TOYO®

INSTRUCTION FOR USE

Troponin I Test

For Troponin I (cTnI) Cardiac Marker
Detection in Whole Blood / Serum / Plasma

in vitro diagnostic test

Only for professional *in vitro* diagnostic use

Product Code: TTI01

Troponin I Test detects cardiac marker Troponin I (cTnI) qualitatively or quantitatively in human whole blood / serum / plasma

BACKGROUND INFORMATION

Cardiac Troponin I (cTnI) is a protein found in cardiac muscle with a molecular weight of 22,5 kDa. Troponin I is a part of a three subunit complex compromising of Troponin T and Troponin C. Along with tropomyosin, this structural complex forms the main component that regulates the calcium sensitive ATPase activity of actomyosin is striated skeletal and cardiac muscle. After cardiac injury occurs, Troponin I is released into the blood 4 - 6 hours after the onset of pain. The release pattern of cTnI is similar to CK-MB, but while CK-MB levels return to normal after 72 hours, Troponin I remain elevated for 6 -10 days, thus providing for a longer window of detection for cardiac injury. The high specificity of cTnI measurements for the identification of myocardial damage has been demonstrated in conditions such as the perioperative period, after marathon runs and blunt chest trauma. cTnI release has also been documented in cardiac conditions other than acute myocardial infarction (AMI) such as unstable angina, congestive heart failure and ischemic damage due to coronary artery bypass surgery. Because of its high specificity and sensitivity in the myocardial tissue, Troponin I has recently become the most preferred biomarker for myocardial infarction. Cardiac isoforms of troponin-I (cTnI) are only expressed in cardiac muscle. Although the cTnI is a structural protein that is found in the striated muscle cell, bound the thin filament, a small percentage (3-4%) exists free in the cytoplasm. The increase in troponins (>0.5 ng/ml) were shown to be very sensitive (100%) in the myocardial infarction (AMI).

INTENDED USE

Troponin I Test is a rapid immunochromatographic assay for qualitative or quantitative detection of human cardiac marker Troponin I (cTnI) in human whole blood / serum / plasma to aid diagnosis of myocardial infarction (MI).

REAGENTS

Anti-cTnI antibodies coated particles and capture reagent immobilized on the membrane.

METHOD

Troponin I Test is a rapid, immunochromatographic assay for the detection of cTnI in human whole blood / serum / plasma samples. There is capture reagent immobilized to "T" test area of the test. While performing the test whole blood / serum / plasma sample dropped to the sample well reacts with the particles coated with anti-cTnI antibodies. This complex migrates to the other end of the membrane by capillary action. If there is cTnI at detectable level in the sample, it binds to capture reagent in the "T" test area and create a visible, colored signal that means the test result is positive. If the sample does not contain cTnI at detectable level, colored line does not appear in the "T" test area. This means the test result is negative. As a procedural control, a colored line always appears in the "C" control area indicating that proper volume of sample has been introduced and membrane wicking has occurred.

For quantitative use, the tecnique is gold absorption immunoassay. In gold absorption immunoassay technique a light source shed light on test membrane and a detector collects reflected light. Colloidal gold particles accumulate in test line according to the concentration of the molecule that is looking for in the sample. These colloidal gold particles absorb the light and amount of the absorption can be correlated to molecule concentration in the sample to get quantitative results.

PRECAUTIONS AND LIMITATIONS

1. For professional and *in vitro* diagnostic use only.
2. Do not use test kit beyond expiry date. The test device is single use. Do not reuse.
3. The test device should remain in its original sealed pouch until usage. Do not use the test if the seal is broken or the pouch is damaged.
4. Wear disposable gloves while performing the test.
5. Use a new pipette for each sample.
6. All patient samples should be handled as taking capable of transmitting disease into consideration. Observe established precautions against microbiological hazards throughout all procedures and follow the standard procedures for proper disposal of samples.
7. The test is compatible with only Toyo ICA-Rapid Test Reader. Read the Test Reader Device's manual carefully before use and follow manual strictly when perform the test.
8. Same samples containing unusually high titers of heterophile antibodies or Rheumatoid Factor (RF) may affect expected results. Even if the test results are positive, further clinical evaluation should be considered with other clinical information available to the physician.
9. This test will indicate only the selectively total cTnI in the sample, and should not be used as the only basis for the diagnosis of myocardial infarction.

As with all diagnostic tests, it should be kept in mind that an identification diagnosis can't be based on a single test result. Diagnosis can only be reached by an expert after the evaluation of all clinical and laboratory findings.

10. If the test result is negative and clinical symptoms persist, additional testing using other clinical methods is recommended. A negative result does not at any time preclude the possibility of myocardial infarction.

STORAGE

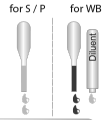
Test device should be kept away from direct sunlight, moisture, heat and radiation sources. Store at 4 - 30°C (39 - 86°F). Do not freeze.
The test in the original packaging retains stable until expiry date at storage conditions. The test device should be used maximum one hour after the foil is opened.
Kit components : Test cassettes, pipettes, diluent (for whole blood samples only) and instructions for use.
Additional materials required but not provided : Sample collection tube, centrifuge and timer.
Additional materials recommended but not provided : Micropipettes to deliver mentioned amount of sample in the test procedure, negative and positive control materials.

SAMPLE COLLECTION AND PREPARATION

The test can be performed using whole blood, serum or plasma. To avoid hemolysis, serum or plasma should be separated from blood as soon as possible.
For Whole Blood Samples: Test should be performed immediately with whole blood samples. Otherwise, whole blood samples should be stored at 2 - 8 °C with anticoagulants (EDTA, heparin, citrate should be used) to avoid coagulation until they are being tested in a period of 2 days after collection.
For Serum Samples: Collect blood into a collection tube without anticoagulant, leave to settle for 30 minutes for blood coagulation and then centrifuge the blood. At the end of centrifuge period remaining supernatant is used as serum.
For Plasma Samples: Collect blood into a collection tube with anticoagulants (EDTA, heparin, citrate should be used) to avoid coagulation of blood sample and then centrifuge the blood. At the end of centrifuge period supernatant is used as plasma.
Do not use turbid, hemolyzed samples. If the sample cannot be tested on the day of collection, store the serum, plasma samples in a refrigerator or freezer. Do not freeze and thaw the serum, plasma samples repeatedly. Do not freeze whole blood sample. Bring the samples to room temperature before testing. Frozen samples must be completely thawed and mixed well prior to testing. Turbid test samples should be centrifuged. Using of frozen and thawed samples should be avoided whenever possible, due to the blocking of the membrane by the debris.

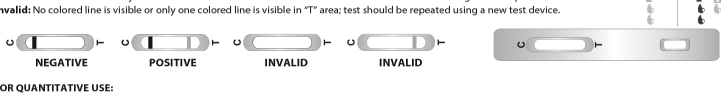
TEST PROCEDURE

1. Bring the tests and whole blood / serum / plasma samples to room temperature. Take the test out of its pouch.
2. **For Whole Blood Samples :** Draw whole blood into pipette and put 2 drops (50 µl) into the sample well of the cassette. Immediately after, 1 drop of diluent is added into the sample well and allowed to soak in.
For Serum / Plasma Samples : Draw serum / plasma into pipette and put 2 drops (50 µl) into the sample well of the cassette. Do not use diluent for serum / plasma samples.
Avoid the formation of any air bubbles.
3. Results should be read at 10 minutes as shown below. Results forming after 20 minutes should be regarded as invalid.



INTERPRETATION OF RESULTS

Negative: Only one colored line is visible in "C" area.
Positive: Two colored lines are visible in "C" and "T" areas.
Low concentration of cTnI may cause a faint line in "T" area. Even such a faint line in "T" area should be regarded as "positive".
Invalid: No colored line is visible or only one colored line is visible in "T" area; test should be repeated using a new test device.



FOR QUANTITATIVE USE:

4. At the end of 10 minutes, put the test into the ICA-Rapid Test Reader and close the cassette chamber slowly. Pay attention to the cassette direction.
5. Perform the measurement process according to the device manual.

Attention: Closing the cassette chamber fast may cause the leftover sample in the sample well after absorption to split and contaminate the reader's optical section resulting with technical malfunctions.
Note: Read the ICA-Rapid Test Reader Device's manual carefully before use. Follow manual strictly when perform the test.

Cut off: 0,5 ng/ml **Linear range:** 0,5-20 ng/ml

Negative : cTnI level is < 0,5 ng/ml
Positive : cTnI level is 0,5 < # < 20 ng/ml or > 20 ng/ml
Invalid : "Control:Invalid" phrase is seen in the result display. Test should be repeated using a new test.

QUALITY CONTROL

Tests have built in procedural quality control features. When the test is complete, the user will see a colored line in the "C" area of the test on negative samples and a colored line in the "T" and "C" area on positive samples. The appearance of the control "C" line is considered as an internal procedural control. This line indicates that sufficient volume of sample was added as well as valid test result. It is recommended that a negative control and a positive control be used to verify proper test performance as an external control. Users should follow appropriate federal, state and local guidelines concerning the external quality controls. In case of using with reader; the user will see "Control:Valid" or "Control:Invalid" phrases in the result display and print to control validity.

PERFORMANCE EVALUATION

Troponin I Test has been evaluated using clinical samples. ELISA methods are used to compare Troponin I Test and following results are obtained.

		Reference	
		+ Result	− Result
Test	+ Result	186	4
	− Result	2	348

Cut off: 0,5 ng/ml
Sensitivity : 99 % Specificity : 98,9 % + Predictive V : 98 % - Predictive V : 99,4 %

Intra Assay

Within-run precision of the same test has been confirmed with samples containing cTnI in the levels 0 ng/ml, 0,2 ng/ml, 0,5 ng/ml, 1 ng/ml, 5 ng/ml. These values were correctly determined for each trial.

Inter Assay

Between-run presicion of the same test has been confirmed with 3 independent assays with the same samples containing cTnI in the levels 0 ng/ml, 0,2 ng/ml, 0,5 ng/ml, 1 ng/ml, 5 ng/ml. These values were correctly determined for each trial.

CROSS REACTIVITY

Serum samples containing 10.000 ng/ml Skletal Troponin I, 2.000 ng/ml Troponin T and 20.000 ng/ml cardiac myosin have been tested with Troponin I Test and no cross reactivity was observed.

INTERFERENCES

Troponin I Test has been tested with potential interfere substances such as; 110 mg/ml human albumin, 6 mg/ml bilirubin, 10 mg/ml hemoglobin 5 mg/ml cholesterol and 15 mg/ml triglycerids trigliserid and no interference was observed.
Troponin I Test has also been tested with following compounds and no interference was observed at a concentration of 50 µg/ml.

Acetominophen	Atorvastatin Calcium	Chlordiazepoxide	Felodipine	Labetalol	Phenobarbital
Acetylsalicylic acid	Bisoproplol Fumarate	Ciazapril	Flunarizine Hydrochloridine	Metoprolol Tartrate	Quinine
Anisodamine	Caffeine	Diclofenac	Furosemide	Moraczine Hydrochloride	Ramipril
Ascorbic acid	Captoprill	Digoxin	Hydrochlorothiazide	Nifedipine	DL-Tyrosine
Atenolol	Chloramphanicol	Erythromycin	Isosorbide Mononitrate	Oxazepam	Trimethoprim
				Pentoxifyline	Verapamil

Hemolytic samples should not be used since they can cause to invalid or false results.

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CE

Manufacturer
Consult instruction for use
Attention, see instruction for use
In vitro diagnostic medical device
For single use only
Number of test
REF Catalog number
Storage temperature
LOT Lot number
Expiry date