



Windsor, Canada, www.bioassayplus.com

ONE STEP ASSAY FOR TROPONIN Strip Form ^{Assay+}

INTENDED USE

Troponin I Serum Test is an immunoassay for the rapid qualitative detection of cardiac troponin I (cTnI) in human serum at a cutoff level of 1.5 ng/ml. It provides an aid in the diagnosis of myocardial infarction in emergency room, point-of-care, and hospital setting.

Troponin I Serum Test provides a qualitative result rather than information about change in the level of cTnI with single testing. Serial testing should be performed to determine a temporal change in the level of cTnI. If desired, a quantitative method should be used to determine the concentration of cTnI. Clinical consideration and professional judgment should be applied when making a diagnostic decision based on this test result.

SUMMARY AND EXPLANATION

Several cardiac markers have been used for the diagnosis of acute myocardial infarction (AMI) in the past decade, such as creatine kinase (CK), the MB isoform of creatine kinase (CK_{MB}), lactate dehydrogenase (LDH) isoforms, myoglobin, and cardiac troponins (cTn). Cardiac Troponins exist as a ternary complex with three subunits: cardiac troponin I (cTnI), T (cTnT) and C (cTnC). Investigations showed that both cTnI and cTnT were superior to any of the other cardiac markers because they demonstrated increased sensitivity (>98% at peak concentration) and specificity (95-100%) for AMI over other traditional markers. 1-3 cTnI is released into the blood circulation with levels exceeding the upper reference limit of normal 4 -6 hours after the onset of AMI and reaches a peak after 12-24 hours. The high level of cTnI remains for up to 5 -7 days. The early release and long duration of cTnI make the test more suitable for the diagnosis of myocardial infarction. 3-5

PRINCIPLE OF THE PROCEDURE

This assay is a double antibody chromatographic lateral flow immunoassay. The test strip in the device consists of 1) a burgundy-colored conjugate pad containing colloidal gold coupled with anti-cTnI antibodies, and 2) a nitrocellulose membrane containing a test line (T line) and a control line (C line). The T line is coated with anti-cTnI antibodies, and the C line is coated with goat anti-mouse antibodies. When cTnI is present in the specimen, the T line will become a burgundy-colored band. If cTnI is not present or present below the detectable level, no T line will develop. The C line should always appear as a burgundy-colored band regardless of the presence of cTnI. The C line serves as an internal qualitative control of the test system to indicate that an adequate volume of specimen has been applied and the liquid migration occurred.

MATERIALS PROVIDED

1. 25 test devices, each pouched with a disposable pipette and a desiccant.
2. One package insert (instructions for use)

MATERIAL REQUIRED BUT NOT PROVIDED

1. Controls: cTnI positive and cTnI negative
2. Specimen collection containers
3. Timer

STORAGE

Store kit at 15-30°C (59-86°F). Kit contents are stable for 2 years or until the expiration date printed on the label, whichever comes first. Exposing the kit to the temperatures over 30°C (86°F) may reduce the shelf life or damage the device.

SPECIMEN COLLECTION AND STORAGE

1. Use human serum sample only.
2. Follow standard laboratory procedures to collect serum specimens.
3. Since cardiac proteins are relatively unstable, it is recommended that fresh samples be tested as soon as possible.
4. Heat activation of samples may lead to hemolysis or protein denaturation and therefore should be avoided.
5. If samples are to be stored for more than 24 hours, they should be stored at -20°C or below, and brought to room temperature before testing. Repeatedly frozen and thawed specimens are not recommended for this assay.
6. Any sediment in serum specimens should be removed by centrifugation. Avoid using any turbid specimens, which may be contaminated by microorganisms.

WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic use only.
2. CAUTION: All human blood products, including serum samples, should be considered potentially infectious. It is recommended that the reagents and patient samples be handled according to the OSHA Standard on Blood borne Pathogens or other appropriate national biohazard safety guidelines or regulations. 7,8
3. Do not use kit beyond the expiration date indicated on the product.
4. The test should remain in its sealed pouch until ready for use.
5. Wear disposable gloves while handling specimens and thoroughly wash hands afterwards.
6. Use separate clean tips for different specimens. Do not pipette by mouth.
7. Do not smoke, eat or drink in areas in which specimens or kit reagents are handled.
8. Observe established procedures for proper disposal of specimens and used test devices.

PROCEDURE

1. Refrigerated specimens or other test materials, including devices, must be equilibrated to room temperature before testing to avoid invalid results.
2. Remove the device from the pouch and place it on a flat surface. Label the device with specimen identification.
3. Add four (4) drops of serum (about 160µl-200µl) into the sample well.

4. Strong positive results may be observed within 5 minutes. Weak positive results may take a longer time. The results should be read within 15-20 minutes. **DO NOT INTERPRET THE RESULTS AFTER 20 MINUTES.**

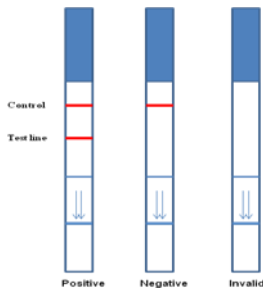
INTERPRETATION OF RESULTS

1. **POSITIVE:** If both the C line and T line appear, the result indicates that the cTnI is detected and the result is positive. Note: The color intensities of C line and T line may not be the same. A faint T line indicates a borderline specimen, which should be re-tested using an alternative method for confirmation.

2. **NEGATIVE:** If only the C line appears, the test indicates that no cTnI is detected or its level is below the detectable and the result is negative.

When the test result is negative or is in conflict with other results, it is imperative to perform a new test approximately one hour later. If the second result is negative and if the last sample was taken more than 6 hours after a suspected AMI case, then the patient has likely not suffered from AMI.

3. **INVALID:** When no control line appears within 5 minutes, the results should be considered invalid. In this case, repeat the test with a new test device.



QUALITY CONTROL

©Built-in Control Features: This test contains a built-in quality control feature, the C line. The appearance of the burgundy C line indicates that an adequate volume of specimen has been applied and the flow occurred.

©External Quality Control: External controls are recommended, positive and negative, to monitor the performance of the assay. Quality controls should be run bimonthly, when the lot is changed, or if the result is suspect.

LIMITATIONS OF THE PROCEDURE

1. The test provides a qualitative test result. The qualitative nature of this assay does not provide information about actual concentration of troponin I at a given time. Interpretation of any test result using

the test should be made together with other clinical information available to physicians using appropriate professional judgment.

2. Human serum samples containing unusually high titers of certain antibodies, such as human anti-mouse or human anti-rabbit antibodies (HAMA or HARA), may influence the test results. The test has been optimized to minimize interference from HAMA-containing specimens ; nevertheless complete elimination of this interference from all patient specimens cannot be guaranteed. Patient samples may contain human anti-mouse antibodies (HAMA) which are capable of giving falsely elevated or depressed results with assays that utilize mouse monoclonal antibodies.

3. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.

4. If the test result is inconsistent with the clinical symptom and patient history, it should be interpreted with caution.

EXPECTED VALUES

1. The test is designed to yield a positive result for free cardiac troponin I concentrations > 1.5 ng/ml or 5 ng/ml ternary complex troponin I.

2. The time required for blood cardiac troponin I levels to reach the upper limit of normal has been found to be 3-10 hours following the onset of symptoms, with maximum concentration being reached after 10-24 hours. The cardiac troponin I level remains elevated for 6 to 10 days in some cases. Therefore, a negative result within the first hours of the onset of symptoms does not rule out acute myocardial infarction with certainty. If AMI is suspected, repeat the test at appropriate intervals.

PERFORMANCE CHARACTERISTICS

1. Analytical Sensitivity: The analytical sensitivity of this device was determined with a panel of forty (40) serum samples. They were evenly distributed into 4 groups, ten (10) samples in each group. The 4 groups were spiked with cTnI at four different levels: 1.5, 1.3, 1.1, and 0.9 ng/ml, separately. The results demonstrated that this device detected all 10 samples at 1.5 ng/ml level as positive, detected 6 out of 10 at 1.3 ng/ml, 5 out of 10 at 1.1 ng/ml, and 3 out of 10 at 0.9 ng/ml. Therefore, the analytical sensitivity of this device is 1.5 ng/ml cTnI.

2. Precision-Proficiency Testing: Between Run Assays at Three (3) Different Testing Sites Threephysician's office laboratories (POLs) and one medical reference laboratory were provided with a panel of eighty (80) blind-labeled serum samples. The samples consisted of four evenly distributed groups and were prepared with normal sera spiked with purified cTnI at four different concentrations: 0, 0.1, 1.5,

and 10 ng/ml. The results obtained by the test demonstrated an agreement of 100% within run as well as between sites.

3. Interference and Cross-Reactivity: The following potentially interfering substances do not appear to interfere or crossreact with cardiac troponin I determinations in the test up to the levels shown below:

In vitro testing of the following commonly-used drugs revealed no interference within the normal therapeutic range: (Analyte (10 µg/ml Final Concentration)

Acetaminophen, Acetylsalicylic acid, Adenine, Albumin (bovine), Aloprinol, Ambroxol, Ampicillin, Ascorbic acid, Atenolol, Atropine, Caffeine, Captopril, Chloramphenicol, Cinnarizine, Cyclophosphamide, Cyclosporine, Digitonin, Digoxin, Dopamine, Erythromycin, Gentistic acid, Nifedipine, Nystain, Oxazepam, Oxytetracycline, Propanolol, Theophiline, L-thyroxine, Urea, Uric acid, Verapamil

4. Recovery Studies Normal human serum was supplemented with purified human cardiac troponin I to yield concentrations of 0, 1.5, and 3 ng/ml. The samples were tested using the testin 6 replicates. As shown in the table below, an agreement of 100% was observed between the expected and the observed results at each cTnI concentration.

5. Clinical Sample Evaluation: A total of 300 clinically confirmed serum samples, 150 positive and 150 negative, were tested with this device, the predicate device, and a quantitative ELISA Troponin I assay for comparison. The results from the predicate device were that, out of the 150 positive specimens, 147 were tested positive, 1 weak positive, and 2 negative; and out of the 150 negative specimens, 144 were tested negative and 6 positive. This device obtained the similar results to the predicate device. Out of the 150 confirmed positive specimens, 147 were tested positive, 2 weak positive, and 1 negative; and out of the 150 confirmed negative specimens, 144 were tested negative and 6 positive. The ELISA test detected 149 positive out of the positive specimens and 146 negative out of the negative specimens. The data demonstrated this device has a sensitivity of 99.3% and a specificity of 96.0%, while the predicate device had a sensitivity of 98.8% and a specificity of 96.0%. The ELISA test gave a sensitivity of 99.3% and specificity of 97.3%. Compared with the ELISA test, the agreement was 97.4% (149/153) for positive results and 95.9% (141/147) for negative results. The overall agreement was 96.7% (290/300).