

RAPYDTEST®

FOR THE DETECTION OF HUMAN HAEMOGLOBIN IN FAECES



RAPYDTEST®
Faecal Occult Blood (FOB)



MICROBIOLOGY

SINGLE USE IN VITRO DIAGNOSTIC DEVICE

Intended Use

The Apacor FOB Rapydtest® is an immunochemical device intended for the qualitative detection of faecal occult blood to be used in laboratories or physicians offices. It is a useful aid to detect bleeding caused by a number of gastrointestinal disorders, e.g., diverticulitis, colitis, polyps, and colorectal cancer. Faecal occult blood tests are recommended for use in:

1. routine physical examinations
2. routine hospital testing
3. screening for colorectal cancer or gastrointestinal bleeding from any source.

Explanation of the Test

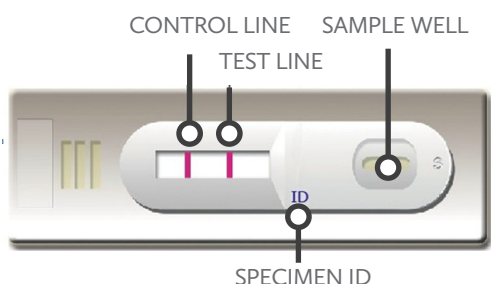
Two types of FOB tests are commercially available: Guaiac Dye and immunochemistry. The Guaiac test is widely used but lacks high accuracy. The Guaiac dye is a naturally occurring phenolic compound that can be oxidized to quinone by hydrogen peroxidase activity of hHb with a detectable colour change. The sensitivity and specificity of Guaiac tests are much lower than those of immunochemical assays. The low accuracy of the Guaiac Dye test is related to dietary peroxidases, including haemoglobin from meat and uncooked fruits and vegetables. Non-cancerous gastrointestinal tract bleeding and iron intake may also cause false positive results from Guaiac test¹.

The Apacor FOB Rapydtest® is designed to specifically detect low levels of human faecal occult blood. It is highly accurate for human haemoglobin (hHb) compared to the Guaiac method. The results of immunochemical FOB rapid tests are not affected by dietary peroxidases, animal blood and ascorbic acid. A Japanese study demonstrated that immunochemical FOB screening test reduced mortality of colorectal cancer by 60%².

Principle

The Apacor FOB Rapydtest® is a sandwich lateral flow chromatographic immunoassay. The test cassette consists of:

1. a burgundy coloured conjugate pad containing monoclonal anti-hHb antibody conjugated with colloidal gold (anti-hHb conjugates) and
2. a nitrocellulose membrane strip containing a test band (T band) and a control band (C band). The T band is pre-coated with another monoclonal anti-hHb antibody, and the C band is pre-coated with goat anti-mouse IgG antibody.



When an adequate volume of test specimen is dispensed into the sample well of the test cassette, the specimen migrates by capillary action across the cassette. hHb if present in the specimen at or higher than 50 ng/ml will bind to the anti-hHb conjugates. The immunocomplex is then captured on the membrane by the pre-coated antibody forming a burgundy coloured T band, indicating a FOB positive test result. Absence of this band suggests that the concentration of hHb in the specimen is below the detectable level, indicating a FOB negative result.

Absence of the T band suggests a negative result. The test contains an internal control (C band) which should exhibit a burgundy coloured band of the immunocomplex of goat anti-mouse IgG/ mouse IgG-gold conjugate regardless of the colour development on the T band. Otherwise, the test result is invalid and the specimen must be retested with another device.

Reagents and Materials Provided

1. Individually sealed foil pouches containing:
 - A. One cassette device
 - B. One desiccant
2. Sample extraction tubes, each containing 1ml extraction buffer
3. One package insert (instruction for use)

Materials may be Required and available for Purchase

1. Positivia FOB Rapydtest® Assay Control Kit contains one vial of positive control and one vial of negative control

Materials Required but not Provided

1. Clock or Timer
2. A container for holding faecal specimen

Warnings and Precautions

For In Vitro Diagnostic Use

1. This package insert must be read completely before performing the test. Failure to follow the insert gives inaccurate test results.
2. Do not open the sealed pouch, unless ready to conduct the assay.
3. Do not use any kit components beyond their stated expiration date.
4. Do not use the components in any other type of test kit as a substitute for the components in this kit.
5. Bring all reagents to room temperature (15°C-30°C) before use.
6. **Do not scoop faecal specimen as this may lead to excess faecal specimen that tends to clot the sample well and interfere with sample migration.**
7. **Do not use specimen with visible blood for the testing.**
8. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
9. Users of this test should follow 'Good Laboratory Practice' for biosafety.
10. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
11. Extraction buffer contains 0.1% NaN₃. Avoid contact with skin or eyes. Do not ingest.
12. Dispose of all specimens and materials used to perform the test as biohazardous waste.
13. The testing results should be read within 10 minutes after a specimen is applied to the sample well of the device. Read result after 10 minutes may give erroneous results.
14. Do not perform the test in a room with strong air flow, ie. electric fan or strong air-conditioning.



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Reagent Preparation and Storage Instructions

All reagents are ready to use as supplied. Store unused test devices unopened at 2°C-30°C. If stored at 2°C-8°C, ensure that the test device is brought to room temperature before opening. The test device is stable through the expiration date printed on the sealed pouch. Do not freeze the kit or expose the kit over 30°C.

Patient Preparation

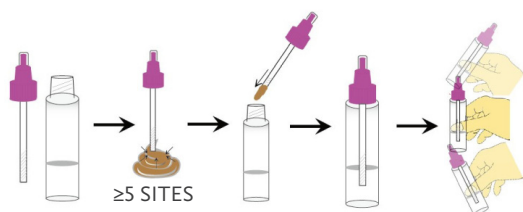
1. A specimen should not be collected from a patient with following conditions that may interfere with the test results:
 - Menstrual bleeding
 - Bleeding haemorrhoids
 - Constipating bleeding
 - Urinary bleeding
2. Dietary restrictions are not necessary.
3. Alcohol and certain medications such as aspirin, indomethacin, phenylbutazone, reserpine, cortocosteroids, and nonsteroidal anti-inflammatory drugs may cause gastrointestinal irritation and subsequent bleeding, thus gives positive reactions. On the advice of the physician, these medicines might be temporarily discontinued for 7 days prior to and during the test period.

Specimen Collection and Handling

Consider any materials of human origin as infectious and handle them using standard biosafety procedures.

1. Collect a random sample of faeces in a clean, dry receptacle.
2. Unscrew the top of the collection tube and remove the applicator stick.
3. Randomly pierce the faecal specimen in at least five (5) different sites. **Do not scoop faecal specimen as this will lead to an invalid test result.**
4. Remove excess sample off the shaft and outer grooves. Be sure sample remains on inside grooves. Specimen on the grooves is sufficient for testing. **Excess amount of faecal specimen can lead to an invalid test result.**
5. Replace the stick in the tube and tighten the cap securely.
6. **Shake the extraction tube vigorously.**

The specimen is now ready for testing, transportation or storage.

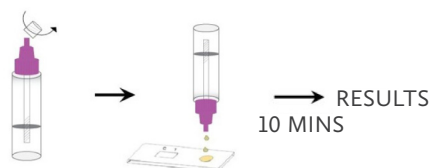


Note: Specimens collected may be stored 3 days at 2°C -8°C, or 1 year at <-20°C.

Test Procedure

- STEP 1: Bring the specimen and test components to room temperature if refrigerated or frozen.
- STEP 2: When ready to test, open the pouch at the notch and remove the test device. Place the test device on a clean, flat surface.
- STEP 3: Shake the sample collection tube vigorously to ensure an effective liquid suspension.

- STEP 4: Hold the tube upright. Twist off the tip. Dispense 2 drops of the solution into the sample well(s) of the cassette. Do not over load samples.



- STEP 5: Start the timer.
- STEP 6: Results can be read in 10 minutes after adding the specimen. Positive results can be visible in as short as 1 minute.

Don't read results after 10 minutes. To avoid confusion, discard the test device after interpreting the result.

Quality Control

1. **Internal Control:** This test contains a built-in control feature, the C band. The C line develops after adding specimen extract. Otherwise, review the whole procedure and repeat test with a new device.
2. **External Control:** Good Laboratory Practice recommends using the external controls, positive and negative, to assure the proper performance of the assay, particularly under the following circumstances:
 - A - New operator uses the kit, prior to performing testing of specimens.
 - B - A new lot of test kits is used.
 - C - A new shipment of kits is used.
 - D - The temperature used during storage of the kit falls outside of 2-30°C.
 - E - The temperature of the test area falls outside of 15-30°C.
 - F - To verify a higher than expected frequency of positive or negative results.
 - G - To investigate the cause of repeated invalid results.

Interpretation of Assay Result

1. **Negative Result:** If only the C band is developed, the test indicates that the hHb in the specimen is below 50 ng/ml FOB buffer. The result is negative.



2. **Positive Result:** If both C and T bands are developed, the test indicates that the concentration of hHb in the specimen is equal to or higher than 50 ng hHb/ml FOB buffer or 50 µg hHb/g faeces. The result is positive.



Samples with positive results should be confirmed with alternative testing method(s) and clinical findings before a diagnostic determination is made.

Interpretation of Assay Result

- Invalid:** If no C band is developed, the assay is invalid regardless of colour development on the T band as indicated below. Repeat the assay with a new device.
If it is caused by an excess amount of faecal specimen collected, re-sample and re-test.



Performance Characteristics

SENSITIVITY

The analytical sensitivity of the test is 50 ng hHb/ml buffer or 50 µg hHB/g faeces.

SPECIFICITY

The Apacor FOB Rapydtest® is specific to human haemoglobin. The following substances, when spiked in both positive and negative specimens, did not interfere with the test results.

SUBSTANCES	CONCENTRATION
Chicken Haemoglobin	100 µg/mL
Pork Haemoglobin	500 µg/mL
Beef Haemoglobin	500 µg/mL
Goat Haemoglobin	100 µg/mL
Horse Haemoglobin	100 µg/mL
Sheep Haemoglobin	100 µg/mL
Fish Haemoglobin	100 µg/mL
Rabbit Haemoglobin	100 µg/mL

HOOK EFFECT OR PROZONE EFFECT

Apacor FOB Rapydtest® cassettes do not show any hook effect or prozone effect up to the concentration of 0.5mg hHb/ml buffer.

REPRODUCIBILITY

Known positive samples were tested in multiple assays and identically positive results were observed. Similarly, known negative samples produced negative results when tested in multiple assays.

Ordering Information

PRODUCT	PACK SIZE	CODE
FOB Rapydtest®	25	1642

Products can be ordered direct from Apacor or from an appointed distributor

Visit our website for all the latest information www.apacor.com or e-mail on: sales@apacor.com

CLINICAL PERFORMANCE

A total of 251 samples were collected from 2 hospitals and tested by the Apacor FOB Rapydtest® and by a leading commercial FOB rapid test. Comparison for all specimens is shown in the following table:

REFERENCE TEST	APACOR FOB RAPYDTEST®		TOTAL
	POSITIVE	NEGATIVE	
POSITIVE	76	0	76
NEGATIVE	1	174	175
TOTAL	77	174	251

Relative Sensitivity: 100.0% , Relative Specificity: 99.4%, Overall Agreement: 99.6%.

Limitations of the Test

- Test Procedure and the Interpretation of Assay Results must be followed closely when testing the presence of occult blood in faeces. Failure to follow the procedure may give inaccurate results.
- The Apacor FOB Rapydtest® is to aid in diagnosis and is not intended to replace other diagnostic procedures such as G.I. fibroscope, endoscopy, colonoscopy, or X-ray analysis. Test results should not be deemed conclusive with respect to the presence or absence of gastrointestinal bleeding or pathology. A positive result should be followed up with additional diagnostic procedures to determine the exact cause and source for the occult blood in the faeces.
- A negative result can be obtained even when a gastrointestinal disorder is present. For example, some polyps and colorectal cancers may bleed intermittently or not at all during certain stages of the disease.

References

- America Cancer Society, Inc. Cancer Reference Information: Can Colon and Rectum Cancer be Found Early? (Online) Available: <http://www.cancer.org>
- Allison JB, Takawa IS, Ransom LJ, Adrian AL. A comparison of fecal occult blood tests for colorectal –cancer screening. N. Eng. J. Med. 1996; 334:155-159.
- Saito H. Screening for colorectal cancer by immunochemical fecal occult blood testing (Review). Jpn J. Cancer Res 1996; 87:1011-1024.