Fecal occult blood test.

**Intended use.** System for qualitative rapid detection of occult blood in fecal samples.

**Test principle.** The Occult Blood iFOBT system is a chromatographic immunooassay which uses a membrane with two immobilized antibodies: a capture anti-human hemoglobin antibody and a control antibody.

The buffered sample is exposed to a colloidal gold-anti-human hemoglobin monoclonal antibody conjugate and the mixture migrates chromatographically through the membrane by capillarity. If there is any hemoglobin in the sample, it binds to the conjugate and a colored line is formed where the anti-hemoglobin antibody is immobilized, yielding a positive result. As the samples continues to migrate through the membrane, a second colored line is formed where the control antibody is immobilized, which confirms the test was performed adequately.

The formation of two colored lines indicates a positive result whereas the formation of one colored line in the control position indicates a negative result.

If no line is formed on the control position, the test was not performed adequately and must be repeated.

**Summary.** The Occult Blood iFOBT system is an immunochromatographic method which detects the presence of occult blood in fecal samples with a simple and rapid procedure. This method does not demand previous dietary restriction, which is necessary for assays based on the stool guaiac test.

The Occult Blood iFOBT test exhibits high sensitivity, being capable of detecting minute quantities of hemoglobin, as little as 0.04 µg/mL, on the buffered feces suspension. The system uses a specific monoclonal antibody, which renders it immunospecific, thus eliminating the occurrence of cross-reactions with bovine, pig, goat, rabbit, horse, shup, and fish hemoglobin. Which may be eventually present in sample due to diet choices. Furthermore, there is no significant interference of substances such as ascorbic acid, bilirubin, and peroxidase.

Therefore, the Occult Blood iFOBT system is convenient for the patient and is practical and safe for the clinical laboratory in fecal occult blood testing.

**Methodology.** Immunochromatography.

**Reagents**

1. **Buffer** - Store at 2 - 30 ºC.
   - Contains sodium chloride 154 mmol/L and sodium azide 3 mmol/L.

2. **Test Device** - Store at 2 - 30 ºC.
   - Contains colloidal gold-anti-hemoglobin, anti-hemoglobin antibody, and control antibody either applied to or immobilized in a membrane.

**Precautions and warnings**

The test device must not be frozen. At the moment of test, it must be at 15 - 30 ºC.

Avoid exposing the test device to room humidity.

The usual security cares should be applied to the reagent handling.

No known test method can offer complete assurance that fecal samples will not transmit infectious diseases. Therefore, all samples should be considered potentially infectious.

The buffer contains sodium azide as preservative. Avoid ingestion. In case of contact with eyes, immediately flush eyes with plenty of water and get medical assistance.

Sodium azide may react with lead and copper plumbing and yield highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide accumulation.

Disposal of all waste material should be in accordance with local guidelines.

**Storage and stability.** Unopened reagents, when stored at indicated temperature, are stable up to the expiration date shown on the label.

**Deterioration.** Microbial or chemical contamination may decrease reagent stability.

**Specimen collection and preparation**

A Standard Operating Procedure (SOP) must be created to establish adequate procedures for sample collection, preparation, and storage. The errors due to bad sampling can be more damaging than the ones which may occur during the analytical procedure.

Fecal samples must be collected in clean and dry containers. Use only fecal samples without preservatives.

The assay must be performed up to 6 hours after sample collection. Optionally, the samples can be stored up to 3 days at 2 - 8 ºC.

Samples must not be collected either during or 3 days after the menstrual period, or if the patient presents hemorrhoid bleeding or hematuria.

Alcohol, medicaments containing acetylsalicylic acid (Aspirin®, AAS®, Somalgin®, Butern®, etc.) and other medicaments in excess can cause gastrointestinal irritation, resulting in occult bleeding. The use of these substances must be discontinued for at least 48 hours prior to sample collection.

Dietary restriction is not necessary to perform the assay.
**Materials required not provided**

1. Timer

**Procedure**

The tube containing the buffer and the test device must be at room temperature (15 - 30 °C) at the moment of testing.

1. Remove the blue stick from the sample tube containing the buffer.

2. Introduce the stick randomly in at least 3 different regions of the fecal sample. This procedure is extremely important, as the blood is distributed unevenly in the sample. Avoid the collection of large amounts of sample. The amount retained in the stick is sufficient for performing the test.

3. Place the stick with the sample back in the tube and close it adequately.

4. Homogenize the tube vigorously to mix the sample with the buffer. After it is prepared, the sample is stable for 15 days at 15 - 30 °C.

5. Remove the test device from the packing, identify it properly, and place it on a horizontal surface.

6. Hold the sample tube with the cap in an upward position, remove the tube cap, and break the top part of the tube with the help of absorbing paper, turning the tube into a dropper.
7. Turn the tube upside down, add 3 drops (0.15 mL) of the mixture in the sample orifice (S) of the test device. Read the results visually after 5 minutes. Do not read the results after 8 minutes.

**Results**

**Positive.** Formation of a red line on the control position (C) and another line on the test position (T). The line color intensity on the test position (T) may vary according to the concentration of hemoglobin present in the sample. Therefore, even a faint red line on the test position (T) indicates a positive result.

**Negative.** Formation of a red line on the control position (C) and absence of red line on the test position (T).

**Invalid.** Absence of red line on the control position (C) indicates either an error in the test procedure or system deterioration. Check the procedure and perform the test again using a new test device.

**Quality control.** The clinical laboratory must keep an internal quality control program, defining clearly all applicable regulations, objectives, procedures, quality specification criteria, tolerance limits, corrective measures, and registration of activities.

The formation of a colored line on the control position indicates an adequate performance of the system. To attest there is no adverse factor affecting the system and it keeps its performance level, it is a best practice to associate a quality control system to it, using a known positive and a known negative sample on a daily basis.

**Performance characteristics**

**Method comparison.** The proposed method was compared to a similar product using 468 positive and negative samples for fecal occult blood. The results are shown on the table below:

<table>
<thead>
<tr>
<th>Comparative method</th>
<th>Occult blood iFOBT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>211</td>
</tr>
<tr>
<td>Negative</td>
<td>1</td>
</tr>
</tbody>
</table>

Relative Sensitivity: 99.5%
Relative Specificity: 99.6%
Efficiency: 99.6%
Kappa Index: 0.99

The Kappa Index (above 0.75) indicates an excellent agreement between the methods, attesting that the Occult Blood iFOBT is substantially equivalent to the comparative method.

**Imprecision - Within run.** The within-run imprecision was assessed by testing 60 replicates of two samples, being one negative and the other with 0.04 μg/mL of hemoglobin in the fecal buffered suspension. Each reaction was read visually between 5 and 8 minutes after the sample was applied to the test device. The results exhibited a perfect agreement with the expected results.

**Imprecision - Run-to-run.** The total imprecision was assessed in 3 independent tests, using 60 replicates of two samples, being one negative and the other with 0.04 μg/mL of hemoglobin in the fecal buffered suspension. Each reaction was read visually between 5 and 8 minutes after the sample was applied to the test device. The results exhibited a perfect agreement with the expected results.

**Prozone.** There was no observable prozone effect in samples with hemoglobin concentrations up to 0.5 mg/mL in the fecal buffered suspension.

Fecal samples with macroscopically perceived blood presence may yield a suspension with hemoglobin concentration above 0.5 mg/mL, which may lead to falsely negative results.

**Analytical sensitivity.** The method is capable of detecting blood amounts as little as 0.04 μg of hemoglobin in a milliliter of fecal buffered suspension.

**References**


**Presentation**

<table>
<thead>
<tr>
<th>Product</th>
<th>Reference</th>
<th>Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Occult Blood iFOBT</td>
<td>112K7-20</td>
<td>20 X 2.0 mL</td>
</tr>
</tbody>
</table>

20 X Test device
Consumer information

[Warranty conditions]

Labtest Diagnóstica warrants the performance of this product under the specifications until the expiration date shown in the label provided that the procedures and storage conditions indicated on the label and in this insert have been followed correctly.

Representante Autorizado na Comunidade Europeia
Authorized Representative in the European Community

Reagente
Reagent

Material Calibrador
Calibrator Material

Controle positivo
Positive control

Limites de temperatura (conserver a)
Temperature limits (conservar a)

Controle negativo
Negative control

Controle
Control

Controle
Control

Data limite de utilização (aaaa-mm-dd ou mm/aaaa)
Use by (yyyy-mm-dd or mm/yyyy)

Controle
Control

Número de lote
Batch code

Válido para < n > testes
Valid for < n > tests

Conteúdo suficiente para < n > testes
Content sufficient for < n > tests

Representante autorizado na Comunidade Europeia
Authorized Representative in the European Community

Corrosivo
Corrosive

Risco biológico
Biological risk

Ref.: 170309

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