One-Step Immunological Fecal Occult Blood Test

Catalog No. DTG-FOB01

INTENDED USE

The Clarity One-Step Immunological Fecal Occult Blood Test is a rapid chromatographic immunoassay for the qualitative detection of human occult blood in human fecal specimens. The device is suitable for professional In-Vitro diagnostic use only.

SUMMARY AND EXPLANATION

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 Clarity One-Step Immunological Fecal Occult Blood 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 50ng/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 OF THE TEST

The Clarity One-Step Immunological Fecal Occult Blood 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 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.

MATERIALS SUPPLIED

- 30 Pouches, each with one FOB test cassette device and one desiccant. The desiccant is for storage purposes only and is not used in the test procedures.
- 35 Specimen collection tubes with extraction buffer.
- 1 Vial of Positive and Negative Control.
- 1 Package Insert.

MATERIALS REQUIRED BUT NOT PROVIDED

Clock or Timer

STORAGE

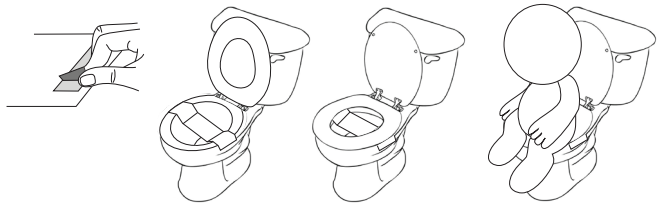
1. Store at 2ºC to 30ºC (35 - 86ºF) in the sealed pouch up to the expiration date.
2. If stored at 2°C to 8°C (35- 46 ºF), ensure that the test device is brought to room temperature before opening.
3. Keep away from sunlight, moisture and heat.
4. DO NOT FREEZE the kit or expose the kit over 30°C

WARNINGS AND PRECAUTIONS

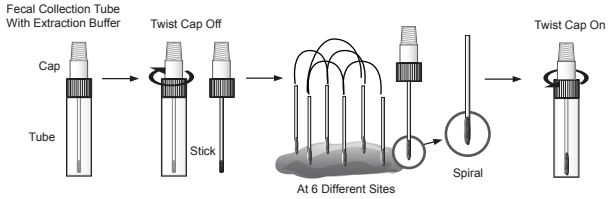
1. This test is designed for "in vitro diagnostic" use.
2. Read instructions carefully before using this test.
3. Warning: the reagents in this kit contain sodium azide which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush 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 handing and discard all used devices according to the local regulations.

SAMPLE COLLECTION AND STORAGE

1. Peel off the tape covering the adhesive strips, located on each end of the sample collection paper.
2. Lift toilet seat, and place sample collection paper across rim of toilet bowl. Use adhesive strips, located on each side of sample collection paper, to secure it to sides of toilet rim, as shown.
3. Replace toilet seat onto toilet bowl.
4. Make bowel movement onto collection paper.
5. Unscrew the cap of the collection tube and remove the applicator stick.
6. Insert the applicator stick into stool sample at 6 different sites. Use only enough fecal material to cover the tip of the applicator stick. Remove excess feces from the stick by gently wiping with an absorbent tissue.
7. Screw the applicator stick into the tube and secure tightly.
8. The specimen is now ready for testing, transportation or storage.
9. The sample specimens and the specimens collected by the patient in the sample tube can be stored at room temperature for up to 10 days and at least for 2 years at -4˚F (-20˚C).



Sample Collection 4



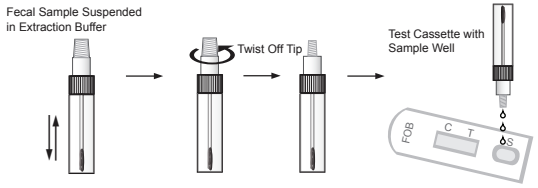
Sample Collection 5 | Sample Collection 6 | Sample Collection 7

Procedure Note:
Do not use stool sample as test specimen if it comes into contact with toilet water.

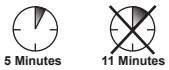
ASSAY PROCEDURE

ASSAY PROCEDURE
1. Remove the cassette from the pouch.
2. Shake the sample collection tube vigorously to ensure a good liquid suspension.
3. Holding the tube upright, unscrew the clear tip cover.
4. Dispense three (3) drops of suspension from the collection tube into sample well.
5. Read the results within 5-10 minutes after adding the extraction solution.

Important: ***Do not read the results after 10 minutes.***



Assay Procedure 2 | Assay Procedure 3 | Assay Procedure 4



Assay Procedure 5

INTERPRETATION OF RESULTS

Positive | Negative | Invalid

Positive: Two red lines appear. One red line should be in the control line 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

1. Internal Controls:
There are two internal control features in the Clarity IFOB test. A colored control band will always appear at the control position (C) if the test has been performed correctly and if the device is working properly. This is considered an internal positive procedural control. A clear background in the result window is considered an internal negative procedural control. If the test has been performed correctly and the Clarity IFOB test is working properly, the background in the result window will be clear, providing a distinct result.

2. External Controls:
"Good laboratory practice" ("GLP") recommends the usage of external controls. Clarity Positive and Negative controls are supplied with the kit.

1. Allow the test and the controls to equilibrate to room temperature 59-86˚F (15-30˚C) prior to testing.
2. To begin the testing, open the sealed pouch of the Clarity IFOB test by tearing the pouch. Remove the test kit from the pouch and use it as soon as possible.
3. Squeeze 3 drops of the Positive control in to the sample well of the Clarity IFOB test cassette.
4. Repeat the same procedure for the Negative control.
5. The results should be read at 5 minutes. Do not interpret results after 10 minutes.
6. Intrepret results the same way as you were to intrepret a patient sample.

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.
4. A number of medications like acetylsalicylic acid, glucocorticoids, non-steriod antiphlogistica / anti-rheumatica or cumarin-derivates may lead to gastrointestinal bleeding. Therefore a test for fecal occult blood should only be started a few days after such medication has been stopped.
5. A negative result can be obtained even when a GI disorder is present. Some bowel lesions, including some polyps and colorectal cancer, may not be uniformly distributed in a fecal sample.
6. The following guidelines apply to fecal occult blood tests: A negative test does not rule out a carcinoma; a positive test need not be verified by another positive test. The test results can only be regarded as preliminary screening or as an aid to diagnosis. It is not intended to replace other diagnostic procedures such as G.I.Fibroscope, endoscopy, colonoscopy or other x-ray studies. A positive test result for fecal occult blood must be re-checked until the bleeding source is detected / located.

EXPECTED RESULTS

Negative results are expected in healthy women and healthy men. The Clarity FOB One Step Rapid Test has a sensitivity of 50ng 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 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. 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 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.
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.

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 Clarity Fecal Occult Blood Rapid Test and compare 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 Clarity FOB One Step Rapid 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 predicated 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.

Tests (tester)	Total Evaluated Samples	Correct Results	Discrepant Results	Agreement
Candidate FOB Device Test Lay user vs. Expected	150	148	2	**98.7%**
Candidate FOB Test Lay user vs. Predicate test	150	147	3	**98.0%**
Candidate FOB Technicians vs Expected	100	99	1	**99.0%**
Candidate FOB Technician vs Predicate test	100	98	2	**98.0%**

Results generated by 3 trained technicians with the candidate FOB One Step Rapid Test as compared to the predicate test:

		Predicate test		Total Results
Candidate FOB 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 + years old, and their educational and ethnic background were recorded. In this study, all the lay users conducted the test with Candidate FOB and the predicate devices, using their own specimens that were collected in the wash rooms at the sites by each lay user.

After the lay user testing, the specimens were sent to professionals to test with Candidate FOB 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
Candidate FOB Test		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%)

		Professional test		Total Results
Candidate FOB Test		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 candidate FOB device 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 prepared specimens of known concentrations is shown below.

		Predicate test		Total Results
Candidate FOB Test		Positive	Negative	
	Positive	33	2	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%)

REFERENCES

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

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