The CLIAwaived, Inc. Rapid Fecal Occult Blood Test is designed to detect lower levels of fecal occult blood than standard guaiac tests. The basis of the test is an immunochromatographic sandwich capture method, which yields results more specific to human hemoglobin and are easier to interpret than those of guaiac-based devices.

**PRINCIPLE OF THE PROCEDURE**

The CLIAwaived, Inc. Rapid Fecal Occult Blood Test is a method that employs a unique combination of polyclonal and monoclonal antibodies to selectively identify hemoglobin in test samples with a high degree of sensitivity. In less than five minutes, elevated levels of human hemoglobin as low as 50 ng/hb/ml can be detected and positive results for higher levels of hemoglobin can be seen in the test as early as two or three minutes.

A fecal sample is collected and prepared for testing using the fecal collection tube; the resulting sample fluid is added directly to the test device. The sample fluid mixes with anti-hemoglobin dye-conjugate in the test membrane forming an antigen-dye complex, which migrates through the test device. The complex is captured in the test (T) zone by immobilized anti-hemoglobin antibodies. The captured dye-complex becomes visible as a rose-pink band within the test zone, which indicates the test has detected human hemoglobin, a positive result. In the absence of hemoglobin, no line will form in the test zone.

A built-in procedural control indicates proper kit performance. The control result is viewable as a rose-pink band in the control (C) zone within the five-minute testing period. The control band is formed by a non-specific sandwich dye conjugate reaction and should appear regardless of the test result.

A rose-pink band in the test zone and in the control zone at or before five minutes is considered a positive result by the criteria of the test. A band only in the control zone at five minutes is a negative result.

**TEST PROCEDURE**

1. Shake the tube vigorously to ensure a good liquid suspension.
2. Hold the tube upright, snap off the tip.
3. Dispense 2-3 drops of solution into the sample (S) well.
4. Wait five minutes and read the result.

**INTERPRETATION OF RESULTS**

1. **Positive:** At five minutes, two rose-pink color bands appear: one in the test (T) zone and one in the control (C) zone. A positive result indicates that the specimen contains human hemoglobin. A positive result may be read sooner than 5 minutes.
2. **Negative:** At five minutes, one rose-pink color band appears in the control (C) zone indicating a negative result and that the specimen does not contain a detectable level of human hemoglobin.
3. **Invalid:** At five minutes, no bands appear, or a test band appears without a control band, disregard the result, indicating the test is invalid. It is recommended that the specimen be re-tested.

**NOTE:** There is no meaning attributed to line color intensity or width. A positive result may be read sooner than 5 minutes; however, a negative result must be read after 5 minutes.

**QUALITY CONTROL**

An internal procedure control has been incorporated into the test to ensure proper kit performance and reliability. The use of an external control is recommended to verify proper kit performance. Quality control samples should be tested according to quality control requirements established by the testing laboratory.

**LIMITATIONS**

1. As with any diagnostic test, the CLIAwaived, Inc. Rapid Fecal Occult Blood Test may not be considered as a conclusive diagnosis for gastrointestinal bleeding or pathology. It is not intended to replace other diagnostic procedures such as G.I. fibre scope, endoscopy, colonoscopy or other x-ray analysis.
2. Although the test is very accurate in detecting human hemoglobin, there is a possibility false result may occur. In addition, because many bowel lesions, including some colorectal cancers and polyps, may bleed intermittently or not at all, occult blood may not be uniformly distributed throughout the fecal sample. Thus test results may be negative even when disease is present.
3. The CLIAwaived, Inc. Rapid Fecal Occult Blood Test has not been tested for toilet water interference and that samples that have touched the toilet water should not be used for testing.
4. The CLIAwaived, Inc. Rapid Fecal Occult Blood Test has not been tested on abnormal blood from Thalassemia and Sickle Cell patients.

**PERFORMANCE CHARACTERISTICS**

1. **Sensitivity.** The analytical sensitivity of the test is 50 ng/hb/ml buffer or 5 ug hgb/g feces.
2. **Accuracy.** There were 120 human hemoglobin free feces extraction specimens collecting over 10 days from in house and grouped these samples into 6 in an evenly distributed number 20. The 6 groups of extraction samples were spiked with human hemoglobin for six different concentrations, respectively, 0.1ng/ml; 10ng/ml; 40ng/ml; 50ng; 100ng/ml; 2000ng/ml. The results obtained agreement 98% with the predicate device.
3. **Specificity.** The CLIAwaived, Inc. Rapid Fecal Occult Blood Test is specific to human hemoglobin. Specimens containing the following substances have no effect on test result.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken Hemoglobin</td>
<td>500 ug/ml</td>
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<tr>
<td>Pork Hemoglobin</td>
<td>500 ug/ml</td>
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<tr>
<td>Beef Hemoglobin</td>
<td>500 ug/ml</td>
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<tr>
<td>Goat Hemoglobin</td>
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<tr>
<td>Horse Hemoglobin</td>
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<tr>
<td>Rabbit Hemoglobin</td>
<td>500 ug/ml</td>
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<tr>
<td>Horseradish Peroxidase</td>
<td>2000 ug/ml</td>
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</tbody>
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REFERENCES