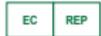


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MI
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Myoglobin/CK-MB/Troponin I

Point-of-care cardiac tests for the simultaneous qualitative detection of Myoglobin, CK-MB and Troponin I in blood, plasma and serum for the rapid diagnosis of myocardial infarction



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LifeSign MI[®] Myoglobin/CK-MB/Troponin I Rapid Test

For *in vitro* Diagnostic Use Only

A Simple and Rapid One-Step Immunoassay for the Simultaneous Qualitative Detection of Myoglobin, CK-MB, and Cardiac Troponin I in Human Whole Blood, Serum, or Plasma.

Intended Use: For the simultaneous qualitative determination of myoglobin, CK-MB, and troponin I in human whole blood, plasma or serum as an aid in the diagnosis of acute myocardial infarction in emergency room, critical care, point-of-care, and hospital settings.

The LifeSign MI[®] Myoglobin/CK-MB/Troponin I Test provides a qualitative analytical test result. The qualitative nature of this assay does not provide information about change - either the rise or fall - in the concentration of myoglobin, CK-MB, and cardiac troponin I with single testing. A quantitative method should be used, if desired, to quantitate the concentration of myoglobin, CK-MB, and cardiac troponin I at any given time. Only with serial testing could a temporal change in the level of myoglobin, CK-MB, and cardiac troponin I be concluded. Clinical consideration and professional judgment should be applied when interpreting the results of the LifeSign MI[®] Myoglobin/CK-MB/Troponin I Test, especially when a single test result is used.

Summary and Explanation: Myoglobin, creatine kinase, and troponin I are proteins found in cardiac muscle cells and are released into the blood upon damage or death of cardiac tissue.^{1,2,3,4} Myoglobin is an oxygen-binding heme protein with a molecular weight of 17,800 daltons, normally found in skeletal as well as cardiac tissue. It constitutes about 2% of the total muscle protein and is located in the cytoplasm of the cell. Also found in the cytoplasm is creatine kinase (ATP: creatine N-phosphotransferase, E.C. No. 2.7.3.2) (CK). CK catalyzes the reversible phosphorylation reaction of creatine with ATP. In humans, isoenzymes of CK have been identified in both the cytosol and mitochondria of cells from a wide variety of tissues.^{5,6} Cytosolic CK exists as a dimeric molecule formed from two types of single-polypeptide subunits, designated "M" and "B". Each subunit has a molecular weight of approximately 41,000 daltons and distinct immunologic epitopes. These two subunits combine to form three different isoenzymes of CK: CK-MM, CK-BB, CK-MB. The relative abundance of the particular isoenzyme is dependent on the tissue being examined. The CK-MM isoenzyme is predominant in skeletal muscle tissue, while the CK-MB isoenzyme is most abundant in cardiac muscle tissue. Troponin I (TnI) is part of the troponin complex which, together with tropomyosin, forms the main component that regulates the Ca⁺⁺-sensitive ATP-ase activity of actomyosin in striated muscle (skeletal and cardiac).⁷ The troponin complex consists of three subunits, troponin T (TnT), troponin I (TnI), and troponin C (TnC). Each subunit has a distinct function with TnC as the site of Ca⁺⁺ binding, TnT the tropomyosin binding, and TnI as the inhibitory subunit.⁸ Different isoforms of TnI exist in the skeletal and cardiac muscles (sTnI and cTnI, respectively) with distinct immunologic epitopes that allow the production of cardiac-specific TnI antibodies.⁹

The cardiac markers myoglobin, CK-MB, and troponin I have been established as useful tools in the diagnosis of acute myocardial infarction (AMI).¹⁰⁻¹⁶ Since the temporal release patterns of the three markers have significant differences, all three are useful tools in the determination of the source and timing of the onset of chest pain. Cell injury from AMI has been shown to result in a level of blood myoglobin above the upper limit of normal in approximately 2-3 hours after the onset of chest pain. Maximum concentrations are generally observed after 9-12 hours. CK-MB and troponin I are found in blood at elevated concentrations approximately 4- 6 hours after the onset of chest pain and peak at 12-24 hours. However, whereas CK-MB levels return to normal values in about 72 hours, troponin I levels remain elevated for up to 5-7 days.¹ The use of these three markers is therefore complementary since they detect cardiac tissue damage over a wide range of times after myocardial infarction.

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.

Reagents

LifeSign MI[®] Myoglobin/CK-MB/Troponin I Test: The test consists of a membrane strip coated with polyclonal goat anti-CK-MM, polyclonal rabbit anti-myoglobin, monoclonal anti-CK-MM antibody as a CK-MM scavenger, and avidin in the Test Area, a dye pad impregnated with complementary monoclonal anti-myoglobin, anti-CK-MB, and anti-TnI and biotinylated polyclonal anti-TnI antibodies in a protein matrix containing 0.05% azide and a red blood cell separating filter. Store at 2-30°C.

Specimen Collection and Preparation

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.

Materials Provided:

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.

Procedure

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.

Procedural Notes

- Do not use this product beyond the expiration date.
- All patient samples should be handled as if they are potentially infectious. Observe established procedures for proper disposal of specimens and the used test device.

LifeSign MI® Myoglobin/CK-MB/Troponin I Test Procedure and Results

1. Add 3 drops (120 µL) of whole blood, serum or plasma sample.



2. Read at 15 minutes.



Myoglobin (+)
CK-MB (+)
Troponin I (+)

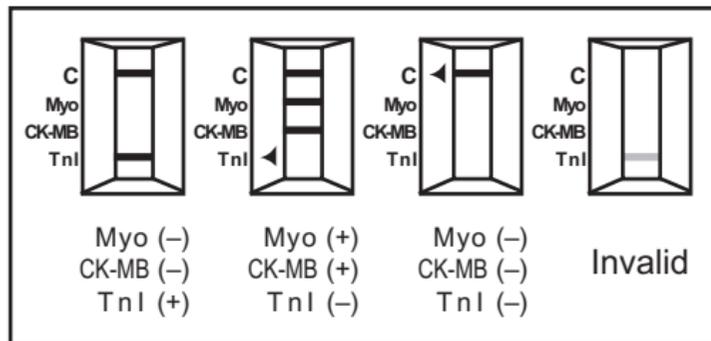
CONTROL (VALIDATION) BAND (C)

The Control/Validation band serves two purposes:

1. Functional test of the dye conjugates: and
2. Proof of sample migration.

If no control band appears, the test is **NOT VALID**.

Repeat the test using a new LifeSign MI® Myoglobin/CK-MB/Troponin I Test, and follow the procedure carefully.



Interpretation of 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 Tnl 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 Tnl bands may appear sooner and darker than the Control band in samples that are very strongly positive.
- The myoglobin, CK-MB, and Tnl 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:

- 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 AMI 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.

User Quality Control: A quality control test using positive and negative controls such as the Princeton BioMeditech LifeSign MI® controls (Myoglobin/CK-MB/Troponin I) should be performed before using a new lot of LifeSign MI® Myoglobin/CK-MB/Troponin I Test and at regular intervals as part of a good quality control (QC) practice. The positive control should be selected to produce a moderate positive result in the myoglobin, CK-MB, and TnI areas as well as the C area. The negative control should produce a negative result (only control band present). Upon confirmation of the expected results, the lot is ready to use with patient specimens. Controls should also be used whenever the validity of test results is questioned and in accordance to local Quality Assurance policy. For information about obtaining controls contact the LifeSign MI® local distributor for assistance.

The presence of a band in the C area acts as an internal procedural control to ensure the valid performance of the test. In the absence of a band in the C area, the test is invalid and must be repeated. If a problem persists, contact the LifeSign MI® Technical Support for assistance, 800-526-2125 or 732-246-3366.

Expected Values: Specimens from 21 healthy adults were tested in the LifeSign MI® Myoglobin/CK-MB/Troponin I Test and all specimens were determined to be negative for all three cardiac markers.

The LifeSign MI® Myoglobin/CK-MB/Troponin I Test has been calibrated against the LifeSign MI® Myoglobin/CK-MB Test and the LifeSign MI® Troponin I Test. The LifeSign MI® Myoglobin/CK-MB Test uses a diagnostic cutoff of 5 ng/mL for CK-MB and 50 ng/mL for myoglobin. The LifeSign MI® Troponin I Test uses a diagnostic cutoff of 1.5 ng/mL. The LifeSign MI® Myoglobin/CK-MB/Troponin I Test is designed to yield a positive result when the concentration of one or more of the cardiac markers, myoglobin, CK-MB, or troponin I, is above these established cut-off values.

Performance Characteristics

Interfering Substances: Substances at the following levels do not interfere with the LifeSign MI® Myoglobin/CK-MB/Troponin I Test :

Human albumin	16 g/dL
Bilirubin (unconjugated)	60 mg/dL
Free hemoglobin	4 g/dL
Triglycerides	1,300 mg/dL

The following drugs were evaluated for potential positive and negative interference by the addition of these materials to (1) a serum sample containing elevated levels of myoglobin, CK-MB, and TnI and (2) a serum sample negative for the 3 cardiac markers. These drugs were tested at approximately twice the recommended therapeutic level. No interference was observed for any of these medications:

Acetaminophen	Digoxin	Nystatine
Acetylsalicylic acid	Dipyridamole	Oxazepam
Allopurinol	Erythromycin	Oxytetracycline
Ambroxol	Furosemide	Phenobarbital
Ampicillin	Glibenclamide	Phenytoin
Ascorbic acid	Hydrochlorothiazide	Probenecid
Atenolol	Indomethacin	Procainamide
Caffeine	Isosorbide dinitrate	D,L - Propanolol
Captopril	L-thyroxine	Quinidine
Chloramphenicol	Methaqualone	Sulfamethoxazol
Chlordiazepoxide	D,L-alpha-Methyldopa	Theophylline
Cinnarizine	Nicotinic acid	Trimethoprim
Cyclosporine	Nifedipine	Verapamil
Diclofenac	Nitrofurantoin	

Cross-Reactivity Studies: Related human proteins were purified and added to normal human plasma to test for their potential reactivity in the LifeSign MI® Myoglobin/CK-MB/TnI Test. A negative result was obtained with the proteins at the following concentrations:

Protein	Concentration (ng/mL)
CK-BB	1000
cardiac myosin light chain	200
cardiac troponin T	5000
cardiac troponin C	1000
fast-twitch skeletal TnI	314

Recovery Study: Normal human blood was supplemented with human myoglobin at concentrations of 125, 200, and 400 ng/mL, CK-MB at concentrations of 6, 10, and 20 ng/mL, and troponin I at concentrations of 2, 4, and 8 ng/mL. The samples were tested using the LifeSign MI® Myoglobin/CK-MB/TnI Test in 6 replicates. There was a 100% agreement between the expected and the observed results at each concentration of cardiac marker.

Proficiency Testing: Four different hospitals were provided with blinded whole blood samples. One group of samples had been supplemented with myoglobin, CK-MB, and TnI at concentrations of 100, 10 and 3 ng/mL, respectively (Level 1). A second set of samples had been supplemented with myoglobin, CK-MB, and TnI at concentrations of 200, 20, and 6 ng/mL, respectively (Level 2). A third set of samples was not supplemented with any of the cardiac markers and thereby served as a negative control group. Each site received five replicates of each sample for a total of 15 samples per site. As shown in the data table below, there was a mean within run agreement of 98.8% and a mean between sites agreement of 98.9% in this study.

LifeSign MI® Myoglobin/CK-MB/Troponin I Test
 (Number of Positive Results/ Total)

Sites	Cardiac Marker Band	No Additions (negative)	Supplemented with Level 1	Supplemented with Level 2	% Agreement of Within Run
Site 1	myoglobin	0/5	5/5	5/5	100
	CK-MB	0/5	5/5	5/5	100
	troponin I	0/5	5/5	5/5	100
Site 2	myoglobin	0/5	5/5	5/5	100
	CK-MB	0/5	4/5	5/5	93.3
	troponin I	0/5	4/5	5/5	93.3
Site 3	myoglobin	0/5	5/5	5/5	100
	CK-MB	0/5	5/5	5/5	100
	troponin I	0/5	5/5	5/5	100
Site 4	myoglobin	0/5	5/5	5/5	100
	CK-MB	0/5	5/5	5/5	100
	troponin I	0/5	5/5	5/5	100
% Agreement Between Sites		100	96.7	100	

Correlation of Assay Results Between Whole Blood and Plasma: Heparinized whole blood from 20 individuals with or without elevated cardiac markers were tested prior to removal of the red cells and after red cell removal. The agreement between the use of whole blood and plasma was 100% as shown in the table below.

Cardiac Marker \ Result	Whole blood(+) and plasma (+)	Whole blood(-) and plasma (-)	Whole blood(+) and plasma (-)	Whole blood(-) and plasma (+)
Myoglobin	11	9	0	0
CK-MB	11	9	0	0
Troponin I	17	3	0	0

Correlation of Assay Results Between Whole Blood and Serum: Whole blood (collected without anti-coagulant) from 20 individuals with or without elevated cardiac markers were tested prior to removal of the red cells and after red cell removal. The agreement between the use of whole blood and serum was 100% as shown in the table below.

Cardiac Marker \ Result	Whole blood(+) and serum (+)	Whole blood(-) and serum (-)	Whole blood(+) and serum (-)	Whole blood(-) and serum (+)
Myoglobin	11	9	0	0
CK-MB	11	9	0	0
Troponin I	17	3	0	0

Method Comparison:

Serum Samples (n = 251) were collected from 183 individuals at different time intervals after being admitted to a hospital emergency department with chest pain. The samples were tested with the LifeSign MI® Myoglobin/CK-MB/Troponin I Test and with the LifeSign MI® Myoglobin/CK-MB Test and the LifeSign MI® Tnl Test. The correlation between the tests is shown below:

		LifeSign MI® Myoglobin/CK-MB Test myoglobin results		LifeSign MI® Myoglobin/CK-MB Test CK-MB results		LifeSign MI® Troponin I Test troponin I results		Total
		Pos	Neg	Pos	Neg	Pos	Neg	
LifeSign MI® Myoglobin/CK-MB/ Troponin I Test	Pos	69	2	89	5	65	2	
	Neg	3	177	3	154	2	182	
Total		72	179	92	159	67	184	251

	Myoglobin	CK-MB	Troponin I
Comparative Sensitivity	95.8% (69/72)	96.7% (89/92)	97% (65/67)
Comparative Specificity	98.9% (177/179)	96.8% (154/159)	98.9% (182/184)
Overall Agreement	98% (246/251)	96.8% (243/251)	98.4% (247/251)

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