

INTEX[®]-FOB Test

Instructions for use

Immunoassay for the qualitative detection of human haemoglobin in fecal samples

1. Intended Use

INTEX[®] Fecal Occult Blood (FOB) Test device is a rapid, visual immuno-chromatographic test for the qualitative detection of human blood in fecal samples. This test is intended as an aid in the diagnosis of lower gastrointestinal (g.i.) disorders. The test is for professional in-vitro diagnostic use only.

The intended use of the FOB assay is to screen for lower gastrointestinal pathologies, such as colorectal cancers and large adenomas that bleed. Colorectal cancer is one of the most commonly diagnosed cancers and a leading cause of cancer death in the United States (Lieberman, 1994; MMWP, 1995). Screening for colorectal cancer probably increases the cancer detection at an early stage, therefore reduces the mortality (Dam et. al., 1995; Miller, 1995; and Lang, 1996).

Earlier commercially available fecal occult blood (FOB) tests utilized the guaiac test, which requires special dietary restriction to minimize false positive and false negative results. The INTEX FOB[®] Test is especially designed to detect human hemoglobin in fecal samples using immunochemical methods, which improved specificity for the detection of lower gastrointestinal disorders, including colorectal cancers and adenomas (Frommer et. al., 1988; St. John et. al., 1993).

2. Test Principle

The INTEX FOB[®] Test is a qualitative, membrane based sandwich immunoassay that has been designed for the detection of human hemoglobin in stool specimen through visual interpretation of color development in the test device.

In this assay fecal occult blood (FOB) is detected with the aid of specific antibodies against hemoglobin.

After the addition of the sample (feces diluted in buffer) a color-labeled antibody specifically binds to hemoglobin if it is present in the sample. When this complex migrates upward on the membrane by capillary action, it is captured with the aid of another specific antibody at the test result line region of the test. A red test result line is generated. If no hemoglobin is present the color labeled antibody cannot bind at the test result line region. No red test result line is formed. So the presence of a colored test result line 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

3. Kit Content

- 5, 20 or 50 individually wrapped test devices
- **Test material for Patients:**
 - sample collection tubes
 - sample collection sachets
 - faeces collector
 - patient's information
- 1 Instruction sheet

4. Materials Required But Not Provided

- Clean dry containers or receptacles for the collection of fecal samples
- Tissue paper to prevent solution from splashing when breaking the tip of the collection tube
- Timer

5. Storage and Stability

The test kit is to be stored refrigerated (2-8°C) or at room temperature (up to 30°C) in the sealed pouch for the duration of the shelf-life. Avoid freezing or storage beyond 30°C.

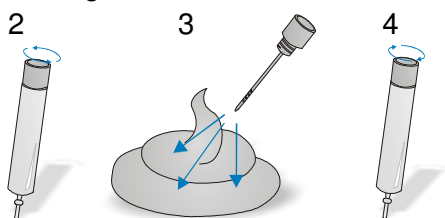
6. Precautions

- *For single in-vitro diagnostic use only*
- *For professional use only.*
- *Do not use the test after the expiration date or if pouch has been damaged.*
- *Do not mix sample collection tubes from different lots.*
- *Do not open the pouch until you are ready to perform the test. Humidity can adversely affect the test performance.*
- *Do not touch or spill liquid onto the white membrane in the test result window.*
- *Avoid cross-contamination of samples by using a new specimen collection container and specimen collection tube for each sample.*
- *All patient's samples should be treated as if capable of transmitting disease. Adequate handling and disposal methods should be established. It is recommended to wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are being tested. Do not eat, drink or smoke in the area where specimens or kits are handled.*
- *The buffer contains low amounts of sodium azide which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of the buffer or extracted samples, always flush with copious quantities of water to prevent azide build up.*
- *Ensure that patients closely follow the specimen collection procedures.*
- *Please follow closely the instructions to ensure reliable results.*

7. Specimen Collection, Preparation and Storage

Patients should **not collect** samples during their menstrual period, if they have bleeding hemorrhoids, blood in the urine, if they are taking rectally administered medication or if they have strained during bowel movement because of constipation. Please note that medication taken as blood diluter or food supplementation with iron might also lead to elevated levels of hemoglobin in stool samples (also see "Clinical Specificity").

1. Collect a random sample of feces in a clean dry container or receptacle.
2. Unscrew and remove the applicator stick from the sample collection tube. Be careful not to spill or spatter solution from container.
3. Collect random sample by using the applicator stick. Take sample from three different spots of the feces specimen. Ensure not to scoop too much material into the tube. The amount of feces that gets stuck on the surface of the applicator stick is sufficient.

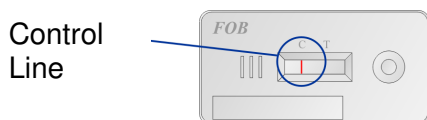


4. Re-insert the applicator stick into the tube and close the cap tightly. Be careful not to break the tip of the sample collection tube.
5. The specimen is now ready to be stored, transported or tested. The specimen should be tested as soon as possible, but may be held up to 7 days prior to testing. The specimen should be transported and stored in an airtight container. It is recommended to store it at +2°C - +8°C until tested. Short time exposure to temperatures up to 30 °C e.g. during transportation do not affect the specimen.

8. Test Procedure

Quality Control/ Internal Procedural Control

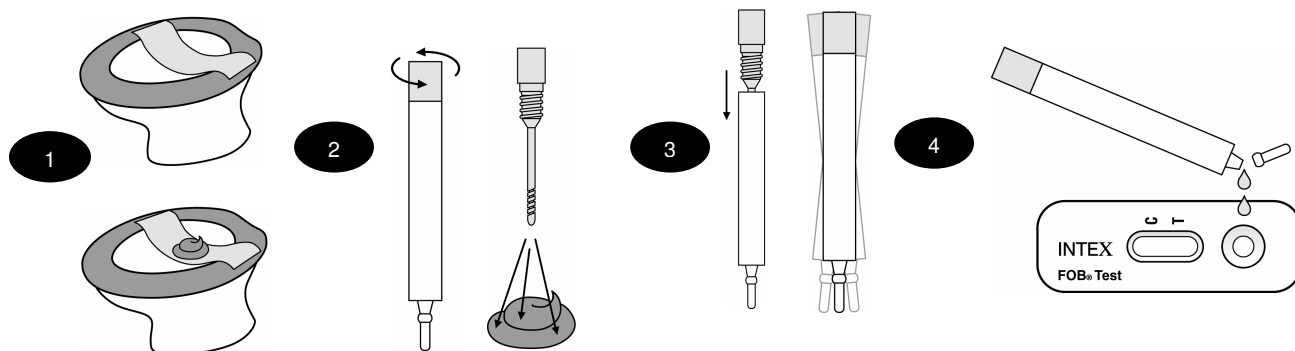
A procedural control is included in the test. A colored line appearing in the control line region (C) of the membrane confirms the proper performance of the test.



IMPORTANT: When testing control material dissolved in buffer the background of the assay is usually clear within 5 minutes. However, when fecal samples are tested, the background may appear slightly yellowish due to the original color of the fecal samples. This is acceptable as long as it does not interfere with the interpretation of test result. The test is invalid if the background fails to clear and obscures the reading of the result.

9. Assay Procedure

- Test device, patient's samples, (extracted sample) should be brought to room temperature (20-30°C) prior to testing.
- Bring the device to room temperature prior to opening to avoid condensation of moisture on the membrane. Remove the test device from the pouch when ready to perform the test. Label the device with patient or control identification.
- Shake the collection tube thoroughly to ensure proper mixing of the fecal sample with the extraction solution.
- Remove the protective cap. Using a piece of tissue paper, break the tip of the collection tube using a twisting motion. Hold the collection tube vertically and dispense 3 drops (app. 120 µL) of solution into the round sample well of the test device by applying a gentle pressure on the tube walls. Start the timer.
- Read the result after 5 minutes. Strong positive results may be interpreted sooner. Do not interpret results after 8 minutes.
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Interpretation of the Results

Positive:

Two pink-red colored lines appear, one in the control line region (C) and one in the test result line region (T). When testing with strong positive samples, the intensity of the control band may be lighter than expected. Comparison of the line intensities is not recommended.

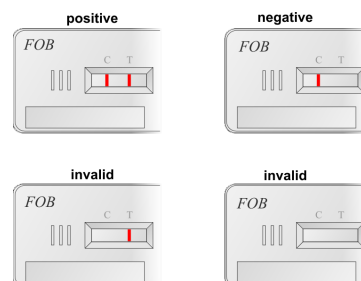
Negative:

Only one pink-red colored line appears in the control line region (C). No apparent faint pink to red colored line appears in the test result line region (T).

Invalid:

The control line (C) is not formed.

In this case the result is invalid even if the test result is visible. The absence of the control line is an indication of procedural error or that test reagents may have deteriorated. Repeat the test with a new test device and if condition persists, contact the manufacturer for technical assistance.



10. Limitations

- This test kit is to be used for the qualitative detection of human hemoglobin in fecal samples. A positive result suggests the presence of human hemoglobin in fecal samples. In addition to intestinal bleeding the presence of blood in stools may have other causes such as hemorrhoids, blood in urine etc. (also see “Specimen Collection”)
- Not all colorectal bleedings are due to precancerous or cancerous polyps. The information obtained by this test should be used in conjunction with other clinical findings and testing methods, such as colonoscopy gathered by the physician.
- Negative results do not exclude bleeding since some polyps and colorectal region cancers can bleed intermittently or not at all. Additionally, blood may not be uniformly distributed in fecal samples. Colorectal polyps at an early stage may not bleed.
- Urine and excessive dilution of sample with water from toilet bowl may cause erroneous test results. The use of a receptacle is recommended.
- Feces specimens should not collect during the menstrual period and not three day before or afterwards, at bleeding due to constipation, bleeding haemorrhoids, or at taking rectally administered medication. It could cause false positive results.
- This test may be less sensitive for detecting upper g.i. bleeding because blood degrades as it passes through the g.i. track.

11. Performance Characteristics

A. Analytical Sensitivity

A sample containing human hemoglobin at concentration equal to 2 µg Hemoglobin/g feces (resp. 40 ng Hemoglobin/ml buffer after extraction) produces a positive result. In some cases sample containing human hemoglobin at concentrations less than 2 µg/g feces can also be tested as positive.

Hook or Prozone effect:

Sample containing as high as 1,250 µg hemoglobin/g feces (0.5 mg/ml buffer) can still test positive. The INTEX FOB[®] Test do not show a Hook or Prozone Effect up to the maximal observed

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physiological concentration (500.000 ng/ml=0,5 mg/ml). Thus, the working range is 40 ng/ml up to 500,000 ng/ml buffer resp. 2 µg up to 1,250 µg Hemoglobin/ g feces

B. Analytical Specificity

The INTEX FOB[®] Test is specific for human hemoglobin and does not show any cross-reaction with the hemoglobin from bovine, pig, horse and sheep up to concentrations of 0.5 mg/ml.

Hemoglobin from rabbit and polecat may cause cross reactions.

The INTEX FOB[®] Test also does not show any cross reaction with billirubin, vitamin C and horse radish peroxidase.

C. Clinical Specificity

The following non-cancer related factors may cause blood in feces samples:

1) Iron

Food supplementation with iron leads to increased release of blood in the colon. Iron itself is not cross-reacting with the test.

2) Acetylsalicylic acid

ASA is main compound in a lot of drugs against headache (e.g. Aspirin[®] from Bayer), and is sometimes used to substitute macumar as a blood diluter. Almost always there are very small amounts of blood in fecal samples in case of healthy humans. This is far below the sensitivity of our test and has nothing to do with cancer or any other serious matter. However, if a patient takes blood diluters bleeding can be more intensive. Therefore the cut-off of INTEX FOB[®] Test may be reached.

3) Coumarin

Coumarines are used as drugs (e.g. Macumar[®]) for prevention of heart attacks, against thrombosis and stroke. Similar to ASA, coumarines are blood diluters. Almost always there are very small amounts of blood in fecal samples in case of healthy humans. This is far below the sensitivity of INTEX FOB[®] Test and has nothing to do with cancer or any other serious matter. However, if a patient takes blood diluters, bleeding can be more intensive. Therefore the cut-off of DIMA FOB test may be reached.

4) Hemorrhoids

Hemorrhoids may bleed. Therefore fecal sample may be contaminated with blood which is not associated with cancer.

5) Monthly period

Small amounts of blood released because of female's period may contaminate the fecal sample. This is blood which is not associated with cancer.

6) Urine samples

Several diseases may cause blood in urine samples. To avoid detection of urine-related blood, stool sample should not get in contact with urine.

Note:









In a recently published study of the Shinshu-University School of Medicine in Japan the cost-value ratio of multiple measurements was examined. It shows that the relative sensitivity increases with amount of test and its relative specificity decreases slightly.



amount of tests	sensitivity	specificity
1	58%	96%
2	89%	95%
3	100%	94%

12. Literature

1. Dam, J.V., et. al.; Fecal Blood Screening for Colorectal Cancer; Archive of Internal Medicine; (1995) 155:2389-2402
2. Frommer, D.J. et. al.; Improved Screening for Colorectal Cancer by Immunological Detection of Occult Blood; British Medical Journal; (1988) 296:1092-1094
3. Lieberman, D.; Screening/Early Detection Model for Colorectal Cancer, Why Screen? Cancer Supplement; (1994) 74(7) : 2023-2027
4. Miller, A.B.; An Epidemiological Perspective on Cancer Screening; Clinical Biochemistry (1995) 28(1) : 41-48
5. Ransohoff, D.F. and Lang, C.A.; Improving the Fecal Occult-Blood Test: The New England Journal of Medicine; (1996) 334 (3) : 189-190
6. Screening for Colorectal Cancer-United States, 1992-1993, and New Guidelines; Mobility and Mortality Weekly Report; (1995) 45 (5) : 107-110
7. St. John, D.J.B., et. al.; Evaluation of New Occult Blood Test for Detection of Colorectal Neoplasia; Gastroenterology; (1993) 104:1661-1668
- 1) Yamamoto M., Nakama H., Cost-effectiveness analysis of immunochemical occult blood screening for colorectal cancer among three fecal sampling methods: Hepatogastroenterology, 2000 Mar-Apr, 47 (32) 396-399.

13. Symbols

	Article number		For single use only
	Lot number		Expiry date
	Storage		Content
	Only for in vitro diagnostics		Instruction sheet

INTEX-FOB[®] Test		
	5 Test cassettes	FO1805
	20 Test cassettes	FO1820
	50 Test cassettes	FO1850



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