



HEMOSURE®

One-step Immunological Fecal Occult Blood Test

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 peroxidase 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 period. 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.^{4,5}

Subsequent developments of latex immunochromatography 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 polyps.² 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.

PN:Y0311103302

Warnings and Precautions:

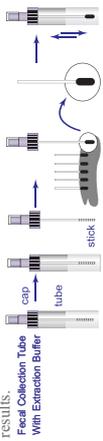
1. The test is intended for IN VITRO DIAGNOSTIC USE ONLY.
2. Read directions for use carefully before performing this test.
3. Do not use the test beyond the expiration date on the pouch label.

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.



- 1) Warm the Fecal Collection Tube with Extraction Buffer to room temperature.
- 2) Loosen cap of the Fecal Collection Tube and remove Applicator Stick.
- 3) Randomly, insert the Applicator Stick into the fecal six (6) times.
- 4) 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.

Figure 1
at 6 different sites

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:

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 as low as 0.05 µg 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 anti-hemoglobin 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.

Material required but not supplied:

Timer, sample container and disposable gloves.

Storage:

Store test device at 2-30°C (room temperature). 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 stored at 4°C for up to six (6) months or at -20°C for up to 12 months.

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, horehound, 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

- 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

3. Interpretation of Results (See Figures 4 - 6)

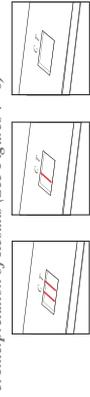


Figure 4

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.



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5. Color blind users may see the Control and Test lines as gray rather than pink-rose lines.

References:

1. Adams, E.C., Layman K.M. Immunohemical confirmation of gastrointestinal bleeding. *Am. J. Clin. Lab. Sci.* 4:343; 1974.
2. Saito, H., et al. An immunological occult blood test for mass screening of colorectal cancer by reverse-passive hemagglutination (RHP-1A). *Japanese J. Gastroenterology.* 61:2831; 1984.
3. Saito H. Screening for colorectal cancer by immunohemical fecal occult blood testing (Review). *Jpn J Cancer Res* 1996; 87:1011-102.
4. Ribbet, A., Fresinos, J., and Escourrou, J. "cult-blood test and Colorectal Tumors." *Lancet.* Vol. 1 (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.

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