One Step Troponin I and Myoglobin test

SD BIO LINE TnI/Myo Duo

Explanation of the test

[Introduction]

Myoglobin and troponin I are proteins found on cardiac muscle cells and are released into the blood upon damage of death of cardiac tissue. Myoglobin is an oxygen-binding heme protein with a molecular weight of 17,800 daltons, normally found on skeletal as well as a cardiac tissue. The troponin complex is formed of three subunits, troponon T (TnT), troponin C (TnC), Troponin I (TnI). The three troponin subunit have distinct functions with TnC as the CA^{++} binding, TnT as the tromysin binding, and TnI as the inhibitory subunits. The troponin complex, together with tropomysin, forms the main component that regulates the Ca^{++}-sensitive ATPase activity of actomyosin in striated muscle (skeletal and cardiac). TnC of cardiac tissues is identical to that in skeletal tissues, but TnI and TnT cardiac isoforms are different from those of skeletal isoforms, which enables the development of cardiac specific antibodies. Recent reports have investigated the utility of determining the serum levels of the different isoforms of TnI.

Detection of cTnI in the serum was investigated as an aid in the determination of myocardial damage in patients with acute myocardial infarction (AMI). Several clinical reports have demonstrated the diagnostic value of determining the serum level of cTnI in identifying patients with AMI. The temporal relation of release of cTnI into the serum has been investigated and compared to the other established cardiac markers such as CK-MB, myoglobin and TnT. Cumulative data from several reports documented that in patients with AMI, cTnI is released into the circulation with levels exceeding the upper reference limit of normal 4-6 hours after the onset of symptoms and peak levels are reached after 12-24 hours. This early release profile is similar to CK-MB. However, CK-MB levels return to normal values after 72 hours, while levels of cTnI remains elevated for up to 5-7 days. Due to the distinct structure of cTnI and the availability of highly-specific detection methods for cTnI, the utility of this marker for the diagnosis of AMI in complex clinical conditions that involve skeletal muscle damage have been investigated. The high specificity of TnI measurements for the identification of myocardial damage has been demonstrated in conditions such as perioperative period, after marathon runs, and blunt chest trauma. The release of cTnI into blood has been documented in clinical conditions that involve myocardial damage, other than AMI, such as unstable angina, congestive heart failure, and ischaemic damage due to coronary artery by-pass surgery. Measurements of cTnI have been investigated and documented to be valuable in identifying patients with AMI presenting to the ED with chest pain.

[Intended Use]

SD BIOLINE TnI/Myo Duo rapid test is a rapid immune-chromatographic assay for the qualitative detection of cardiac troponin I (cTnI) and Myoglobin in human serum,plasma or whole blood as an aid in the diagnosis of myocardial infarction in emergency room, critical care, and hospital settings. SD BIOLINE TnI/Myo Duo rapid test provides a qualitative analytical test result which cannot monitor the rise and fall of cTnI and myoglobin in single testing. Single testing is not recommended for AMI monitoring. Test result should be interpreted by the physian in conjunction with other test results and patient clinical findings.

[Principle]

The membrane was pre-coated with mouse monoclonal anti-myoglobin (T2), goat polyclonal anti-troponin I (T1) and goat anti-mouse IgG (C), respectively, for the test line and control line. The conjugate pad is placed at the end of the membrane containing colloidal gold particles coupled with myoglobin and cTnI antibody. When a specimen has been dispensed into the sample well myoglobin and cTnI in the specimen bind to the specific antibodies coupled with colloidal gold to form complexes. The complex migrate along the membrane and are captured by immobilized myoglobin (T2) and cTnI (T1) antibodies on the test lines. Unbound complexes are captured in the control lines. If the concentration of myoglobin and cTnI in the specimen is lower than cutoff level, only the colored control lines can be seen in the test window. If no band is present in the control lines, a test result is no valid and another test must be run.

Materials Provided / Active ingredients of main components

1. Materials Provided
   - Test devices individually foil pouched with desiccant
   - 80$\mu\ell$ Disposable droppers
   - Instruction for use
2. Active ingredients of main components
   - 1 test device included ; Gold conjugate (as main component) : Mouse monoclonal anti-troponin I – gold colloid (0.26±0.005μg), Mouse monoclonal anti-myoglobin – gold colloid (0.104±0.020μg), Test line "1" (as main component) : Goat polyclonal anti-troponin I (0.48±0.096μg), Test line "2" (as main component) : Mouse monoclonal anti-myoglobin (0.32±0.064μg), Control line(as main component) : Goat anti-mouse immunoglobulin G (0.64±0.128μg)

Kit storage and stability

1. The test device should be stored at 1~30°C. Do not store at refrigerator.
2. The test device is sensitive to humidity as well as to heat.
3. Perform the test immediately after removing the test device from foil pouch.
4. Do not use it beyond the expiration date.
5. The shelf-life of the kit is as indicated on outer package.
6. Do not use the test kit if the pouch is damaged or the seal is broken.
7. Do not re-use the test device.

Warnings

1. For in vitro diagnostic use only. DO NOT RE-USE test device.
2. Do not eat or smoke while handling specimens.
3. Wear protective gloves while handling specimens. Wash hands thoroughly afterwards.
4. Avoid splashing or aerosol formation.
5. Clean up spills thoroughly using an appropriate disinfectant.
6. Decontaminate and dispose of all specimens, reaction kits and potentially contaminated materials, as if they were infectious waste, in a biohazard container.
7. Do not use the test kit if the pouch is damaged or the seal is broken.
8. The instruction must be followed exactly to get accurate results.

Specimen Collection, Storage and Precaution

1. Whole blood
   - Collect the whole blood into the collection tube (containing anticoagulants such as heparin, EDTA and sodium citrate) by venipuncture.
   - Optimal results were obtained when patient samples were tested immediately after collection. Whole blood samples should be used within 24 hours after collection.
2. Plasma or Serum
   - [Plasma] Collect the whole blood into the collection tube (containing anticoagulants such as heparin, EDTA and sodium citrate) by venipuncture and then centrifuge blood to get plasma specimen.
     [Serum] Collect the whole blood into the collection tube (NOT containing anticoagulants) by venipuncture, leave to settle for 30 minutes for blood coagulation and then centrifuge blood to get serum specimen of supernatant.
   - If plasma or serum specimens are not tested immediately, they should be refrigerated at 2~8°C. For storage period longer than 2 weeks, freezing is recommended. They should be brought to room temperature (1~30°C) prior to use.
   - Plasma or serum specimens containing a precipitate may yield inconsistent test results. Such specimens must be clarified prior to assaying.
3. Precaution
   - Anticoagulants such as heparin, EDTA and sodium citrate do not affect the test result.
   - As known relevant interference, hemolytic samples, rheumatoid factors-contained samples and lipaemic, icteric samples can lead to impair the test results.
   - Use separately disposable capillary pipettes or pipette tips for each sample in order to avoid cross-contamination of either samples which could cause erroneous results.

Procedure of the test (Refer to figure)

1. Collect specimen according to instructions in **Specimen Collection, Storage and Precaution.**
2. Test device and sample should be brought to room temperature (15~30°C) prior to testing. Do not open pouches until ready to perform the assay.
3. Remove the test device from the sealed pouch immediately before use.
4. [Using a micropipette]
   Add 80$\mu\ell$ of serum, plasma or whole blood specimen into the sample well(S).

   OR,

   [Using a disposable dropper]
   Take serum, plasma or whole blood specimen up to the Fill Line (about 80$\mu\ell$) and then add the drawn specimen into the sample well(S).
5. For interpretation of this test results, please see the downside (letter of "C", "2" and "1") of the result window.
   Caution : Do not read the results after 15 minutes. Reading too late can give false results.

Interpretation of the test (Refer to figure)

1. A color band will appear at left section of the result window to show that the test is working properly. This band is the "Control Band".
2. The middle section of the result window indicates the test result of Myoglobin and right section indicates the test result of Troponin I.

Negative Result

The presence of only one purple color band within the result window indicates a negative result.

Positive Result

The presence of two or three color bands ("T1" and/or "T2" band and "C" band) within result window, no matter which band appear first, indicates a positive result.

Note :

- If bands are present in the cardiac Troponin I (cTnI, "T1") area and Control line ("C"), the Troponin I concentration is 1ng/ml or greater.
- If bands are present in the Myoglobin (Myo, "T2") area and Control line ("C"), the Myoglobin concentration is 50 ng/ml or greater.

Invalid Result

If the Control band is not visible within the result window after performing the test, the result is considered invalid. The directions may not have been followed correctly or the test may have deteriorated. It is recommended that the specimen be re-tested.

Limitations of the test

1. The test is for professional and in-vitro diagnostic use only.
2. A positive test result may only be used as an indicator of myocardial damage and requires further confirmation. Serial sampling of patients suspected of AMI at multiple time points is also recommended due to the delay between onset of symptoms and the release of cardiac marker proteins into the blood stream.
3. The test is a qualitative screening assay and is not suggested for use in determining the quantitative levels. As with all diagnostic tests, a definitive clinical diagnosis should not made based on the results of a single test. The test results should be used in conjunction with other clinical information such as clinical signs and symptoms and other test results to diagnose AMI. Confirmation of test results should only be made by a physician after all clinical and laboratory findings have been evaluated.
4. Samples containing unusually high titers of certain antibodies such as human anti-mouse or human anti-rabbit antibodies have been known to affect the performance of these devices. However these studies using the SD BILINE TnI/Myo Duo rapid test have not been tested.

Internal Quality Control

The SD BIOLINE TnI/Myo Duo test device has "Test lines" and "Control Line" on the surface of the cassette. All the Test lines and Control Line in result window are not visible before applying any samples. The Control Line is used for procedural control. Control line should always appear if the test procedure is performed properly and the test reagents of control line are working.

Performance Characteristics

SD BIOLINE TnI/Myo Duo Rapid test has been tested with TnI/Myo positive confirmed by laboratory reference assay and negative clinical samples. In a comparison of the SD BIOLINE TnI/Myo Duo Rapid kit compared to laboratory reference assay. Results gave sensitivity of 96.2% (50/52) for TnI, 100% (52/52) for Myo and specificity of 100% (380/380) for TnI, 97.4% for Myo(370/380).

Test Method		SD BIOLINE TnI/Myo Rapid test kit				Total	
		Troponin I		Myoglobin			
		Positive	Positive	Positive	Positive		
Laboratory Reference assay	Positive	50	2	52	2	52	
	Negative (Serum)	0	360	10	350	360	380
	Negative(Whole Blood)	0	20	0	20	20	
Sensitivity		96.2% (50 / 52)		100% (52 / 52)		-	
Specificity		100% (380 / 380)		97.4% (370 / 380)		-	

Expected Values

The cut-off levels are 50 ng/ml for Myoglobin and 1 ng/ml for cTnI. The specimens containing cTnI and/or myoglobin, at the concentration of equal or above established cutoff levels will give positive results of the SD BIOLINE TnI/Myo Duo rapid test.

Bibliography of suggested reading

1. Structural studies of interactions between cardiac troponin I and actin in regulated thin filament using forster resonance energy transfer. Xing J, Chinnaraj M, Zhang Z, Cheung HC, Dong WJ. Biochemistry. 2008 December 16; 47(50): 13383–13393.
2. Troponin elevation in patients with various tachycardias and normal epicardial coronaries. Kanjwal K, Imran N, Grubb B, Kanjwal Y. Indian Pacing Electrophysiol J. 2008 Jul–Sep; 8(3): 172–174.
3. Profile of patients with acute heart failure and elevated troponin I levels. Sukova J, Ostadal P, Widimsky P. Exp Clin Cardiol 2007;12(3):153-156.
4. Use of troponin for the diagnosis of myocardial contusion after blunt chest trauma. Jackson L, Stewart A. Emerg Med J. 2005 March; 22(3): 193–195.
5. A rapid point-of-care cardiac marker testing strategy facilitates the rapid diagnosis and management of chest pain patients in the emergency department.
   Straface AL, Myers JH, Kirchick HJ, Blick KE. Am J Clin Pathol. 2008 May;129(5):788-95.
6. Short term effects of milrinone on biomarkers of necrosis, apoptosis, and inflammation in patients with severe heart failure.
   Lanfear DE, Hasan R, Gupta RC, Williams C, Czerska B, Tita C, Bazari R, Sabbah HN. J Transl Med. 2009 Jul 29;7:67.
7. Early positive biomarker in relation to myocardial necrosis and impaired fatty acid metabolism in patients presenting with acute chest pain at an emergency room.
   Nagahara D, Nakata T, Hashimoto A, Takahashi T, Kyuma M, Hase M, Tsuchihashi K, Shimamoto K.
   Circ J. 2006 Apr;70(4):419-25.

product. Disclaimer:
Whilst every precaution has been taken to ensure the diagnostic ability and accuracy of this product, the product is used outside of the control of the Manufacturer and Distributor and the result may accordingly be affected by environmental factors and / or user error. A person who is the subject of the diagnosis should consult a doctor for further confirmation of the result.

Warning:
The Manufacturers and Distributors of this product shall not be liable for any losses, liability, claims, costs or damages whether direct or indirect or consequential arising out of or related to an incorrect diagnosis, whether positive or negative, in the use of this product.

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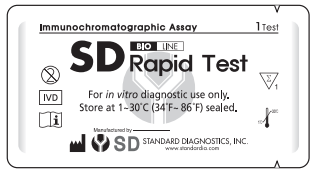
SD BIOLINE TnI/Myo Duo Rapid Test Procedure

1 **Now, open the package and look for the following;**

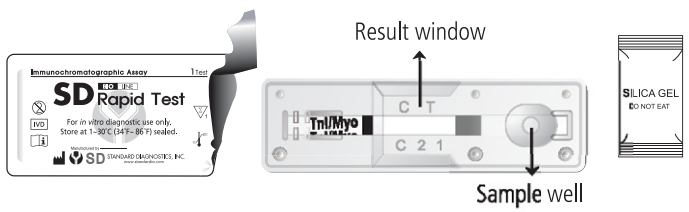
1) Test device individually foil pouched with a desiccant.
2) 80$\mu\ell$ Disposable droppers.
3) Instruction for use.

1



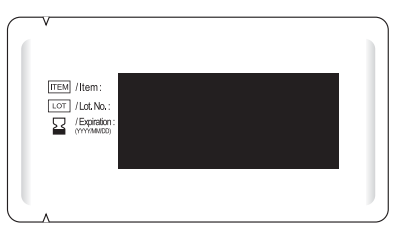
2

Open the foil pouch and look for the following.



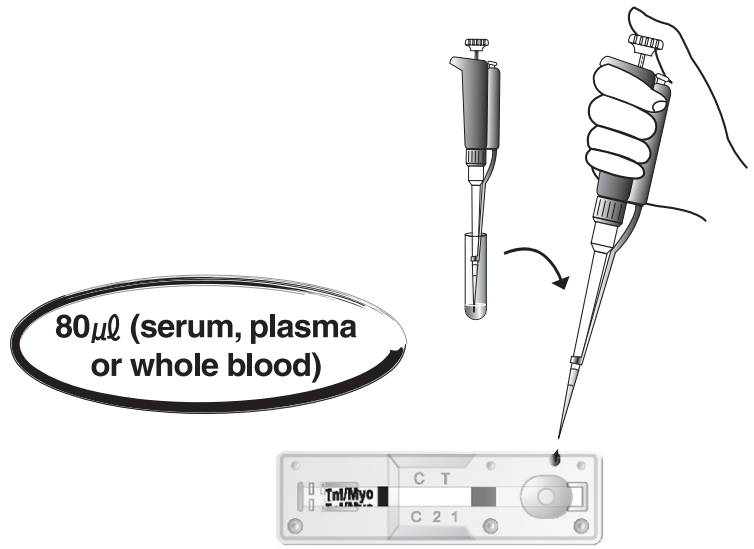
2 **FIRST, read carefully the instruction on how to use the SD BIOLINE TnI/Myo Duo test kit.**

3 **Next, look at the expiry date at the back of the foil pouch.**
Use another kit, if expiry date has passed.



I. Using a micropipette

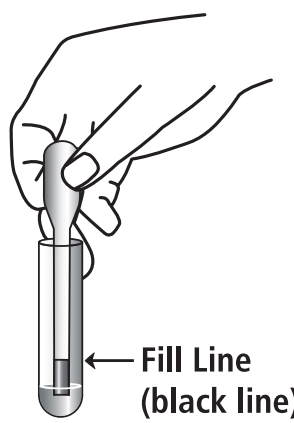
4



Add 80$\mu\ell$ of serum, plasma or whole blood specimen into the sample well (s) using a micropipette.

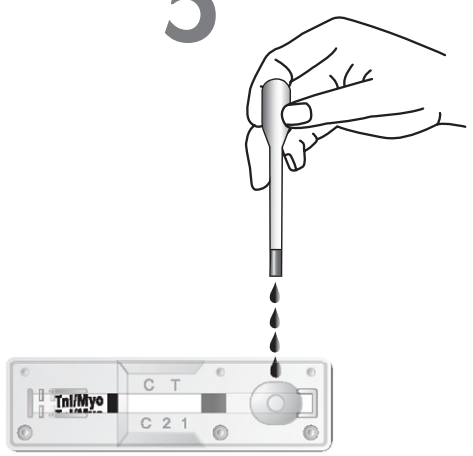
II. Using a disposable dropper

4



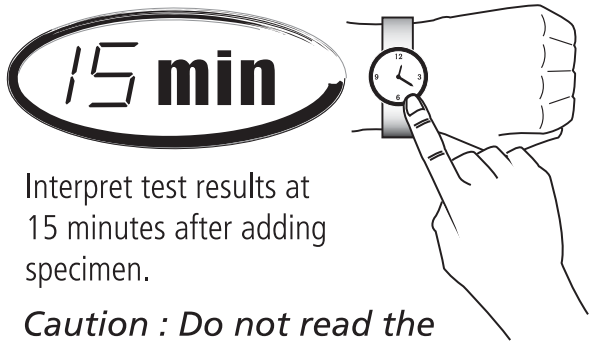
Take serum, plasma or whole blood specimen up to the Fill Line (about 80$\mu\ell$).

5



Add the drown specimen into the sample well (s).

Interpretation

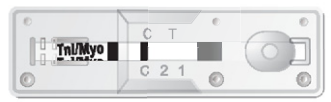


Interpret test results at 15 minutes after adding specimen.

Caution : Do not read the results after 15 minutes.
Reading too late can give false results.

How to interpret test results.

Negative



Positive

1. TnI positive

2. Myo positive

3. TnI and Myo positive

Invalid