The kidneys rapidly eliminate myoglobin from the system, hours after the onset of chest pains and peak within 5-12 hours. Since the protein rapidly clears from the system, myoglobin is a diagnostic marker that is helpful in distinguishing AMI from other causes of chest pain, such as pulmonary embolism or aortic dissection. Serial testing for myoglobin and other biochemical markers is now accepted as the standard of care for diagnosing AMI.

### 1.0 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 (19 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 other tissues, it is difficult to use it as a plasma marker for muscle cell viability to discriminate between heart or skeletal muscle injury. The Myoglobin content of human heart, however, is lower than that of skeletal muscle.

Serial measurement of biochemical markers is now accepted universally as an important determinant in ruling in or ruling out acute myocardial infarction. Myoglobin is one of the most important markers of acute myocardial infarction (AMI) within 2h of admission because of chest pain. AMI disrupts cardiac cell membranes, releasing intracellular cardiac enzymes into the blood. For example, dehydrogenase type-1 (LD1) and cardiac troponin subunits I and T (Troponin I and T) have been shown to have diagnostic utility in the detection of AMI.

### 2.0 SUMMARY AND EXPLANATION OF THE TEST

Myoglobin has been shown to be a useful marker for the diagnosis of acute myocardial infarction. The assay is based on the principle that intact myoglobin binds to a solid surface and that any unbound myoglobin can be separated from the antibody-conjugated myoglobin by aspiration or decantation. The activity of the enzyme present on the surface of the solid matrix is assayed with a suitable substrate to produce color.

The employment of several serum references of known Myoglobin levels permits the construction of a dose response curve, an unknown specimen's activity can be correlated with Myoglobin concentration.

### 3.0 PRINCIPLE

Immunoenzymometric assay (TYPE 3):
The essential reagents required for an immunoenzymometric assay include a monoclonal antibody directed against myoglobin (unlabelled), and an enzyme labeled antibody to myoglobin (directed against distinct and specific epitopes of myoglobin). The monoclonal antibody is allowed to react with the unbound enzyme-myoglobin conjugate separately is separated from the bound fraction by aspiration or decantation. The activity of the enzyme present on the surface of the solid matrix is assayed with a suitable substrate to produce color.

The optimal dose response curve can be generated from which the antigen concentration can be accurately determined.

### 4.0 REAGENTS AND MATERIALS PROVIDED

- **Myoglobin Calibrators** - 2.0 ml/vial (Dried) Icons A – F
- **Working Substrate Solution**
- **Stop Solution** – 8.0ml/vial
- **Sample vial(s)**
- **Buffer concentrate**
- **Concentrate reagents**
- **Components of test kit**
- **User’s guide**

### 5.0 PRECAUTIONS

For In Vitro Diagnostic Use

Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface antigen, HIV 1& 2 and HCV antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous. Local and institutional guidelines for handling blood products can be found in the Center for Disease Control / National Institute of Health, " Biosafety in Microbiological and Biomedical Laboratories," Second Edition, 1988, HHS.

Safe disposal of kit components must be according to local regulatory and statutory requirement.

### 6.0 SPECIMEN COLLECTION AND PREPARATION

- Blood samples should be collected from the patient while fasting. Discontinue any sedative or analgesic medications at least 24 hours before the test.
- Samples should be collected in a plain red-top tube. Avoid blood samples containing anticoagulants other than EDTA.
- Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for a maximum period of five (5) days.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight, to avoid interference.

### 7.0 QUALITY CONTROL

Each laboratory should assay control at levels in the low, normal and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in each test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate a change in experimental conditions or degradation of kit reagents.

### 8.0 REAGENT PREPARATION

1. Wash Buffer
   - Dilute contents of wash solution to 1000ml with distilled or deionized water in a suitable storage container. Diluted buffer can be stored for 2-30°C for up to 4 weeks.

2. Working Substrate Solution – Stable for one year
   - Prepare as described in the kit insert. Pour solutions from the amber vial labeled Solution A’ into the clear vial for ease of identification. Mix and label accordingly. Store at 2 - 8°C.

### 9.0 SPECIFIC IMMUNOASSAY PROCEDURES

#### 1. Pipette(s) capable of delivering 0.025ml (25µl) and 0.100ml (100µl) volumes with a precision of better than 1.5%

- **Buffer concentrate**
- **Components of test kit**
- **User’s guide**

#### 2. Pipette(s) capable of delivering 0.025ml (25µl) of the appropriate calibrators, controls and samples into the assigned wells.

- **Buffer concentrate**
- **Components of test kit**
- **User’s guide**

#### 3. Add 0.100 ml (100µl) of the Myoglobin Enzyme Reagent to each well.

- **Buffer concentrate**
- **Components of test kit**
- **User’s guide**

#### 4. Add 0.100 ml (100µl) of the Working Substrate Solution to all wells.

- **Buffer concentrate**
- **Components of test kit**
- **User’s guide**

#### 5. Incubate for 15 minutes at 37°C.

- **Buffer concentrate**
- **Components of test kit**
- **User’s guide**

#### 6. Add 0.050ml (50µl) of stop solution to each well and mix gently

- **Buffer concentrate**
- **Components of test kit**
- **User’s guide**

### 10.0 CALCULATION OF RESULTS

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

1. Results are obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding myoglobin concentration in ng/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of myoglobin for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph; find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (0.532) dictates the dose response curve at 43 ng/ml myoglobin concentration (See Figure 1).

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

<table>
<thead>
<tr>
<th>EXAMPLE 1</th>
</tr>
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<tbody>
<tr>
<td>SAMPLE</td>
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<tr>
<td>A1</td>
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<tr>
<td>B1</td>
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<td>C1</td>
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<td>J1</td>
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<tr>
<td>K1</td>
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<tr>
<td>L1</td>
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</tbody>
</table>

*The data presented in Example 1 and Figure 1 is for illustration only and should not be used in lieu of a dose response curve prepared with each assay.

11.0 QC PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:
1. The absorbance (OD) of calibrator 'A' should be < 0.1.
2. The absorbance (OD) of calibrator 'F' should be ≥ 1.3.
3. Four out of six quality control pools should be within the established range.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form 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 achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, myoglobin or grossly contaminated specimen(s) should not be used.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

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

<table>
<thead>
<tr>
<th>TABLE 2</th>
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<tbody>
<tr>
<td><strong>Within Assay Precision (Values in ng/ml)</strong></td>
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<tr>
<td>SAMPLE</td>
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<tr>
<td>Pool 1</td>
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<tr>
<td>Pool 2</td>
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<tr>
<td>Pool 3</td>
</tr>
</tbody>
</table>

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

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 0.178 ng/ml.

14.3 Accuracy

The Myoglobin AccuBind® ELISA Test System was compared with a predicate radioimmunnoassay assay. Biological specimens included, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.

14.4 Specificity

The cross-reactivity of the Myoglobin Elisa method to selected substances was evaluated by adding the interfering substance(s) to a serum matrix at the following concentration(s). The antibody (x) value ranged from N/D – 118 ng/ml, whereas a value obtained by the dilution factor to obtain the corrected concentration of myoglobin in the sample.

15.0 REFERENCES