In vitro Nano-Check™ AMI cTnI Cardiac Marker Test

One Step Immunoassay for Cardiac Troponin I (cTnI)

For in vitro Diagnostic Use

One–step immuno-chromatographic assay for the detection of cTnI in human whole blood, serum, and plasma

1. INTENDED USE

The Nano-Check™ AMI cTnI Test is a rapid immunoassay for the determination of Cardiac Troponin I (cTnI) in human whole blood, serum and plasma specimen at the cutoff concentration of 0.5 ng/ml as an aid in the diagnosis of Acute Myocardial Infarction (AMI). In conjunction with Nano-Checker 710 reader, the Nano-Check™ cTnI Test can monitor the rise and fall of cTnI. Test results should be interpreted by the physician along with other test results and patient clinical symptoms findings.

2. SUMMARY AND EXPLANATION OF THE TEST

When a myocardial infarction (MI) occurs in the hypoperfused region of the myocardium, oxygen can no longer be supplied to the cells in the region. Cell death is inevitable if oxygen is not restored within 10-15 minutes and will result in the release of certain proteins from within cytoplasm into the blood stream. Some proteins are exclusive to and predominant in the cardiac muscle cells; they can function as cardiac makers and be detected in the blood specimens of AMI patients by specialized immunoassays.[1-3] Cardiac troponin I is one of the specific biochemical maker for detecting early stage of AMI , unstable angina(UA), and congestive heart failure(CHF).

Troponin I

Troponin is a contractile regulatory protein complex found in skeletal and cardiac muscle. The troponin complex consists of three distinctive polypeptide components, troponin I (TnI), troponin T (TnT), and troponin C (TnC), and plays a fundamental role in the transmission of intracellular calcium signal actin-myosin interaction.[5] TnC of cardiac tissues is identical to that in skeletal tissues, but TnI and TnT of cardiac isoforms are distinctive to those of skeletal isoforms, which enables the development of cardiac specific antibodies.[6] Moreover, cTnI level becomes elevated in the blood as a result of myocardial injury or necrosis. Therefore, cTnI is used as an aid in the diagnosis of myocardial infarction.[7-8] Studies on the release kinetics indicate that cTnI is not early marker of myocardial necrosis. It appears in serum within 3-6 hours after symptom onset, similar to the release of CK-MB. However, cTnI remains elevated for 4-9 days post-AMI and 13 times more abundant in the myocardium than CK-MB.[9-10] In addition to its utility in diagnosis, elevated cTnI levels convey prognostic information and has been shown to identify patients having an increased risk of death.[11]

3. PRINCIPLE

The Nano-Check™ AMI cTnI Test is an immuno-chromatography assay for the determination of cTnI in whole blood, serum and plasma. The membrane strip contains one test line and one control line; streptavidin for biotinylated cTnI antibody and rabbit anti-goat IgG antibody for the control line. A dye pad is placed at the end of the membrane containing biotinylated cTnI antibody and gold colloidal particles coupled with cTnI antibody. When a sample is applied into the sample well, the cardiac makers present in the sample bind to the specific antibodies coupled with gold particles. cTnI in a sample binds to both cTnI specific dye coupled antibody and biotinylated antibody. The immune complexes move along the nitrocellulose membrane through the test lines and bind to streptavidin immobilized on the test line. Unbound immune complexes pass through the test line and are captured by rabbit anti-goat IgG antibody in the control line.

If the concentration of this marker in the sample is above the cutoff level, red bands appear at the corresponding test lines and the control line. If the concentration of the marker in the sample is lower than the cutoff level, only the colored control line can be seen in the test window. This colored control band must always appear at the control line position (Con) for valid test results. A test result is not valid if the colored control line does not appear in the test window.

To measure the concentration of cTnI, the tested device should be read by Nano-Checker 710 Reader. The reader can analyze color intensity of the test line and convert it to concentration of the cTnI in the specimen by the predetermined equation.

4. REAGENT

The Nano-Check™ AMI cTnI Test contains all the reagents necessary for the detection of cTnI in human whole blood, serum and plasma. The device contains a membrane strip coated with streptavidin on the test line and dye pad infused with biotinylated polyclonal goat anti-cTnI antibody and gold colloidal particles coupled with anti-cTnI specific antibody. A stabilizer containing 0.05% sodium azide and BSA protein are deposited on the dye pad in dried form

5. MATERIALS

Provided

- Nano-Check™ cTnI Test device containing membrane strip in a sealed pouch with desiccant
- Instructions for Use
- Sample transfer pipette (if applicable)

Required but not provided

- Whole blood, Serum or Plasma Collection Container
- Positive and negative quality control materials
- Timer
- Nano-Checker 710 or equivalent Nano-Checker Reader (For quantitative analysis)

6. STORAGE AND STABILITY

The test kit should be stored at 2°C - 30°C in the original sealed pouch for the duration of shelf life.

7. PRECAUTIONS

- For *in-vitro* diagnostic and professional use only.
- Handle all specimens as potentially infectious. Proper handling and disposal methods should be established.
- To avoid cross contamination, use a fresh transfer device for each clinical sample tested.
- Do not use test kit if the pouch is damaged or improperly sealed.
- Do not use test kit beyond expiration date.

8. SPECIMEN COLLECTION AND PREPARATION

- This test can be used for whole blood, plasma, and serum samples. If serum samples are to be used, collect the blood in a tube without anticoagulant and allow clotting for at least 25 minutes before centrifugation. Whole blood or plasma samples using heparin or EDTA as the anticoagulant can be used for testing with this product. Other blood anticoagulants have not been evaluated. Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products. Variation in these products may exist between manufacturers and, at times, from lot-to-lot.
- The samples should be collected under standard laboratory conditions.
- Optimal results were obtained when patient samples were tested immediately after collection. Whole blood samples should be used within 4 hours after collection. Plasma or serum samples may be refrigerated for 24 hours at 2-8°C. If testing cannot be performed within 24 hours, or for shipment of samples, freeze at -20°C or colder.[12,13]
- Sodium azide can be added as a preservative up to 0.1% without affecting the test results.
- Refrigerated or frozen serum or plasma specimen should reach room temperature and be homogeneous prior to testing

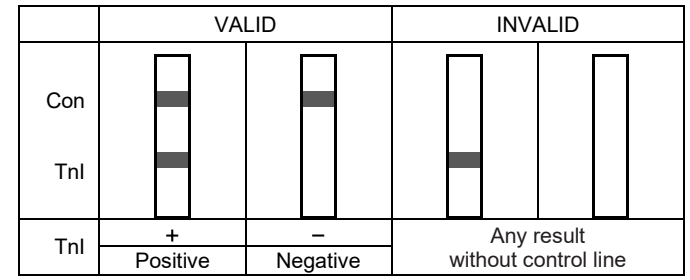
9. TEST PROCEDURE AND PROTOCOL

1. Collect specimen according to instructions in “Specimen Collection”.
2. Test device and sample should be brought to room temperature (20°C-30°C) prior to testing.
3. Remove the test device from the sealed pouch immediately before use. Label the device with patient or control identification.
4. Using sample transfer pipette, deliver dropper contents (80µl) of sample into the sample well.
5. Read the results at 15 minutes. For qualitative interpretation of results, please see section below, “Interpretation of Results”. Do not interpret results after 15 minutes. For the quantitative result, the tested device should be analyzed by the Nano-Checker 710 reader following by the instruction manual.

10. INTERPRETATION OF RESULTS

Qualitative Analysis

The results of the Nano-Check™ cTnI Test are determined visually and interpreted according to the predetermined cutoff values of 0.5 ng/ml for cTnI. The cutoff level of 0.5 ng/ml was determined by comparison to the quantitative assay system of Beckman Coulter, Access AccuTnI Assay. This cutoff level may be different if compared to a quantitative assay system other than Beckman Coulter. We recommend that users should establish a correlation if a quantitative assay system other than Beckman Coulter Access is used.



Negative: A single red colored band at the control area (Con) without a band at test line (cTnI) is a valid negative result and indicates the concentrations of cTnI in the sample is below the cutoff levels.

Positive: Appearance of red colored band at the control area (Con) and appearance of red colored band at the test line indicate that concentrations of cTnI in the sample is at or above the cutoff level. The intensity of red color in the test line may be weaker or stronger than that in the control line.

Invalid: If no colored band appears in the control area in 15 minutes (Con), the test result is invalid. The test result is inconclusive, and the assay should be repeated.

Note:

- Very faint band in the test line indicate that the cTnI in the specimen is near the cutoff level. The samples should be re-tested 1-2 hours later or test results should be confirmed by Nano-Checker 710 Reader or other quantitative assay.
- Do not interpret the results after 15 min.

Quantitative Analysis

The signal intensity of test line can be analyzed by Nano-Checker 710 Reader and reported as a concentration of analytes in the tested specimen. When the test result is valid and measured value is in the range of reference value, the result can be interpreted as a negative. The value is above the reference range but below cutoff value, the specimen should be retested with the sample collected later. The reading value is above the cutoff value, the result can be interpreted as a positive.

11. LIMITATIONS

- The test is for professional and in-vitro diagnostic use only.
- A positive test result may only be used as an indicator of myocardial damage and requires further confirmation. Serial sampling of patients suspected of AMI at multiple time points is also recommended due to the delay between onset of symptoms and the release of cardiac marker proteins into the blood stream.
- As with all diagnostic tests, a definitive clinical diagnosis should not be made based on the results of a single test. The test result should be used in conjunction with other clinical information such as clinical signs and symptoms and other test results to diagnose AMI. Confirmation of test results should only be made by a physician along with clinical symptoms and laboratory findings.
- Samples containing unusually high titers of certain antibodies such as human anti- mouse or human anti-rabbit antibodies have been known to affect the performance of this device.[14] However, these studies using the Nano-Check™ AMI cTnI Test have not been performed.
- Patients taking more than 30 µg/day of biotin may have falsely negative results and should not use this test, unless it is confirmed that the patient is not taking more than 30 µg/day of biotin.

12. QUALITY CONTROL

The presence of a reddish colored band in the Control area of the window acts as an internal control to ensure that an adequate volume of sample has been added. In the absence of this Control band, the test is invalid and must be repeated. Good laboratory practice recommends the use of control materials to ensure proper kit performance. Quality control specimens are available from commercial sources and should be assayed using the same procedures followed when running patient samples. Controls should minimally be run before using each new lot of Nano-Check™ AMI cTnI Test, at regular intervals afterwards and any time the validity of the test results are questioned. For the calibration of Nano-Checker 710 Reader, two

different levels of calibration cards are supplied with the reader. The reader should be calibrated periodically with the provided calibration card. If the reading value of calibration card is out of the described range, it should be recalibrated.

13. EXPECTED VALUES

The cutoff values of the Nano-Check™ AMI cTnI Test were determined by comparison to the Beckman Coulter quantitative assay, Access AccuTnI Assay. The cutoff level of TnI is 0.5 ng/ml. The specimens containing cTnI at the concentration of equal or above established cutoff level will give positive result using the Nano-Check™ AMI cTnI Test. The cutoff level may be different if a quantitative assay system other than Beckman Coulter Access is used.

14. PERFORMANCE CHARACTERISTICS

1. Assay Cutoff

Patient plasma containing cTnI, were diluted in normal human serum to the concentration at or near the cutoff level. Analyte concentrations in the diluted samples were confirmed by Quantitative Assay, Access AccuTnI of Beckman Coulter. Fifteen devices were tested for each sample at concentrations of 0.31, 0.57 and 1.07 ng/ml cTnI. The results are shown in following table. The cutoff concentration was assigned as 0.5 ng/ml for cTnI.

Analyte	Conc. determined by Access Assay (ng/ml)	Test Result Negative(n)	Test Result Positive(n)	Cutoff level
cTnI	0.31	7	8	0.5 ng/ml
	0.57	0	15	
	1.07	0	15	

2. Recovery Studies

Recovery studies were performed with patient serum diluted in normal human serum. Patient serum containing high levels of cTnI was sequentially diluted with normal human serum to yield different concentrations. Each diluted sample was tested using Nano- Check™ AMI cTnI Test in 3 replicates. The data shown in the table below demonstrates recovery rate between observed results and expected results at each concentration of cTnI.

Analyte	Expected Concentration (ng/ml)	Determined Average Concentration	Agreement % of Expect Values	Total Recovery (%)
cTnI	22.4	24.17	107.8	104.2
	11.2	10.17	90.8	
	5.6	5.00	89.3	
	2.8	3.60	128.6	
	1.4	1.87	133.3	
	0.7	0.80	114.3	
	0.35	0.37	105.6	
	0.18	0.13	76.2	

3. Limit of Detection for Quantitative assay

The analytical sensitivity for each analyte was determined according to CLSI guidance, EP17-A.

Analyte	LoB (ng/ml)	LoD (ng/ml)	LoQ (ng/ml)
cTnI	0.08	0.1	0.1

4. Reportable range for Quantitative assay

Reportable range of each analyte, cTnI or Myoglobin was determined by Linearity study. Samples prepared by serial dilution was tested in triplicate, and data was analyzed by regression analysis.

Analyte	Reportable range (ng/ml)
cTnI	0.1 - 30

5. Analytical Specificity

Potentially interfering substances were spiked into normal serum and patient serum containing cTnI about 1.5 times of the cutoff concentration. The substances at the following level do not interfere with the performance of the Nano-Check™ AMI cTnI Test.

	Substance	Concentration
Endogenous substances	Bilirubin	50 mg/dl
	Hemoglobin	4000 mg/dl
	Human serum albumin	10 g /dl
	Triglycerides	1,250 mg/dl
	Biotin, Vitamin B7	300 ng/ml

The device was tested for interference by potentially cross-reacting endogenous proteins. Potentially cross-reacting proteins, added into normal human serum up to the following concentrations, do not interfere with test result.

	Substance	Concentration
Cross-reacting endogenous proteins	Cardiac myosin light chain	1,000 ng/ml
	Cardiac Troponin T	1,000 ng/ml
	Cardiac Troponin C	1,000 ng/ml
	Skeletal Troponin I	1,000 ng/ml

6. Reproducibility / Precision Test for Qualitative Assay

Two Clinical sites and one in-house operator were provided with blindly labeled serum samples. Patient serum samples, containing high levels of cTnI were diluted in normal human serum to make positive samples of different concentrations. A normal human serum sample was also provided as negative control of the test. Five aliquots from each sample were tested at each testing site. The results shown in the table below demonstrate 100% agreement for between run as well as for within run.

Analyte	Testing Site	Concentration of each analyte (ng/ml) in each sample and Nano-Check™ cTnI Test Precision Testing Result						
		Sample 1		Sample 2		Sample 3		% agreement within run
		cTnI	0	cTnI	1.03	cTnI	2.05	
		-	+	-	+	-	+	
cTnI	Site I	5	0	0	5	0	5	100%
	Site 2	5	0	0	5	0	5	100%
	Site 3	5	0	0	5	0	5	100%
	% agreement between run	100%		100%		100%		

7. Precision / Reproducibility Test for Nano-Check™ AMI cTnI Quantitative Assay

Reproducibility of the Nano-Check™ AMI cTnI quantitative assay system with Nano-Checker 710 Reader was determined in a study using plasma based in-house control materials. Specimens at each level were tested in duplicate for 10 days. The within run and total standard deviation were calculated by the analysis of variance method.

Analyte	Samples	Mean (ng/ml)	SD within- run	Total CV (%)
CTnI	Level 1	0.66	0.15	22.85
	Level 2	2.54	0.32	12.76
	Level 3	18.21	2.31	12.67

8. Correlation Assay between Plasma and Serum Sample

Patient samples were prepared for matched samples of serum and heparinized plasma. Samples were grouped as four different levels; 1.2-1.8, 0.5-1.0, 0.3-0.5 and <0.06 ng/ml of cTnI. Ten samples in each group were tested using Nano-Check™ AMI cTnI Test, and the results demonstrated in the following table:

Analyte	Conc. range (ng/ml)	Serum Positive	Serum Negative	Plasma Positive	Plasma Negative
TnI	1.2-1.8	10	0	10	0
	0.5-1.0	10	0	10	0
	0.3-0.5	9	1	7	3

9. Correlation Assay between Whole blood and Plasma Sample.

To test correlation of assay results between plasma and whole blood samples in Nano- Check™ AMI cTnI qualitative test, normal whole blood samples were spiked with clinical specimens containing cTnI to make three different desired levels between negative and 4 times then cut off values of cTnI. Five samples in each group were tested using Nano-Check™ AMI cTnI Test, prior to removal of cells and after removal of cells by centrifugation. The test results are demonstrated in the following table:

Analyte	Conc. range (ng/ml)	Plasma Positive	Plasma Negative	Whole Blood Positive	Whole Blood Negative
TnI	1.3-2.4	5	0	5	0
	0.5-1.0	5	0	5	0
	0.1-0.47	3	2	2	3

To perform matrix comparison study between plasma and whole blood in Nano-Check™ AMI cTnI quantitative test, ten different levels of analyte concentrations in reportable range by spiking analyte proteins into whole blood collected from healthy volunteers. Corresponding plasma specimens were prepared from each level of whole blood specimens by centrifugation. Each sample was tested on Nano-Check™ cTnI device in triplicates. The concentrations in matched sample matrix were analyzed using correlation and regression methods. The values of correlation coefficients (0.94-0.95) suggest strong correlation between plasma and whole blood specimens or between plasma specimens treated with different anticoagulants.

Matrix	Intercept	Slope	Correlation Coefficient
Lithium Heparin Plasma vs. Whole Blood	0.0321	0.9575	0.951
EDTA Plasma vs. Whole Blood	-0.0436	0.9413	0.936
Lithium Heparin Plasma vs. EDTA Plasma	0.0267	0.9175	0.948

10. Method Comparison Study Qualitative test

Plasma samples were collected from 206 emergency room patients who were admitted because examination results suggested a cardiac event. Additionally, 50 samples were collected from outpatients who were not suspected of having a cardiac event. The 256 clinical samples were tested using the Nano-Check™ AMI cTnI Test and the Beckman Coulter Access test system. Results are summarized below.

AMI cTnI Test Results Compared to Quantitative Access Results (ng/ml)

		Access Accu TnI Test Result			
		<0.01 - 0.29	0.3-0.47	0.52-0.6	0.61->100
Nano-Check™ cTnI	Positive	1	7	6	91
	Negative	143	6	2	0

Quantitative test

A comparison of cTnI values of heparin plasma samples with Nano-Check™ AMI cTnI assay system using Nano-Checker 710 Reader and Beckman Coulter Access system was carried out. The results of cTnI values obtained by two different methods were analyzed to give the following statistical data.

Analyte	n	Ranges of Observation (ng/ml)	Intercept	Slope Coefficient	Correlation
cTn I	67	0.06-44.80	0.5348	0.9175	0.948

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