ColoSignal™

One Step iFOB Test

For immunological detection of fecal occult blood

INTENDED USE

ColoSignal™ is a rapid immunochemical Fecal Occult Blood (iFOB) test device intended for the qualitative detection of fecal occult blood in feces by professional laboratories and physician offices. The test is able to be used for the determination of 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, (2) monitoring any bleeding in patients, and (3) screening for colorectal cancer or gastrointestinal bleeding.

SUMMARY AND EXPLANATION

Colorectal cancer is the third most common cancer in the world. The presence of occult blood in feces could be an early warning sign of colorectal cancer and polyps. Other gastrointestinal disorders such as diverticulitis, Crohn's disease, colitis ulcer, etc. may also be associated with the presence of fecal occult blood. The European Union and the U.S. Preventive Services Task Force (USPSTF) recommend screening for colorectal cancer using fecal occult blood test in adults, beginning at age 50 years.

There are two different types of FOB tests available, the traditional guaiac FOB test and antibody based immunochemical FOB test. The traditional guaiac FOB tests do not provide a high degree of accuracy. ColoSignal™ is an antibody based immunochemical FOB test with higher sensitivity and specificity in detecting low levels of human fecal occult blood, especially from the lower gastrointestinal bleeding. It does not require special dietary restrictions prior to the specimen collection compared to the traditional guaiac FOB method.

TEST PRINCIPLE

ColoSignal™ is a one-step lateral flow chromatographic immunoassay utilizing two monoclonal antibodies to specifically detect the presence of human hemoglobin (hHb) in feces. The stool specimen is collected into the collection tube containing extraction solution. When the moderate volume of specimen extract is dispensed into the sample well of the test device, the specimen flows by capillary action through the conjugate pad where the colloidal gold labeled antibody-conjugate binds to hHb present in the specimen, forming an immuno-complex. This immuno-complex will then bind to the membrane coated monoclonal anti-hHb antibody in test region, producing a visible test line (T line) when the concentration of hHb is at or above 50 ng/ml. Then the reaction mixture flows further along the strip until it reaches the control region where the excess or unbound conjugate binds to the membrane coated goat anti-rabbit IgG antibody producing a second visible control line (C line) regardless of the presence of hHb. This control line indicates the test strip is functioning properly and the result is valid.

MATERIALS PROVIDED

Content/Catalog#	CS020	TCS020
Instruction for use	1	1
Foil pouches, each pouch containing 1 test cassette and 1 desiccant	20	20
Specimen collection tube with extraction buffer and attached patient label	20	20
Specimen collection paper	20	20
Specimen bag	20	20
Absorbent sleeve	20	-
Return mail box	20	-
Patient instructions	20	-

MATERIALS REQUIRED BUT NOT PROVIDED

1. Timer.
2. External controls.

WARNINGS AND PRECAUTIONS

1. For In Vitro Diagnostic use only.
2. Do not use test kit beyond expiration date or the pouch that is damaged.
3. Read the entire procedure carefully prior to performing any tests.
4. Improper temperature and humidity levels may adversely affect test results.
5. Do not eat, drink or smoke in the area where the specimens and kits are handled. Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout the procedure and follow the standard procedures for proper disposal of specimens. Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.
6. When the assay procedure is completed, dispose specimens carefully after autoclaving them at 121°C (250°F) for at least 20 minutes. Alternatively, they can be treated with 0.5% sodium hypochloride (or house-hold bleach) for one hour before disposal. The used testing materials should be discarded in accordance with local, state and/or federal regulations.
7. Do not reuse the test device.

STORAGE AND STABILITY

Store the kit at room temperature 8-35°C (46-95°F) in a cool and dry place away from the heat and direct sunlight. The kit is stable until the expiration date printed on the label. DO NOT FREEZE or expose kit components to temperature over 35°C (95°F). The test device should remain sealed in the foil pouch until ready for use.

SPECIMEN COLLECTION AND PREPARATION

Patient preparation

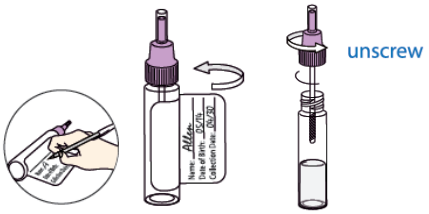
1. Specimen should not be collected during or within three days of a menstrual period, or if the patient suffers from bleeding hemorrhoids or blood in the urine.
2. Alcohol and certain medications such as aspirin, indomethacin, reserpine, phenylbutazone, corticosteroids, and nonsteroidal anti-inflammatory drugs (NSAIDs) may cause gastrointestinal irritation and subsequent bleeding in some patients.
3. Dietary restrictions are not necessary.

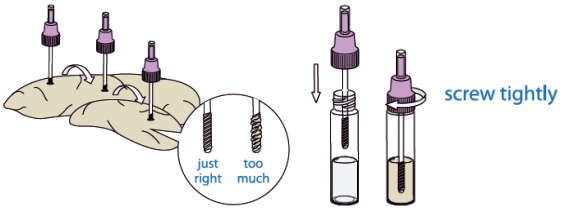
Specimen collection

The patients can follow the illustrated patient instructions inserted in the kit to collect the stool specimen, the collection procedures are outlined below. The stool specimens can be collected at any time of the day.

1. Prior to specimen deposit, urinate first if possible. Flush the toilet bowl several times, and remove all toilet cleaners, disinfectants or deodorizers.
2. Remove tape cover from each end of the collection paper, and position the paper onto rear half of toilet bowl. Allow the paper to sag just above water.
3. Lower the toilet seat, and make bowel movement on collection paper. If need to urinate, then shift body forward on the toilet seat so as to avoid urinating on the paper.

4. Fill out the attached label, then remove the back paper, and attach the label to the specimen collection tube.
5. Unscrew the cap from collection tube, and keep it in a vertical position. Do not pour out the buffer that is inside the tube.
6. Randomly **Poke** spiral applicator into feces at least 3 different sites to collect a moderate amount of stool specimen. *DO NOT SCOOP the stool specimen.*
7. Insert and screw the applicator back into collection tube and secure tightly. Flush away the collection paper and remaining feces.





8. The specimen is ready for testing, transportation or storage. The specimen collected can be stored at room temperature 8-35°C (46-95°F) for up to 3 days and at 2-8°C (36-46°F) for up to 14 days.

TEST PROCEDURE

All test cassettes and clinical specimen should be at room temperature before beginning the test procedure. Wear gloves to perform the following steps.

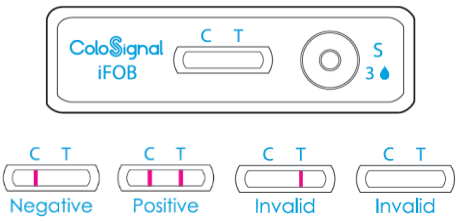
1. Remove the test cassette from its foil pouch by tearing along the notch and place it on a clean, flat, and dry surface. Use the test cassette as soon as possible.
2. Remove absorbent sleeve from specimen bag, then remove collection tube from absorbent sleeve.
3. Shake the collection tube several times to mix the specimen and buffer.
4. Hold the collection tube upright, and then unscrew the transparent tip of collection tube.
5. Squeeze **3~4 drops** of specimen solution from the collection tube into the sample well (S) of test cassette.
6. Read results at **5-10 minutes**. It is important that the background is clear before the result is read. Some positive results may appear sooner.
   Do not read results after 10 minutes.

INTERPRETATION OF RESULTS

Positive: If both burgundy colored C line and T line are present, the test result is positive and valid. A positive result indicates the level of hHb in the specimen is at or above 50 ng hHb/ml stool specimen extract or 5 µg hHb/g stool.

Negative: If only the burgundy colored C line appears in the control region, the result is negative and valid. A negative result indicates the level of hHb in the specimen is below 50 ng hHb/ml stool specimen extract or 5 µg hHb/g stool.

Invalid: If there is no visible C line appears in the control region regardless of the presence of T line, the result is invalid. The assay should be repeated with a new test device.



QUALITY CONTROL

Internal Quality Control

ColoSignal™ one step iFOB test contains a built-in control feature, the Control (C) line. The presence of this burgundy colored C line indicates the sufficient specimen volume was used and the reagents migrated properly. If a C line does not develop in the control region, the test is considered invalid. In this case, review the whole procedure and repeat the testing with a new device.

External Quality Control

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

LIMITATIONS

1. This test is for the detection of human hemoglobin in feces only, not for use in testing urine, gastric specimens or other body tissues or fluids.
2. A number of conditions, as mentioned in the "Patient Preparation" section, may cause false positive results. The patients with the mentioned conditions may be considered for testing after such bleeding ceases.
3. Intermittent tumor bleeding and irregular distribution of blood in the feces also contribute to false negative results. Repeating testing is recommended if a pathological condition is suspected.
4. Urine and excessive dilution of stool specimens with water from toilet bowl may cause erroneous results.
5. As with all diagnostic tests, the conclusive clinical diagnosis cannot 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. ColoSignal™ one step iFOB test is designed for the preliminary screening and cannot replace other diagnostic procedures such as colonoscopy or sigmoidoscopy, etc.

PERFORMANCE CHRATERISTICS

1. **Sensitivity**: The analytical sensitivity of this test is 50 ng hHb/ml stool specimen extract, which is about 5 µg hHb/g stool.
2. **Reproducibility**: Positive and negative stool specimen spiked to target hHb concentrations of 0 ng/ml, 37.5 ng/ml, 50 ng/ml, 62.5 ng/ml, 200 ng/ml, 1,000 ng/ml, and 2,000ng/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 test by POL was 96.7%.
3. **Accuracy**: A validation study using 620 hemoglobin negative stool specimen extracts and 28 positive extracts, was performed with this iFOB test and another CE approved commercial iFOB test. It was found that this iFOB test has a 99.7% test Accuracy.
4. **Specificity**: This test is specific for the detection of hHb, hHb-S, hHb-C, from whole human blood at a concentration of 50 ng/mL. This test does not detect hemoglobin from a cow, horse, pig, sheep, chicken, or rabbit.
5. **Interference Testing**: Positive and negative specimens were added with 1 mg/mL of interference factors extracted from ground raw meat from beef, pork, goat, rabbit, chicken & horseradish peroxidase and assayed with this test. It was found that there was no cross-reaction with test results for both the negative and positive stool specimens. Acetaminophen, Aspirin, Ampicillin, Vitamin C, Atropine, Caffeine, Gentisic acid, Glucose, Human albumin, Urea and Uric acid were added to both negative and positive stool specimens and assayed with this test. The results showed that there is no change of the interpretation of the test results before and after the addition of these additives. No interference was found with any of the substances at the following concentrations:

Substance	Concentration	Substance	Concentration
Aspirin	20 mg/dL	Acetaminophen	20 mg/dL
Ampicillin	40 mg/dL	Glucose	2000 mg/dL
Vitamin C	40 mg/dL	Human albumin	2000 mg/dL
Atropine	40 mg/dL	Urea	4000 mg/dL
Caffeine	40 mg/dL	Uric acid	10 mg/dL
Gentisic acid	40 mg/dL		

REFERENCES

1. U.S. Preventive Services Task Force. Screening for colorectal cancer: U.S. Preventive Services Task Force recommendation statement. Ann Intern Med 2008 Nov 4; 149(9):627-37.
2. N. Segnan, J. Patnick, L. von Karsa. European guidelines for quality assurance in colorectal cancer screening and diagnosis. First edition. European Commission 2010; 1-21
3. L.G. van Rossum et al. Random comparison of guaiac and immunochemical fecal occult blood tests for colorectal cancer in a screening population. Gastroenterology 2008; 135:82-90.
4. H. Saito. Screening for colorectal cancer by immunochemical fecal occult blood testing. Jpn J Cancer Res 1996; 87:1011-24.

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CE METAS Medical Total Solutions

V3. 201607

IVD	In Vitro Diagnostic Device		Consult Instruction for Use
REF	Catalogue Number	Σ	No. of Tests in Package
LOT	Lot Number		Use by
	Keep Away from Sunlight		Keep Dry
	Do Not Reuse		Temperature Limitation
	Manufacturer	EC REP	Authorized Rep. in EC