

EpiTuub[®] FOB Test - Instructions for Fecal Sample Collection

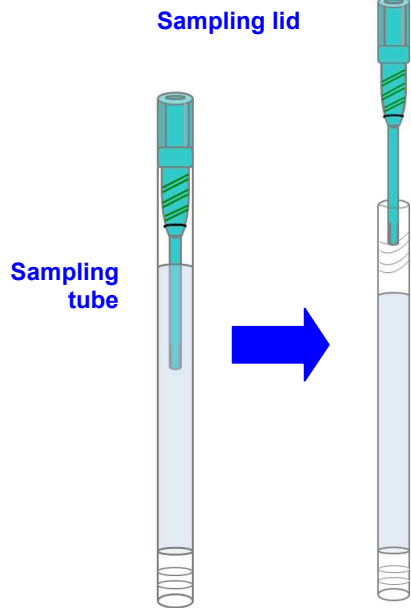
FOR REFERENCE USE ONLY

Monoclonal antibody based immunoassay test for the detection of fecal occult blood

Version 12

1

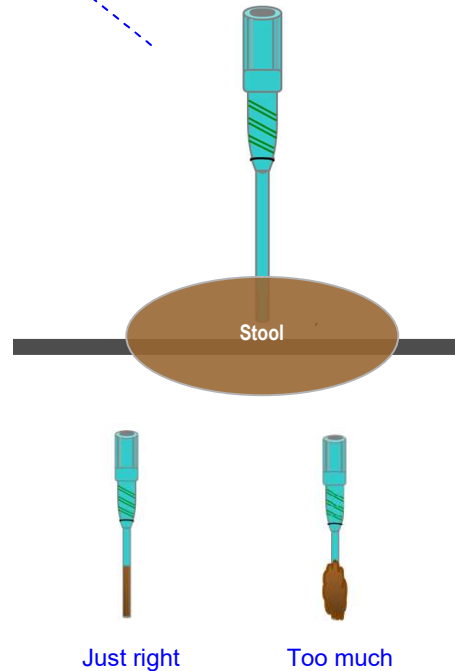
1. Collect a stool sample using the enclosed stool sampling paper:
 - a. Clean the bowl and flush the toilet two times. Unfold and lay the Sample Collection Paper directly on the top of the water in the toilet bowl (the paper should float above the water).
 - b. After bowel movement, take the sampling tube and unscrew the sampling lid, keeping the sampling tube in a **vertical position** to prevent loss of solution.



2

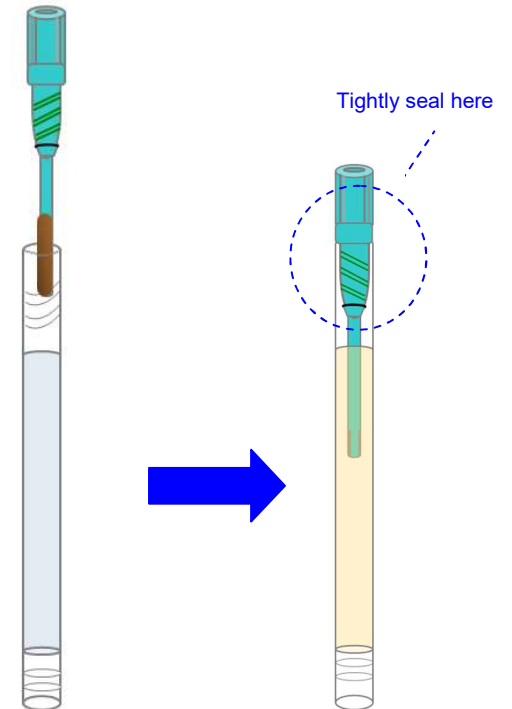
1. Hold the sampling lid by the **Thumb Grip**.
2. Use the tip of the sampling lid to collect a small amount of fecal sample at two or more sites. Only take the fecal sample that sticks to the sampling lid tip (never intentionally place any separate piece of fecal sample into the tube). The total amount of stool collected should be less than one grain of cooked rice. For liquid stool, collect 0.1mL into the sampling tube.

Thumb Grip



3

1. Insert and screw the sampling lid back into the sampling tube **in a vertical position**. Do not spill any solution from the tube.
2. Tightly seal the lid with the tube.
3. Flush toilet.



READ ALL THE INFORMATION IN THIS LEAFLET BEFORE SAMPLING

Store at 2-8°C. Do not freeze. Keep out of reach of children. For in-vitro diagnostic use. Not to be taken internally. Not to be sampled directly from anus. If you have any questions, please contact your physician or laboratory staff or call Epitepe Diagnostics at 858-693-7877 from 8:00 a.m. to 5:00 p.m. PST



Manufactured by Epitepe Diagnostics, Inc. San Diego CA 92121, USA

(V12/2019-06)

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US Patent: 7,780,915

EpiTuub[®] FOB Test — Instructions for Test Procedures

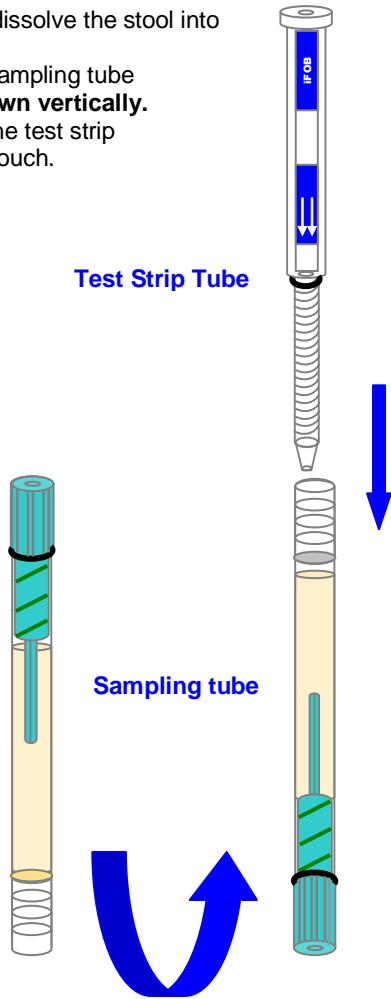
Monoclonal antibody based immunoassay test for the detection of fecal occult blood

A

1. Shake to dissolve the stool into solution.
2. Turn the sampling tube **upside down vertically**.
3. Remove the test strip from foil pouch.

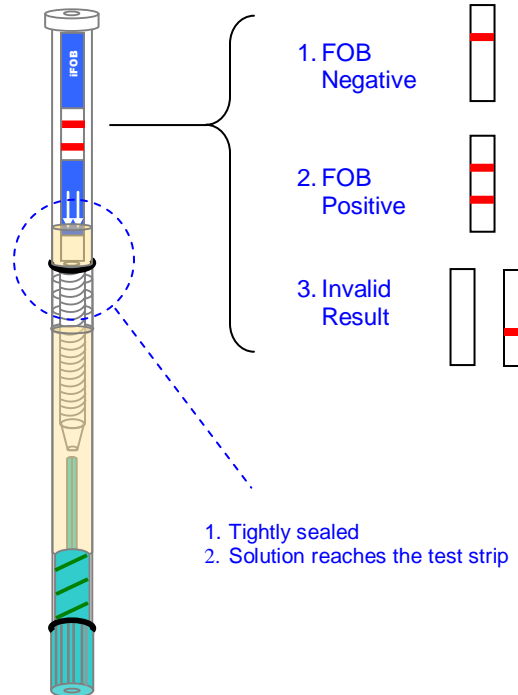
Test Strip Tube

Sampling tube



B

1. Insert and screw the test strip in a **vertical position** into the sampling tube by breaking the bottom seal of the sampling tube.
2. Allow the solution to flow into the bottom space of test strip, keeping the device in a **vertical position**.
3. You may soon see a red fluid moving across the white area of the test strip. Read test result after 5 minutes.



1. Tightly sealed
2. Solution reaches the test strip

For In-Vitro Diagnostic Use.

Catalog Number: KT-313

INTENDED USE

This EpiTuub[®] Fecal Occult Blood (FOB) Test Device is a rapid immunological test intended for the qualitative detection of fecal occult blood in feces by professional laboratories and physician office laboratories. The test is intended for the determination of gastrointestinal (GI) bleeding, found in a number of gastrointestinal disorders, e.g., diverticulitis, colitis, polyps, and colorectal cancer. This FOB test device is recommended for use in (1) routine physical examinations, (2) monitoring any bleeding in patients, and (3) screening for colorectal cancer or gastrointestinal bleeding.

SUMMARY OF PHYSIOLOGY

Colorectal cancer is the third most common cancer in the world. The appearance of fecal occult blood is often the first, if not the only, indicator associated with colorectal cancer and polyps. Other gastrointestinal disorders such as diverticulitis, Cohn's disease, colitis ulcer, etc. may also be associated with the presence of fecal occult blood. There are two different types of FOB tests available, the traditional guaiac FOB test and antibody based immunological FOB test. The traditional guaiac FOB tests do not provide a high degree of accuracy. Immunological FOB tests are more accurate and do not require special dietary restrictions prior to the test sample collection.

TEST PRINCIPLE

The EpiTuub[®] FOB test is a "sandwich" immunoassay utilizing two monoclonal antibodies to specifically detect the presence of human hemoglobin (h-Hb) in feces. It consists of two units, a fecal sampling device and a test strip. A stool specimen is collected into the sampling tube containing extraction solution. After mixing the stool sample, a test strip is screwed into the sampling tube by breaking the bottom seal of the sampling tube while maintaining a vertical position. The extracted fecal solution flows into the bottom space of the test strip and triggers the start of the FOB immunoassay. If human hemoglobin is present at a level of greater than 50 ng/mL in a fecal sample extract, an immuno-complex of "labeled monoclonal anti-human hemoglobin antibody – human hemoglobin – membrane coated monoclonal anti-human hemoglobin antibody" is formed. A red colored band appears in the test region, which is located in the lower half of the test membrane. A similar colored band must appear in the control region located in the upper-half of the test membrane, indicating the test strip is functioning properly and the result is valid.

REAGENTS AND MATERIALS PROVIDED

1. Fecal specimen collection device (30159): containing sampling tube, sampling lid and pre-added extraction solution in the sampling tube. This device should be stored at 2 to 8°C. Do not freeze.

READ ALL THE INFORMATION IN THIS INSERT BEFORE TESTING

Store at 2-8°C. Do not freeze. Keep out of reach of children. For in-vitro diagnostic use. Not to be taken internally. Not to be sampled directly from anus. If you have any questions, call customer information staff of Epitope Diagnostics at 1-858-693-7877, 8:00 a.m. to 5:00 p.m. PST.

EpiTuub[®] FOB Test - Instructions for Test Procedures

Monoclonal antibody based immunoassay test for the detection of fecal occult blood

2. Test strip (30158): one dipstick for the fecal occult blood test is assembled in a transparent housing and sealed in a foil pouch with desiccant. It should remain in its original sealed pouch until ready for use. The test strip should be stored at 2 to 8°C. Do not freeze.
3. Instruction for use.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Timer or clock.
2. External controls (30160, 30161 Recommended)

PRECAUTIONS

1. For in-vitro diagnostic use only. Not to be taken internally.
2. Do not use product beyond the expiration date.
3. Handle all specimens as potentially infectious.
4. Do not reuse the test.

PATIENT PREPARATION

1. Specimen should not be collected during or within three days of a menstrual period, or if the patient suffers from bleeding hemorrhoids or blood in the urine.
2. Alcohol, aspirin, indomethacin, reserpin, phenylbutazone, corticosteroids and other medications may cause gastrointestinal irritation resulting in occult bleeding. With the physicians approval, such medication should be discontinued for 7 days before and throughout the test period. Rectal medication should be discontinued.
3. Dietary restrictions are not necessary.

SPECIMEN COLLECTION

1. Stool specimens can be collected at any time of the day.
2. Collect a random sample of feces in a clean, dry cup or toilet paper or as indicated in the figure 1.
3. Unscrew the sampling lid and keep the sampling tube in a vertical position to prevent the loss of any extraction solution.
4. Insert and twist the tip of the sampling lid into the stool specimen at two or more different sites (figure 2).
5. Collect fecal sample that is stuck to the surface of the sampling lid. Do not intentionally collect any separate and large pieces of fecal sample into the tube.
6. Replace the sampling lid into the tube and secure tightly (figure 3).
7. The specimen is ready for testing, transportation or storage. It can be stored at 2-8°C for up to 14 days and at room temperature for up to 5 days.

Note: Two specimens from three consecutive bowel movements are recommended from American Cancer Society. Specimen should not be collected during digital rectal examination.

TEST PROCEDURE

1. Bring the sealed foil pouch test strips and collected specimens to room temperature.
2. Shake the sampling tube vigorously to ensure a good liquid suspension.
3. Position the sampling tube upside down vertically and let it settle for about 1 minute.
4. Remove the test strip from the sealed foil pouch.

5. Screw the test strip in a vertical position into the sampling tube by breaking the bottom seal of the sampling tube. Secure tightly! (Figure A)
6. Allow the solution to flow into the bottom space of the test strip and keeping the device in a vertical position.
7. Read test result at 5 minutes. Do not interpret test result after 10 minutes.

INTERPRETATION OF RESULTS

- **Positive:**
If two red/pink colored bands are visible within 5 minutes, the test result is positive and valid (Figure B).
- **Negative:**
If test area has no red/pink colored band and the control area displays a red colored band, the test result is negative (Figure B).
- **Invalid:**
If a colored band does not form in the control area regardless of there being any band in the test area, the test result is invalid (Figure B).

QUALITY CONTROL

Good laboratory practices recommend the use of appropriate controls. There are two types of controls for the EpiTuub[™] FOB test, the internal procedural control and external controls.

1. **Internal procedural control:** Each EpiTuub[™] FOB test consists of a built in procedural control. It will appear if the test has been performed correctly, sample wicking has occurred and the reagents are reactive. It does not ensure that the test line antibody is accurately detecting the presence or absence of occult blood in the test fecal sample.
2. **External controls:** It is recommended to use external positive controls. The external positive controls are not provided with this kit, but are commercially available from Epitope Diagnostics. External controls are used to assure that the test line antibody is reactive. However, external controls will not detect an error in performing the patient sample test procedure. It is recommended that the external control be tested once per kit.

Follow local, state, and federal guidelines for running quality control.

LIMITATION OF THE PROCEDURE

1. A number of conditions, as mentioned in the "Patient Preparation" section, can cause false positive results.
2. Intermittent tumor bleeding and irregular distribution of blood in the feces also contribute to false negative results.
3. Urine and excessive dilution of fecal samples with water from toilet bowl may cause erroneous results.
4. EpiTuub[™] FOB test is not for use in testing urine, gastric specimens or other body fluids.
5. As with all diagnostic tests, the definitive clinical diagnosis must not be based on the result of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated. EpiTuub[™] FOB test is designed for the preliminary screening and should not replace other diagnostic procedures such as colonoscopy or sigmoidoscopy, etc.

PERFORMANCE CHARACTERISTICS

1. **Sensitivity:** The analytical sensitivity of this test is 50 ng h-Hb/ml fecal sample extract, which is about 1 µg h-Hb/gram stool.
2. **Reproducibility:** Positive and negative fecal samples spiked to target h-Hb concentrations of 0 ng/ml, 37.5 ng/ml, 50 ng/ml, 62.5 ng/ml, 200 ng/ml, 1,000 ng/ml and 10,000 ng/ml were repeated tested in multiple assay (30x) by both laboratory professionals and staff from physician office laboratories (POL). The test results were compared and found to be highly consistent with a 99.0% agreement between the results from POL and results from laboratory professional. The overall accuracy of this FOB test by POL was 96.6%.
3. **Accuracy:** A validation study using 120 hemoglobin negative fecal sample extracts and 150 positive extracts, was performed with this FOB test and another FDA approved commercial immunological FOB test. It was found that this FOB test has a 99% test sensitivity and 100% test specificity.
4. **Prozone Effect:** It is observed that this FOB test detects 100,000 ng h-Hb/ml fecal sample extract, which equals to about 2 mg h-Hb/gram stool.
5. **Specificity:** This FOB test is specific for the detection of h-Hb, h-Hb-S, h-Hb-C, from whole human blood at a concentration of 50 ng/ml and 100,000 ng/ml. This FOB test does not detect hemoglobin from a cow, horse, pig, fish, chicken, or rabbit.
6. **Interference Testing:** Positive and negative samples added with interference factors extracted from ground raw meat of beef, pork, goat, rabbit, chicken and assayed with this FOB test. It was found that there was no cross-reaction with test results for both the negative and positive fecal samples. Extracts from raw broccoli, cauliflower, cantaloupe, horseradish, turnip and vitamin C pills were added to both negative and positive fecal samples and assayed with this FOB test. The results showed that there is no change of the interpretation of the FOB test results before and after the addition of these extracts.

REFERENCES

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3. CDC. Screening for colorectal cancer - United States, 1997. MMWR 1999;48:116
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5. Sieg A, et al. Validity of new immunological human fecal hemoglobin and albumin tests in detecting colorectal neoplasia - an endoscopy controlled study. Z Gastroenterol. 1998;36:485



Manufacturer	No. of tests
Catalog Number	Keep away from heat and direct sun light
Lot No.	Store at
In Vitro Diagnostic Device	Use by