



Core™ Troponin I
Rapid assay for detection of Human Cardiac Troponin I
in serum/plasma and whole blood
CAT N°: TR-170002

INTRODUCTION

Core Troponin I is a rapid, two-site sandwich immunoassay for the detection and semi quantification of human cardiac Troponin I (cTnI) levels in human serum, plasma and whole blood.

SUMMARY

Discovered by Ebashi, Troponins are regulatory proteins in cardiac muscle that modulate the interaction between actin and myosin, during the calcium-mediated contraction of cardiac muscle. Three distinct tissue specific isoforms of Troponin I have been identified, two in skeletal muscle and one in cardiac muscle. The cardiac isoform of Troponin I (cTnI) has an additional sequence of 31 amino acids at the N terminal end that accounts for cardiac specificity, with a molecular weight of 22.5 kDa. This absolute specificity of Troponin I for cardiac tissue makes it an ideal biomarker for myocardial injury.

Clinical study results have demonstrated that elevated serum levels of cardiac Troponin I (cTnI) are detectable within 4 to 6 hours after the onset of chest pain, reach peak concentration in approximately 12 hours and remain elevated for 3-10 days following acute myocardial infarction. Thus cardiac Troponin I (cTnI) meets all the criterion laid down by National Academy of Clinical Biochemistry (NACB) for an ideal cardiac biomarker in early identification and risk stratification of patients with chest pain suggestive of ischaemia and identification of patients that present after infarction.

PRINCIPLE

Core Troponin I test utilizes the principle of immunochromatography, with a unique two-site sandwich immunoassay on a nitrocellulose membrane. The conjugate pad contains two components – monoclonal anti-cTnI conjugated to colloidal gold and rabbit IgG conjugated to colloidal gold. As the test sample flows through the membrane assembly of the device, the highly specific anti-cTnI antibody - colloidal gold conjugate complexes with cTnI in the sample and travels on the membrane due to capillary action along with rabbit IgG-colloidal gold conjugate. This sample moves further on the membrane to the test region (T) where it is immobilized by another specific anti-cTnI antibody coated on the membrane leading to the formation of a pink-purple band. A detectable coloured band is formed if cTnI level is equal to or greater than 0.3 ng/ml. The absence of this coloured band in the test region indicates cTnI concentration < 0.3 ng/ml.

The rabbit IgG-colloidal gold conjugate and unbound complex, if any, moves further to the reference region (R) that contains pre-calibrated anti rabbit IgG antibodies, corresponding to 1ng/ml cTnI, immobilised on the membrane. The intensity of the pink purple coloured band at the reference region (R) corresponds to a cTnI concentration of 1 ng/ml. The reference band would form even in a negative specimen. Semi-quantitative information about the concentration of cTnI can be deduced by comparing the intensity of the test band against the reference band. If the intensity of test band is less than the reference band, cardiac Troponin I (cTnI) concentration is equal to or above 0.3 ng/ml and less than 1 ng/ml. If the intensity of the test band is equal to or greater than reference band, cardiac Troponin I (cTnI) concentration is equal to or greater than 1 ng/ml.

The unreacted conjugate along with unbound complex if any, move further on the membrane and are subsequently immobilized by the anti-rabbit antibodies coated on the membrane at the control region (C), forming a pink-purple coloured band. This control band acts as a procedural control and serves to validate test results.

REAGENTS AND MATERIAL SUPPLIED:

Core Troponin I kit contains :

A. Individual pouches each containing-

1. Test device: Membrane assembly pre-dispensed with monoclonal anti-cTnI colloidal gold conjugate, rabbit IgG colloidal gold conjugate, monoclonal anti-cTnI antibody and anti-rabbit antiserum coated at the respective regions.

2. Desiccant pouch .

3. Sample dropper.

B. Sample Running buffer in a dropper bottle.

C. Package insert.

OPTIONAL MATERIAL REQUIRED:

Calibrated micropipettes capable of delivering 25 µl sample accurately

STORAGE AND STABILITY:

The sealed pouches in the test kit and the kit components may be stored between 4-30°C for the duration of shelf life as indicated on the pouch/carton.

DO NOT FREEZE.

NOTE:

1. For in vitro diagnostic use only. NOT FOR MEDICINAL USE.
2. Do not use beyond expiry date.
3. Read the instructions carefully before performing the test.
4. Do not inter mix reagents from different lots.
5. Handle all specimens as potentially infectious.
6. Follow standard biosafety guidelines for handling and disposal of potentially infective material.

SPECIMEN COLLECTION AND PREPARATION:

1. **Core Troponin I** uses human serum, plasma or whole blood as specimen.
2. No special preparation of the patient is necessary prior to specimen collection by approved techniques.
3. Fresh anticoagulated whole blood should be used as test specimen. EDTA or Heparin or oxalate can be used as a suitable anticoagulant.
4. Whole blood should be used immediately and should not be frozen. Do not use haemolysed, clotted or contaminated whole blood specimens.
5. Preferably fresh serum is to be used as specimen, allow blood to clot completely. Centrifuge to obtain clear serum. Do not use turbid, lipaemic and haemolysed serum/plasma.
6. In case of delay in testing, sample may be stored at 2-8°C for maximum upto 24 hours. Only one freeze thaw cycle is advisable for frozen specimen.

Refrigerated specimen must be brought to room temperature prior to testing.
 7.Specimen containing precipitates or particulate matter must be centrifuged and clear supernatant only be used for testing.

IMPORTANCE OF SEQUENTIAL TESTING:

Immediately after a cardiac event, the damaged myocardial cells start releasing cardiac Troponin I (cTnI) in circulation and their level rises in a time specific manner. Since patients present at varying times for testing following the onset of chest pain in a cardiac event, it is necessary to perform sequential testing for optimal diagnostic accuracy.

A protocol for measuring cardiac Troponin I (cTnI) levels requires testing at admission or 3 hours after onset of chest pain and at 6 and 9 hours. Modification may be necessary depending upon specific clinical situation. Hence sequential testing of cardiac Troponin I (cTnI), together with ECG results and patient history and symptoms are necessary for differential diagnosis between acute myocardial infarction and unstable angina pectoris.

The positive and negative likelihood ratios correspond to the clinical concepts of ruling in and ruling out disease. Thus, a higher positive likelihood ratio means that a test result is better for ruling in disease when positive, and a lower negative likelihood ratio means that a test result is better for ruling out disease when negative. Examination of likelihood ratios reveals that levels of cardiac Troponin I (cTnI) are very useful at ruling out AMI when the value is negative at 10 or more hours from the onset of chest pain. However, a negative test value early in the course of episode of chest pain does very little to reduce the likelihood of AMI. A positive cardiac Troponin I (cTnI) value after 6 or more hours after the onset of chest pain appears to be very useful at ruling in AMI. Thus a negative cardiac Troponin I (cTnI) level identifies patient at low risk for adverse cardiac events.

TESTING PROCEDURE AND INTERPRETATION OF RESULTS:

1. Bring the **Core Troponin I**- kit components to room temperature before testing.
2. Open the pouch by tearing along the notch.
3. Retrieve the device, sample dropper and desiccant. Check the colour of the desiccant. It should be blue. If it has turned colourless or pink, discard the device and use another device.
4. Once opened the device must be used immediately.
5. Tighten the vial cap of the sample running buffer provided with the kit in clockwise direction to pierce the dropper bottle nozzle.
6. Label the device with specimen identity.
7. Place the testing device on a flat horizontal surface.
8. Holding the sample dropper vertically, carefully dispense **four (4) drops** of serum/plasma/whole blood into the sample port 'A'.
9. Add **four (4) drops** of sample running buffer in buffer port 'B'.
10. At the end of 15 minutes read results as follows:

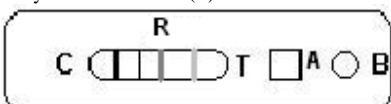
Negative Result

Presence of two coloured bands at Reference (R) and Control (C) regions indicate absence of cTnI or the concentration of cTnI in the specimen is below 0.3 ng/ml.



Positive Result

1.If intensity of the Test band (T) is less than the Reference band - cTnI concentration is $\geq 0.3\text{ng/ml}$ and $<1\text{ng/ml}$.



2.If intensity of the Test band is equal to or greater than the Reference band - cTnI concentration is $\geq 1\text{ng/ml}$.



Invalid Result

The test is invalid if the Control band and/or Reference band is not visible at fifteen minutes. Verify the test procedure and repeat the test with a new device.

PERFORMANCE CHARACTERISTICS:

Core Troponin I detects cardiac Troponin I at a concentration of $\geq 0.3\text{ ng/ml}$.

Internal Evaluation

The performance of **Core Troponin I** was evaluated using a panel of 50 samples in comparison with a commercially available rapid test. The results of the evaluation are as follows:

SPECIMEN DATA	TOTAL	Core Troponin I	Commercially Available Rapid Test
No. of specimen tested	50	50	50
No. of Positive specimens	12	12	12
No. of Negative specimens	38	38	38

Based on this evaluation :

Sensitivity and Specificity of Core Troponin I -100%

External Evaluation

In an independent study conducted performance of **Core Troponin I** was evaluated using a panel of 20 samples in comparison with a commercially available automated chemiluminescence assay. The results of the evaluation are as follows:

SPECIMEN DATA	TOTAL	Core Troponin I	Chemiluminescence Assay
No. of specimen tested	20	20	20
No. of Positive specimens	11	11	11
No. of Negative specimens	9	9	9

Based on this evaluation :

Sensitivity and Specificity of Core Troponin I -100%







REMARKS:

1. Sequential testing of cTnI is important for diagnosing patients presenting with an evolving AMI. Diagnosis should not be made based on a single test result.
2. Samples with normal CK-MB levels and positive **Core Troponin I** result may occur in a patient with unstable angina pectoris and probably reflects a micro infarct not detected by CK-MB test .
3. Unstable angina pectoris and Non ST segment elevation myocardial infarction (NSTEMI) are closely related cardiac manifestations. Samples with normal CK-MB level and non-diagnostic ECG change, but positive test results indicates a subset of high-risk acute coronary syndrome patients and are classified under NSTEMI.
4. All serum cardiac enzyme markers may be positive with rhabdomyolysis, however cTnI is only slightly elevated despite significant elevations in both CK and CK-MB test.
5. cTnI levels may rarely rise in skeletal muscle disorders and renal failure.
6. cTnI levels may rise in other cardiac conditions causing myocardial damage namely myocarditis, cardiac contusion, recent cardiac surgery or catheterization.
7. cTnI is present only in cardiac tissue; serum levels are extremely low in normal healthy individuals.
8. cTnI levels are elevated upto 8 days, hence reinfarction may not be detected.
9. Interference due to heterophile antibodies, rheumatoid factors and other nonanalyte substances in patient's serum, capable of binding antibodies multivalently and providing erroneous analyte detection in immunoassays, has been reported in various studies. Though **Core Troponin I** uses sufficient amount of blocking agents to inhibit majority of these interference, nevertheless, some vigilance to this possibility of antibodies interference. Results that appear to be internally inconsistent or incompatible with the clinical presentation should invoke suspicion of the presence of an endogenous artifact and lead to appropriate investigation.
10. The membrane is laminated with an adhesive tape to prevent surface evaporation. Air pockets or patches may appear, which do not interfere with the test result. Presence of a band at the test region even if low intensity or formation is a positive result .
11. The deliberate slow reaction kinetics of **Core Troponin I** is designed to maximize and enhance reaction time between sample capture and tracer elements to improve test sensitivity.
12. As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical laboratory findings have been evaluated.

BIBLIOGRAPHY:

1. Troponin testing: An audit in three metropolitan hospitals, Richard X Davey, JMA, Vol.179, 81-83, 21st July 2003.
2. Clinical laboratory in emergency medicine, cardiac Troponins, John Sarko, Charles V.Pollack, The journal of emergency medicine, Vol.23, 57-65, 2002.
3. A systematic review of Troponin T and I for diagnosing Acute Myocardial Infarction, Mark H.Ebell, Cheryl A. Flynn, The Journal of Family Practice, April 16, 2000.
4. Analytical performance and clinical application of a new rapid bedside assay for the detection of serum cardiac Troponin I, Christopher Heeschen, Brita U. Goldmann, Robert. H. Moeller, Christian W. Hamm, Clinical Chemistry, 44:9, 1925-1930, 1998.
5. National Academy of Clinical Biochemistry Standards of Laboratory Practice: Recommendations for the use of cardiac markers in coronary artery diseases, Alan H. B. Wu, Fred S. Apple, W. Brian Gibler, Robert L. Jesse, Myron M. Warshaw, Roland Valdes, Jr., Clinical Chemistry 45:7, 1104-1121, 1999.
6. Cardiac specific Troponin I levels to predict the risk of mortality in patients with acute coronary syndromes, Elliott M. Antman, Milenko J. Tanasijevic, Bruce Thompson, Mark Schactmann, The New England Journal of Medicine, 1342-1349, October 31, 1996.
7. IFCC Committee on Standardization of markers of cardiac damage: Premises and Project Presentation, Abstract, Vol.11 No.2, 19-22, JIFCC, 1999.
8. Clinical Laboratory Diagnostics, Use and Assessment of Clinical laboratory results, Edited by Lothar Thomas, First Edition, TH Books Verlagsgesellschaft mbH, Frankfurt, Germany, 1998.

SYMBOLS USED

	Consult instructions for use
	Storage temperature
	Use by
LOT	Batch code
REF	Catalogue number
IVD	In vitro diagnostic medical device
CARD	Test Device
PIPETTE	Disposable Plastic Dropper
BUF	Sample running buffer
	Manufactured By
	Date of Manufacture
	Contains sufficient <n> tests



Version En2 - 12/2006.

Aspect Court, 4 Temple Row
Birmingham B2 5HG
UNITED KINGDOM