One Step Myoglobin/CK-MB/Troponin I Combo Test Device (Whole Blood/Serum/Plasma)

**A rapid, one step test for the qualitative detection of Myoglobin, CK-MB, and Troponin I in whole blood, serum or plasma.**

**PRINCIPLE**

The One Step Myoglobin/CK-MB/Troponin I Combo Test Device (Whole Blood/Serum/Plasma) is a qualitative, membrane based immunoassay for the detection of Myoglobin, CK-MB and Troponin I in whole blood, serum or plasma. The membrane is pre-coated with specific capture antibodies in each of the test line regions of the test. During testing, the whole blood, serum or plasma specimen reacts with the particle coated with specific antibodies. The mixture migrates upward on the membrane chromatographically by capillary action to react with specific capture reagents on the membrane and generate a colored line. The presence of this colored line in the specific test line region 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.

**MATERIALS PROVIDED**

- Test devices (contain anti-Myoglobin antibody coated particles, anti-CK-MB antibody coated particles, anti-Troponin I antibody coated particles, and capture reagents coated on the membrane)
- Buffer.
- Droppers.
- Package insert.

**MATERIALS NEEDED BUT NOT PROVIDED**

- Specimen collection container.
- Lancets (for finger stick whole blood only).
- Centrifuge.
- Timer.

**PRECAUTIONS**

- For professional in vitro diagnostic use only. Do not use after expiration date.
- The test must remain in the sealed pouch until use.
- Do not eat, drink or smoke in the area where the specimens or kits are handled.
- Do not use if pouch is damaged.
- Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout the procedure and follow the standard procedures for proper disposal of specimens.

- Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are being tested.
- Humidity and temperature can adversely affect results.
- The used test should be discarded according to local regulations.

**STORAGE AND STABILITY**

- Store as packaged in the sealed pouch either at room temperature or refrigerated (2-30°C).
- The test is stable through the expiration date printed on the sealed pouch.
- The test must remain in the sealed pouch until use.
- Do not freeze.
- Do not use beyond the expiration date.

**SPECIMEN COLLECTION AND PREPARATION**

- The One Step Myoglobin/CK-MB/Troponin I Combo Test Device (Whole Blood/Serum/Plasma) can be performed using whole blood (from venipuncture or fingerstick), serum or plasma.
- To collect Fingerstick Whole Blood specimens:
  - Wash the patient’s hand with soap and warm water or clean with an alcohol swab. Allow to dry.
  - Massage the hand without touching the puncture site by rubbing down the hand towards the fingertip of the middle or ring finger.
  - Puncture the skin with a sterile lancet. Wipe away the first sign of blood.
  - Gently rub the hand from wrist to palm to form a rounded drop of blood over the puncture site.
  - Position the patient’s finger so that the drop of blood is just above the specimen well (S) of the test device.
  - Allow 3 hanging drops of fingerstick whole blood to fall into the specimen well (S) of the test device, or move the patient’s finger so that the hanging drop touches the specimen well (S). Avoid touching the finger directly to the specimen well (S).
  - Separate serum or plasma from blood as soon as possible to avoid hemolysis. Use only clear, non-hemolyzed specimens.
  - Testing should be performed immediately after specimen collection. Do not leave the specimens at room temperature for prolonged periods. Serum and plasma specimens may be stored at 2-8°C for up to 3 days. For long-term storage, specimens should be kept below -20°C. Whole blood collected by venipuncture should be stored at 2-8°C if the test is to be run within 2 days of collection. Do not freeze whole blood.
specimens. Whole blood collected by fingerstick should be tested immediately.

- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly.
- If specimens are to be shipped, they should be packed in compliance with local regulations covering the transportation of etiologic agents.

**PROCEDURE**
Allow the test, specimen and/or controls to reach room temperature (15–30℃) prior to testing.

1. Bring the pouch to room temperature before opening it. Remove the test device from the sealed pouch and use it as soon as possible. Best results will be obtained if the test is performed immediately after opening the foil pouch.
2. Place the test device on a clean and level surface.
3. For **Serum or Plasma** specimens: Hold the dropper vertically and transfer 2 drops of serum or plasma (approximately 50 μL) to the specimen well (S) of the test device, then start the timer. See illustration below.
4. For **Venipuncture Whole Blood** specimens: Hold the dropper vertically and transfer 3 drops of venipuncture whole blood (approximately 75 μL) to the specimen well (S) of the test device, then add 1 drop of buffer (approximately 40 μL) and start the timer. See illustration below.
5. For **Fingerstick Whole Blood** specimens: Allow 3 hanging drops of fingerstick whole blood specimen (approximately 75 μL) to fall into the center of the specimen well (S) on the test device, then add 1 drop of buffer (approximately 40 μL) and start the timer. See illustration below.
6. Wait for the colored line(s) to appear. Read results at 10 minutes. Do not interpret results after 20 minutes.

**INTERPRETATION OF RESULTS**
(Please refer to the illustration)

**POSITIVE:**
A colored line in the control line region (C) and the presence of one or more colored lines in the test line regions indicates a positive result. This indicates that the concentration of Myoglobin, CK-MB and/or Troponin I is above the minimum detection level.

**NEGATIVE:**
One colored line appears in the control line region (C). No apparent colored lines appear in any of the test line regions. This indicates that the concentration of Myoglobin, CK-MB and Troponin I are below the minimum detection levels.

**INVALID:**
Control line (C) fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

**EXPECTED VALUES**
The One Step Myoglobin/CK-MB/Troponin I Combo Test Device (Whole Blood/Serum/Plasma) has been compared with a leading commercial Myoglobin/CK-MB/T EIA test, demonstrating an overall accuracy of 98.0% with Myoglobin, 99.8% with CK-MB, and 98.5% with Troponin I.

**QUALITY CONTROL**
- An internal procedural control is included in the test. A colored line appearing in the control line region (C) is an internal procedural control. It confirms sufficient specimen volume, adequate membrane wicking and correct procedural technique.
- Control standards are not supplied with this kit; however it is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

**LIMITATIONS**
1. The One Step Myoglobin/CK-MB/Troponin I Combo Test Device (Whole Blood/Serum/Plasma) is for in vitro diagnostic use only. This test should be used for the detection of Myoglobin, CK-MB, and Troponin I in whole blood, serum or plasma specimens only. Neither the quantitative value nor the rate of increase in Myoglobin, CK-MB and Troponin I can be determined by this qualitative test.
2. The One Step Myoglobin/CK-MB/Troponin I Combo Test Device (Whole Blood/Serum/Plasma) will only indicate the qualitative level of Myoglobin, CK-MB and Troponin I in the specimen and should not be used as the sole criteria for the diagnosis of myocardial infarction.
3. The One Step Myoglobin/CK-MB/Troponin I Combo Test Device (Whole Blood/Serum/Plasma) cannot detect less than 50 ng/mL Myoglobin, 5 ng/mL CK-MB and 0.5 ng/mL Troponin I in specimens. A negative result at any time does not preclude the possibility of myocardial infarction.
4. As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.
5. Unusually high titters of heterophile antibodies or rheumatoid factor (RF) may affect the results. Even if test results are positive, further clinical evaluation should be considered with other clinical information available to the physician.
6. There is a slight possibility that some whole blood specimens with very high viscosity or which have been stored for more than 2 days may not run properly on the test device. Repeat the test with a serum or plasma specimen from the same patient using a new test device.

**PERFORMANCE CHARACTERISTICS**

**Sensitivity and Specificity**
The One Step Myoglobin/CK-MB/Troponin I Combo Test Device (Whole Blood/Serum/Plasma) has been evaluated with a leading commercial Myoglobin/CK-MB/Troponin I EIA test using clinical specimens. The results show that relative to leading EIA tests, the One Step Myoglobin/CK-MB/Troponin I Combo Test Device (Whole Blood/Serum/Plasma) exhibits 100% sensitivity and 97.7% specificity for Myoglobin, 100% sensitivity and 98.8% specificity for CK-MB, and 98.7% sensitivity and 98.4% specificity for Troponin I.

<table>
<thead>
<tr>
<th>One Step Myoglobin Test vs. EIA</th>
<th>Method</th>
<th>EIA</th>
<th>Total Results</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>One Step Myoglobin Test</strong></td>
<td>Positive</td>
<td>60</td>
<td>383</td>
</tr>
<tr>
<td>Total Results</td>
<td>60</td>
<td>383</td>
<td>443</td>
</tr>
</tbody>
</table>

Relative Sensitivity: 100% (94.0%-100.0%)*
Relative Specificity: 97.7% (95.6%-98.9%)*
Accuracy: 98.0% (96.2%-99.1%)*
*95% Confidence Interval

<table>
<thead>
<tr>
<th>One Step CK-MB Test vs. EIA</th>
<th>Method</th>
<th>EIA</th>
<th>Total Results</th>
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</thead>
<tbody>
<tr>
<td><strong>One Step CK-MB Test</strong></td>
<td>Positive</td>
<td>54</td>
<td>1</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>422</td>
<td>422</td>
</tr>
<tr>
<td>Total Results</td>
<td>54</td>
<td>422</td>
<td>477</td>
</tr>
</tbody>
</table>

Relative Sensitivity: 100% (93.4%-100.0%)*
Relative Specificity: 99.8% (98.7%-99.9%)*
Accuracy: 99.8% (98.8%-99.9%)*
* 95% Confidence Interval

One Step Troponin I Test vs. EIA

<table>
<thead>
<tr>
<th>One Step Troponin I Test Method</th>
<th>EIA</th>
<th>Total Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Results</td>
<td>Positives</td>
<td>Negatives</td>
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<tr>
<td>Positive</td>
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<tr>
<td>Total Results</td>
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<td>513</td>
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</tbody>
</table>

Relative Sensitivity: 98.7%
Relative Specificity: 98.4%
Accuracy: 98.5% (97.4%-99.3%)*
* 95% Confidence Interval

Precision
Intra-Assay
Within-run precision has been determined by using replicates of 10 tests for each of three lots using Myoglobin specimen levels at 0 ng/mL, 50 ng/mL, 100 ng/mL, 200 ng/mL and 400 ng/mL, CK-MB specimen levels at 0 ng/mL, 5 ng/mL, 10 ng/mL, 20 ng/mL and 40 ng/mL and Troponin I specimen levels at 0 ng/mL, 2 ng/mL, 5 ng/mL, 10 ng/mL and 20 ng/mL. The specimens were correctly identified >99% of the time.

Inter-Assay
Between-run precision has been determined by 3 independent assays on the same fifteen specimens: 0 ng/mL, 50 ng/mL, 100 ng/mL, 200 ng/mL and 400 ng/mL of Myoglobin, 0 ng/mL, 5 ng/mL, 10 ng/mL, 20 ng/mL and 40 ng/mL of CK-MB and 0 ng/mL, 2 ng/mL, 5 ng/mL, 10 ng/mL and 20 ng/mL of Troponin I. Three different lots of the One Step Myoglobin/CK-MB/ Troponin I Combo Test Device (Whole Blood/Serum/Plasma) have been tested using these specimens. The specimens were correctly identified >99% of the time.

Cross-Reactivity
Serum containing known amounts of antibodies to Myoglobin, CK-MB and Troponin I have been tested with 10,000 ng/mL Skeletal Troponin I, 2,000 ng/mL Troponin T, 1,390 ng/mL CK-MM, 1,000 ng/mL CK-BB and 20,000 ng/mL Cardiac Myosin. No cross-reactivity was observed, indicating that the One Step Myoglobin/CK-MB/Troponin I Combo Test Device (Whole Blood/Serum/Plasma) has a high degree of specificity for Myoglobin, CK-MB and Troponin I.

Interfering Substances
The One Step Myoglobin/CK-MB/Troponin I Combo Test Device (Whole Blood/Serum/Plasma) has been tested and no interference was observed in specimens containing 110 mg/dL human albumin, 6 mg/dL bilirubin, 10 mg/dL hemoglobin, 5 mg/dL cholesterol and 15 mg/dL triglycerides. The following compounds have also been tested using the One Step Myoglobin/CK-MB/Troponin I Combo Test Device (Whole Blood/Serum/Plasma) and no interference was observed:

REFERENCES