Fluoro-Check™ AMI cTnI Test

For in vitro Diagnostic Use

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

1. INTENDED USE

The Fluoro-Check™ AMI cTnI Test is a time resolved fluorescence immunoassay for the quantitative determination of Cardiac Troponin I (cTnI) in human whole blood, serum, and plasma specimen at detection limit concentration of 0.03 ng/mL as an aid in the diagnosis of acute myocardial infarction (AMI) and cardiac muscle damage. In conjunction with Fluoro-Checker™ TRF Reader, Fluoro-Check™ AMI 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 cytoplasm into the blood stream. Some proteins are exclusive to and predominant in the cardiac muscle cells; they can function as cardiac markers and be detected in the blood specimens of AMI patients by specialized immunoassays.[1-4] Cardiac troponin I is one of the specific biochemical markers for detecting early stage of AMI, unstable angina (UA), and congestive heart failure (CHF).

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 cTnI and cTnT 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 Fluoro-Check™ AMI cTnI Test is an immuno-chromatography assay for the quantitative 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 goat anti-chicken IgY antibody for the control line. A dye pad is placed at the end of the membrane containing biotinylated cTnI antibody and fluorescence particles coupled with cTnI antibody. When a sample is applied into the sample well, the cardiac markers present in the sample bind to the specific antibodies coupled with fluorescence particles. cTnI in 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 IgY coupled with fluorescence particles are captured by goat anti-chicken IgY antibody in the control line.

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

4. REAGENT

The Fluoro-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 monoclonal anti-cTnI antibody and fluorescence 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

- 20 Test devices in sealed aluminum foil pouch with desiccant
- 20 Disposable droppers
- Instructions for Use
- QR card

Required but not provided

- Whole blood, Serum, or Plasma Collection Container
- Positive and negative quality control materials
- Timer
- Fluoro-Checker™ TRF Reader

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, serum, and plasma 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 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. Plasma or serum samples may be refrigerated for 24 hours at 2-8ºC. If testing cannot be performed within 24 hours, or for shipment of samples, freeze at -20ºC 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

a) Collect specimen according to instructions in "Specimen Collection".
b) Test device and sample should be brought to room temperature prior to testing.
c) Remove the test device from the sealed pouch immediately before use. Label the device with patient or control identification.
d) Using sample transfer pipette, deliver dropper contents (80 µL) of sample into the sample well.
e) Read the results at 15 minutes. The tested device should be analyzed by the Fluoro-Checker™ TRF Reader following by the instruction manual.

10. INTERPRETATION OF RESULTS

The signal intensity of test line can be analyzed by Fluoro-Checker™ TRF Reader and the results are expressed as TnI concentration using predetermined calibration curves specific for Fluoro-Check™ AMI cTnI Test. A cutoff of 0.50 ng/mL cTnI is recommended for diagnosis of AMI. A test result lower than the laboratory reference range, 0.06 ng/mL, is considered "negative" for AMI[15]. Values between higher than laboratory reference range (≥ 0.06 ng/mL) and less than cutoff for AMI (< 0.5 ng/mL) is considered "intermediate". However, diagnosis of AMI should be determined with other test results and symptoms of patients, including serial testing of cTnI for monitoring rise and fall of cTnI value.

11. LIMITATIONS

- The test is for professional and *in-vitro* diagnostic use only.
- Test result obtained by current device 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 Fluoro-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 conformed that the patient is not taking more than 30 µg/day of biotin.

12. QUALITY CONTROL

The presence of fluorescence 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 Fluoro-Check™ AMI cTnI Test, at regular intervals afterwards and any time the validity of the test results are questioned.
For the calibration of the assay performance, QR card is supplied with the assay kit. Refer to the Fluoro-Checker™ TRF Reader for QR card.

13. CLLINICAL CUTOFF AND REFERENCE RANGE

The clinical cutoff of the Fluoro-Check™ AMI cTnI Test, 0.5 ng/mL, was determined by feasibility study of ROC analysis, and performance comparison with the Access AccuTnI™ Assay (Beckman Coulter). The reference range of the assay was determined as < 0.06 ng/mL, which is the laboratory reference range of TnI[15].

14. PERFORMANCE CHARACTERISTICS

1. Detection limits

Limit of Blank (LoB), Limit of Detection (LoD) and Limit of quantification (LoQ) studies were performed according to NCCLS guideline EP17-A. The LOB determination was determined by testing 120 replicates of a blank sample. Plasma samples were collected from 10 cardiac asymptomatic individuals. To determine LoD, seven levels of low positive samples (concentration of 0.0125 ng/mL – 0.0275 ng/mL) were tested with 14 replicates per each level. Tentative concentration that is calculated to exceed the LoB was verified in 8 replicates per run across 5 days. The LoQ was determined to be reliably detected concentration (equal to or greater than LoD) and assay precision criteria of less than 20%.

The determined LoB, Verified LoD and determined LoQ are summarized below:

	LoB	LoD	LoQ
Concentration(ng/mL)	0.01 ng/mL	0.03 ng/mL	0.03 ng/mL

2. Linearity / assay reportable range

Linearity studies of Fluoro-Check™ AMI cTnI Test were conducted as instructed from the NCCLS guideline, EP6-A. Data set was collected with samples covering dynamic range of Fluoro-Check™ AMI cTnI assay system and it was confirmed that the linear model was capable of interpolating between the experimental points.

Fluoro-Check™ AMI cTnI Test was demonstrated to be linear from 0.03 ng/mL to 30 ng/mL, with 12.7% repeatability within this interval.

3. Interference & specificity test

Potentially interfering substances were spiked into normal serum and patient serum containing cTnI about 2 times of the LoQ concentration. The substances at the following level do not interfere with the performance of the Fluoro-Check™ AMI cTnI Test.

	Substances	Concentration
Endogenous substances	Human serum albumin	5 g/dL
	Hemoglobin	4 g/dL
	Trinitroglycerin	1.25 g/dL
	Bilirubin	50 mg/dL
	Biotin, Vitamin B7	300 ng/mL
Potentially cross-reacting endogenous proteins	TnC	1,000 ng/ mL
	Skeletal TnI	1,000 ng/ mL

The following medicines and chemicals were proven to be not interfering to Fluoro-Check™ AMI cTnI performance.

Acetaminophen	Erythromycin	PCP
Acetylsalicylic Acid	Fluoxetine	Phenobarbital
Allopurinol	Furosemide	Phenytoin
Ampicillin	Hydrocodone	Probenecid
Ascorbic Acid	Ibuprofen	Procainamide
Caffeine	Indomethacin	Propranolol
Captopril	Metoprolol	Quinidine
Chloramphenicol	Morphine	Sulfamethoxazole
Digoxin	Nicotine	Theophylline
Diltiazem	Nitrofurantoin	Verapamil
Dipyridamole	Oxytetracycline	Warfarin
Dopamine		

4. Precision Test

Precision of the Fluoro-Check™ AMI cTnI Test with Fluoro-Checker™ TRF Reader was determined according to NCCLS guideline EP5-A. The study included 3 plasma samples, with concentration of 0.1 ng/mL, 0.4 ng/mL and 4 ng/mL. Test was conducted with 150 replicates per sample across 10 replicates per run for 15 days. The within-run and total precision data are summarized in the table below. The results shown in the table below show that the total imprecision range for the three tested level of TnI controls were located between 11.5% and 13.6%.

Assay precision was also investigated using whole blood samples.

Analyte	Mean (ng/mL)	Within Run		Total Run	
		SD (ng/mL)	CV (%)	SD (ng/mL)	CV (%)
cTnI	0.111	0.014	12.8	0.015	13.6
	0.402	0.049	12.1	0.049	12.4
	4.173	0.487	11.7	0.481	11.5

Whole blood collected from 3 healthy subjects was aliquoted as 3 vials, and each vial was spiked with different amounts of standard cTnI material. Expected cTnI concentration in each vial was 0 ng/ mL (negative), 0.5 ng/mL (positive - low level), or 6.0 ng/ mL (positive - high level). Each sample was tested on Device, Fluoro-Check™ AMI cTnI Test, by running 8 replicates for negative sample or each 16 replicates for each positive samples. The coefficient of variation (CV) of within run (within subject) was 13.0% (low level) or 13.9% (high level), and total precision was 13.4% (low level) and 14.0% (high level). The data of whole blood sample was comparable to precision data using plasma sample.

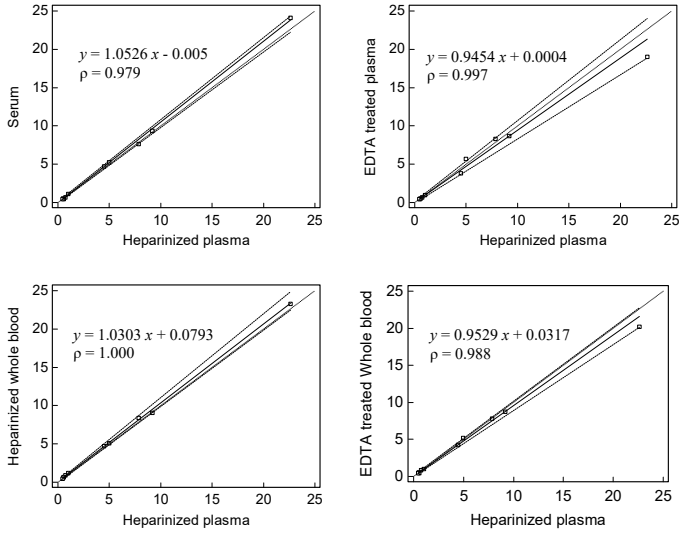
Analyte	Mean (ng/mL)	Within Subject		Total	
		SD (ng/mL)	CV (%)	SD (ng/mL)	CV (%)
cTnI	< 0.03	N/A	N/A	N/A	N/A
	0.515	0.067	13.0	0.069	13.4
	6.230	0.866	13.9	0.872	14.0

5. Matrix Comparison Study

Available sample type was investigated by matrix comparison study. Whole blood was collected from five subjects, each specimen was divided into two vials, and spiked with cTnI of different amount. The target concentration of cTnI were set differently for each specimen, ranges between 0.5 ng/mL – 10 ng/mL. Then, each vial of cTnI spiked specimen was treated differently to prepare 5 sample types: serum, heparinized whole blood, heparinized plasma, EDTA treated whole blood and EDTA treated plasma. Each sample of matched matrices was tested on Fluoro-Check™ AMI cTnI Test, and the results were evaluated by Passing-Bablok regression and Spearman's correlation method. The data of regression and correlation analysis demonstrated strong correlation between matrices (correlation coefficient between matrices as 0.979 to 1), which indicated that samples in different matrices, heparinized whole blood, heparinized plasma, EDTA treated whole blood, EDTA treated plasma and serum, perform equivalently for cTnI test on Fluoro-Check™ AMI cTnI Test Device.

		SERUM	EDTA TREATED PLASMA	HEPARINIZED WHOLE BLOOD	EDTA TREATED WHOLE BLOOD
REGRESSION EQUATION*	Intercept A	-0.005	0.0004	0.0793	0.0317
	Slope B	1.0526	0.9454	1.0303	0.9529
RANKED CORRELATION*	rho	0.979	0.997	1	0.988

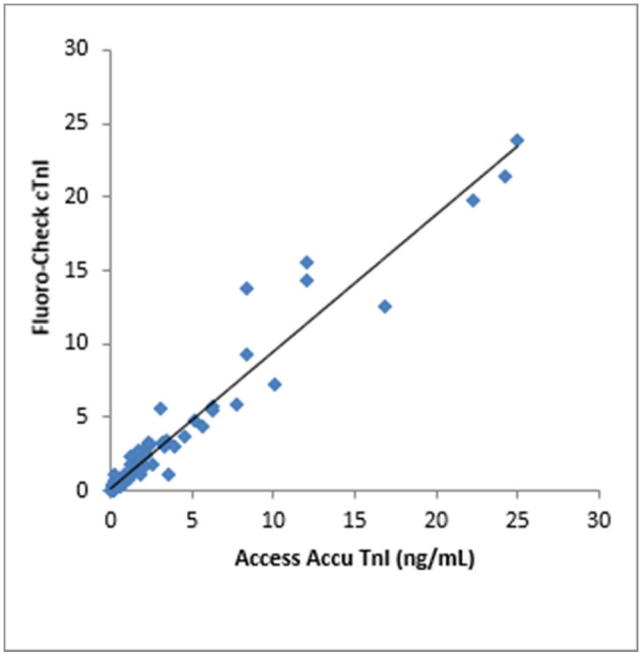
* Regression analysis and correlation analysis was conducted referring to heparinized plasma.



6. *Method Comparison Study*

Method comparison study was performed for Fluoro-Check™ AMI cTnI Test in conjunction with Fluoro-Checker™ TRF Reader versus Access AccuTnI™ (Beckman Coulter Inc). Plasma samples were collected from 133 emergency room patients who had chest pain. The comparison result was regressed using Passing-Bablok model and correlation was analyzed using Spearman's ranked correlation. Results were summarized below. The results showed that the slope of 0.9691 and linear up to 25 ng/mL with ρ=0.959 indicating a good correlation between two systems. Therefore, concentration of TnI measured by Fluoro-Checker™ TRF Reader strongly correlated with Beckman Coulter Access.

n	Range of observation (ng/mL)	Intercept (ng/ml)	Slope	Correlation Coefficient
133	0.03 – 30	0.0149	0.9691	0.959



15. REFERENCES

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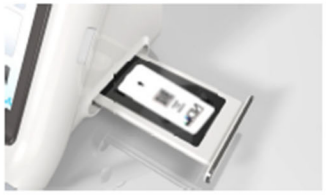
Quick Reference Instruction for Fluoro-Check™ AMI cTnI with Fluoro-Checker™ TRF analyzer

Read the complete test procedure, including recommended QC before performing the test. Refer to the IFU for complete information about the test. Ensure ALL components are at room temperature when running the test.

QR card registration

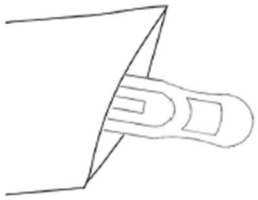
① On test page, Insert the QR card for automatic registration of lot information



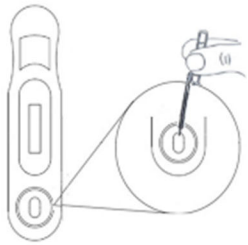
Sample preparation

② Collect whole blood, plasma, or serum specimen. Both the test cassette and sample should be brought to room temperature prior to testing.

③ Remove the test cassette from the sealed pouch immediately before use.



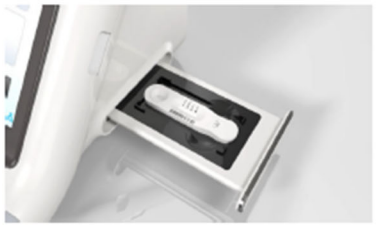
④ Deliver 80 µL of whole blood or plasma or serum sample into the sample well.



Using Fluoro-Checker™ TRF analyzer to read the cassette

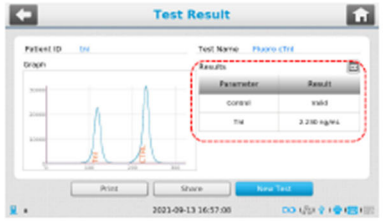
Select "Sample Type"
Set "Default" Mode for Incubation

⑤ Insert the cassette immediately and press Run.



⑥ 15mins of Incubation will automatically start.

⑦ Result will appear on screen in 15mins.



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