**Purpose**

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

**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 (sheep or goat) 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.

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.

**Materials**

# Storage:

Store test device at 39.2°F - 86°F (4°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 98.6ºF (37 ºC), 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).

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| **Reagents** | **Supplies** | **Equipment** |
| • Test Cassette individually sealed in a foil pouch. Each Test Cassette contains a combination of mouse monoclonal antibodies and polyclonal antibodies   * Fecal Collection Tube containing 2.0 mL of extraction buffer. * iFOBT Control Set | * Disposable gloves * Sample collection container * Mailers (Outpatient and Clinic specimens) | * Test Cassette * Collection tube * Clock or Timing Device |

# Warnings and Precautions:

* The test is intended for **IN VITRO DIAGNOSTIC USE ONLY**.
* Read directions for use carefully before performing this test.
* Do not use the test beyond the expiration date on the pouch label.
* For professional use only.
* 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

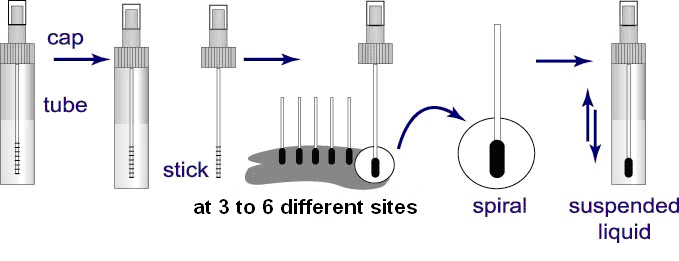
**Safety**

* 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.

**Specimen Sample Collection and Preparation (See Figure 1)**

**NOTE:**

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.



Fecal Collection Tube

With Extraction Buffer

Figure 1

1. Prior to collection, if Fecal Collection Tube has been 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 up to six (6) times to obtain ample amount of sample.
4. Do not clump, scoop, or fill the tube.
5. Return the Applicator Stick into the Fecal Collection Tube and tighten the cap thoroughly.
6. Shake the tube to mix the sample with the Extraction Buffer.

**Quality Control** (CLIA Complexity: Waived)

Internal Quality Control

1. 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.

External Quality Control

1. Run external controls on each new lot or shipment of Hemosure test cassettes.
2. Expected results:

* The Positive Control contains purified human hemoglobin and gives a positive result.
* The Negative Control contains a buffer and no human hemoglobin and gives a negative result.
* The test is positive (+) if two lines (Control and Test) are visible in the viewing window. Any trace of a pink line in the Test Line area is a positive test result.
* The test is negative (-) if only the Control line is visible and there is no trace of a pink line in the Test line area.
* The test is invalid if the control line does not appear, if this occurs, the test should be repeated.

1. Hemosure® iFOBT Controls includes a positive control containing stabilized human hemoglobin and negative control containing a buffer matrix. Handle all control material as if potentially infectious. For **IN VITRO DIAGNOSTIC USE ONLY.**

**PROCEDURE**

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| **Step** | **Action** |
| 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. |
| 4 | Add three (3) drops of the Extraction Buffer mixture to the Sample Well. |
| 5 | Start timer. |
| 6 | Read results within five (5) to ten (10) minutes. Do not read after ten (10) minutes. |
| **Interpretation**  **Interpretation of Results (See Figures 2 – 4)**    Figure 2 Figure 3 Figure 4   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. | |
| **Result Reporting**   * **Positive –** Screening for Human blood present * **Negative –** Screening for Human blood absent | |

**Restrictions/Limitations**

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 screen, 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.

**Procedure Notes**

**Sensitivity:**

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

**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.

**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%.

**References**

1. *Hemosure Pkg insert, Hemosure, Inc., El Monte, CA. Web:* [*www.hemosure.com*](http://www.hemosure.com)*.*
2. *CLSI Hemosure iFOBT Procedure, prepared in accordance with guidelines recommended by CLSI Document GP2-A4.*
3. *Adams, E.C., Layman K.M. Immunochemical confirmation of gastrointestinal bleeding. Ann.ehn. Lab. Sci. 4:343; 1974.*
4. *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.*
5. *Saito H. Screening for colorectal cancer by immunochemical fecal occult blood testing (Review). Jpn J Cancer Res 1996; 87:1011-102.*
6. *Ribet, A., Frexinos, J., and Escourrou, J. “Occult-blood test and Colorectal Tumors.” Lancet, Vol. I (1980):417.*
7. *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.*