LIMING BIO

Human Fecal Occult Blood Rapid Test Device

REF 501060	Specimen: Feces
Language: English	Version: 01
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For professional *in vitro* diagnostic use only.

INTENDED USE

The *StrongStep*® FOB Rapid Test Device (Feces) is a rapid visual immunoassay for the qualitative presumptive detection of human hemoglobin in human fecal specimens. This kit is intended to be used as an aid in the diagnosis of lower gastrointestinal (g.i.) pathologies.

INTRODUCTION

Colorectal cancer is one of the most commonly diagnosed cancers and a leading cause of cancer death in the United States. Screening for colorectal cancer probably increases the cancer detection at an early stage, therefore reduces the mortality.

Earlier commercially available FOB tests utilized the guaiac test, which requires special dietary restriction to minimize false positive and false negative results. The FOB Rapid Test Device (Feces) are especially designed to detect human hemoglobin in fecal samples using Immunochemical methods, which improved specificity for the detection of lower gastrointestinal. disorders, including colorectal cancers and adenomas.

PRINCIPLE

The FOB Rapid Test Device (Feces) has been designed to detect human hemoglobin through visual interpretation of color development in the internal strip. The membrane was immobilized with anti-human hemoglobin antibodies on the test region. During the test, the specimen is allowed to react with colored anti-human hemoglobin antibodies colloidal gold conjugates, which were precoated on the sample pad of the test. The mixture then moves on the membrane by a capillary action, and interact with reagents on the membrane. If there were enough human hemoglobin in specimens, a colored band will form at the test region of the membrane. Presence of this colored band indicates a positive result, while its absence indicates a negative result. Appearance of a colored band at the control region serves as a procedural control. This indicates that proper volume of specimen has been added and membrane wicking has occurred.

KIT COMPONENTS

Individually packed test devices	Each test contains colored conjugates and reactive reagents precoated at the corresponding regions.
Specimens dilution tube with buffer	0.01 M Phosphate buffered saline (PBS) and 0.02% sodium azide.
Package insert	For operating instructions

MATERIALS REQUIRED BUT NOT PROVIDED

Timer	For timing use.
Centrifuge	For treatment of specimens in special circumstances

PRECAUTIONS

- For professional in vitro diagnostic use only.
- Do not use after the expiration date indicated on the package. Do not use the test if the foil pouch is damaged. Do not reuse tests.
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not completely guarantee the absence of transmissible pathogenic agents. It is therefore, recommended that these products be treated as potentially infectious, and handled by observing usual safety precautions (e.g., do not ingest or inhale).
- Avoid cross-contamination of specimens by using a new specimen collection container for each specimen obtained.
- Read the entire procedure carefully prior to testing.
- Do not eat, drink or smoke in any area where specimens and kits are handled. Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout the procedure and follow standard procedures for proper disposal of specimens. Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.
- The specimen dilution buffer contains sodium azide, which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of specimen dilution buffer or extracted samples, always flush with copious quantities of water to prevent azide buildup.
- Do not interchange or mix reagents from different lots.
- Humidity and temperature can adversely affect results.
- Used testing materials should be discarded according to local regulations.

STORAGE AND STABILITY

- The kit should be stored at 2-30°C until the expiry date printed on the sealed pouch.
- The test must remain in the sealed pouch until use.
- **Do not freeze.**
- Cares should be taken to protect components in this kit from contamination. Do not use if there is evidence of microbial contamination or precipitation. Biological contamination of dispensing equipments, containers or reagents can lead to false results.

SPECIMEN COLLECTION AND STORAGE

- The FOB Rapid Test Device (Feces) is intended only for use with human fecal specimens.
- Patients should not collect samples during or within 3 days of their menstrual period, if they have bleeding hemorrhoids, blood in the urine, or if they have strained during bowel movement.
- Alcohol, aspirin and other medications taken in excess may cause gastrointestinal irritation resulting in occult bleeding. Such substances should be discontinued at least 48 hours prior to testing.
- No dietary restrictions are necessary before testing.
- Perform the testing immediately after the specimen collection. Do not leave the specimens at room temperature for prolonged periods. Specimens may be stored at 2-8°C for up to 72 hours.
- Bring specimens to room temperature prior to testing.
- Pack the specimens in compliance with applicable regulations for transportation of etiological agents, in case they need to be shipped.

PROCEDURE

Bring tests, specimens, buffer and/or controls to room temperature (15-30°C) before use.

1. Specimen collection and pre-treatment:
   1) Use the specimens collection cards provided in the kit for specimens collection. Follow the operation procedure written on it for instructions. Other clean dry containers could also be used for the same purpose. Best results will be obtained if the assay is performed within 6 hours after collection.
   2) Unscrew and remove the dilution tube applicator. Be careful not to spill or spatter solution from the tube. Collect specimens by inserting the applicator stick into at least 3 different sites of the feces.
   3) Place the applicator back into the tube and screw the cap tightly. Be careful not to break the tip of the dilution tube.
   4) Shake the specimen collection tube vigorously to mix the specimen and the extraction buffer. Specimens prepared in the specimen collection tube may be stored for 6 months at -20°C if not tested within 1 hour after preparation.
2. Testing
   1) Remove the test from its sealed pouch, and place it on a clean, level surface. Label the test with patient or control identification. To obtain a best result, the assay should be performed within one hour.
   2) Using a piece of tissue paper, break the tip of the dilution tube. Hold the tube vertically and dispense 3 drops of solution into the specimen well (S) of the test device.
   Avoid trapping air bubbles in the specimen well (S), and do not drop any solution in observation window.
   As the test begins to work, you will see color move across the membrane.

1. Wait for the colored band(s) to appear. The result should be read at 5 minutes. Do not interpret the result after 10 minutes.

Note: If the specimen does not migrate due to the presence of particles, centrifuge the extracted specimens contained in the extraction buffer vial. Collect 100 µL of supernatant, dispense into the specimen well (S) of a new test device and start again, following the instructions described above.

INTERPRETATION OF RESULTS

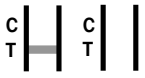
POSITIVE RESULT:

Two colored bands appear on the membrane. One band appears in the control region (C) and another band appears in the test region (T).

NEGATIVE RESULT:

Only one colored band appears in the control region (C). No apparent colored band appears in the test region (T).

INVALID RESULT:



Control band fails to appear. Results from any test which has not produced a control band at the specified reading time must be discarded. Please review the procedure and repeat with a new test. If the problem persists, discontinue using the kit immediately and contact your local distributor.

NOTE:

1. The intensity of the color in test region (T) may vary depending on the concentration of aimed substances present in the specimen. Therefore, any shade of color in the test region should be considered positive. Besides, the substances level can not be determined by this qualitative test.
2. Insufficient specimen volume, incorrect operation procedure, or performing expired tests are the most likely reasons for control band failure.

QUALITY CONTROL

- Internal procedural controls are included in the test. A colored band

appearing in the control region (C) is considered an internal positive procedural control. It confirms sufficient specimen volume and correct procedural technique.

- External controls are not supplied with this kit. It is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

LIMITATIONS OF THE TEST

1. The FOB Rapid Test Device (Feces) is for professional *in vitro* diagnostic use, and should be used for the qualitative detection of human hemoglobin only.
2. The presence of blood in stools may be due to several causes, besides colorectal bleeding, such as hemorrhoid, blood in urine or stomach irritations.
3. Negative results do not exclude bleeding since some polyps and colorectal region cancers can bleed intermittently or not at all. Additionally, blood may not be uniformly distributed in fecal samples. Colorectal polyps at an early stage may not bleed.
4. Urine and excessive dilution of sample with water from toilet bowl may cause erroneous test results.
5. This test may be less sensitive for detecting upper g.i. bleeding because blood degrades as it passes through the g.i. track.
6. All colorectal bleedings may not be due to precancerous or cancerous polyps. Like with all diagnostic tests, a confirmed diagnosis should only be made by a physician after all clinical and laboratory findings have been evaluated.

PERFORMANCE CHARACTERISTICS

Table: FOB Rapid Test vs. Another Commercially Available Rapid Test

			FOB Rapid Test		
			+	-	Total
Relative Sensitivity: 96.2% (93.2%-98.2%)*	Other RT	+	255	10	265
Relative Specificity: 98.7% (97.3%-99.5%)*		-	7	514	521
Overall Agreement: 97.8% (96.6%-98.7%)*			262	524	786
***95% Confidence Interval**					

A. Analytical Sensitivity
A sample containing human hemoglobin at concentration equal to or higher than 40 ng/ml produces a positive result. In some cases sample containing human hemoglobin at concentrations less than 40 ng/ml can also be tested as positive.

Hook or Prozone effect:
Sample containing as high as 0.5 mg/ml hemoglobin can still test positive. The tests do not show a Hook or Prozone Effect up to the maximal observed physiological concentration (0.5 mg/ml). Thus, the working range is 40 ng/ml up to 0.5 mg/ml.

B. Analytical Specificity:
The test is specific for human hemoglobin and does not show any cross-reaction with the hemoglobin from bovine, pig, horse and sheep concentrations up to 0.5 mg/ml.

The test also does not show any cross reaction with billirubin, vitamin C and horse radish peroxidase.

C. Clinical Specificity:
The following non-cancer related factors may cause blood in feces samples:

1) Iron
Food supplementation with iron leads to increased release of blood in the colon. Iron itself is not cross-reacting with the test.

2) Acetylsalicylic acid
ASA is main compound in a lot of drugs against headache (e.g. Aspirin® from Bayer), and is sometimes used to substitute macumar as a blood diluter. Almost always there are very small amounts of blood in fecal samples in case of healthy humans. This is far below the sensitivity of our test and has nothing to do with cancer or any other serious matter. If a patient takes blood diluters bleeding can be more intensive. Therefore the cut-off of the test may be reached.

3) Coumarin
Coumarines are used as drugs (e.g. Macumar®) for prevention of heart attacks, against thrombosis and stroke. Similar to ASA, cumarines are blood diluter. Almost always there are very small amounts of blood in fecal samples in case of healthy humans. This is far below the sensitivity of the test and has nothing to do with cancer or any other serious matter. If a patient takes blood diluters, bleeding can be more intensive. Therefore the cut-off of the test may be reached.

4) Hemorrhoids
Hemorrhoids may bleed. Therefore fecal sample may be contaminated with blood which is not associated with cancer.

5) Monthly period
Small amounts of blood released because of female's period may contaminate the fecal sample. This is blood which is not associated with cancer.

6) Urine samples
Several diseases may cause blood in urine samples. To avoid detection of urine-related blood, stool sample should not get in contact with urine.

LITERATURE REFERENCES

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GLOSSARY OF SYMBOLS

REF	Catalog number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	In vitro diagnostic medical device		Use by
	Manufacturer	Σ	Contains sufficient for <n> tests
	Do not reuse	EC REP	Authorized representative in the European Community
CE	CE marked according to IVD Medical Devices Directive 98/79/EC		

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