Jant Pharmacal Corporation

Accutest® Dual Sample Immunological Fecal Occult Blood (IFOB) Test CLIA Waived

FOB PACKAGE INSERT INSTRUCTION (PI)

For Professional Use

INTENDED USE

The Accutest® Dual Sample Immunological Fecal Occult Blood (IFOB) Test is a rapid, qualitative, sandwich immunoassay for the determination of fecal occult blood in human stool sample by laboratories or physician offices. It is useful to determine gastrointestinal (GI) bleeding found in a number of gastrointestinal (GI) 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) hospitals monitoring for bleeding in patients, 3) Screening for colorectal cancer or gastrointestinal bleeding from any source.

SUMMARY AND EXPLANATION OF THE TEST

The American Cancer Society and Centers for Disease Control recommend a fecal occult blood feces test annually after age 50 to aid in the early detection of colorectal cancer. Three types of assays for fecal occult blood testing are commercially available: 1) Gum Guaiac method, 2) Hemoporphyrin and 3) Sandwich Immunochemical method.

The gum Guaiac method is widely available but lacks high accuracy. Guaiac is a naturally occurring phenolic compound that can be oxidized to quinine by hydrogen peroxidases with a detectable color change. The sensitivity and specificity of Guaiac methods 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 Methods.

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 immunochemical 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 immunochemical tests are not affected by dietary peroxidases, animal blood and ascorbic acid.

PRINCIPLE OF THE PROCEDURE

The Accutest® Dual Sample IFOB Test is a qualitative, sandwich colloidal gold conjugate immunoassay for the determination of human hemoglobin in feces. The method employs a unique combination of monoclonal and polyclonal antibodies to selectively identify hemoglobin in test sample with a high degree of sensitivity. In less than 5 minutes, elevated levels of human hemoglobin as low as 50 ng/ml can be detected, and positive results for high levels of hemoglobin can be seen in the test as early as two to three minutes.

As the test sample flows through the absorbent device, the Colloidal Gold labeled antibody- conjugate binds to the hemoglobin in the specimen forming an antibody-antigen complex. This complex binds to the Antihemoglobin antibody in the positive reaction zone and produces a pink – rose color band when the hemoglobin concentration is greater than the 50 ng/ml. In the absence of hemoglobin, there is no line in the positive reaction zone. The reaction mixture continues flowing through the absorbent device past the positive reaction zone and negative control zone. Unbound conjugate binds to the reagents in the negative control zone, producing a pink rose color band, demonstrating that the reagents and device are functioning correctly. A **NEGATIVE** specimen produces one distinct color band in the control area. A **POSITIVE** specimen produces two color bands, one in the control and one in the test area. There is no meaning attributed to color or its intensity for either line.

REAGENTS

1. The Accutest® Dual-Sample IFOB Test consists of a chromatographic absorbent device in which the hemoglobin in the stool sample binds with an antibody immobilized on a porous membrane. The method employs unique monoclonal (mouse) antibodies to selectively identify Fecal Occult Blood hemoglobin in test samples at the cutoff level of 50ng/ml hHb/ml buffer. The control zone of the membrane of the test device is coated with goat anti-rabbit antibody. The sample pad contains a colloidal gold-labeled mouse monoclonal anti- Fecal Occult Blood hemoglobin antibody and colloidal gold labeled rabbit IgG. A **POSITIVE** specimen produces two distinct color bands in both the test area and control area. A **NEGATIVE** specimen produces only one color band in the control area. There is no meaning attributed to color or its intensity for either line.

MATERIALS PROVIDED

1. Test device in foil wrapper (**Do Not** open before use. If the foil package is damaged do not use the device. Call Jant Pharmacal Corp. or your distributor).

2. Instructions for testing.

3. Specimen Collection Tube (2.5 ml): Contains 2.5 ml of hemoglobin extraction buffer for stool sample, pH 7.2, stabilizers, and 0.1 % sodium azide as preservative.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Specimen collection containers.
2. Clock or timer.
3. Latex gloves

WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic use only.
2. For professional use only.
3. Do not use the test cassette beyond the expiration date imprinted on the outside of the foil pouch.
4. Use a new specimen collection tube for each test to avoid cross contamination of fecal samples.
5. Visually inspect the foil package to ensure it is intact. If the package is not intact discard the device.

STORAGE AND STABILITY

The test device and sample collection tube are stable when stored in a controlled environment at 2° – 30°C (35.6° – 86° F) until the expiration date printed on the label. Do not expose the test device to temperature over 30°C (86° F). However the sample collected may be stored up to eighteen (18) days from 9° - 37°C (48.2°F - 98.6° F) , six (6) months at refrigerated temperature 2° – 8°C (35.6° – 46.4°F) and two years (2) at ≤20°C (≤-4°F).

PATIENT LIMITATION

1.A specimen should not be collected from a patient with the following conditions that may interfere with the test results:

•Menstrual bleeding

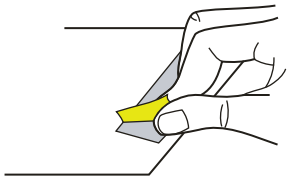
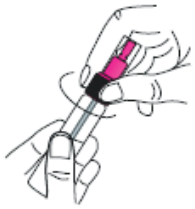
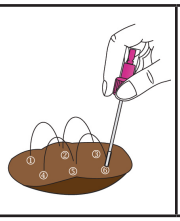
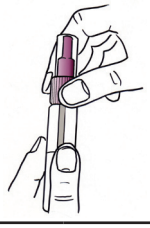
•Bleeding hemorrhoids

•Constipation bleeding

•Urinary bleeding

2.Alcohol and certain medications such as aspirin, indomethacin, reserpine, phenylbutazone, corticosteroids and non-steroidal anti-inflammatory drugs may cause gastrointestinal irritation and subsequent bleeding in some patients. Such substances should be discontinued at least 48 hours prior to testing.

SAMPLE COLLECTION AND STORAGE

1.Peel off the tape covering the adhesive strips, located on each end of the sample collection paper.	
2.Lift toilet seat. and place sample collection paper across rim of toilet bowl. Use adhesive strips, located on each side of sample collection paper, to secure it to sides of toilet rim, as shown.	
3.Replace toilet seat onto toilet bowl	
4.Make bowel movement onto collection paper.	
5.Unscrew the cap of the collection tube and remove the applicator stick.	
6.Insert the applicator stick into stool sample at 6 different sites. Use only enough fecal material to cover the tip of the applicator stick.	 
7.Screw the applicator stick into the tube and secure tightly. 8.The specimen is now ready for testing, transportation or storage. 9.Collect the second sample and place into the second sample collection tube (collection tube 2). The collection procedure can follow points 5, 6, 7, 8 above.	

Procedural Note:

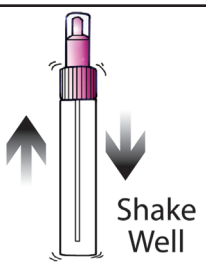
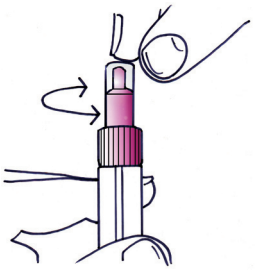
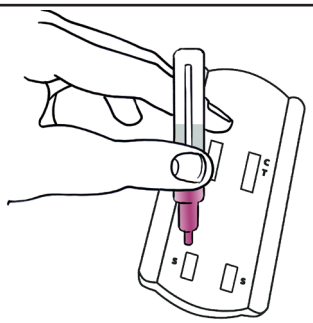
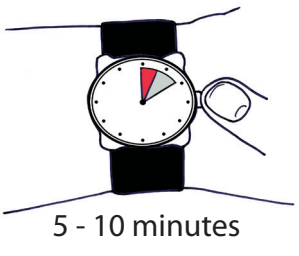
1.Do not use stool sample as test specimen if it comes into contact with toilet water.

2. The second stool sample can be collected from a different site on the same stool, or a stool sample collected from a different day (preferred).

3. If you are collecting stool samples from different days, you must save the first stool sample at room temperature (2°-39°C).

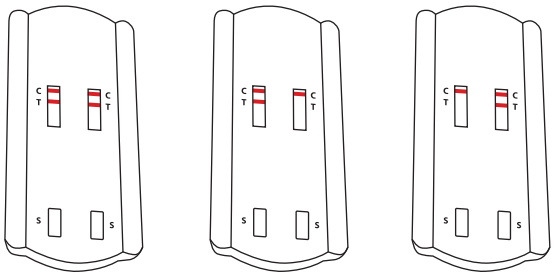
4. Tests that use specimens from two different days must be performed at the same time.

ASSAY PROCEDURE

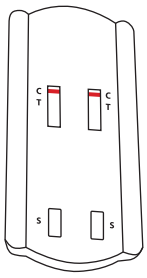
1.Remove the cassette from the pouch.	
2.Shake the sample collection tube vigorously to ensure a good liquid suspension.	
3.Holding the tube upright, unscrew the clear tip cover.	
4.Dispense four (4) drops of suspension from each tube into appropriate sample well: a)Four (4) drops from sample collection tube one (1) should be placed into sample well one (S1). b)Four (4) drops from sample collection tube two (2) should be placed into sample well two (S2).	
5.Read the results within 5-10 minutes after adding the extraction solution. ***Important: Do not read the results after 10 minutes.***	

READING THE TEST RESULTS

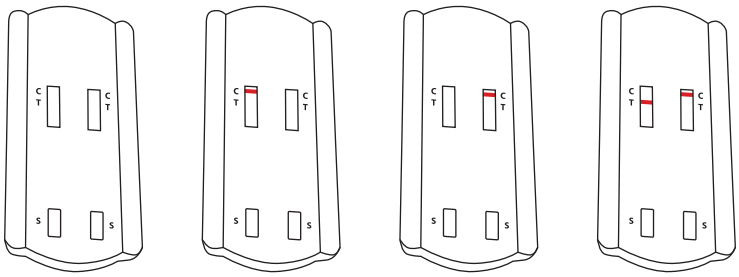
1.Positive: In addition to the control band, a magenta band also appears on the test region (lower portion of the read area). If this is the case, the Accutest® Dual-Sample Immunological Fecal Occult Blood level is above the detection cutoff level of 50 ng/ml extraction buffer or 50 µg hHB/g of feces for the sample tested. Note: Positive results for either sample 1 and/or sample 2 would indicate positive results for occult blood in the stool.



2.**Negative:** One magenta band appears on the control region with no visible band on the test region (lower portion of the read area). This is an indication that the sample tested is below the Accutest® Dual-Sample IFOB detection sensitivity level of 50 ng/ml extraction buffer or 50 µg hHB/g of feces.



3. **Invalid:** If, after performing the test, no lines are visible in either or both control areas of the cassette, then the results are invalid. It is recommended that the specimens be retested.



QUALITY CONTROL

1.Internal Quality Control

Each reaction cassette has its own built-in quality control indicator. If, after performing the test, no line is visible on the cassette, the device may have been over or under loaded with specimen or the test cassette may have deteriorated. The assay will have to be repeated using a new Accutest® Dual-Sample IFOB test cassette. Re-read the instructions carefully or call Jant Pharmacal Corporation or their local distributor for assistance.

2.External Quality Control

Good laboratory practice recommends the use of control material to test each product batch or whenever it is necessary to validate reagent performance and reliability.

AFTER TESTING

Stool specimens may be infectious. Properly handle and dispose of all used reaction devices into an approved biohazard container. Residual specimens should be disposed in a medically approved manner after completion of all testing including confirmatory testing.

LIMITATION 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.Analytical Sensitivity:

Accutest® Dual-Sample IFOB Test has been designed for the detection of Fecal Occult Blood test in feces at the detection cutoff of 50 ng hHb/ml buffer or 50 µg hHb/g feces.

2. Interfering Substances and Cross Reactivity:

A study was conducted with Accutest® Dual-Sample IFOB Test to determine the cross-reactivity of non human hemoglobin, dietary peroxidases and other interfering substances. The following substances, were spiked in both positive and negative specimens. No false results were obtained.

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	500
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

3.Reproducibility:

The reproducibility of Accutest® Dual-Sample IFOB Test was determined using replicate assays of samples from human hemoglobin free fecal extraction buffer, spiked with human hemoglobin at the following concentration levels: 0, 25, 50, 75, 200 and 2000 ng/ml. Tests were conducted with kits from three different production lots. Each sample was run through fifteen parallel assays. The data showed 100% precision for the duplicates of each sample and 100% precision from different lots.

4.Method Comparison:

Study

The study on 180 spiked samples were blind labeled and tested with the Accutest® Dual-Sample IFOB Test and the predicate device at each hospital laboratory site. Three different people in each laboratory carried out the analysis. The results (N=3 x 180 samples) were obtained from three different hospital laboratory sites. Stool samples were spiked with hHb at 0, 37.5, 50, 62.5, 200 and 2000 ng hHb/ml.

When compared to expected result (ELISA) the positive percent agreement (PPA) is 99.2% (95% CI: 97.6% - 99.8%) and the negative percent agreement (NPA) is 96.7 % (95% CI: 92.9% - 98.8%). The total percent agreement was 98.3% (95% CI: 96.9% - 99.2%).

When compared to the predicate device result, the positive percent agreement (PPA) is 98.3% (95% CI: 96.4% - 99.4%) and the negative percent agreement (NPA) is 94 % (95% CI: 89.4% - 96.9%). The total percent agreement was 96.8% (95% CI: 95%-98.2%).

LIMITATIONS OF THE TEST

1.This product is designed to be used for the detection of human hemoglobin from human feces only.

2.Results can not 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.

3. 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.

4. 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.

5. The American Cancer Society recommends home collection of two samples from three consecutive bowel movements.

BIBLIOGRAPHY

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4.Ribet,A., Frexinos,J., Escourrou,J., Delpu (1980). Occult-blood test and colorectal tumors. Lancet 1:417.