LifeSign MI® Myoglobin/Troponin I Test

New Myoglobin/Troponin I Test for
the Rapid Diagnosis of Myocardial Infarction in Human Whole Blood, Serum or Plasma

For in vitro Use Only

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

PBM

Intended Use

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

The LifeSign MI® Myoglobin/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 and cardiac troponin I with single testing. A quantitative method should be used, if desired, to quantitate the concentration of myglobin and cardiac troponin I at any given time. Only with serial testing could a temporal change in the level of myoglobin and cardiac troponin I be concluded. Clinical consideration and professional judgement should be applied when interpreting the results of LifeSign MI® Myoglobin/Troponin I Test, especially when single testing results are used.

Summary and Explanation

Myoglobin 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. 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 con-

sists 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 and troponin I have been established as useful tools in the diagnosis of acute myocardial infarction (AMI).[10-14] Since the temporal release patterns of the two markers have significant differences, both of them are useful tools in the determination of the source 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. Troponin I is found in blood at elevated concentrations approximately 4–6 hours after the onset of chest pain and peak at 12-24 hours, and remains elevated for up to 5-7 days.[1] The use of these two markers is therefore complementary as an aid in the diagnosis of AMI given the different release times and half-lives after myocardial infarction.

Principle

The LifeSign MI® Myoglobin/Troponin I Test employs a solid-phase chromatographic immunoassay technology to qualitatively detect the elevation of myoglobin 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 plasma is allowed to migrate to the dye pad. Myoglobin and troponin I present in the sample bind to specific antibody-dye conjugates and migrate through the Test area containing immobilized anti-myoglobin antibody and avidin. The cardiac marker-antibody-dye complexes bind to the corresponding immobilized antibodies or avidin 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 both of cardiac markers, myoglobin, or troponin I, are above established cutoff values. 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 and Troponin I concentrations are 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/Troponin I Test: The test consists of a membrane strip coated with polyclonal rabbit anti-myoglobin antibody and avidin in the Test Area, a dye pad impregnated with complementary anti-myoglobin and anti-troponin I antibodies, and biotinylated polyclonal anti-TnI antibody in a protein matrix containing 0.05% azide, 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 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 clotting. Since cardiac proteins are relatively unstable, it is recommended that fresh samples be used as soon as possible. Whole blood samples should be tested within 4 hours of collection. If specimens must be stored, the red 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/Troponin I Rapid Test sealed in a foil pouch with desiccant and a dropper
Results 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/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, serum or plasma into the Sample well.
4. Read the test results at 15 minutes.

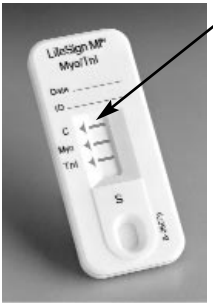
Procedural Notes

- Do not use this product beyond the expiration date.
- If a micropipettor is used, use separate clean tips for each specimen.
- 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/Troponin I Test Procedure and Results

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

2. Read at 15 minutes.



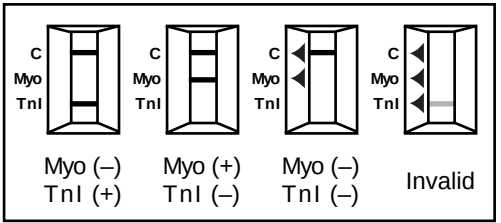
Myoglobin (+)
Troponin I (+)

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/Troponin I Test, and follow the procedure carefully.



Interpretation of results

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 and the concentration of troponin I is below 1.5 ng/ml and the test result is negative.

Positive (+)
The presence of a pinkish purple colored band in the C area and the presence of one or two distinct bands in the Test area indicates a positive result. The presence of a band in the myoglobin or TnI test areas indicates that the sample contains myoglobin or TnI, respectively in a concentration above the established cut-off for that marker.

Notes:
- A positive test result for myoglobin 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 and TnI bands may appear sooner and darker than the Control band with samples that are very strongly positive.
- The myoglobin and TnI bands may appear after the appearance of the Control band and be fainter with samples that are weakly positive.
- The LifeSign MI® Myoglobin/Troponin I Test has been optimized to insure 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 myo globin and troponin I of 50,000, and 1,100 ng/mL, respectively, were demonstrated to produce the expected positive test results in the LifeSign MI® Myoglobin/Troponin I Test.

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/Troponin I Test are to be used in conjunction with other clinical information such as clinical signs and symp

toms and other test results to diagnose cardiac ischemia. A positive test result from a patient suspected of AMI may be used as an indicator of myocar dial damage and requires further confirmation. Serial sampling of patients suspected of AMI is recommended due to the delay between onset of symp toms and the release of cardiac protein markers into the bloodstream.

- Samples containing an unusually high titer of certain antibodies, such as human anti-mouse immunoglobulin or human anti-rabbit immunoglobulin anti bodies, may affect the performance of the test.
- Hematocrit values in the range of 20 - 60% did not significantly affect test results.

User Quality Control

A quality control test using positive and negative controls such as the LifeSign MI® Controls (MyoglobinTroponinI) should be performed at regular intervals as part of a good quality control (QC) practice and before using a new lot of LifeSign MI® Myoglobin/Troponin I Rapid Test to confirm that the tests produce the expected results. The positive control should be selected to produce a moderate positive result in the myoglobin and TnI areas as well as the C area. The negative control should produce a negative result (control band present only). 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 insure 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 Services for assistance. The intensity of control line, weak or strong, does not have any bearing on the test result or the kit performance. Any visible line at the C area should be considered as valid confirmation.

Expected results

Specimens from 21 healthy adults were tested on the LifeSign MI® Myoglobin/Troponin I Test and all were negative for both cardiac markers.

LifeSign MI® Myoglobin/Troponin I Test has been calibrated against the LifeSign MI® Myoglobin/CK-MB Test and the LifeSign MI® Troponin I Test. LifeSign MI® Myoglobin/CK-MB Test uses a diagnostic cutoff of 50 ng/ml for myoglobin and LifeSign MI® Troponin I Test uses a diagnostic cutoff of 1.5 ng/ml for troponin I. LifeSign MI® Myoglobin/Troponin I Test is designed to yield a positive result if the concentration of one or both of cardiac markers, myoglobin, or troponin I, are above these establised cutoff values.

Performance Characteristics

Interfering substances: Levels of the following endogenous substances do not interfere with the LifeSign MI® Myoglobin/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 and TnI, and (2) a serum sample negative for the both cardiac markers. These drugs were tested at approximately twice the recommended therapeutic level. No interference was observed for any of these exogenous substances:

Acetaminophen	Digoxin	Nystatine
Acetylsalicylic acid	Dipyridamole	Oxazepam
Allopurinol	Erythromycin	Oxytetracycline
Ambroxol	Furosemide	Phenobarbital
Ampicillin	Glibenclamide	Phenytoin
Ascrobic 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 added to normal human plasma to test for their potential reactivity in the LifeSign MI® Myoglobin/Troponin I Test. A negative result was obtained with the proteins at the following concentrations:

Protein	Concentration (ng/ml)
CK-BB	1,000
cardiac myosin light chain	200
cardiac troponin T	5,000
cardiac troponin C	1,000
fast-twitch skeletal TnI	314

Recovery Study: Normal human blood was supplemented with myoglobin at concentrations of 125, 200, 400 ng/mL, and troponin I at concentrations of 2, 4, and 8 ng/mL. The samples were tested using the LifeSign MI® Myoglobin/Troponin I 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, and TnI at concentrations of 200 and 3 ng/mL, respectively (Level 1). A second set of samples had been supplemented with myoglobin and TnI at concentrations of 400 and 6 ng/mL, respectively (Level 2). A third set of samples was not supplemented with the cardiac markers and thereby served as the negative control group. Each site received five replicates of each sample for a total of 15 samples per site. As shown in the following data table, 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/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 Troponin I	0/5 0/5	5/5 5/5	5/5 5/5	100 100
Site 2	Myoglobin Troponin I	0/5 0/5	5/5 4/5	5/5 5/5	100 93.3
Site 3	Myoglobin Troponin I	0/5 0/5	5/5 5/5	5/5 5/5	100 100
Site 4	Myoglobin Troponin I	0/5 0/5	5/5 5/5	5/5 5/5	100 100
% Agreement Between Sites		100	97.5	100	

Correlation of Assay Results Between Whole Blood and Plasma: Heparinized whole blood from 20 individuals with and 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 following table.

Cardiac Markers / Results	Whole blood(+) and plasma (+)	Whole blood(–) and plasma (–)	Whole blood(+) and plasma (–)	Whole blood(–) and plasma (+)
Myoglobin	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 and 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 Markers / Results	Whole blood(+) and serum (+)	Whole blood(–) and serum (–)	Whole blood(+) and serum (–)	Whole blood(–) and serum (+)
Myoglobin	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/Troponin I Test and with the LifeSign MI® Myoglobin/CK-MB Test and the LifeSign MI® TroponinI Test. The correlation between the tests is shown below:

	Myoglobin	Troponin I
Comparative Sensitivity	95.8% (69/72)	97% (65/67)
Comparative Specificity	98.9% (177/179)	98.9% (182/184)
Overall Agreement	98% (246/251)	98.4% (247/251)

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P.O. Box 7139, Princeton, N.J. 08543 **Printed in USA**