

ONE STEP ASSAY FOR QUALITATIVE *IN VITRO* DIAGNOSTIC USE

INTENDED USE

The Troponin I Whole Blood/Serum Test is an immunoassay for the rapid qualitative detection of cardiac troponin I (cTnI) in human whole blood or 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 settings.

The Troponin I 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 results from this test.

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 (CKMB), 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). Investigation has shown that both cTnI and cTnT are superior to other cardiac markers because they demonstrate increased sensitivity (>98% at peak concentration) and specificity (95-100%) for AMI over other traditional markers, or even compared to the WHO criteria.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. High levels of cTnI remain for up to 5-7 days. The early release and long duration of cTnI make the test 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 mouse 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 mouse anti-cTnI antibodies, and the C line is coated with goat anti-mouse antibodies. The sample pad of the test strip is pretreated with rabbit anti human red blood cell antibodies to separate red blood cells from the blood specimen.

If cTnI is present in the specimen, a T line will appear as 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 liquid flow has occurred.

REAGENTS AND MATERIALS SUPPLIED

- 25 test devices, each pouched with a disposable pipette.
- One package insert (instructions for use)

MATERIALS REQUIRED BUT NOT PROVIDED

- Controls: cTnI positive and cTnI negative
- Specimen collection containers
- Timer

STORAGE

- Store kit at 15-30°C (59-86°F). **Do not freeze.** 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. Serum

- Follow standard laboratory procedures to collect serum specimens.
- Serum specimens can be stored at 9-30°C (48-86°F) for 8 hours, at 2°-8°C (36-46°F) for one week, and at -20°C (-4°F) or lower for prolonged storage. Repeatedly frozen and thawed specimens are not recommended for this assay.
- Any sediment in serum specimens should be removed by centrifugation. Avoid using turbid specimens, which may be contaminated by microorganisms.

2. Whole Blood

- Follow standard laboratory procedures to collect whole blood specimens. Collect blood in a tube containing citrate anticoagulant.
- Fresh specimens are recommended since cardiac troponin proteins are unstable. Whole blood samples should be tested within four (4) hours. Do not freeze whole blood specimens, as this can lead red blood cells to break, which may cause hemolysis. If specimens are to be stored, the red blood cells should be removed.
- Use the provided pipette to pick up the blood, and apply four drops to the sample well of the device.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- 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 Bloodborne Pathogens or other appropriate national biohazard safety guidelines or regulations.⁶⁻⁸
- Do not use the kit beyond the expiration date indicated on the product.
- The device should remain in its sealed pouch until ready for use.

- Wear disposable gloves while handling specimens and thoroughly wash hands afterwards.
- Use separate, clean tips for different specimens. Do not pipette by mouth.
- Do not smoke, eat or drink in areas in which specimens or kit reagents are handled.
- Observe established procedures for proper disposal of specimens and used test devices.

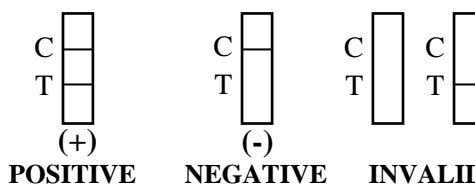
According to EU regulation 2017/746, any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the EU Member State in which the user and/or patient is established.

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 whole blood or serum to the sample well.
4. Strong positive results may be observed within 5 minutes. Weak positive results may take longer. The results should be read within 15-20 minutes.

DO NOT INTERPRET THE RESULTS AFTER 20 MINUTES.

INTERPRETATION OF RESULTS



POSITIVE: If both the C line and T line appear, cTnI is detected and the result is positive. **Note:** The color intensities of the C and T lines may not be the same.

A faint T line indicates a borderline specimen, which should be re-tested using an alternative method for confirmation.

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

If 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.

INVALID: If 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 a burgundy C line indicates that an adequate volume of specimen has been applied and flow has occurred.

External Quality Control

External positive and negative controls are recommended 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

- This 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 should be made together with other clinical information available to physicians using appropriate professional judgment.
- 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.
- Serum samples demonstrating gross lipemia, gross hemolysis or turbidity should not be used with this test.
- Test results that are inconsistent with clinical symptoms and patient history should be interpreted with caution.

EXPECTED VALUES

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.

The time required for blood cardiac troponin I levels to reach the upper limit of normal has been found to be 4-6 hours following the onset of symptoms, with maximum concentrations being reached after 12-24 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 2 panels, one for serum samples and one for whole blood samples. Each consisted of 40 members evenly distributed into 4 groups, ten (10) samples in each group. The 4 groups were separately spiked with cTnI at different levels: 1.5, 1.3, 1.1 and 0.9 ng/mL.

For the serum panel, this device detected all 10 samples at 1.5 ng/mL level as positive, detected 7 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. For the whole blood panel, this device detected all 10 samples at 1.5 ng/mL 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.

Serum Panel		Troponin I Sample Concentration (ng/mL)			
		1.5	1.3	1.1	0.9
Test Result	Positive (+)	10	7	5	3
	Negative (-)	0	3	5	7
Total		10	10	10	10

Whole Blood Panel		Troponin I Sample Concentration (ng/mL)			
		1.5	1.3	1.1	0.9
Test Result	Positive (+)	10	6	5	3
	Negative (-)	0	4	5	7
Total		10	10	10	10

2. Precision-Proficiency Testing: Between Run Assays at Four (4) Different Testing Sites

Three physician's office laboratories (POLs) and one medical reference laboratory were provided with two panels of samples spiked with purified cTnI. One panel contained eighty (80) blind-labeled whole blood samples and the other, eighty (80) blind-labeled serum samples. Samples in each panel consisted of four evenly distributed groups with 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 for spiked whole blood samples, and an agreement more than 98% for spiked serum samples.

Whole Blood Panel	cTnI Concentration (ng/mL) and Test Result								Agreement Within Run
	0		0.1		1.5		10		
	-	+	-	+	-	+	-	+	
Site I	20	0	20	0	0	20	0	20	100%
Site II	20	0	20	0	0	20	0	20	100%
Site III	20	0	20	0	0	20	0	20	100%
Site IV	20	0	20	0	0	20	0	20	100%
Between-Site Agreement	100%		100%		100%		100%		

Serum Panel	cTnI Concentration (ng/mL) and Test Result								Agreement Within Run
	0		0.1		1.5		10		
	-	+	-	+	-	+	-	+	
Site I	20	0	19	1	0	20	0	20	98.8%
Site II	20	0	20	0	0	20	0	20	100%
Site III	20	0	20	0	0	20	0	20	100%
Site IV	19	1	20	0	0	20	0	20	98.8%
Between-Site Agreement	98.8%		98.8%		100%		100%		

3. Interference and Cross-Reactivity

The following potentially interfering substances do not appear to interfere or cross-react with cardiac troponin I determinations in the test up to the levels shown below:

Analyte	Test Level
Biotin	200 ng/mL
Bilirubin	1 mg/mL
Hemoglobin	2 mg/mL
Rabbit skeletal muscle troponin C	2.5 µg/mL
Human cardiac troponin T	2.5 µg/mL
Human skeletal muscle troponin T	2.5 µg/mL
Human skeletal muscle troponin I	2.5 µg/mL
Cholesterol	8 mg/mL
Triglyceride	12.5 mg/mL

In vitro testing of the following commonly-used drugs revealed no interference within normal therapeutic ranges:

Analyte (10 µg/mL Final Concentration)		
Acetaminophen	Chloramphenicol	Nifedipine
Acetylsalicylic acid	Cinnarizine	Nystatin
Adenine	Cyclophosphamide	Oxazepam
Allopurinol	Cyclosporine	Oxytetracycline
Ambroxol	Digitonin	Propranolol
Ampicillin	Digoxin	Theophylline
Ascorbic acid	Dopamine	l-Thyroxine
Atenolol	Erythromycin	Urea
Atropine	Gentistic acid	Uric acid
Caffeine	Isoproterenol	
Captopril	Isosorbide dinitrate	

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 test in 6 replicates. As shown in the data table below, an agreement of 100% was observed between the expected and the observed results at each cTnI concentration.

cTnI added (ng/mL)	Test Results		Agreement with Expected Results
	Negative	Positive	
0	6	0	100%
1.5	0	6	100%
3	0	6	100%

5. Clinical Sample Evaluation

A total of 300 clinically confirmed serum samples, 150 positive and 150 negative, were tested with this device, a 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; 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; 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).

Assay Values	ELISA Assay Results			
	Positive (≥1.5 ng/mL)	Negative (<1.5 ng/mL)	Total	Agreement
	Positive	149	6	155
Negative	4	141	145	95.9%
Total	153	147	300	96.7%

REFERENCES

- Bodor GS. Cardiac Troponins: A Decade of Change in Cardiac Marker Testing. *LabMedica International* 2004; 21(2): 12-14.
- Bodor GS, Porter S, Landt Y, Ladenson JH. Development of monoclonal antibodies for an assay of cardiac troponin-I and preliminary results in suspected cases of myocardial infarction. *Clin Chem.* 1992 Nov;38(11):2203-14.
- Adams JE 3rd, Sicard GA, Allen BT, Bridwell KH, Lenke LG, Dávila-Román VG, Bodor GS, Ladenson JH, Jaffe AS. Diagnosis of perioperative myocardial infarction with measurement of cardiac troponin I. *N Engl J Med.* 1994 Mar 10;330(10):670-4.
- Cummins B, Auckland ML, Cummins P. Cardiac-specific troponin-I radioimmunoassay in the diagnosis of acute myocardial infarction. *Am Heart J.* 1987 Jun;113(6):1333-44.
- Larue C, Calzolari C, Bertinchant JP, Leclercq F, Grolleau R, Pau B. Cardiac-specific immunoenzymometric assay of troponin I in the early phase of acute myocardial infarction. *Clin Chem.* 1993 Jun;39(6):972-9.
- U.S. Department of Labor, Occupational Safety and Health Administration, 29 CFR Part 1910.1030, Occupational Exposure to Bloodborne Pathogens; Final Rule. *Federal Register*; 1991; 56(235): 64175.
- USA Center for Disease Control/National Institute of Health Manual, "Biosafety in Microbiological and Biomedical Laboratories", 1984.
- National Committee for Clinical Laboratory Standards. Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids, and Tissue: 1997 Approved Guideline. *NCCLS Document M29-A.*



Temperature limitation



Use by
YYYY-MM



Batch/Lot code



In vitro diagnostic medical device



Manufacturer



Catalog number



Contains sufficient for
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Consult instructions for use



Do not reuse



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