**Introduction:**

Myoglobin is an enzyme, found primarily in cardiac and skeletal muscle. It is an oxygen binding protein and exists as a monomeric form of hemoglobin. Being a monomer of hemoglobin— which is a tetramer—myoglobin has one fourth the molecular weight (18 kDa) of hemoglobin. Since Myoglobin, like FABP (fatty acid binding protein) is a low molecular mass cytoplasmic protein present not only in heart but also in skeletal muscles, it is used as a plasma marker for muscle cell viability to discriminate between heart or skeletal muscle injury. The Myoglobin content of human heart, however, is largely dependent on the degree of fibrosis.

Serial measurement of biochemical markers is now accepted universally as an important determinant in ruling in or ruling out AMI. Myoglobin, with a metabolic half-life of 12-20 minutes, is released into the vascular system. Some of the proteins second two hours later, negatively predict AMI in nearly 100% of patients with high levels of myoglobin levels as well. Most complications are muscle traumas. Renal failures and other kidney problems exhibit abnormalities on plasma determination and enzyme labeled antibodies (directed against distinct and different epitopes of Myoglobin) are added to the unbound enzyme-Myoglobin conjugate by agitation or decantation. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce light.

The employment of several serum references of known (Myoglobin) levels permits the construction of a dose response curve. Importantly, the dose-response curve is not demonstrated.
within thirty (30) minutes of adding the Working Signal reagents. 

NOTE: Always add reagents in the same order to minimize reaction time differences between wells.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of myoglobin in unknown specimens.

1. Record the RLUs obtained from the printout of the microplate luminometer as outlined in Example 1.
2. Plot the light intensity data for each duplicate serum calibration curve against the known concentration of myoglobin (greatest light output). This conversion eliminates differences in light output and should be established for each instrument before using it as a standard.
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of myoglobin for an unknown, locate the standard curve of average RLUs of the calibrators (the duplicates of the unknown may be averaged as indicated). In the following example, the average RLUs (60424) of control serum contain 150 ng/ml myoglobin concentration (See Figure 1).

Note: Computer data reduction software designed for chemiluminescence assays may be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

In order for the assay results to be considered valid the following criteria should be met:

1. The Dose Response Curve should be within established parameters.
2. Four out of six quality control wells should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis for this product are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to avoid reproducible results.
2. Pipetting of samples should not extend beyond ten (10) seconds to allow an accurate reading.
3. Highly hemolytic, hemoglobin or grossly contaminated specimen(s) should not be used.
4. If a value is not used, it is recommended to repeat the dose response curve.
5. The addition of signal reagent initiates a kinetic reaction, therefore the duplicate samples should be added in the same sequence to eliminate any time-deviation during reaction.
6. Failure to observe a sufficient solution adsorbed in the aspiration or desorption wash step(s) may result in poor replication and spurious results.
7. Use components from the same lot. No intermixing of reagents should be performed by a skilled individual or trained professional.
8. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind IFU may yield inaccurate results.
9. All applicable national, and international standards, regulations, and local including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper usage.
10. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.

12.2 Interpretation

1. Measurements and interpretation of results must be performed by a skilled individual or trained professional.
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system have been formulated to eliminate maximally intrinsic interference, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. "Heterophilic antibodies: a problem for all immunoassays" Clin. Chem. 1988;34:27-33). For diagnostic purposes, the results from this assay should be combined with clinical examination, patient history and all other clinical circumstances.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by use of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

13.0 EXPECTED VALUES

Myoglobin values are consistently the same in plasma and serum. However, a serum sample that has been quickly separated from the red cells is preferred. Myoglobin levels are higher in trained athletes or people who are used to a daily regimen of strenuous exercise.

Based on the clinical data gathered by Monobind in concordance with the published literature the following ranges have been assigned. These ranges should be used as guidelines only:

<table>
<thead>
<tr>
<th>Sample I.D.</th>
<th>Well Number</th>
<th>RLU(A)</th>
<th>Mean RLU(B)</th>
<th>Value (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>A1</td>
<td>82</td>
<td>83</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>C1</td>
<td>2028</td>
<td>2099</td>
<td>10</td>
</tr>
<tr>
<td>D</td>
<td>D1</td>
<td>2169</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>E1</td>
<td>8500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>F1</td>
<td>9051</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>G1</td>
<td>21088</td>
<td>21289</td>
<td>50</td>
</tr>
<tr>
<td>H</td>
<td>H1</td>
<td>21289</td>
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</tr>
</tbody>
</table>

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precision of the Myoglobin AccuLite® CLIA Test System were determined by analysis on three different levels of pool control sera. The mean, number, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

14.2 Sensitivity

The sensitivity (detection limit) was ascertained by determining the variability of the 0 ng/ml serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose. The assay sensitivity was found to be 0.03 ng/ml.

14.3 Specificity

The cross-reactivity of the Myoglobin AccuLite® CLIA test system to selected substances was evaluated by adding interfering substance(s) to a serum matrix at the following concentration(s): the assay system used did not detect any hemoglobin, CK-MB, TnI or FPAB when tested at very high concentrations. The presence of lipemia (25 mg/ml), hemoglobin (4.0 mg/ml) and bilirubin (2.5 mg/ml) did not affect the assay precision.

14.4 Accuracy

The Myoglobin AccuLite® CLIA test system was compared with a predicate ELISA assay. Biological specimens from population (cardiac and asymptomatic) were used. (The values ranges from N/D – 185 ng/ml). The total number of such specimens was 85. The data obtained is displayed in Table 4.

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>α</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>10</td>
<td>41.5</td>
<td>2.7</td>
</tr>
<tr>
<td>Medium</td>
<td>10</td>
<td>131.2</td>
<td>7.2</td>
</tr>
<tr>
<td>High</td>
<td>10</td>
<td>238.9</td>
<td>10.1</td>
</tr>
</tbody>
</table>

*As measured in ten experiments in duplicate over ten days.

15.0 REFERENCES


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