Procedures: LifeSign MI® Myoglobin/CK-MB/Troponin I Test

Prepared by	Date Adopted	Supersedes Procedure #

Review Date	Revision Date	Signature

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PRINCIPLE:

The LifeSign MI® Myoglobin/CK-MB/Troponin I Test employs a solid-phase chromatographic immunoassay technology to qualitatively detect the elevation of myoglobin, CK-MB, and troponin I in human blood samples. When a sample of blood is dispensed into the sample well, red blood cells are removed by the separation filter and the plasma migrates into the test membrane. Myoglobin, CK-MB and troponin I present in the sample bind to specific antibody-dye conjugates and migrate through the Test area containing immobilized anti-CK-MB, anti-myoglobin, and streptavidin. The cardiac marker-antibody-dye complexes bind to the corresponding immobilized antibodies or streptavidin in the Test area. Unbound dye complexes migrate out of the Test area and are later captured in the Control (C) area.

Visible pinkish-purple bands will appear in the Test and Control (C) areas if the concentrations of one or more of cardiac markers, myoglobin, CK-MB, or troponin I, are above established cutoff values. If the CK-MB concentration in the specimen is 5 ng/mL or greater, a band is present in the CK-MB area. If the myoglobin concentration in the specimen is 50 ng/mL or greater, a band is present in the myoglobin area. If the troponin I concentration in the specimen is 1.5 ng/mL or greater, a band is present in the troponin I area. If a band is present only in the Control (C) area, the test result is read as negative, indicating that the myoglobin, CK-MB, and troponin I concentrations are all below the cutoff values. If no band is present in the Control (C) area, the test is invalid and another test must be run, regardless of the presence or absence of band(s) in the Test Area.

SPECIMEN:
Whole blood, plasma or serum may be used as samples for this procedure. For whole blood or plasma, collect blood in a tube containing heparin as the anticoagulant. If serum samples are to be used, collect the blood in a tube without anticoagulant and allow to clot. Since cardiac proteins are relatively unstable, it is recommended that fresh samples be used as soon as possible. Blood samples should be tested within 4 hours of collection. If specimens must be stored, the blood cells should be removed. Plasma or serum samples may be refrigerated for 24 hours at 2-8°C. If plasma or serum samples must be stored for more than 24 hours, they should be frozen at -20°C or below.

EQUIPMENT AND MATERIALS:

Each box contains the following:
LifeSign MI® Myoglobin/CK-MB/Troponin I Test sealed in a foil pouch with desiccant and dropper
Result sticker
Directions for use
Materials Required But Not Provided:
1. Vacutainer® (Becton Dickinson) tube, or equivalent, containing heparin as the anticoagulant or a tube designed for collection of serum.
2. Timer
3. Micropipettor and disposable pipet tips which are necessary only if the dropper provided is not used.

Storage Requirements:

Store at 2-30°C. Kit is stable until the date on outer box or pouch.

QUALITY CONTROL:

Internal Quality Control:
- Each LifeSign MI device has built in controls. The control line in the Control window is an internal positive procedural control, i.e., a proper amount of sample is used; sample is added to the sample well, and not through the reading window; and the reagent system worked properly. A distinct pinkish-purple control line will always appear if the test has been performed correctly. If the control line does not appear, the test is invalid and a new test should be performed. If the problem persists, contact LifeSign for technical assistance.

- The Myoglobin/CK-MB/Troponin I Control line is considered an internal positive procedural control since it will only appear if 1. The anti-myoglobin/CK-MB/troponin I antibody is active; 2. A sufficient amount of sample is present to migrate up the test

strip; and 3. The wicking chemistry is working properly. If there is no Control line, the test should be considered invalid and should be repeated with a new device.

- A clear background in the Test Result Window (T) is considered an internal negative procedural control. If the test is performed correctly and the LifeSign MI device is working properly, the background in the Test Result Window (T) should be clear within 15 minutes.

- The positive and negative procedural controls in each LifeSign MI device satisfy the requirements of testing a positive and negative control on a daily basis, and shall be recorded daily by the laboratory.

User Quality Control:

- Good laboratory practice includes the use of external controls to ensure proper kit performance. Before using a new lot or shipment of **LifeSign MI** kits, a quality control test using two levels of controls shall be performed to confirm the expected Q.C. results. Upon confirmation of the expected results, the kit is ready for use with patient specimens. If external controls do not perform as expected, repeat the tests or contact LifeSign Technical Assistance. The built-in purplish-red Control line indicates only the integrity of the test device and proper fluid flow.

PROCEDURE - STEPWISE:

1. Open the foil pouch, remove the LifeSign MI® Myoglobin/CK-MB/Troponin I Test and lay the test on a level surface.
2. Label the test with the patient's identification.
3. Using the dropper provided, add 3 drops (120 µl) of whole blood or plasma or serum into the sample well.
4. Read the test results at 15 minutes.

REPORTING RESULTS:

1. Negative (-)
A single pinkish purple colored band in the Control (C) area, with the absence of a distinct colored band in the Test area, indicates that the concentration of myoglobin is below 50 ng/ml, the concentrations of CK-MB is below 5 ng/ml, and the concentration of troponin I is below 1.5 ng/ml and the test result is negative.

2. Positive (+)
The presence of a pinkish purple colored band in the C area and the presence of one or more distinct bands in the Test area indicates a positive test result. If a band is present in the Myo area, the myoglobin concentration is 50 ng/ml or greater. If a band is present in the CK-MB area, the CK-MB concentration is 5 ng/ml or greater. If a band is present in the TnI area, the troponin I concentration is 1.5 ng/ml or greater.

Notes:

- A positive test result for myoglobin, CK-MB, or troponin I can be read as soon as a distinct colored band appears in both the C area and in the Test area for that cardiac marker.
- Positive test results from strong positive samples may appear within 5 minutes.
- The myoglobin, CK-MB, and TnI bands may appear sooner and darker than the Control band in samples that are very strongly positive.
- The myoglobin, CK-MB, and TnI bands may appear after the appearance of the Control band and be fainter in samples that are weakly positive.
- The LifeSign MI® Myoglobin/CK-MB/Troponin I Test has been optimized to ensure that high concentrations of the cardiac markers will not result in false negative test results which are commonly referred to as a “high dose hook” or “prozone effect” for quantitative immunoassays. Concentrations of myoglobin, CK-MB, and troponin I of 50,000, 50,000, and 1,100 ng/mL, respectively were demonstrated to produce the expected positive test results in the LifeSign MI® Myoglobin/CK-MB/Troponin I Test.

3. Invalid
A distinct colored band in the C area should always appear. If no pinkish purple band is present in the C area at the end of the 15 minute test period, the test is invalid, and the sample must be retested using a new test.

LIMITATIONS OF THE PROCEDURE:

- The results of the LifeSign MI® Myoglobin/CK-MB/Troponin I Test are to be used in conjunction with other clinical information such as clinical signs and symptoms and electrocardiographic test results to diagnose cardiac ischemia. A positive test result from a patient suspected of Acute Myocardial Infarction may be used as an indicator of myocardial damage and requires further confirmation. Sampling of patients suspected of AMI at multiple time points is recommended due to the delay between onset of symptoms and the release of cardiac protein markers into the bloodstream.
- Samples containing unusually high titers of certain antibodies, such as human anti-mouse or human anti-rabbit antibodies, may affect the performance of the test.
- Hematocrit values in the range of 20 - 60% did not significantly affect the LifeSign MI® Test results.

REFERENCES:

1. Adams, J.E., et al, Biochemical markers of myocardial injury. Is MB creatine kinase the choice for the 1990's. Circulation 88:750 (1993)
2. Cairns, J. et al., usefulness of serial determinations of myoglobin and creatine kinase in serum compared for assessment of acute myocardial infarction. Clin.Chem. 29:469 (1983)
3. Ohman, E.M. et al., Early detection of acute myocardial infarction: Additional diagnostic information from serum concentrations of myoglobin in patients without ST elevation. Br. Heart J. 63:335 (1990)
4. McComb, J.M. et al., Myoglobin in the very early phase acute myocardial infarction. Ann. Clin. Biochem. 22:162 (1985)
5. Neumeier, D., Tissue specific and subcellular distribution of creatine kinase isoenzymes. In Lang, H. (ed):Creatine Kinase Isoenzymes, Springer Verlag p.85 (1981)
6. Lott, J.A. and Abbott, L.B., Creatine kinase isoenzymes Clin.Lab.Med. 6:547 (1986)
7. Mehegan, J.P. And Tobacman, L.S. Cooperative interaction between troponin molecules bound to the cardiac thin filament. J.Biol.Chem. 266:966 (1991)
8. Ebashi,S., Ca2+ and the contractile proteins. J.Mol.Cell.Cardiol. 16:129 (1984)
9. Bodor, S.G., et al., Development of monoclonal antibody for an assay of cardiac troponin I and preliminary results in suspected cases of myocardial infarction. Clin.Chem. 38:2203 (1992)
10. Hamm,C.W., Cardiac-specific Troponins in Acute Coronary Syndromes, in Heart Disease, a textbook of Cardiovascular Medicine, ed. Braunwald, E. ,W.B. Saunders Co. Update 3 (1977)

11. Panteghini, M., et al., Comparison of the Diagnostic Performance of Two Rapid Bedside Biochemical Assays in the Early Detection of Acute Myocardial Infarction, Clin.Cardiol. 21:394 (1998)
12. Tucker, J.F., et al., Value of serial myoglobin levels in the early diagnosis of patients admitted for acute myocardial infarction, Ann.Emerg.Med. 24:704 (1994)
13. Hamm, C.W., et al., Emergency room triage of patients with acute chest pain by means of rapid testing for cardiac troponin T or troponin I. New Eng.J.Med. 337:1648 (1997)
14. Antman, E.M., et al., Cardiac-specific troponin I levels to predict the risk of mortality inpatients with acute coronary syndromes. New Eng.J.Med. 335:1342 (1996)
15. Brogan, G.X. Jr., et al., Evaluation of Cardiac STATus™ CK-MB/Myoglobin device for rapidly ruling out acute myocardial infarction, Clin.Lab Med. 17(4):655 (1997)
16. Sylven,C. et al., Excellent reliability of nurse-based bedside diagnosis of acute myocardial infarction by rapid dry-strip creatine kinase, myoglobin, and troponin T. Amer.Heart J. 135(4):677 (1998)

ALTERNATIVE METHOD:

List alternative method (or reference laboratory) to be used should the LifeSign MI Myoglobin/CK-MB/Troponin I become unavailable.

Alternative Method:

Reference Laboratory

Name:
Address:
Address:
Phone:
Contact:

CRITERIA FOR REFERRAL OF SPECIMENS:

When referring specimens for outside testing, the following procedures are recommended:

1. Verify that the testing laboratory possesses a valid CLIA certification authorizing performance of the referred test.
2. Follow laboratory guidelines for specimen shipment.
3. Report results exactly as received (no alteration or revision either of results or interpretive information provided by the testing laboratory).
4. Permit direct test report from the testing laboratory to the authorized person or entity that ordered the test.
5. Retain or be able to produce an exact duplicate of each testing laboratory's report.
6. Provide the name and address where the test was performed and indicate this information on the test report.

TECHNICAL ASSISTANCE:

Technical assistance is available from LifeSign, LLC, Somerset, New Jersey, between the hours of 8:30 a.m. and 4:45 p.m. E.S.T.

1-800-526-2125

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For more information please contact CLIAwaived™ Inc. at:

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