An *in vitro* immunoassay for the rapid, qualitative detection of human hemoglobin in feces.

CLIA Complexity: Waived

**INTENDED USE**
The Polymedco OC-Light FOB test is an immunological test intended for the detection of fecal occult blood in feces by professional laboratories and physician office labs. The test is useful for the determination of gastrointestinal (GI) bleeding, found in a number of gastrointestinal (GI) disorders, e.g., diverticulitis, colitis, polyps, and colorectal cancer.
The OC Light test is recommended for use in Routine physical examinations.
1. Monitoring for bleeding in patients.
2. Screening for colorectal cancer or gastrointestinal bleeding.

**SUMMARY**
The presence of fecal occult blood in the stool is associated with gastrointestinal disorders such as diverticulitis, polyps, and Crohn’s disease, that may lead to colorectal cancer if not treated. Early diagnosis by fecal occult blood screening and treatment of these problems has been shown to significantly reduce mortality from colorectal cancer. Conventional test methods used for the detection of fecal occult blood do not provide a high degree of accuracy. Immunological tests developed to detect human hemoglobin are more accurate and do not require special dietary restrictions on patients.

**PRINCIPLE**
The OC-Light iFOBT is an immunoassay utilizing a blend of a polyclonal and monoclonal antibodies to specifically detect the presence of Hb in feces. The sample end of the test strip is dipped in the feces extract. The liquid feces wicks through a series of absorbent materials and contacts latex particles conjugated to an antibody specific to Hb. The sample and latex conjugate then wick through a membrane that contains zones of immobilized antibodies - a patient test zone of anti-Hb capture antibody and a control zone of anti-mouse antibody. If Hb is present in the sample it serves to link the latex conjugate to the capture antibody in the patient test zone. The control zone antibody binds the monoclonal antibody on the latex. The buildup of latex particles in the zones leads to the development of visible blue bands.

**PRECAUTIONS**
- For in vitro diagnostic use only.
- For professional and laboratory use.
- Do not reuse the test.
- Do not use test strips if canister is damaged; does not seal. The directions for use must be followed carefully for accurate results.
- Treat feces extraction samples and used test strips as if they are potentially infectious.
- Do not use beyond the labeled expiration date. The expiration date can be found on the carton and vial labels.

**STORAGE AND STABILITY**
Store the OC-Light iFOBT at 2°-30°C (36°-86°F) in their original canister. DO NOT FREEZE. Test strips are stable when stored at these temperatures until the expiration date printed on the labeling.

**REAGENTS AND MATERIALS SUPPLIED**

<table>
<thead>
<tr>
<th>OC-Light iFOBT Complete Kit (FOB50)</th>
<th>OC-Light iFOBT Test Kit (FOBSTR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 Test Strips</td>
<td>50 Test Strips</td>
</tr>
<tr>
<td>50 Sampling Bottles</td>
<td>OC-Light iFOBT Bottle Kit (FOBBTL)</td>
</tr>
<tr>
<td>50 Collection Papers</td>
<td>50 Sampling Bottles</td>
</tr>
<tr>
<td></td>
<td>OC-Light iFOBT Personal Use Kit (FBPU)</td>
</tr>
<tr>
<td></td>
<td>20 Personal Use Packs</td>
</tr>
</tbody>
</table>

**MATERIALS REQUIRED BUT NOT PROVIDED**
- Timing Device
- External Controls (FBT-POC Recommended)
- Rack (FOB-RACK)
SPECIMEN COLLECTION AND HANDLING
Collect feces from the collection paper or from specimen caught in a clean cup. Contamination from toilet water should be avoided.

1. Fill in all required information on the sampling bottle.

2. Open green cap by turning to the left and pulling upwards.

3. Scrape the surface of the fecal sample with the sample probe.

4. Cover the grooved portion of the sample probe completely with stool sample.

5. Close sampling bottle by inserting the sample probe and screwing cap on tightly to the right. Do not reopen.

6. Extracted feces may be stored at room temperature for up to 15 days or can be refrigerated at 2-8ºC for up to 30 days.

7. Bring extractions to room temperature prior to assaying and mix well before sampling.

TEST PROCEDURE
Refer to Figure 1.
2. Remove an OC-Light iFOBT strip from the canister. Minimize the amount of time that the canister is left open and assure that the canister is securely closed after opening.
3. Remove the white cap on the extraction vial. Drop the sample end of the dipstick into the extraction vial.
4. Start timer.
5. When the timer reaches 5 minutes, read results. Read results as shown under “Interpretation of Results”
NOTE: Specimens with high concentrations of Hb may produce positive results in as little as 1 minute. Confirm negatives at 5 minutes. Do not read after 10 minutes.

FIGURE 1
INTERPRETATION OF RESULTS

POSITIVE
Carefully look for the appearance of a test line in the Test Region. ANY blue colored line, NO MATTER HOW FAINT, in the Test Region with a colored line in the Control Region is a positive result. Neither the intensity nor the color should be compared to that of the Procedural Control line.

NEGATIVE
If no blue line appears in the Test Region and one line in the Procedural Control Region the result is negative.

INVALID
If no line appears in the Procedural Control Region, the test is invalid and must be repeated with a new strip.

QUALITY CONTROL
Good laboratory practices recommend the use of appropriate controls. There are two types of controls for the OC-Light iFOBT, the internal procedural control and external controls.

Procedural Control
The Procedural Control is found in the Procedural Control Region of the test strip. This control assures the operator that (A) sample addition and migration through the test strip has occurred and that (B) the control anti-mouse antibody and the reporter MAb are intact and functional. This control does not ensure that the capture antibody is accurately detecting the presence or absence of Hb in the sample.

External Control
External controls are used to assure the operator that the capture and conjugated antibodies are present and reactive. External controls will not detect an error in performing the patient test procedure. Controls should be assayed once per kit. To use, unscrew the white cap on the sample bottle. Add four drops of the control. Replace the white cap and shake vigorously. Follow step three of the patient test procedure. If controls do not perform as expected, do not use the test results. Repeat the test or call Polymedco Technical Services at 800-431-2123.

LIMITATIONS
- The OC-Light iFOB Test is intended only for the detection of hemoglobin in feces. It is not advised for use in patients suspected of upper GI bleeding.
- Patients with the following conditions should not be considered for testing as these conditions may interfere with the test results:
  - Bleeding hemorrhoids
  - Constipation bleeding
  - Urinary bleeding
- Certain medications such as aspirin and non-steroidal anti-inflammatory drugs may cause gastrointestinal irritation and subsequent bleeding in some patients and cause positive results.
- As with any occult blood test, results obtained with the OC-Light iFOB Test should not be considered conclusive evidence of the presence of absence of g.i. bleeding or pathology. The OC-Light iFOB Test is designed for preliminary screening. It is not intended to replace other diagnostic procedures such as colonoscopy or sigmoidoscopy in combination with double contrast barium x-ray.
- Because gastrointestinal lesions may bleed intermittently and blood in feces is not distributed uniformly, a negative test result does not assure absence of lesion.
- Urine and excessive dilution of samples with water from the toilet bowl may cause erroneous test results. For best result, use the collection paper in the collection kit.
OC-Light iFOB Test is not for use in testing urine, gastric specimens or other body fluids. FOB testing is recommended annually by the American Cancer Society (2008) for average-risk women and men, 50 years of age and older. However, patients with significant risk factors such as family history of colorectal cancer should be screened earlier and more often.

EXPECTED VALUES

Positive rates with immunochemical fecal occult blood tests have been shown to vary in each patient population depending on the test used, age and ethnicity of the patient and the predisposition to colorectal disease and other factors that may be associated with lower gastrointestinal bleeding. The OC-Light iFOBT will detect Hb in feces at levels as low as 50 ng/ml.

PERFORMANCE CHARACTERISTICS

Sensitivity

Reproducibility studies were conducted at three Physician Office Labs (POL). 100 human hemoglobin-free stool extracts were collected and separated in five groups of twenty. Each group of specimens were spiked with a known level of human hemoglobin to result in the following concentrations: 0 ng/mL, 30 ng/mL, 50 ng/mL (at the cut-off), 62.5 ng/mL (just above the cut-off), and 2000 ng/mL (prozone). The total number of determinations per level of hemoglobin was 60. The reads were compared with results from a reference laboratory. There was 99.3% agreement between the results obtained from the POL and the results obtained from the reference laboratory. The overall accuracy of the OC-Light iFOBT by the POL users was 98.9%.

<table>
<thead>
<tr>
<th>Concentration of Hb in Extracted Feces in ng/mL</th>
<th>0</th>
<th>30</th>
<th>50</th>
<th>62.5</th>
<th>2000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Results</td>
<td>0</td>
<td>22</td>
<td>47</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>Negative Results</td>
<td>60</td>
<td>38</td>
<td>13</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>Agreement</td>
<td>100%</td>
<td>36%</td>
<td>78%</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Specificity

The specificity of the OC-Light iFOBT was evaluated from cross reactive studies with known amounts of hemoglobin (Hb), Hb-S, Hb-C, Hb-F, at concentrations of 50 ng/mL. All of the OC-Light iFOBT results were positive.

Interference Testing

Cross reactivity studies were completed to investigate the cross reactivity of other species of hemoglobin (Hb) and tissue extracts on the OC-Light iFOBT and another commercially available FOB tests. Hb of bovine, equine, pig, rabbit, sheep, fish, chicken and goat origin was added to the test device to determine the cross reactivity of the test with Hb of other species. Each Hb species was added to normal stool extracts at both 0 and 50 ng/mL human hemoglobin (hHb). The results were as expected. The negative results continued to be negative and the positive results continued to be positive after the addition of the animal hemoglobins. The study was repeated with tissue extracts of beef, pig, rabbit, sheep, fish and chicken and no cross reactivity was evident.

Dietary Testing

A potential interference of dietary substances on the OC-Light iFOBT was assessed. Aqueous extracts of raw broccoli, cauliflower, cantaloupe, horseradish, red radish, parsnip and turnip were added to the test device to determine if vegetable extracts cross react with the test. The extracts were prepared by homogenizing raw vegetable in a food processor and then subsequently centrifuging the extract to separate the solid and liquid phases. Dietary Iron and Vitamin C supplements and horseradish peroxidase were also tested for cross reactivity. No cross reactivity was evident.

REFERENCES