Rapid Response™

Fecal Immunochemical Test
(Feces)

REF FOB-9C, FOB-9C36

Additional Information for Laboratory and Physician Office

Read all the instructions before performing the Rapid Response™ Fecal Immunochemical Test.

Principle of Test

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 particles coated with anti-hemoglobin antibodies. The mixture migrates upward on the membrane chromatographically by capillary action to react with anti-hemoglobin antibody on the membrane and generate a colored line. The presence of this colored line in the test region indicates a positive result, while its absence indicates a negative result. To serve as a procedural control, a colored line will always appear in the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred.

Collection of Stool from a Toilet Bowl

If using a receptacle,

1. Prepare a dry and clean receptacle that can be conveniently placed into and removed from the toilet bowl (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. Urinate first if necessary.
3. Have a bowel movement and remove the receptacle with stool from the toilet bowl.

If not using a receptacle,

1. Do not contaminate specimen with urine. 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 contacts water or does not contact water can be used for the test procedure.

Quality Control

A red 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, the test should be discarded. Do not report the results. Repeat the test with a new cassette and follow the procedure carefully. If the second test does not show lines, refer to the Assistance section for Technical Support.

Performance Characteristics

Detection limit

hHb concentration (ng/mL)	Total	Positive	Negative
0	20	0	20
37.5	20	0	20
50	20	20	0
62.5	20	20	0
2000	20	20	0

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

Potential Interferences

An 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. Substances tested included horseradish peroxidase (20 mg/mL), aqueous extracts of red radish, raw turnip, cauliflower and broccoli, dietary supplements of chloride, fluoride, Vitamin C (ascorbic acid) and iron, and toilet water with and without cleaner and deodorizer.

Analyte	Rapid Response™ Fecal Immunochemical Test	
	0ng	50ng
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, chicken hemoglobin, fish hemoglobin, horse hemoglobin, goat hemoglobin, pig hemoglobin, rabbit hemoglobin and sheep hemoglobin, respectively, at the concentration of 200 ng/mL.

Hemoglobin Type	Rapid Response™ Fecal Immunochemical Test	
(200ng/mL)	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:

3 Sites	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
2,000 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
2,000 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:

3 days	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
2,000 ng/mL	30	10 / 0	10 / 0	10 / 0

Accuracy

A study was conducted to evaluate the Rapid Response™ Fecal Immunochemical Test and compare the results with a commercially available Fecal Occult Blood Rapid Test at three physician office laboratories by technical personnel, and one medical laboratory by non-technical personnel with diverse educational backgrounds and ages.
Consumer results using the Rapid Response™ Fecal Immunochemical Test compared to both the professional and Predicate test results were evaluated:

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 the predicate device.

Study of non-technical personnel

150 human stool extraction samples were spiked with hHb at the following concentrations: 0, 37.5, 50, 62.5, and 500ng/ml (30 at each concentration). Thirty (30) participants without technical background were enrolled to conduct the study in a medical laboratory. Each participant tested 5 samples (1 at each concentration) with the two FOB devices. Results obtained from lay users agreed 98.7% with the expected results and 98.0% with results of predicated device.

Test (tester)	Total Evaluated Samples	Correct Results	Discrepant Results	Agreement
Rapid Response™ Fecal Immunochemical Test Lay User vs. Expected	150	148	2	98.7%
Rapid Response™ Fecal Immunochemical Test Lay User vs. Predicate test	150	147	3	98.0%
Rapid Response™ Fecal Immunochemical Test Technician vs. Expected	100	99	1	99.0%
Rapid Response™ Fecal Immunochemical 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™ Fecal Immunochemical 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%)

Lay User Study

An additional U.S. study was performed with 20 lay users from a general population at each of 3 sites and were asked to participate in the OTC and comparison studies. All the lay users were at ages of 50 plus years old, and their educational and ethnic background were recorded. In this study, all the lay users conducted the test with the Rapid Response™ Fecal Immunochemical Test and the predicate devices, using their own specimens that were collected in the washrooms at the sites by each lay user.
After the lay user testing, the specimens were sent to professionals to test with Rapid Response™ Fecal Immunochemical Test and predicate devices for comparison. The professionals were blinded by masking the stool sample receptacles before being sent to them. The results of the testing performed by the lay users with their own specimen and the professional testing are shown below.

Part 1: Study with users' own specimen

		Predicate test		Total Results
Rapid Response™ Fecal Immunochemical Test	Results	Positive	Negative	
	Positive	5	1	6
	Negative	2	52	54
Total Results		7	53	60

Percent Positive Agreement= 2/7= 71.4% (95% C.I. =38 % - 94%)
Percent Negative Agreement= 1/53= 98.1 % (95% C.I. = 91 % - 100%)
Overall Agreement= 57/60= 95.0% (95% C.I. = 88% - 97%)

		Predicate test		Total Results
Rapid Response™ Fecal Immunochemical Test	Results	Positive	Negative	
	Positive	6	0	6
	Negative	1	53	54
Total Results		7	53	60

Percent Positive Agreement= 6/7 = 85.7% (95% C.I. =50% - 99%)
Percent Negative Agreement =0/53 = 100% (95% C.I. = 94% - 100%)
Overall Agreement= 59/60= 98.3% (95% C.I. = 91% -100%)

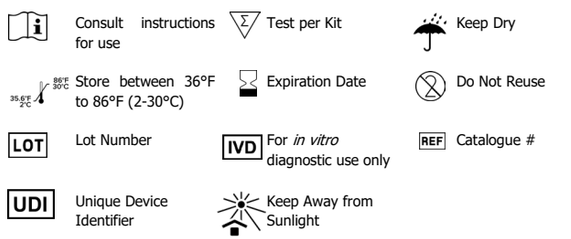
Part 2: Study with prepared specimens

A study was performed to evaluate the ability of a lay user to interpret results at concentrations around the cutoff and obtain interpretation equivalent to the predicate test. A series of negative human stool samples were spiked with human hemoglobin (hHb) at the following concentrations: 0, 37.5, 50, 62.5, and 500ng/ml and tested by the lay users with both devices.
Specimens at each concentration were divided into 12 containers (60 total), and each lay user picked one randomly and tested it with the Rapid Response™ Fecal Immunochemical Test and the predicate device.
The lay users were blinded by masking the stool sample receptacles before being sent to them for testing. The results of the lay user testing using pre- pared specimens of known concentrations is shown below.

		Predicate test		Total Results
Rapid Response™ Fecal Immunochemical Test	Results	Positive	Negative	
	Positive	33	0	35
	Negative	2	23	25
Total Results		35	25	60

Percent Positive Agreement= 33/35 = 94.3% (95% C.I. = 82% - 98%)
Percent Negative Agreement= 23/25 = 92% (95% C.I. = 80% - 98%)
Overall Agreement= 56/60= 93.3% (95% C.I. = 84% - 97%)

Glossary of Symbols



Assistance:
If you have any questions regarding the use of this product, please call Technical Support at 1-888-339-9964 (9:00 a.m. to 5:30 p.m. EST)

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