Principle of the Procedure
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/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 absorption 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-orange color band. In the absence of hemoglobin, there is no line in the positive test reaction zone. The presence of a pink-orange color band in the control reaction zone demonstrates 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 includes:
   - Reagents and materials provided
   - A combination of monoclonal and polyclonal antibodies to selectively identify 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 and Stability
Store test device at 2°F–36°F (2°C–20°C). The test device is stable until the date printed on the labeling. It’s recommended to use the fecal collection tube immediately after sampling. Otherwise, the tube may be stored up to six (6) days at room temperature and up to thirty (30) days in refrigerator at 36°F–46°F (2°C–8°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 sample.

Patient Limitations
A specimen should not be collected from a patient with the following conditions that may interfere with the test results:
- Menstrual bleeding
- Constipation bleeding
- Bleding hemmoroids
- Urinary bleeding

Assay Procedure
1. Sample Collection and Preparation (See Figure 1)
   - Collect a sample of feces within one (1) hour of defecation directly into the fecal collection tube. Avoid contamination of fecal samples.
   - A specimen should not be collected from a patient with the following conditions that may interfere with the test results:
     - Constipation bleeding
     - Urinary bleeding
     - Menstrual bleeding
     - Constipation bleeding
     - Bleding hemmoroids
     - Urinary bleeding
   - 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 sample.

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. With any occult blood test, Hemosure® One Step Immunological Fecal Occult Blood Test may not be considered as a conclusive diagnostic test for intestinal 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. endoscopy, endoscopy, colonoscopy or other x-ray studies.
4. Abnormal human hemoglobin were not tested for potential cross-reactivity.
5. Color blind users may see the control and est lines as gray rather than pink-orange lines.

Reference

Quality Control
1. Internal Quality Control
   - Hemosure® FOB 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

Performance Characteristics
1. Sensitivity: The sensitivity of the test is 50ng hHb/mL buffer or 50µg 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® antibodies (sheep or goat) directed against human hemoglobin.
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: 0.375, 50, 62.5 and 2000hHb/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 99% 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 99%.
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%.