Core Troponin I
Rapid assay for the detection of Human Cardiac Troponin I in serum/plasma and whole blood.

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
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 the membrane. As the test sample flows through the membrane assembly of the device, the coloured anti-cardiac Troponin I antibody-colloidal gold conjugate complexes with cardiac Troponin I (cTnI) in the sample. This sample moves further on the membrane to the test region where it is immobilized by the anti-cardiac Troponin I antibody coated on the membrane leading to the formation of a pink-purple band. The complex moves further on the reference region, where appearance of light pink purple band, corresponding to cardiac Troponin I (cTnI) concentration of 1 ng/ml. If the intensity of test band is less than the reference band, cardiac Troponin I (cTnI) concentration is at least 0.1 ng/ml but 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 absence of coloured band in the test region indicates a negative test result. The unreacted conjugate along with unbound complex if any, move further on the membrane at the control region, forming a pink-purple band. This control band serves to validate test performance.

Contents
Each kit contains:
A. Individual pouches each containing
   1) Test device: Membrane assembly predispensed with monoclonal anti-cardiac Troponin I colloidal gold conjugate, rabbit globulin colloidal gold conjugate, monoclonal anti-cardiac Troponin I antibody and anti-rabbit antibody at the respective regions
   2) Desiccant pouch
   3) Sample dropper
C. Clearing buffer in a dropper bottle
B. Package insert
C. Optional material required:
   Calibrated micropipettes capable of delivering 25 μl sample accurately

Storage And Stability
The sealed pouches in the test kit the kit components may be stored between 4-30°C for the duration of shelf life as indicated on the pouch. DO NOT FREEZE.

Note
Read the instructions carefully before performing the test. For in vitro diagnostic use only. NOT FOR MEDICINAL USE. Do not use beyond expiry date. Do not inter mix reagents from different lots. Handle all specimens as potentially infectious. Follow standard biosafety guidelines for handling and disposal of potentially infective material.
Specimen collection and preparation:
Core Troponin I uses human serum, plasma or whole blood as specimen. No special preparation of the patient is necessary prior to specimen collection by approved techniques.

1) Fresh anticoagulated whole blood should be used as test sample, EDTA or Heparin or oxalate can be used as a suitable anticoagulant. Do not use haemolysed, clotted or contaminated blood specimens.
2) 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.
3) In case of delay in testing sample can be stored at 2-80C for maximum upto 24 hours or frozen at –200C. Only one freeze thaw cycle is advisable for frozen sample. Refrigerated specimen must be brought to room temperature prior to 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 and 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. Once opened the device must be used immediately.
3) Tighten the vial cap of the clearing buffer provided with the kit in clockwise direction to pierce the dropper bottle nozzle.
4) Label the device with specimen identity.
5) Place the device on a flat horizontal surface.
6) Holding the sample dropper vertically, carefully dispense four (4) drops of serum/plasma/whole blood into the sample port ‘A’.
7) Add four (4) drops of sample clearing buffer in buffer port ‘B’.
8) At the end of 15 minutes read results as follows.

Negative Result:
Presence of two pink-purple coloured bands at Reference (R) and Control (C) regions indicate absence of cardiac Troponin I (cTnI) or the concentration of cardiac Troponin I (cTnI) in the specimen is below 0.1 ng/ml.

Positive Result:
If the intensity of the Test band is less than the reference band, cardiac Troponin I (cTnI) concentration is at least 0.1 ng/ml but less than 1 ng/ml.

If the intensity of the test band is equal to or greater than the Reference band, cardiac Troponin I (cTnI) concentration is ≥ 1 ng/ml.

9) The test result should not be interpreted after 15 minutes
10) The test should be considered invalid if no bands appear on the device. Repeat the test with a new device ensuring that the test procedure has been followed accurately.
Performance Characteristics

In an in-house study, the performance of Core Troponin I was evaluated using a panel of 50 samples (32 serum/plasma and 18 whole blood samples) in comparison with commercially available rapid test. The results of the evaluation are as follows:

<table>
<thead>
<tr>
<th></th>
<th>Core Troponin I</th>
<th>Commercial Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of samples tested</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Number of Positive results</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Number of Negative results</td>
<td>38</td>
<td>38</td>
</tr>
</tbody>
</table>

Based on the above study the specificity and sensitivity of Core Troponin I is 100%.

Remarks:

1) Sequential testing of Core Troponin I 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 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 a non-diagnostic ECG change, but positive Core Troponin I test result 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 cardiac Troponin-I (cTnI) is only slightly elevated despite significant elevations in both CK and CK-MB test.
5) Cardiac Troponin-I (cTnI) may rarely give positive result in skeletal muscle disorders and renal failure.
6) Cardiac Troponin I (cTnI) levels may rise in other cardiac conditions causing myocardial damage namely myocarditis, cardiac contusion, recent cardiac surgery or catheterization.
7) Cardiac Troponin-I (cTnI) is present only in cardiac tissue; serum levels are extremely low in normal healthy individuals.
8) Cardiac Troponin-I(cTnI) levels are elevated upto 8 days, hence re infarction cannot be detected.
9) No diagnosis should be made based on a single test result.

Bibliography