Rapydtest®

FOR THE DETECTION OF HUMAN HAEMOGLOBIN IN FAECES

APACOR

FOB Rapydtest®

MICROBIOLOGY

SINGLE USE IN VITRO DIAGNOSTIC DEVICE

FOB Rapydtest®

Intended Use

The Apacor FOB Rapydtest® is an immunochemical test 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

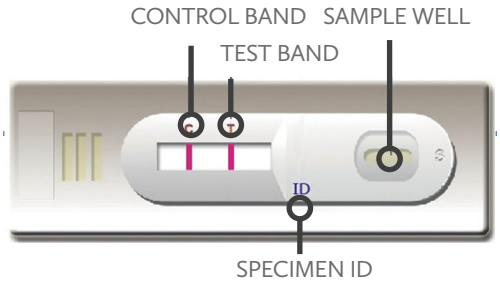
Two types of FOB tests are commercially available: guaiac dye and immunochemistry. Guaiac tests are widely used but lack accuracy. Guaiac dye is a naturally occurring phenolic compound that can be oxidized to quinone by hydrogen peroxidase activity of human haemoglobin (hHb) resulting in a detectable colour change. The sensitivity and specificity of guaiac tests are much lower than those of immunochemical assays. The low accuracy of the guaiac dye tests is related to dietary peroxidases, including haemoglobin from meat and uncooked fruits and vegetables. Non-cancerous gastrointestinal tract bleeding and iron intake may calso cause false positive results with guaiac tests[1].

Immunochemical tests are highly accurate for the detection of hHb compared to the guaiac method. The results of immunochemical FOB tests (iFOBT) are not affected by dietary peroxidases, animal blood or ascorbic acid. A Japanese study demonstrated that iFOBT screening tests reduced mortality of colorectal cancer by 60%[2]. The Apacor FOB Rapydtest® is an iFOBT designed to specifically detect low levels of human faecal occult blood. It can be performed within 10 minutes by minimally skilled personnel and without the use of laboratory equipment.

Principle

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

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



A trace amount of haemoglobin (hHb) is first extracted from the faecal specimen with the sample extraction tube. 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 concentrations equal to or higher than 50ng/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 positive test result. Absence of the T band suggests that the concentration of hHb in the specimen is below the detectable level, indicating a negative result.

The test contains an internal control (C band) which should exhibit a burgundy coloured band of the immunocomplex of control antibodies regardless of any colour development on the T band. If the C band does not develop, 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 2ml extraction buffer
3. Patient ID stickers
4. One package insert (instruction for use)

Materials Required but not Provided

1. Clock or Timer
2. Faecal specimen container

Warnings and Precautions

For In Vitro Diagnostic Use

1. This package insert must be read completely before performing the test. Failure to follow the instructions may give 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 from any other type of test kit as a substitute for the components from 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 may block the sample well and result in an invalid test result.**
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 0.3% Proclin-300. 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 test result should be read 5 to 10 minutes after a specimen is applied to the sample well of the device. Reading results 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.

Reagent Preparation and Storage Instructions

Store unused or remaining test kits at 2-30°C. If stored at 2-8°C, ensure that the test kit is brought to room temperature (15-30°C) before opening. The test kit is stable through the expiration date printed on the sealed pouch. Do not freeze the kit or expose the kit to temperatures 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

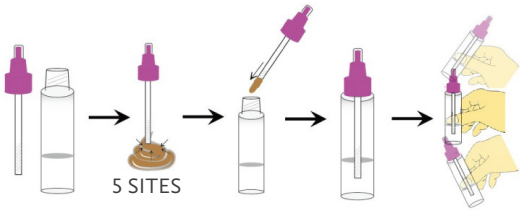
2. Dietary restrictions are not necessary.

3. Alcohol and certain medications such as aspirin, indomethacin, phenylbutazone, reserpine, corticosteroids, and nonsteroidal anti-inflammatory drugs may cause gastrointestinal irritation and subsequent bleeding, and produce positive reactions. On the advice of a physician, these medicines may 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 stool specimen in a clean, dry receptacle.
2. Fill in all required information on the patient ID sticker and apply to the sample extraction tube.
3. Open the sample extraction tube by unscrewing the top and use the collection stick to randomly pierce the stool specimen in five different sites. Do not scoop stool specimen. Ensure that stool specimen is only in the grooves of the collection stick. Excess stool specimen may lead to an invalid test result.
4. Replace the collection stick and tighten securely to close the sample extraction tube.
5. Shake the stool collection device vigorously to extract the hHb in the specimen.



The specimen is now ready for testing, transportation or storage. **Note:** It is recommended to test the specimen immediately after extraction. If not tested immediately, the extracted specimen may be stored at room temperature (20-37°C) for up to 10 days or at 2-8°C for up to 21 days. For longer storage, the extracted specimen may be frozen at -20°C. Avoid multiple freeze-thaw cycles.

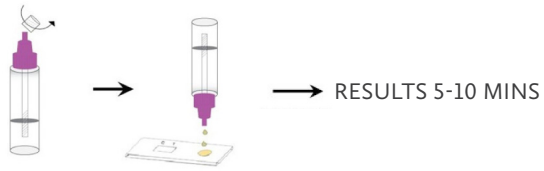
Test Procedure

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 cassette. Place the test cassette on a clean, flat surface.

STEP 3: Shake the sample extraction tube vigorously to ensure an effective liquid suspension.

STEP 4: Position the sample extraction tube upright and twist off the dispenser cap. Holding the sample extraction tube vertically, dispense 2 drops (70-90 µl) into the sample well of the cassette. Do not overload the sample well.



STEP 5: Start the timer.

STEP 6: Result can be read 5 -10 minutes after adding the sample. Positive results may be visible in a time period as short as 1 minute.

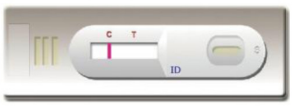
Do not read results after 10 minutes. To avoid confusion, discard the test device after interpreting the result.

Quality Control

1. **Internal Control:** This test contains a built-in control feature, the C band. The C band develops after adding specimen extract. If the C band does not develop, review the entire procedure and repeat the test with a new device.
2. **External Control:** Good Laboratory Practice recommends using external positive and negative controls to assure the proper performance of the assay, particularly under the following circumstances:
   - **A** - 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 storage temperature 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 develops, the test indicates that the concentration of hHb in the specimen is below 50 ng/ml buffer. The result is negative or non-reactive.



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

3. **Invalid**: If no C band develops, the assay is invalid regardless of colour development on the T band as indicated below. Repeat the assay with a new device. **If caused by an excess amount of faecal specimen collected, collect a new specimen and retest.**

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Performance Characteristics

SENSITIVITY

The analytical sensitivity of the test is 50 ng hHb/ml buffer or 7 mg hHb/g faeces approximately.

SPECIFICITY

The Apacor FOB Rapydtest® is specific for hHb. The following substances do not interfere with test results when spiked into negative specimens and weak positive specimens.

		APACOR FOB RAPYDTEST®	
SUBSTANCE	CONCENTRATION	NEGATIVE	POSITIVE
CONTROL	N/A	NEGATIVE	POSITIVE
BOVINE HAEMOGLOBIN	2 mg/ml	NEGATIVE	POSITIVE
CHICKEN HAEMOGLOBIN	2 mg/ml	NEGATIVE	POSITIVE
FISH HAEMOGLOBIN	2 mg/ml	NEGATIVE	POSITIVE
GOAT HAEMOGLOBIN	2 mg/ml	NEGATIVE	POSITIVE
HORSE HAEMOGLOBIN	2 mg/ml	NEGATIVE	POSITIVE
PORK HAEMOGLOBIN	2 mg/ml	NEGATIVE	POSITIVE
RABBIT HAEMOGLOBIN	2 mg/ml	NEGATIVE	POSITIVE
SHEEP HAEMOGLOBIN	2 mg/ml	NEGATIVE	POSITIVE
TURKEY HAEMOGLOBIN	2 mg/ml	NEGATIVE	POSITIVE
ASCORBIC ACID	20 mg/dl	NEGATIVE	POSITIVE
BILIRUBIN	100 mg/dl	NEGATIVE	POSITIVE
CAFFEINE	40 mg/dl	NEGATIVE	POSITIVE
GLUCOSE	2,000 mg/dl	NEGATIVE	POSITIVE
HORSERADISH PEROXIDASE	20 mg/ml	NEGATIVE	POSITIVE
IRON (Fe3+/Fe2+))	5 mg/ml	NEGATIVE	POSITIVE

Aqueous extracts of cooked and uncooked beef, broccoli, cabbage, cantaloupe, cauliflower, chicken, fish, horseradish, lamb, parsnip, pork, red radish, turkey and turnip were spiked into negative and positive specimens. No interference was detected. Additionally, toilet bowl cleansers do not interfere with the results of the Apacor FOB Rapydtest®.

DOSE HOOK EFFECT

No false negative results due to the dose hook effect were observed for specimens containing hHb at concentrations up to 4 mg/ml.

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.

CLINICAL PERFORMANCE

A total of 175 specimens were collected for the performance study. The Apacor FOB Rapydtest® was compared to a leading commercial iFOB Rapid Test marketed in the U.S. and other regions. Comparison for all specimens is shown in the following table:

	APACOR FOB RAPYDTEST®		
REFERENCE TEST	POSITIVE	NEGATIVE	TOTAL
POSITIVE	47	1	48
NEGATIVE	1	126	127
TOTAL	48	127	175

Relative Sensitivity: 97.9%, Relative Specificity: 99.2%, Overall Agreement: 98.9%

Limitations of the Test

1. Test Procedure and the Interpretation of Assay Results must be followed closely when testing for the presence of occult blood in faeces. Failure to follow the procedure may give inaccurate results.
2. 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.
3. A negative or non-reactive 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. A negative or non-reactive result can also be obtained if the quantity of occult blood present in the specimen is below the detection limit of the assay.
4. The Apacor FOB Rapydtest® has not been validated for testing of patients with hemaglobinopathies.
5. Specimens containing visible blood may produce negative results due to the hook effect.

References

1. 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.
2. Saito H. Screening for colorectal cancer by immunochemical fecal occult blood testing (Review). Jpn J. Cancer Res 1996; 87:1011-1024.

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: orders@apacor.com

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