Rapid Response®

Fecal Immunochemical Test
(Feces)
REF FOB-9C36 Product Insert

A rapid test for the qualitative detection of human occult blood in human fecal specimens.

For laboratory *in vitro* diagnostic use only.

Intended Use

The Rapid Response® Fecal Immunochemical Test is a rapid chromatographic immunoassay for the qualitative detection of human occult blood in human fecal specimens. The device is suitable for use in laboratories.

Introduction

Gastrointestinal diseases may cause occult (hidden) blood in stool. In the early stages, gastrointestinal problems such as colon cancer, ulcers, polyps, colitis, diverticulitis, and fissures may not show any visible symptoms, only occult blood. Traditional guaiac-based method lacks sensitivity and specificity to test for fecal occult blood and has diet-restriction prior to the testing.

The Rapid Response® Fecal Immunochemical Test is a rapid test to qualitatively detect low levels of fecal occult blood in feces. The test uses double antibody sandwich assay to selectively detect as low as 50 ng/mL of hemoglobin or 6 µG hemoglobin/g feces. In addition, unlike the guaiac assays, the accuracy of the test is not affected by the diet of the patients.

Principle

The Rapid Response® Fecal Immunochemical Test is a lateral flow chromatographic immunoassay based on the principle of the double antibody-sandwich technique. The membrane is pre-coated with anti-hemoglobin antibody on the test line region of the device. During testing, the specimen reacts with the particle coated with anti-hemoglobin antibody. The mixture migrates upward on the membrane chromatographically by capillary action to react with anti-hemoglobin antibody on the membrane and generate a coloured line. The presence of this coloured line in the test region indicates a positive result, while its absence indicates a negative result. To serve as a procedural control, a coloured line will always appear in the control line region indicating that proper volume of specimen has be added and membrane wicking has occurred.

Warnings and Precautions

1. This test is designed for laboratory *in vitro diagnostic* use.
2. Read instructions carefully before using this test.
3. **Warning:** the reagents in this kit contain sodium azide which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up.
4. Do not use it if the tube/pouch is damaged or broken.
5. Test is for single use only. Do not re-use under any circumstances.
6. Do not use the test device or collection tube beyond the expiration date.
7. Do not use the kit if the pouch is punctured or is not well sealed.
8. Keep out of the reach of children.
9. Fecal specimens may be infectious; ensure proper handling and discard all used devices according to the local regulations.

Storage and Stability

- Store at 2°C to 30°C (36 - 86°F). in the sealed pouch up to the expiration date.
- If stored at 2°C to 8°C (36 - 46°F), ensure that the test device is brought to room temperature before opening.
- Keep away from sunlight, moisture, and heat.
- **DO NOT FREEZE** the kit or expose the kit to temperatures over 30°C (86°F).

Materials

Materials provided:

- 12 individual pouches each with a Rapid Response® Fecal Immunochemical Test cassette per box (Kit contains 3 boxes)
- 12 Specimen collection tubes with extraction buffer per box (Kit contains 3 boxes)
- 1 Instruction for Use per box (Kit contains 3 boxes)

Materials required but not provided:

- Timer
- Collection Container

Collection of Stool from a Toilet Bowl

If Using a Receptacle

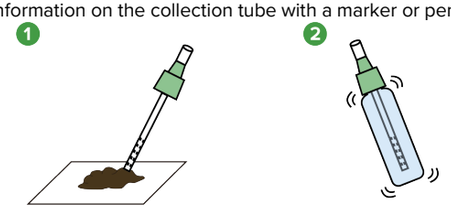
1. Prepare a dry and clean receptacle that can be placed into and taken out from a toilet bowl conveniently (above the water surface). Do not use toilet paper (toilet paper may contain substances which are inhibitory for some fecal specimens).
2. Do not contaminate specimen with urine. Please urinate first, if necessary.
3. Have a bowel movement and remove the receptacle with stool out of the toilet bowl.

If not Using a Receptacle

1. Do not contaminate specimen with urine. Please urinate first, if necessary.
2. Flush the toilet bowl twice before excreting. If necessary, clean the toilet bowl.
3. Have a bowel movement. Stool that has or has not come in contact with water can be used for the following procedure.

Collection and Storage of Specimens

1. Unscrew the green cap of the collection tube and remove the applicator stick.
2. Insert the stick into the fecal specimen at 6 different sites. (See illustration 1).
3. Insert the sampled applicator back into the tube and tighten the green cap securely.
4. Shake the tube with the cap vigorously for about 5 seconds to release and disperse the stool sample into the collection buffer. (See illustration 2).
5. If necessary, it is recommended to write identifying information on the collection tube with a marker or pen.

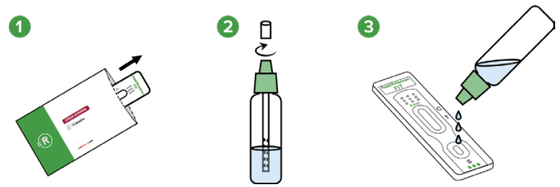


- Specimen should not be collected during or within three days of menstrual period, or if the patient suffers from bleeding hemorrhoids or blood in the urine.
- The specimen may be collected from stool in a toilet bowl with or without contact to flushing water, or stool from a receptacle before coming into a toilet bowl.
- 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.
- Dietary restrictions are not necessary.
- The sample can be stored at room temperature 8°C to 30°C (46 - 86°F) up to 24 hours or in a refrigerator 2°C to 8°C (36 - 46°F) for up to 72 hours.

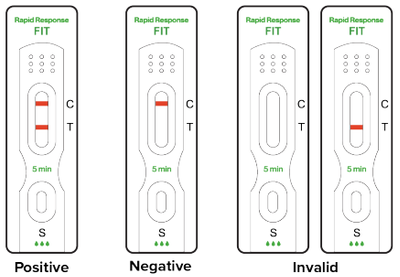
Test Procedure

Bring all materials and specimens to room temperature 8°C to 30°C (46 - 86°F).

1. Remove the test device from its foil pouch by tearing along the notch and use it as soon as possible. (See illustration 1).
2. Holding the sample collection device upright, carefully unscrew the small white cap of collection device. (See illustration 2).
3. Squeeze 3 drops (~75 µL) of the sample solution in the sample well of the cassette, as in the illustration. (See illustration 3).
4. Read the test results between 5-10 minutes. Test results read earlier than 5 minutes and later than 10 minutes are not valid. Before reading results, please put device on a clear and single coloured background to avoid visual disturbances.



Results Interpretation



POSITIVE: Two red lines appear. One red line should be in the control region (C) and another red line should be in the test line region (T).
NEGATIVE: One red line appears in the control line region (C). No line appears in the test line region (T).
INVALID: Control line fails to appear.
NOTE: if the test line is weak, it is recommended that the test be repeated in 48 hours.

Quality Control

A read line appearing in the control region (C) is the internal procedural control. It confirms sufficient specimen volume and correct procedural technique. External controls should be tested at regular intervals as part of the laboratory quality control process.
Users should follow the appropriate local guidelines concerning the running of external quality controls.
It is recommended that a positive Hb control (containing 50 ng/mL) and a negative Hb control (containing “0” ng/mL) be evaluated to perform quality control testing with each new lot, each new shipment, or every 3 months (whichever comes first).
If the test does not show any Control or Test line in the window or a smudged or partial line appears, the test should be discarded. Do not report the results. Run the test again with a new cassette and follow the procedure exactly. If the second test does not show lines, please contact Technical Services at 1-888-339-9964.

Limitations

1. As with all diagnostic tests, all results must be considered with other clinical information available to the physician. A definite clinical diagnosis should only be made by the

physician after all clinical and laboratory findings have been evaluated.

2. This test is limited to the detection of fecal occult blood in human stool sample only.
3. Although the test is highly accurate in detecting human hemoglobin, a low incidence of false positive results may occur. In addition, because many bowl lesions, including some polyps and colorectal cancers, may bleed intermittently or not at all, occult blood may not be uniformly distributed throughout a fecal sample. Thus test results may be negative even when disease is present.

Expected Results

Negative results are expected in healthy women and healthy men. The Rapid Response® Fecal Immunochemical Test has a sensitivity of 50 ng hHb/mL of buffer solution.

Performance Characteristics

Detection Limit

The sensitivity of the device was tested by spiking 100 hemoglobin-free stool samples with varying concentrations (0, 37.5, 50, 62.5 and 2000 ng hHb/ml) of human hemoglobin. The test shows a cut-off of 50 ng hHb/mL, and no pro-zone effect was seen up to 2000 ng hHb/ml. Result are summarized below:

hHb concentration (ng/mL)	Total	Positive	Negative
0	20	0	20
37.5	20	0	20
50.0	20	20	0
62.5	20	20	0
2000	20	20	0

Potential Interferences

An interference study was carried out by adding known amounts of potential interfering analytes to aqueous fecal samples that contain 0 and 50 ng/ml of human hemoglobin. The expected result at 0 ng/ml if no interference has occurred is negative. The expected result at 50 ng/mL is positive if no interference has occurred. The results of the interference study are summarized below:

Analyte	Concentration	Rapid Response® FIT Test Result	
		0ng/mL	50ng/mL
Acetoaminophen	25 mg/dL	-	+
Acetoaceto Acid	2000 mg/dL	-	+
Ascorbic Acid	20 mg/dL	-	+
B-hydroxybutyrate	20 mg/dL	-	+
Caffeine	20 mg/dL	-	+
Ephedrine	20 mg/dL	-	+
Gentisic Acid	20 mg/dL	-	+
Phenylpropanolamine	20 mg/dL	-	+
Salicylic Acid*	20 mg/dL	-	+
Phenothiazine	20 mg/dL	-	+
EDTA	20 mg/DL	-	+
Acetosalicylic Acid	20 mg/dL	-	+
Cannabinol	10 mg/dL	-	+
Codeine	10 mg/dL	-	+
Ethanol	1.0 %	-	+
Methanol	10 %	-	+
Albumin	2000 mg/dL	-	+
Glucose	2000 mg/dL	-	+
Bilirubin	1000 mg/dL	-	+
Hemoglobin	2000 mg/dL	-	+

A second interference study was carried out by adding known amounts of potential interfering substances to aqueous fecal samples that contain 0 and 50 ng/ml of human hemoglobin. The expected result at 0 ng/ml if no interference has occurred is negative. The expected result at 50 ng/mL is positive if no interference has occurred. The results of the interference study are summarized below:

Interfering substances	Rapid Response® FIT Test Result	
	0ng/mL	50ng/mL
Horseradish peroxidase (20 mg/mL)	-	+
Extract of red radish	-	+
Extract of raw turnip	-	+
Extract of cauliflower	-	+
Extract of broccoli	-	+
Dietary supplements with chloride, fluoride, and iron	-	+
Dietary supplements with Vitamin C	-	+
Toilet water with cleaner and deodorizer	-	+
Toilet water without cleaner and deodorizer	-	+

Potential Cross Reactors

A cross-reactivity study of animal hemoglobin was carried out by spiking negative (0 ng hHb/mL) and positive (50 ng hHb/mL) fecal samples with beef hemoglobin, pig hemoglobin, rabbit hemoglobin and sheep hemoglobin, respectively, at the concentration of 200 ng/mL.

Hemoglobin Type (200 ng/mL)	Rapid Response® FIT Test Result	
	0ng	50ng
Beef hemoglobin	-	+
Chicken hemoglobin	-	+
Fish hemoglobin	-	+
Horse hemoglobin	-	+
Goat hemoglobin	-	+
Pig hemoglobin	-	+
Rabbit hemoglobin	-	+
Sheep hemoglobin	-	+

Reproducibility

Inter-Site

To evaluate reproducibility of the test, 75 hemoglobin-free fecal samples spiked with varying levels (0, 37.5, 50, 62.5, and 2000 ng hHg/mL) of human hemoglobin were tested at 3 medical laboratories with 3 lots of tests and run 5 times for each lot at each site. The results are summarized below:

	Total Results	Lot 1	Lot 2	Lot 3
Hb concentrations		P / N	P / N	P / N
0 ng/mL	45	0 / 15	0 / 15	0 / 15
37.5 ng/mL	45	0 / 15	0 / 15	0 / 15
50 ng/mL	45	15 / 0	15 / 0	15 / 0
62.5 ng/mL	45	15 / 0	15 / 0	15 / 0
2000 ng/mL	45	15 / 0	15 / 0	15 / 0

Intra-Run

Intra-Run reproducibility was determined by testing 3 lots of test cassettes by spiking 50 hemoglobin-free fecal samples with varying levels (0, 37.5, 50, 62.5, and 2000 ng hHg/mL) of human hemoglobin and run 10 times each. The results are summarized below:

	Total Results	Lot 1	Lot 2	Lot 3
Hb concentrations		P / N	P / N	P / N
0 ng/mL	30	0 / 10	0 / 10	0 / 10
37.5 ng/mL	30	0 / 10	0 / 10	0 / 10
50 ng/mL	30	10 / 0	10 / 0	10 / 0
62.5 ng/mL	30	10 / 0	10 / 0	10 / 0
2000 ng/mL	30	10 / 0	10 / 0	10 / 0

Inter-Day

Day to day reproducibility study was carried out by testing cassettes from the same lot with 50 fecal samples spiked with varying levels (0, 37.5, 50, 62.5, and 2000 ng hHg/mL) of human hemoglobin on 3 consecutive days with 10 replicates each day. The results are summarized below:

	Total Results	Lot 1	Lot 2	Lot 3
Hb concentrations		P / N	P / N	P / N
0 ng/mL	30	0 / 10	0 / 10	0 / 10
37.5 ng/mL	30	0 / 10	0 / 10	0 / 10
50 ng/mL	30	10 / 0	10 / 0	10 / 0
62.5 ng/mL	30	10 / 0	10 / 0	10 / 0
2000 ng/mL	30	10 / 0	10 / 0	10 / 0

Accuracy

A study was conducted to evaluate the Rapid Response® Fecal Immunochemical Test and compare results with a commercially available Fecal Occult Blood Rapid test at three physician office laboratories by technical personnel.

Study of Technical Personnel

In each POL site, 100 human stool extraction samples were spiked with human hemoglobin at the following concentrations: 0, 37.5, 50, 62.5, and 500 ng/mL (20 replicates at each concentration). Results obtained from 3 sites agreed 99.0% with the expected results and 98.0% with results of predicated device.

Tests (testers)	Total Evaluated Samples	Correct Results	Discrepant Results	Agreement
Rapid Response® FIT Test Technicians vs. Expected	100	99	1	**99.0%**
Rapid Response® FIT Test Technician vs. Predicate Test	100	98	2	**98.0%**

Results generated by 3 trained technicians with the Rapid Response® Fecal Immunochemical Test as compared to the predicate test:

		Predicate Test		Total Results
Rapid Response® FIT Test	Results	Positive	Negative	
	Positive	59	1	60
	Negative	1	39	40
Total Results		60	40	100

Percent Positive Agreement = 59/60 = 98.3%
(95% C.I. = 91.1%-100%)
Percent Negative Agreement = 39/40 = 97.5%
(95% C.I. = 86.8%-99.9%)
Overall Agreement = 98/100 = 98.0% (95% C.I. = 93.0%-99.8%)

Bibliography

1. Simon J.B. Occult Blood Screening for Colorectal Carcinoma: A Critical Review, Gastroenterology, Vol. 88: 820. 1985.
2. Blebea J. and McPherson RA. False-Positive Guaiac Testing With Iodine, Arch, Pathol, Lab, Med. 1985;109:437-40

Glossary of Symbols


Consult instructions for use


Test per Kit

EC REP Authorized Representative


Store between 36°F to 86°F


Use by


Do Not Reuse

LOT Lot Number

IVD For laboratory *in vitro* diagnostic use only

REF Catalogue #

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