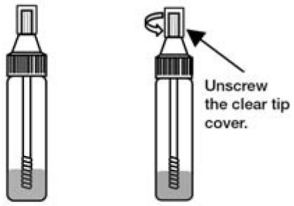
5. Shake the tube well to mix the specimen and the FOB buffer.
   NOTE: Samples collected may be stored at least eight (8) days at ambient temperatures below 35°C (95°F), six (6) months at 2-8°C (36-46°F) and two (2) years at ≤ -20°C (≤ -4°F).

ASSAY PROCEDURE

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

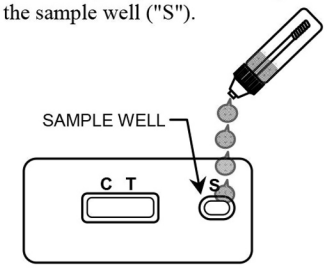


3. Holding the collection tube upright, unscrew the clear tip cover.



ASSAY PROCEDURE, CONTINUED

4. Squeezing the collection tube, dispense **3 drops** of the FOB buffer in the collection tube into the sample well ("S").

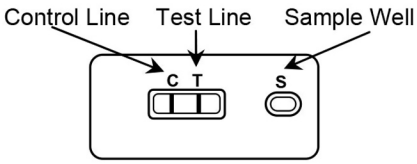


5. Read the result within **5-10 minutes** after adding the FOB buffer.
   IMPORTANT: Do not read the test results after ten (10) minutes.

INTERPRETATION

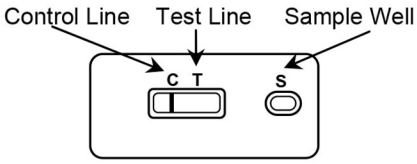
POSITIVE:

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.



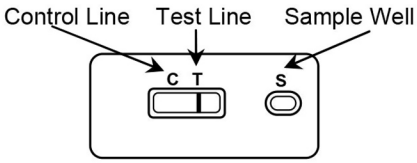
NEGATIVE:

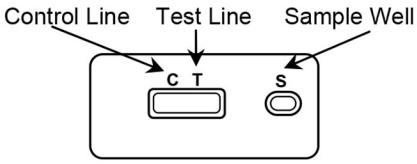
If only the C-line develops in the control region of the test strip, the result is negative. A negative result indicates the hHb in the specimen is below 50 ng/ml.



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.

Second Generation FIT ™ Procedure Guide

INTENDED USE

The Pinnacle BioLabs Second Generation FIT Fecal Immunochemical Test is a rapid chromatographic immunoassay for the qualitative detection of human occult blood in feces.

INTRODUCTION

Most of diseases can cause hidden blood in the stool. In the early stages, gastrointestinal problems such as colon cancer, ulcers, polyps, colitis, diverticulitis, and fissures may not show any visible symptoms, only occult blood. Traditional guaiac-based method lacks sensitivity and specificity, and has diet-restriction prior to the testing.

The Pinnacle BioLabs Second Generation FIT Fecal Immunochemical Test is a rapid test to qualitatively detect low levels of fecal occult blood in feces. The test uses double antibody sandwich assay to selectively detect as low as 50ng/mL of hemoglobin or 6μg hemoglobin/g feces. In addition, unlike the guaiac assays, the accuracy of the test is not affected by the diet of the patients.

PRINCIPLE

The Pinnacle BioLabs Second Generation FIT Fecal Immunochemical Test is a lateral flow chromatographic immunoassay based on the principle of the double antibody–sandwich technique. The membrane is pre-coated with anti-hemoglobin antibody on the test line region of the device. During testing, the specimen reacts with the particle coated with anti-hemoglobin antibody. The mixture migrates upward on the membrane chromatographically by capillary action to react with anti-hemoglobin antibody on the membrane and generate a colored line. The presence of this colored line in the test region indicates a positive result, while its absence indicates a negative result. To serve as a procedural control, a colored line will always appear in the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred.

PRODUCT CONTENTS

The Pinnacle BioLabs Second Generation FIT Fecal Immunochemical Test contains anti-hemoglobin antibody particles and anti-hemoglobin antibody coated on the membrane.

MATERIALS SUPPLIED

Each test contains : 1. One cassette device
2. Specimen collection tube with extraction buffer
3. One desiccant

MATERIAL REQUIRED BUT NOT PROVIDED

Clock or Timer

STORAGE AND STABILITY

All reagents are ready to use as supplied. Store unused test device unopened at 2°C-30°C.

If stored at 2°C-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 over 30°C.

WARNINGS AND PRECAUTIONS

1. For In Vitro diagnostic use only.
2. Warning: the reagents in this kit contain sodium azide which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up.
3. Do not use it if the tube/pouch is damaged or broken.

SPECIMEN COLLECTION

Collect stool sample by using the special sample collection device. First, unscrew the top of the sample collection device, take out the sample collection stick, and collect the sample by dipping the stick into 6 different places of the stool sample. Then, put the sample collection stick back in the sample collection device and screw together tightly.

Specimen should not be collected during or within three days of menstrual period, or if the patient suffers from bleeding hemorrhoids or blood in the urine.

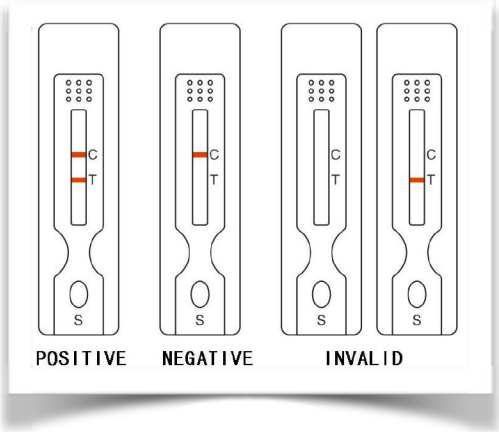
Alcohol, aspirin and other medications taken in excess may cause gastrointestinal irritation resulting in occult bleeding. Such substances should be discontinued at least 48 hours prior to testing.

Dietary restrictions are not necessary.

TEST PROCEDURE

1. Remove the test device from its foil pouch by tearing along the notch and use it as soon as possible.
2. Specimen collection. See also specimen collection.
3. Shake the sample collection device several times.
4. Holding the sample collection device upright, carefully unscrew the small cap on the top of the collection device. Note this is a separate cap from the one used to collect specimen.
5. Squeeze 3 drops (~75uL) of the sample solution in the sample well of the cassette, as in the illustration.
6. Read the test results in 5 to 10 minutes. It is important that the background is clear before the result is read. Do not read results after 10 minutes.

INTERPRETATION OF RESULTS



Positive: Two lines appear. One colored line should be in the control line region (C) and another apparent colored line should be in the test line region (T).

Negative: One colored line appears in the control line region(C). No line appears in the test line region (T).

Invalid: Control line fails to appear.

QUALITY CONTROL

A pink line appearing in the control region(C) is the internal procedural control. It confirms sufficient specimen volume and correct procedural technique. External controls be tested at regular intervals as good laboratory testing process.

Users should follow the appropriate local guidelines concerning the running of external quality controls.

LIMITATIONS

1. As with all diagnostic tests, all results must be considered with other clinical information available to the physician. A definite clinical diagnosis should only be made by the physician after all clinical and laboratory findings have been evaluated.
2. This test is limited to the detection of FOB in human stool sample only.
3. Although the test is highly accurate in detecting human hemoglobin, a low incidence of false positive results may occur. In addition, because many bowl lesions, indulging some polyps and colorectal cancers, may bleed intermittently or not at all, occult blood may not be uniformly distributed throughout a fecal sample. Thus rest results may be negative even when disease is present.

PERFORMANCE CHARACTERISTICS

Sensitivity

The Pinnacle BioLabs Second Generation FIT Fecal Immunochemical Test can detect the levels of human occult blood as low as 50 ng/mL hemoglobin or 6μg hemoglobin/g feces.

Specificity

The Pinnacle BioLabs Second Generation FIT Fecal Immunochemical Test is specific to human hemoglobin. Specimen containing the following substances at the standard concentration were tested on both positive and negative controls with no effect on test results.

Substances	Concentrations(Diluted with the extraction buffer)
Bovine hemoglobin	1 mg/mL
Chicken hemoglobin	1 mg/mL
Pork hemoglobin	1 mg/mL
Goat hemoglobin	1 mg/mL
Horse hemoglobin	1 mg/mL
Rabbit hemoglobin	1 mg/mL
Turkey hemoglobin	1 mg/mL

REFERRENCE

1. Simon J.B. *Occult Blood Screening for Colorectal Carcinoma: A Critical Review,* Gastroenterology, Vol. 1985; 88: 820.
2. Blebea J. and Nepherson RA. *False-Positive Guaiac Testing With Iodine, Arch Pathol Lab Med,* 1985;109:437-40

Index of Symbols

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

CE FDA
CLIA WAIVED