

INTENDED USE

The D.D. Troponin I test is a rapid one-step chromatographic immunoassay for the qualitative detection of human cardiac Troponin I (cTnI) in whole blood, serum or plasma as an aid in the diagnosis of myocardial infarction (MI). The test is for professional in vitro diagnostic use only.

A positive result indicates a high risk for myocardial infarction (MI). A negative result, however, does not exclude MI and further follow-up is required including quantitative cardiac troponin testing.

SUMMARY

Cardiac Troponin I (cTnI) is a protein found in cardiac muscle with a molecular weight of 22.5 kDa.¹ Troponin I is part of a three subunit complex further comprising Troponin T and Troponin C. Along with tropomyosin, this structural complex forms the main component that regulates the calcium sensitive ATPase activity of actomyosin in striated skeletal and cardiac muscle.² After cardiac injury occurs, cTnI 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, cTnI levels remain elevated for 6-10 days, thus providing 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.³ 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.⁴ Because of its high specificity and sensitivity in the myocardial tissue, Troponin I has recently become the most preferred biomarker for myocardial infarction.⁵

The D.D. Troponin I test is a simple test that utilizes a combination of anti-cTnI antibody coated particles and capture reagent to detect cTnI in whole blood, serum or plasma. The minimum detection level is 0.5ng/mL.

TEST PRINCIPLE

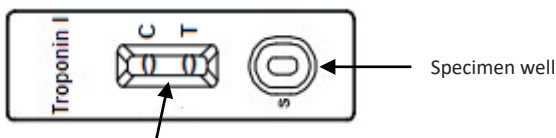
The D.D. Troponin I test is a qualitative, membrane-based immunoassay for the detection of cardiac Troponin I (cTnI) in whole blood, serum or plasma. The membrane is pre-coated with capture reagent on the test line region of the test. After specimen is added to the specimen well of the cassette, it reacts with anti-cTnI antibody coated colloid gold particles in the test. This mixture migrates chromatographically along the length of the test and interacts with the immobilized capture reagent. The test format can detect cTnI in specimens. If the specimen contains cTnI, a colored line will appear in the test line region, indicating a positive result. If the specimen does not contain cTnI, or if the cTnI level is below the detection level of the D.D. Troponin I test, a colored line will not appear in this region, indicating a negative result. To serve as a procedural control, a colored line will always appear in the control line region, indicating that proper volume of specimen has been added and membrane wicking has occurred.

REAGENTS

The test contains anti-cTnI antibody coated colloid gold particles and capture reagent coated on the membrane.

SET UP OF THE TEST DEVICE

In the plastic mould of the test cassette there is a test strip with the coated membrane. On one side you see the round sample well and in the middle the reaction zone with the test line region (T) and the control line region (C).



Reaction zone with test line region (T) and control line region (C) (marked with ovals)

MATERIALS PROVIDED

- Single sealed test devices
- Disposable pipettes (inside the test pouch)
- Dropper vial with buffer
- Instructions for use

MATERIALS REQUIRED BUT NOT PROVIDED

- Specimen collection containers
- Centrifuge (for plasma/serum)
- Heparinized capillary tubes and dispensing bulb (for whole blood from fingertip)
- Lancets (for whole blood from fingertip)
- Timer

STORAGE AND STABILITY

Store the test components cooled or at room temperature (2-30°C / 36-86 °F). DO NOT FREEZE. The D.D. Troponin I test kit is stable until the expiration date printed on the kit label. The test device must remain in the sealed pouch until use. Do not use after the expiration date.

PRECAUTIONS

- For professional in vitro diagnostic use only.
- Read carefully the instruction for use before starting the test procedure.
- Test devices and disposable pipettes for single use only.

- Do not use the test components beyond the printed expiration date.
- The test device is humidity sensitive and must remain in the sealed pouch until ready to use.
- Do not use the test device if the foil pouch is damaged.
- Humidity and high temperature can adversely affect results.
- To avoid any contamination, it is requested to use a new specimen container for any new specimen.
- Do not spill specimen fluid into the reaction zone of the test cassette.
- Do not touch the test membrane to avoid any contamination.
- Do not mix components from different test kits.
- Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout all procedures and follow the standard procedures for proper disposal of specimens.
- By a suspicion of myocardial infarction, the test result should only be a first, additional hint. The patient should be submitted in hospital for further investigation, because it is possible that cTnI concentration in blood is too low for a positive test result or is disturbed by other factors.
- High levels of Biotin (such as supplements marketed for hair, skin, and nail growth) may interfere with the test result. Please consider Biotin interference as a possible error when a test result doesn't match the clinical presentation.

SPECIMEN COLLECTION AND PREPARATION

The D.D. Troponin I test can be performed using whole blood (from venipuncture or fingertip), serum or plasma.

Whole blood from fingertip:

- Wash the hand of the patient with soap and warm water or clean the puncture site thoroughly with alcohol.
- Massage the hand in direction of the fingertip of the middle finger or ring finger without touching the puncture.
- Prick the fingertip with a sterile lancet. Wipe the first drop of blood.
- Rub the hand from the wrist to the palm and to the finger to form a round drop at puncture.
- Take a blood sample filling the capillary with 75 µl of the sample. Avoid air pockets.
- Put the dispensary bulb at the top of the capillary and press it to dribble blood sample in the specimen well (S) of the test device.

General comments

- Separate serum or plasma from blood as soon as possible to avoid hemolysis.
- Use only clear, non-hemolyzed specimens.
- Heparin or citrate blood can be used for the plasma extraction.
- EDTA K2, Heparin sodium, Citrate sodium and Potassium Oxalate can be used as the anticoagulant for collecting the specimen.
- Testing should be performed immediately after specimen collection. Do not leave the specimens at room temperature for prolonged periods. Serum and plasma specimens may be stored at 2-8° C for up to 3 days. For long-term storage, specimens should be kept below -20°C. Whole blood collected by venipuncture should be stored at 2-8°C if the test is to be run within 2 days of collection. Do not freeze whole blood specimens. Whole blood collected by fingertip should be tested immediately.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly.
- If specimens are to be shipped, they should be packed in compliance with local regulations covering the transportation of etiologic agents.

TESTING PROCEDURE

Allow the D.D. Troponin I test device, buffer, specimen and/or controls to equilibrate to room temperature (15-30°C / 59-86 °F) prior to testing.

1. Adjust the timer to **10 minutes**.
2. Remove the test device from the sealed pouch and use it as soon as possible. Best results will be obtained if the assay is performed within one hour. Place the test device on a clean and level surface.
3. **Serum or plasma:**
Hold the provided pipette vertically and transfer **3 drops of serum or plasma** into the round specimen well (S) of the Troponin I test and start the timer.

Venipuncture Whole Blood:

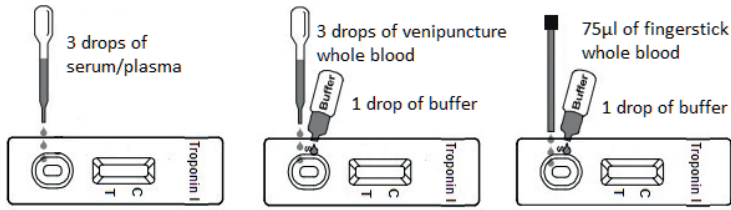
Hold the provided pipette vertically and transfer 3 drops of whole blood (or Heparin or citrate whole blood) into the round specimen well (S) of the Troponin I test. Add 1 drop of buffer and start the timer.

Fingertip Whole Blood:

Fill the capillary tube and transfer approximately 75 µl of fingertip whole blood specimen to the specimen well of test cassette. Add 1 drop of buffer and start the timer.

Note: It is suggested not to use the buffer beyond 6 months after opening the vial.





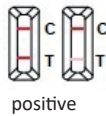
4. Wait for the red line(s) to appear. The result should be read at 10 minutes.
Do not interpret the result later than 20 minutes after sample addition.

INTERPRETATION OF RESULTS

Negative:
One red line appears in the control line region (C).
No apparent red line appears in the test line region (T).
Negative results should be confirmed after 20 minutes.

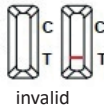


Positive:
Two distinct red lines appear. A line forms in the control line region (C) and another line forms in the test line region (T).



Note: The intensity of the red color in the test line region (T) will vary depending on the concentration of cTnI present in the specimen. Therefore, also faint reddish test lines (T) should be considered positive.

Invalid:
The control line (C) is not formed. In this case the test result is invalid even if the test line is visible. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the test procedure and repeat the test with a new test device. If the problem persists, discontinue using the test kit immediately and contact your local distributor.



QUALITY CONTROL

As internal procedural control the D.D. Troponin I test includes the control line. It forms only if sufficient specimen volume has been added and the chromatography has been finished successfully. External controls are not supplied with this kit.

LIMITATIONS

- The D.D. Troponin I test is for professional in vitro diagnostic use and should only be used for the qualitative detection of cardiac Troponin I in whole blood, serum or plasma specimen. The colour intensity or width of the lines must not influence the test evaluation at all. Neither the quantitative value nor the rate of increase in cTnI can be determined by this qualitative test.
- The Troponin I test will only indicate the presence of cTnI in the specimen and should not be used as the sole criteria for the diagnosis of myocardial infarction.
- If the test result is negative and clinical symptoms persist, additional testing using other clinical methods is recommended. The minimum detection limit of the assay is 0.5ng/ml of cTnI in specimens. Thus, a negative result does not at anytime rule out the existence of Troponin I in blood, because the protein concentration may be below the minimum detection level of the test. Please keep in mind that the rise of Troponin I takes place several hours after the onset of pain. If the testing takes place too early, cTnI concentrations might still be too low to be detected by the assay. A negative test result does not exclude the possibility of myocardial infarction at any time.
- Like with all diagnostic tests, a final diagnosis should only be made by a physician, after all clinical and laboratory findings have been evaluated.
- Some specimens 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.
- In rare cases it is possible that the antigen-antibody reaction of the test is inhibited by the presence of autoantibodies in the patient's blood, which block the binding sites. False negative test results might be the consequence. Please note that this is a general problem with all detection methods based on an antigen-antibody reaction for the detection of proteins.
- Before starting the test procedure, please check if the patient regularly uses supplements marketed for hair, skin, and nail growth. Supplements containing Biotin may interfere with the test result. Please consider Biotin interference as a possible error when a test result doesn't match the clinical presentation.
- There is a slight possibility that some whole blood specimens with very high viscosity or which have been stored for more than 1 day may not run properly on the test cassette. Repeat the test with a serum or plasma specimen from the same patient using a new test cassette.
- The hematocrit of the whole blood should be between 25% and 65%.

EXPECTED VALUES

The D.D. Troponin I Test has been compared with a leading commercial cTnI Chemiluminescence immune assay, demonstrating an overall accuracy of 99.1%. With a cTnI value >0.4ng/ml a myocardial disease or an acute myocardial infarction cannot be excluded.

PERFORMANCE CHARACTERISTICS

Sensitivity and Specificity

The D.D. Troponin I Test has been evaluated with a leading commercial cTnI Chemiluminescence immune assay using clinical specimens. The results show that the sensitivity of the D.D. Troponin I Test is 97.6% and the specificity is 99.4% relative to the CLIA test.

Method	CLIA		Total Results
	Results		
D.D. Troponin I Test (Whole Blood/ Serum/Plasma)	Positive	83	85
	Negative	2	
Total Results		358	360

Relative sensitivity: 97.6% (95%CI*: 91.8%~99.7%);
Relative specificity: 99.4% (95%CI*: 98.0%~99.9%);
Accuracy: 99.1%(95%CI*: 97.7%~99.8%).

*Confidence Intervals

Precision

Intra-Assay

Within-run precision has been determined by using 15 replicates of five specimens: a negative, cTnI 1.0ng/mL positive, cTnI 5.0ng/mL positive, cTnI 10ng/mL positive and cTnI 40ng/mL positive. All cTnI negative and positive values were correctly identified >99% of the time.

Inter-Assay

Between-run precision has been determined by 15 independent assays on the same five specimens: a negative, cTnI 1.0ng/mL positive, cTnI 5.0ng/mL positive, cTnI 10ng/mL positive and cTnI 40ng/mL positive specimens. Three different lots of the Cardiac Troponin I Rapid Test Cassette (Whole Blood/Serum/Plasma) have been tested over a 3-day period using negative, cTnI 1.0ng/mL positive, cTnI 5.0ng/mL positive, cTnI 10ng/mL positive and cTnI 40ng/mL positive specimens. The specimens were correctly identified >99% of the time.

Cross-reactivity

The Troponin I test has been tested by 10,000ng/mL Skeletal Troponin I, 2,000ng/mL Troponin T, 20,000ng/mL Cardiac Myosin, HBsAg, HBsAb, HBeAg, HBeAb, HBcAb, syphilis, anti-HIV, anti-H.pylori, MONO, anti-CMV, anti-Rubella and anti-Toxoplasmosis positive specimens. The results showed no cross-reactivity.

Interfering Substances

The following potentially interfering substances were added to cTnI negative and positive specimens.

Acetaminophen: 20 mg/dL	Albumin: 10,500mg/dL	Caffeine: 20 mg/d
Acetylsalicylic Acid: 20 mg/dL	Bilirubin: 1,000mg/dL	Creatin: 200 mg/dL
Ascorbic Acid: 20mg/d	Gentisic Acid: 20 mg/dL	Oxalic Acid: 600mg/dL
Cholesterol: 800mg/dL	Hemoglobin 1,000 mg/dL	Triglycerides: 1,600mg/dL

None of the substances at the concentration tested interfered in the assay

LITERATURE

- Adams, et al. Biochemical markers of myocardial injury, Immunoassay Circulation 88: 750-763 (1993).
- Mehegan JP, Tobacman LS, Cooperative interaction between troponin molecules bound to the cardiac thin filament. J. Biol. Chem. 266:966 (1991)
- Adams, et al. Diagnosis of Perioperative myocardial infarction with measurements of cardiac troponin I. N.Eng.J. Med 330:670 (1994).
- Hossein-Nia M, et al. Cardiac troponin / release in heart transplantation. Ann.Tho rac. Surg. 61 : 227 (1996).
- Joint European Society of Cardiology/American College of Cardiology: J.Am. Coll. Cardio., 36(3), „Myocardial Infarction Redefined,“ 2000.
- FDA. The FDA warns that biotin may interfere with lab tests; FDA safety communication.

SYMBOLS

	CE mark according to the requirements of Annex III of the European Directive 98/79 EC		Catalogue number
	In vitro diagnostic medical device		For single use only
	Content sufficient for <n> tests		Use by
	Batch code		Temperature limitation
	Manufacturer		Consult instruction for use

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