		Predicate test		Total Results
iFOB One Step Rapid 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 yrs old, and their educational and ethnic background were recorded. In this study, all the lay users conducted the test with Orient Gene 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 Orient Gene 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 Result
iFOB One Step Rapid 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
iFOB One Step Rapid 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 Orient Gene 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
iFOB One Step Rapid Test	Results	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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TANNER scientific®

iFOB ONE STEP RAPID TEST

IMMUNOCHEMICAL FECAL OCCULT BLOOD TEST

INTENDED USE

The iFOB One Step Rapid 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 and physician's offices as well as for Over The Counter use.

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 iFOB One Step Rapid 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 iFOB One Step Rapid 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

- 36 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
- 36 ea. Specimen collection tubes with extraction buffer
- 1 Instruction for Use

MATERIALS REQUIRED BUT NOT PROVIDED

Clock or Timer

STORAGE

1. Store at 2°C to 30°C (35 - 86°F) in the sealed pouch up to the expiration date.
2. If stored at 2°C-8°C, 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.

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. So 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 contacts with water or not can be used for following procedures.

COLLECTION AND STORAGE OF SPECIMENS

1. Unscrew the bottom cap (red end) of the collection tube and remove the applicator stick.
2. Insert the stick into the fecal specimen at 6 different sites.
3. Insert the sampled applicator back into the tube and tighten the bottom (red end) securely. The narrow hole only allows the stick to go through and will prevent the excess sample from getting into the tube.
4. Shake the tubes with bottom cap (red end) vigorously for about 5 seconds to release and disperse the stool sample into the collection buffer.
5 If necessary, it is recommended to write identifying information on collection tube with a marker pen.

SPECIMEN COLLECTION STEPS 2 AND 3

SPECIMEN COLLECTION STEPS 4 AND 5

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 (8-30°C) up to 24 hours or in a refrigerator (2-8°C) for up to 72 hours.

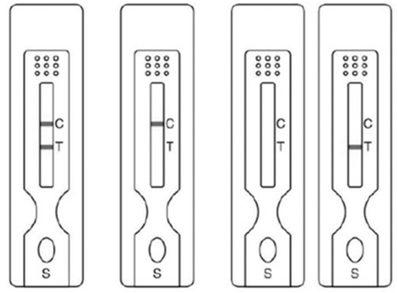
TEST PROCEDURE

1. Bring all materials and specimens to room temperature (8–30°C).
2. Remove the test device from its foil pouch by tearing along the notch and use it as soon as possible.
3. Holding the sample collection device upright, carefully break off the tip of collection device.
4. Squeeze 3 drops (~75uL) of the sample solution in the sample well of the cassette, as in the illustration.
5. 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-colored background to avoid visual disturbances.

TEST PROCEDURE 3

TEST PROCEDURE 4

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

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. 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 (866) 982-3818 (hours: 8 AM to 5 PM Central Time; Mon. - Fri.).

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 bowel 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 iFOB 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.	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 hHb concentration (ng/mL)	Total Results	LOT 1 P/N	LOT 2 P/N	LOT 3 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:

hHb concentration (ng/mL)	Total Results	LOT 1 P/N	LOT 2 P/N	LOT 3 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 hHb concentration (ng/mL)	Total Results	LOT 1 P/N	LOT 2 P/N	LOT 3 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 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 iFOB 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
iFOB One Step Rapid Test Layuser vs. Expected	150	148	2	**98.7%**
iFOB One Step Rapid Test Layuser vs. Predicate test	150	147	3	**98.0%**
iFOB One Step Rapid Test Technician vs Expected	100	99	1	**99.0%**
iFOB One Step Rapid Test Technician vs Predicate test	100	98	2	**98.0%**

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

		Predicate test		Total Results
iFOB One Step Rapid 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%)