

Lab Name: _____

Procedure #: _____

Procedure: CLIA Complexity: Waived

Prepared By	Date Adopted	Supersedes Procedure #

Review Date	Revision Date	Signature

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This Procedural Bulletin is intended to provide a ready outline reference for performance of the assay. These abbreviated directions for use are not intended to replace the complete package insert. It is the obligation of every manufacturer of medical devices labeled FOR *IN VITRO* DIAGNOSTIC USE to provide a complete package insert in accordance with FDA labeling regulation (21 CFR 809.10). Prepared in accordance with the guidelines recommended by the Clinical and Laboratory Standards Institute, Wayne, PA 19087; CLSI Document GP2-A4.

Hemosure, Inc. provides CLSI procedures for your use. The procedures are required to include the same information as listed in the package insert. Any modifications to this document are the sole responsibility of the Laboratory.

Lab Name: _____**CLIA Complexity: WAIVED****Intended Use:**

Hemosure® One Step Immunological Fecal Occult Blood Test is a rapid, immunochemical device for the qualitative determination of Fecal Occult Blood by laboratories or physician's offices. It is useful in determining gastrointestinal (GI) bleeding in a number of GI disorders, e.g. diverticulitis, colitis, polyps, and colorectal cancer.

Summary and Explanation:

The guaiac method was developed by Van Deen in 1864 to detect occult blood. Boas began to use this method in 1901 to diagnose gastric bleeding. Since that time, numerous improvements have been introduced which utilize the peroxides activity of heme. However, in order to get accurate test results, guaiac-based tests require that certain foods, drugs, vitamins and other substances should be avoided before and during the sample collection period¹. Several authors have also found that some patients with colorectal cancer or adenoma tested negative for occult blood because of the lack of sensitivity of guaiac-based methods^{4,1}.

Subsequent developments of latex immunochemical agglutination and of the single radial immunodiffusion (SRID) and of the reverse-passive hemagglutination (RDHAA) have produced test methods more sensitive to low concentrations of human hemoglobin in feces^{2,3}. The results of clinical studies indicate that test results are positive in only about 50-60 percent of patients with colorectal cancers and only 25-30 percent of patients with polyps⁵. Therefore, a more sensitive means for detecting fecal occult blood is important for the diagnosis of diseases that result in gastrointestinal bleeding. Hemosure® One Step Immunological Fecal Occult Blood Test actually detects lower levels of fecal occult blood than the standard guaiac tests by employing an immunospecific, sandwich assay that is not affected by dietary peroxidases, animal blood, or ascorbic acid.

Principle :

Hemosure® One Step Immunological Fecal Occult Blood Test is a qualitative, sandwich dye conjugate immunoassay and employs a unique combination of monoclonal and polyclonal antibodies to selectively identify hemoglobin in test samples with a high degree of sensitivity. In less than five minutes, elevated levels of human hemoglobin (hHB) as low as 50ng hHB/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. (Please Note: Test Procedure instructions state the following "Read results within five (5) to ten (10) minutes. Do not read after ten (10) minutes.")

As the test sample flows up through the absorbent device, the labeled antibody-dye conjugate binds to the hemoglobin in the specimen forming an antibody-antigen complex. This complex binds to antihemoglobin antibody in the positive test reaction zone and produces a pink-rose color band. In the absence of hemoglobin, there is no line in the positive test reaction zone. The pink-rose color bands in the control reaction zone demonstrate that the reagents and devices are functioning correctly.

Reagents and Materials Provided:**Hemosure® One Step Immunological Fecal Occult Blood Test contains:**

1. One test Cassette individually sealed in a foil pouch. Each Test Cassette contains a combination of mouse monoclonal antibodies and polyclonal antibodies (sheep or goat) directed against human hemoglobin.
2. One Fecal Collection Tube containing 2.0 mL of extraction buffer.

Materials Required but Not Supplied:

1. Clock or timing device
2. Sample collection container
3. Disposable gloves

Storage:

Store test device at 35.6°F-86°F (2°C -30°C). The test device is stable until the date printed on the pouch label. If Fecal Collection Tube is not used immediately after sampling, it may be safely stored up to fourteen (14) days at ambient room temperature as high as 98.6°F (37 °C), up to six (6) months in refrigerator at 39.2°F (4°C) or for twelve (12) months in freezer at -4°F (-20°C).

Warnings and Precautions:

1. The test is intended for *IN VITRO* DIAGNOSTIC USE ONLY.
2. Read directions for use carefully before performing this test.
3. Do not use the test beyond the expiration date on the pouch label.
4. For professional use only.
5. Use a new specimen collection tube for each test to avoid cross contamination of fecal samples.

Patient Limitations:

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

Assay Procedure:**1. Sample Collection and Preparation (See Figure 1)**

NOTE: Handle all specimens as if potentially infectious. Proper precautions in handling should be maintained according to good laboratory practice. Fecal Samples should be collected using disposable gloves. Although no interference was noted with the toilet water testing, it is advisable to avoid samples coming in contact with toilet bowl water. If this is unavoidable, recommend that the user flush the toilet thoroughly, before sample collection, to avoid possible contamination from residual hHb, which may lead to false positive results.

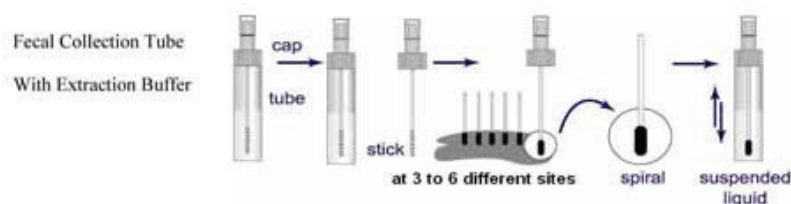


Figure 1

- 1) If refrigerated, allow to come to room temperature.
- 2) Unscrew cap of the Fecal Collection Tube and remove Applicator Stick.
- 3) Randomly insert the Applicator Stick into the fecal sample from three (3) to six (6) times.
- 4) Do not clump, scoop, or fill the tube.
- 5) Return the Applicator Stick into the Fecal Collection Tube and tighten the cap thoroughly. Shake the tube to mix the sample with the Extraction Buffer.

2) Test Procedure (See Figure 2-3)

- 1) Remove the Test Cassette from its foil wrapper by tearing along the slice.
- 2) Shake the Fecal Collection Tube to ensure that the fecal sample is well mixed.

- 3) Twist off the tip of the cap on the Fecal Collection Tube. Add three (3) drops of the Extraction Buffer mixture to the Sample Well.
- 4) Start timer.
- 5) Read results within five (5) to ten (10) minutes. Do not read after ten (10) minutes.



Figure 2

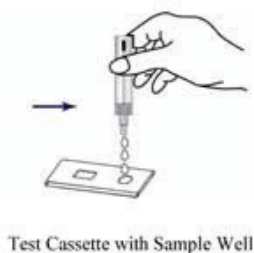


Figure 3

3) Interpretation of Results (See Figures 4 – 6)

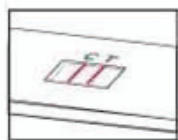


Figure 4

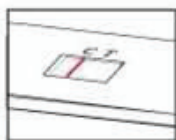


Figure 5



Figure 6

- 1) Positive: One band appearing in the "C" region, the other in the "T" region.
- 2) Negative: Only one color band appearing in the "C" region.
- 3) Invalid: No color bands appearing in the window at all, the test result is invalid.
The test should be repeated with a new Test Cassette.

Quality Control

1. Internal Quality Control

Hemosure iFOB test contains a built-in Control--C Line. This line appears next to the C on the Test Window. The presence of C line indicates that an adequate sample volume was used and that the Test Cassette worked properly. If no C Line appears the test is invalid and must be repeated.

2. External Quality Control

The appropriate local, state and federal guidelines regarding external quality controls should be followed.

Performance Characteristics:

1. Sensitivity:

The sensitivity of the test is 50ng hHb/mL buffer or 50 µg hHb/g feces.

2. Specificity:

Hemosure® One Step Immunological Fecal Occult Blood Test is specific for human hemoglobin. Hemoglobin from horse, pigs, fish, beef, chicken, rabbit, rat, goat, and mouse do not react with Hemosure® One Step Immunological Fecal Occult Blood Test. Aqueous extracts of broccoli, cantaloupe, cauliflower, horseradish, parsnip, raw turnip, and red radish were tested with and without human hemoglobin present in the samples. Additionally, a 20mg/mL solution of horseradish peroxidase, with and without human hemoglobin present, was also tested. No interference was observed. Toilet bowl deodorizers/fresheners, cleaners also did not interfere with Hemosure® One Step Immunological Fecal Occult Blood Test.

3. Accuracy:

Reference Laboratory and Physicians Office Laboratory (POL) Studies One hundred (100) hHb-free feces extraction specimens collected in-house were divided into five (5) groups of 20 each. The five groups of extractions sample were spiked with hHb at the following concentrations: 0, 37.5, 50, 62.5 and 2,000 ng hHb/mL. The specimens were blinded and tested with Hemosure® One Step Immunological Fecal Occult Blood Test at a Physicians Office Laboratory and a Reference Laboratory.

The results obtained from the POL site, by persons with diverse education background and work experience, agree 97% with the expected results. The result obtained from the Reference Laboratory agreed 99% with expected. Overall, the accuracy of Hemosure® One Step Immunological Fecal Occult Blood Test is 97%.

4. Comparison Studies:

Fifty (50) specimens were also tested in-house with Hemosure® One Step Immunological Fecal Occult Blood Test and a predicate device. The correlation between Hemosure® One Step Immunological Fecal Occult Blood Test and the predicate device was over 99%.

Limitation for the Procedure:

1. A negative result can be obtained even when a GI disorder is present. Some bowel lesions, including some polyps and colorectal cancer, may not bleed at all or may bleed intermittently, or the blood may not be uniformly distributed in a fecal sample.
2. Certain medications may cause gastrointestinal irritation resulting in occult bleeding. This may result in a false positive test result.
3. As with any occult blood test, Hemosure® One Step Immunological Fecal Occult Blood Test may not be considered as a conclusive diagnostic for gastrointestinal bleeding or pathology. The test results can only be regarded as a preliminary screening or as an aid to diagnosis. It is not intended to replace other diagnostic procedures such as G.I. fibroscope, endoscopy, colonoscopy or other x-ray studies.
4. Abnormal hemoglobins were not tested for potential cross-reactivity.
5. Color blind users may see the Control and Test lines as gray rather than pink-rose lines.

Reference:

1. Adams, E.C., Layman K.M. *Immunochemical confirmation of gastrointestinal bleeding.* *Ann. Clin. Lab. Sci.* 4:343; 1974.
2. Salto, H., et al. *An immunological occult blood test for mass screening of colorectal cancer by reverse-passive hemagglutination (RPI-IA).* *Japanese J. Gastroenterology.* 61:2831; 1984.
3. Saito H. *Screening for colorectal cancer by immunochemical fecal occult blood testing (Review).* *Jpn J Cancer Res* 1996; 87:1011-102.
4. Ribet, A., Frexinos, J., and Escourrou, J. "Occult-blood test and Colorectal Tumors." *Lancet, Vol. I* (1980):417.
5. Allison JB, Takawa IS, Ransom LJ, Adrian AL. *A comparison of fecal occult blood test for colorectal-cancer screening.* *N Engl J Med* 1996; 334:155-159.

Manufactured for:

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Lab Name: _____

Log Sheet

Record Built-in Procedural Controls on the first patient each day

	Date	Patient Name	Positive Procedural Control (Burgundy Line)	Test Results At 5-10 minutes Do not read the test results after ten (10) minutes	Lot Number and Exp. Date	Technician
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						

Lab Name: _____

Log Sheet

HEMOSURE®
Record Built-in Procedural Controls on the first patient each day.

	Date	External Positive Control Result	External Negative Control Result	Lot Number and Exp. Date	Technician
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					