CLSI PROCEDURE

Product Name: **Status iFOBT**	
Item Number: 38030-DTM	**Waived**

Institution:	
Prepared By:	Date:
Title:	

Accepted By:	Date:
Title:	

Accepted By:		Date:	

Discontinued By	Date:

CONTENTS

SECTION 1 - TEST NAME

Status iFOBT

Immunochemical Fecal Occult Blood Test

SECTION 2 - INTENDED USAGE

Status iFOBT is a rapid qualitative test for the immunochemical detection of fecal occult blood/human hemoglobin (hHb) in human fecal specimens as an aid in the diagnosis of gastrointestinal disorders such as: diverticulitis, colitis, polyps, and colorectal cancer. The device is suitable for use in laboratories and physician's offices as well as for home use. The test is recommended for use in routine physical examination, new patient screening at admission and screening and monitoring any suspected colorectal cancer and/or gastrointestinal bleeding from any source.

SECTION 3 - SUMMARY AND EXPLANATION OF TEST

The American Cancer Society recommends that average-risk men and women over age 50 should be tested for fecal occult blood yearly or as often as their physician recommends.[1] Normally, a person may lose up to 1.5 mL of blood per day in the stool. Blood loss greater than 3 mL may be indicative of an abnormal condition. Patients with a family history of intestinal disorders or colorectal cancer, as well as those with personal histories of inflammatory bowel diseases such as polyps, colitis, and diverticulitis, should be monitored more frequently for occult blood in the stool.[2,3] Digital examination, proctosigmoidoscopy, or colonoscopy is also recommended. Polyps, the precursors of colorectal cancer, can be treated successfully if detected early.

Status iFOBT (Fecal Occult Blood Test) is designed to detect fecal occult blood that can be caused by a number of conditions such as ulcers, hemorrhoids, polyps, colitis, diverticulitis, cancer and fissures. Since these disease conditions may not produce visible symptoms in their early stages, the test may act as an early warning signal. Patients testing positive with the test must be examined thoroughly with other medical procedures.[2,3]

SECTION 4 - PRINCIPLE OF TEST

Status iFOBT employs solid-phase chromatographic immunoassay technology to qualitatively detect the presence of FOB in human feces. The sample is collected in a tube with extraction buffer. The tube is shaken to mix the fecal sample in the extraction buffer. Then, 3 drops (about 110 µL) of the mixed sample solution is added into the sample well of the test device. After the sample has been dispensed into the sample well, the extracted sample migrates into the pad containing detector antibody conjugated dye label. The hHb in the sample will bind to the detector antibody and migrate onto the membrane where the test and control lines are located. On the membrane, immobilized capture antibodies form the invisible Test line.

On the membrane, immobilized capture antibodies form the invisible Test line. When the complex of hHb and detector antibodies reaches the Test line, the complex binds to the capture antibodies to form a visible reddish Test line indicative of a positive result; i.e., hHb

is present. When no hHb is present in the sample, no reddish Test line forms. In addition to the Test line, a Control line on the membrane provides an internal quality control of the test device. Anti-species specific IgG antibodies are immobilized at the Control line.

These antibodies will capture any unreacted/excess antibody-gold conjugates, forming a distinct Control line. The Control line serves to demonstrate that: lyophilized antibodies in the dye pad have been hydrated; sufficient sample has been applied to allow for migration to the Test line and beyond; chemicals are working properly; and the proper procedure was followed. If a Control line does not appear within the designated incubation time, the test result is invalid and the test should be repeated using a new test device.

SECTION 5 - KIT CONTENTS AND STORAGE

Materials Provided:

- **Status iFOBT** device in a sealed foil pouch. The test device contains a combination of monoclonal antibodies directed against the globin of human hemoglobin.
- Sample collection tube, with sampler, containing extraction buffer solution (PBS) Warning: Do not swallow – if spilled, wash with plenty of water.
- Sample collection paper
- Mailing Envelope
- Instructions for use
- Patient instructions for use
- Plastic bag with absorbent pad

STORAGE REQUIREMENTS

Store the kit or kit contents 2-30ºC (36-86°F) until use. Do not freeze. The test device is stable until the expiration date printed on the pouch

SECTION 6 - MATERIALS REQUIRED BUT NOT PROVIDED

- Timer
- External controls
- Latex gloves

SECTION 7 - WARNINGS AND PRECAUTIONS

- In vitro diagnostic use only
- Read directions for use carefully before performing this test procedure
- Treat fecal samples and used test materials as if they are potentially infectious
- Do not reuse the test device
- Do not use the test beyond the expiration date indicated on the pouch

SECTION 8 - PATIENT PREPARATIONS AND SPECIMEN COLLECTION

- To avoid false results, specimens should not be collected while the patient has bleeding hemorrhoids or constipation, cuts on hands, during menstrual period or immediately after menstrual period.

- Hands and test area should be kept clean and free from blood to avoid false positive results.
- The American Cancer Society recommends home collection of two samples from three consecutive specimens for a total of 6 samples.[4]

Sample Collection

1. Deposit the fecal sample on collection paper or in a clean container.
   NOTE: Do not allow collection paper or feces to come into contact with toilet water or urine. The sample should not be tested if it becomes wet or contaminated. If this happens, place a clean tissue on the collection paper, then collect new feces sample for testing.
2. Unscrew the sampler from the sample collection tube with the fecal sample still on collection paper; randomly insert the grooved end of the sampler into the fecal sample in at least 6 different sites. Ensure the grooved end of the sampler is completely covered with fecal sample.
3. Insert the sampler into the sample collection tube and firmly tighten it. Do not reopen.
4. Shake the tube vigorously to mix the sample and the extraction buffer.
   NOTE: The extracted fecal sample may be stored at room temperature for up to 10 days.

SECTION 9 - QUALITY CONTROL AND ASSURANCE

Internal Procedural Control

The **Status iFOBT** device contains a built-in Control line that serves as an internal procedural control. The presence of the Control line indicates that: an adequate sample volume was used; the reagents migrated properly; and the control antibody reagents are working properly.

External Quality Control

Good laboratory practice recommends the use of external positive and negative controls to assure the test reagents are working properly and that the user has performed test correctly. If the controls do not perform as expected, review the instructions for use to see if the test was performed correctly; repeat the test or contact LifeSign Technical Services before performing patient specimens. Contact your supplier or LifeSign Technical Services (800-526-2125) for information about purchasing controls.

SECTION 10 - TEST PROCEDURE

Sample Testing

1. Shake the sample collection tube vigorously for 20-30 seconds to ensure the sample is well mixed with the buffer. Some small amount of sample may not dissolve; this is normal.
2. Remove test device from its foil pouch by tearing at the notch.
3. Tap the collection tube on a hard surface to dislodge any trapped air in top of cap.
4. Unscrew tip cap on the sample collection tube. Holding the tube vertically above the sample well of the Status iFOBT device, squeeze the tube gently to dispense three (3) drops into the sample well.
5. Read the result in 5-10 minutes. Important: **DO NOT READ RESULTS AFTER 10 MINUTES**.

SECTION 11 - INTERPRETATION OF RESULTS

POSITIVE: A reddish line appearing in the Control Position (C), and another reddish line in the Test Position (T), indicates that human hemoglobin has been detected in the sample. One line may be darker or lighter than the other, but even very faint reddish lines remain valid. Any positive result should be followed up with further examination to establish the source of bleeding.

NEGATIVE: Only one red line appearing in the Control Position (C), with NO reddish line in the Test Position (T), indicates that human hemoglobin has not been detected in the sample.

INVALID: If no reddish line appears in the Control Position (C), the test result is invalid and the sample remaining in the sample collection Tube should be re-tested with a new test device.

SECTION 12 - LIMITATIONS

- Results are not conclusive evidence of the presence or absence of gastrointestinal bleeding caused by cancer or pathology.
- As with any immunochemical test, a positive **Status iFOBT** result should not be considered a conclusive diagnosis for gastrointestinal bleeding or pathology. Immunochemical FOB testing has been shown to be valuable in preliminary screening, particularly of asymptomatic populations, or as an aid to diagnosis. The test is not intended to totally replace other diagnostic procedures such as colonoscopy, flexible sigmoidoscopy, or other imaging studies such as double contrast barium enema or CT colonography.
- Because gastrointestinal lesions may bleed intermittently and blood in feces is not distributed uniformly, a negative test result does not assure absence of lesions.
- A test result may be negative even when disease is present, because bowel lesions, including some colorectal cancers and significant polyps, may not bleed at all or may bleed intermittently, or the blood may not be uniformly distributed in a fecal specimen and therefore missed during sampling.
- Results may be positive for samples from patients without significant bowel pathology. Usually, the reasons for such false positive results are obscure, but in some cases, certain medications may cause gastrointestinal irritation resulting in occult bleeding.
- FOB testing is recommended annually by the American Cancer Society (2001) for average-risk women and men, 50 years of age and older. However, patients with significant risk factors such as family history of colorectal cancer should be screened earlier and more often.
- The intended use of **Status iFOBT** for pediatric patients (21 years and below) has not been established.

SECTION 13 - EXPECTED RESULTS

- Read results at 5 minutes
- Do not read results beyond 10 minutes
- Even if a very faint color band appears in the test region, it should be considered a positive result

SECTION 14 - PERFORMANCE CHARACTERISTICS

Sensitivity (Analytical Detection Limit)

Using dilutions of material with known hemoglobin concentration assayed by standard reference methodology, the analytical detection limit, or sensitivity, of **Status iFOBT** has been set at 50 ng hHb/mL buffer or 50 µg hHb/g feces.

Method Comparison Study

The performance of **Status iFOBT** was evaluated in comparison with a commercially available predicate device in a reference laboratory study. Five (5) different sample concentrations, 0, 37.5, 50, 62.5, and 500 ng hHb/mL, were tested using the Status iFOBT device and the predicate device using 8 replicates for each concentration, for a total of 40 samples. At each concentration, all results with the **Status iFOBT** agreed 100% with expected results and the results of the predicate.

Reproducibility Study

The performance of ***Status iFOBT*** was also evaluated in a study at three physicians' office laboratory (POL) sites. Five (5) different concentrations, 0, 37.5, 50, 62.5, and 500 ng hHb/mL, were prepared by spiking hHb into the extraction buffer. Each concentration was divided into 60 vials for each test. These vials were labeled for blind study. Each site tested a total of 100 tests with blinded samples of 5 concentrations, 20 samples per concentration, for a total of 300 samples. The results obtained from the three POL sites agreed 99.7% with the expected results.

SECTION 15 - REFERENCECES

1. American Cancer Society. Colorectal Cancer Screening Guidelines: http://www.cancer.org/docroot/CRI/content/CRI_2_4_3X_Can_colon_and_rectum_cancer_be_found_early.asp?sitearea=
2. Mitchell SH, Schaefer DC, Dubagunta S. Am Fam Physician. 2004 Feb 15;69(4):875-81.
3. Rockey DC. Occult gastrointestinal bleeding. N. Engl. J. Med. 1999;341:38-46.
4. Smith RA, von Eschenbach AC, Wender R, Levin B, Byers T, Rothberger D, Brooks D, et al. American Cancer Society Guidelines on Screening and Surveillance for the Early Detection of Adenomatous Polyps and Colorectal Cancer. CA-A Cancer Clin. 2001; 51:44-54.

SECTION 16 - TECHNICAL ASSISTANCE

For technical assistance, contact LifeSign Technical Service Department at 1-800-526-2125

Helpful CLIA brochure links to explain Clinical Laboratory Improvement Amendments (CLIA) regulation requirements

Individualized Quality Control Plan- IQCP

http://www.cms.gov/Regulations-and-Guidance/Legislation/CLIA/Downloads/CLIAbrochure11.pdf

Proficiency Testing

https://www.cms.gov/Regulations-and-Guidance/Legislation/CLIA/downloads/CLIAbrochure8.pdf

Proficiency Testing Providers

http://www.cms.gov/Regulations-and-Guidance/Legislation/CLIA/Downloads/ptlist.pdf

Personnel Competency Assessment

http://www.cms.gov/Regulations-and-Guidance/Legislation/CLIA/Downloads/CLIA_CompBrochure_508.pdf

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Certification of Training

This is to verify that personnel responsible for running _____ test at _____ have been thoroughly in-serviced on the test and the test procedure(s).

This has included:

Review of the package insert
Demonstration of the product assay
Successful performance of the test and interpretation of results

Names of the personnel who have been trained with the above test and are responsible for reporting patient results:

Print Name	Signature	Date

Signature(s) of those responsible for personnel and testing:

_____ Signature _____ Date

_____ Signature _____ Date

_____ Signature _____ Date

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Test Validation Form

Account Name: _____

Address: _____

_____

Telephone: _____

Test Name: _____ Lot # : _____

Start Date: _____

Supervisor Signature: _____

Sample Number	Expected Results	Test Result	Tester's Initials	Comments

Reviewed by: _____

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Corrective Action Form

Problem /Error	Corrective Action

Laboratory Technologist: _____ Date: _____

Laboratory Director: _____ Date: _____

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Quality Control

Name of Facility _____

Use this cover sheet with each new shipment and/or with each new kit lot

Product _____ Lot# _____ Exp Date _____

Date Received _____ Rec'd By _____

	Date	Positive Control	Negative Control	Initials
Initial QC				
Additional QC				
Additional QC				
Additional QC				
Additional QC				
Additional QC				
Additional QC				

Reviewed by _____ Date _____

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Testing Personnel Competency Assessment

Test _____

Procedure	Satisfactory	Unsatisfactory	Not Applicable	Comments/Corrective Action(s)
Observation of Test performance				
Patient Sample Preparation				
Specimen Handling/Processing				
Testing				
Recording/Reporting Results				
Assessment of Test Performance Using Known Samples				
Review of Records				
Patient/Quality Control Log Sheet Records				
Proficiency Testing Records				
Assessment of Problem Solving Skills				

(Attach all supporting documents)

Evaluator: _____ Date: _____

Testing Personnel: _____ Date: _____