Fluoro-Check™ AMI 3 IN 1 Test

For in vitro Diagnostic Use

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

1. INTENDED USE

The Fluoro-Check™ AMI 3 IN 1 Test is a time resolved fluorescence immunoassay for the quantitative determination of Cardiac Troponin I (cTnI), Creatine Kinase MB (CK-MB), and Myoglobin (Myo) in human whole blood, serum, and plasma specimen at detection limit concentration of 0.03 ng/mL, 2.0 ng/mL, and 10 ng/mL respectively, 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 3 IN 1 Test can monitor the rise and fall of cTnI, CK-MB, and Myoglobin. 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 makers and be detected in the blood specimens of AMI patients by specialized immunoassays.[1-3] Unfortunately none of cardiac markers that have been discovered show early release, have 100% cardiac specificity, and a substantial life time in circulation. This situation has led to a panel approach for the utilization of markers in patients with AMI. The constituents of this cardiac panel should include a marker that rapidly increases after cardiac injury and is highly cardiac tissue specific. The combination of cTnI, CK-MB and Myoglobin are widely used in panel assays intended for the determination of AMI in chest pain patients.[4]

Cardiac 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 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]

CK-MB

Creatine Kinase (CK) is present in most tissues and is primarily concerned with ATP regeneration. This enzyme is dimeric and exists as three isozymes: MM (muscle), MB (hybrid), and BB (brain). [12] The MB isozyme has its highest concentration in the heart muscle, thus its level in the serum has diagnostic value. The CK-MB level in normal serum is less than 5 ng/mL. In cases of uncomplicated AMI, CK-MB level becomes elevated within 4-8 hours after the onset of chest pain, reaching a peak between 12-24 hours and then drops down to normal by 48 hours. The peak level of CK-MB is 21 ng/mL or higher.[13-14] CK-MB has been considered the gold standard for the diagnosis of AMI because of its cardio-specificity. However, CK-MB is not an ideal marker to use alone because its level does not increase early enough to make a rapid diagnosis and may also be increased in other conditions. Although CK-MB is more concentrated in the myocardium (approximately 15% of the total CK), it is also present in skeletal muscle. False-positive elevations occur in a number of clinical settings, including trauma, heavy exertion, and myopathies.[15-16]

Myoglobin

Myoglobin, an oxygen binding heme protein present in muscle tissue including cardiac, skeletal and smooth muscle, has attracted considerable interest as an early marker of MI.[2,17] Following injury to any of these muscles, myoglobin appears in the blood more rapidly than any other marker[4]. Levels may be elevated as early as one hour following the onset of chest pain when CK-MB levels are still in the range of normal.[2,18,19] This rapid appearance is due to the location of myoglobin in the cell and its low molecular weight. Myoglobin typically rises 2-4 hours after the onset of infarction, peaks at 6-12 hours, and returns to normal within 24-36 hours. Normally the level of myoglobin in serum is 30-80 ng/mL. In patients with MI, the level could increase approximately 10 times above the upper limit of normal. Myoglobin exhibits high clinical sensitivity for AMI but poor specificity.[1, 3] Many studies suggest that myoglobin may be a good screening assay in Emergency Rooms for the early diagnosis of AMI. However, elevated myoglobin values should be cautiously interpreted if the patient has renal dysfunction or skeletal muscle injury. Because of these limitations, detection of myoglobin in a patient suspected of AMI may need to be supplemented by the presence of a more definitive cardiac maker. However, a negative result in a patient admitted within 2-9 hours after onset of chest pain may help in ruling out AMI.

3. PRINCIPLE

The Fluoro-Check™ AMI 3 IN 1 Test is an immuno-chromatography assay for the quantitative determination of three biochemical markers (cTnI, CK-MB, and Myoglobin) simultaneously in whole blood, serum, and plasma. The membrane strip contains three test lines and one control line; immobilized antibody against CK-MB, antibody against Myoglobin, and streptavidin for biotinylated cTnI antibody for test line and anti-chicken IgY antibody for control line. A dye pad containing biotinylated cTnI antibody and fluorescence particles coupled with cTnI, CK-MM, Myoglobin, and IgY antibodies is placed at the end of the membrane. 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 or biotinylated antibody. The immune complexes move along the nitrocellulose membrane through the test lines and bind to streptavidin or immobilized specific antibody on the test line. Unbound immune complexes pass through the test line and IgY antibody coupled with fluorescence particles are captured by anti-chicken IgY in the control line.

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

4. REAGENT

The Fluoro-Check™ AMI 3 IN 1 Test contains all the reagents necessary for the detection of cTnI, CK-MB and Myoglobin in human whole blood,

serum, and plasma. The device contains a membrane strip coated with CK-MB, Myoglobin antibody and streptavidin on the test line and dye pad infused with biotinylated monoclonal cTnI antibody and fluorescence particles coupled with cTnI, CK-MM, and Myoglobin 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 containing membrane strip in a sealed 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.
- Do not use hemolyzed specimens as hemolysis may affect test results.
- 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 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. 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

1. Collect specimen per 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 (80 µl) of sample into the sample well.
5. 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 and control line can be analyzed by Fluoro-Checker™ TRF Reader and reading results are expressed as a concentration of analytes using predetermined calibration curves specific for Fluoro-Check™ AMI 3 IN 1 Test.

The cutoff of 0.50 ng/mL for cTnI, 5 ng/mL for CK-MB, and 80 ng/mL for Myoglobin are recommended for diagnosis of AMI.

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.[22] However, these studies using the Fluoro-Check™ AMI 3 IN 1 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 3 IN 1 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. CLINICAL CUTOFF AND REFERENCE RANGE

The clinical cutoff values of the Fluoro-Check™ AMI 3 IN 1 Test were determined by performance comparison with the Access® (Beckman Coulter). The clinical cutoff level of each cardiac maker is 0.5 ng/mL for cTnI, 5 ng/mL for CK-MB, and 80 ng/mL for Myoglobin, which were determined in a feasibility study by ROC analysis. The cutoff level may be different if a quantitative assay system other than Beckman Coulter Access is used.

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 determined LoB, Verified LoD and determined LoQ are summarized below.

Ananlyte	Concentration (ng/mL)		
	LoB	LoD	LoQ
cTnI	0.01	0.03	0.03
CK-MB	0.8	2	2
Myoglobin	3.6	10	10

2. Linearity / Reportable range

Linearity studies of Fluoro-Check™ AMI 3 IN 1 were conducted as instructed from the NCCLS guideline, EP6-A. Data set was collected with samples covering dynamic range of Fluoro-Check™ AMI 3 IN 1 test and it was confirmed that the linear model was capable of interpolating between the experimental points. Linearity ranges of 3 analytes were determined as follow, with 11.0% ~13.8% repeatability within this interval.

Analyte	Reportable range	Correlation coefficient (r^2)	CV (%)
cTnI	0.03 ng/mL - 30 ng/mL	0.916	13.8
CK-MB	2 ng/mL - 200 ng/mL	0.926	13.1
Myoglobin	10 ng/mL – 500 ng/mL	0.958	11.0

3. Interference & specificity test

The following endogenous substances do not interfere with the performance of the Fluoro-Check™ AMI 3 IN 1 Test at the levels below (less than 10% bias).

	Substances	Concentration
Endogenous substances	Human serum albumin	5 g/dL
	Hemoglobin	5 g/dL
	Triglyceride	1 g/dL
	Cholesterol	0.5 g/dL
	Bilirubin	50 mg/dL
	Biotin, Vitamin B7	300 ng/mL
Potentially cross-reacting endogenous proteins	Cardiac TnC	1 µg/mL
	Cardiac TnT	1 µg/mL
	Skeletal TnI	1 µg/mL
	CK-BB	1 µg/mL
	Cardiac myosin light chain	1 µg/mL

The following substances do not show any significant interference with this assay at the level tested, 10 µg/mL.

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

4. Precision Test

Precision study was performed at 3 levels of analyte, cTnI, CK-MB, and Myoglobin plasma sample with 200 replicates per each level of sample for 20 days, based on NCCLS guideline EP5-A. The within-run and total precision data are summarized in the table below.

Analyte	Mean (ng/mL)	Within Run		Total Run	
		SD (ng/mL)	CV (%)	SD (ng/mL)	CV (%)
cTnI	0.10	0.012	12.2	0.013	12.8
	0.54	0.047	8.8	0.053	9.9
	4.03	0.446	11.1	0.466	11.6
CK-MB	9.59	1.40	14.6	1.52	15.8
	23.17	2.78	12.0	3.07	13.3
	76.37	10.0	13.1	10.3	13.5
Myo	31.26	5.44	17.4	5.81	18.6
	101.77	17.4	17.1	18.5	18.2
	302.77	51.9	17.1	53.8	17.8

Precision data of whole blood sample is comparable to that of plasma sample. Whole blood specimen from 3 donors were spiked with 3 analytes, cTnI, CK-MB, and Myoglobin to prepare three levels of samples, and tested in 15 replicates.

Analyte	Mean (ng/mL)	Within Subject		Total Subject	
		SD (ng/mL)	CV (%)	SD (ng/mL)	CV (%)
cTnI	<0.01	N/A	N/A	N/A	N/A
	0.56	0.07	12.9	0.07	13.2
	4.95	0.68	13.7	0.66	13.3
CK-MB	<2	N/A	N/A	N/A	N/A
	33.57	4.32	12.9	4.28	12.8
	116.99	17.32	14.8	18.80	16.1
Myo	<10	N/A	N/A	N/A	N/A
	73.15	11.82	16.2	11.93	16.3
	241.27	42.80	17.7	43.44	18.0

5. Matrix Comparison Study

Performance of the assay using samples of five matrices-whole blood and plasma, in heparin or EDTA as anticoagulant, and serum were compared at 3 analytes, cTnI, CK-MB, and Myoglobin ranges of 0.15 ng/mL – 25 ng/mL, 15 ng/mL – 150 ng/mL, 10 ng/mL – 400 ng/mL, respectively. The results were evaluated by Passing-Bablok regression and Spearman's correlation method.

Data presented by heparinized plasma as reference in table below, demonstrate that the assay performs equivalently in those matrices.

	Correlation coefficient (ρ)			
Analyte	Serum	EDTA plasma	Heparinized whole blood	EDTA whole blood
cTnI	0.951	0.951	0.986	0.986
CK-MB	0.979	0.993	0.993	0.993
Myoglobin	0.982	0.955	0.991	0.991

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

*6. **Method Comparison Study***

Method comparison study was performed for Fluoro-Check™ AMI 3 IN 1 in conjunction with Fluoro-Checker™ TRF reader versus Access® (Beckman Coulter Inc.). Samples were collected from patients who had a chest pain. The comparison result was regressed using Passing-Bablok model and correlation was analyzed using Spearman's ranked correlation. The table below summarizes the results, which demonstrate that concentration of TnI, CK-MB, and Myoglobin measured by Fluoro-Checker™ TRF Reader were strongly correlated with Access® (Beckman Coulter).

Analyte	Range (ng/mL)	Intercept (ng/mL)	Slope	Correlation Coefficient
TnI	0.03 – 30	0.0149	0.9691	0.959
CK-MB	2.0 - 200	-0.3202	0.9733	0.921
Myoglobin	10 -500	1.7265	1.0373	0.887

15. REFERENCES

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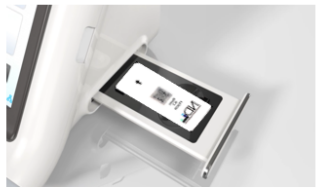
Quick Reference Instruction for Fluoro-Check ™ AMI 3 IN 1 with Fluoro-Checker ™ TRF analyzer

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 (20℃-30℃) when running the test.

QR card registration

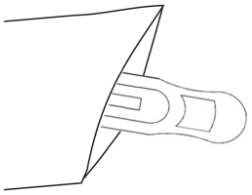
① On test page, Insert the QR card for automatic registration of lot information



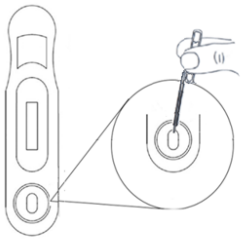
Sample preparation

② Collect whole blood, plasma, or serum specimen. Both the test cassette and sample should be brought to room temperature (20℃~30℃) prior to testing.

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

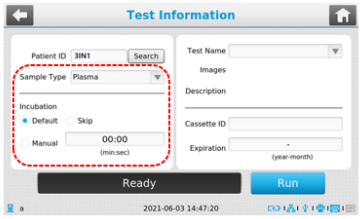


④ Deliver 80 µl of whole blood or plasma or serum sample into the sample well.

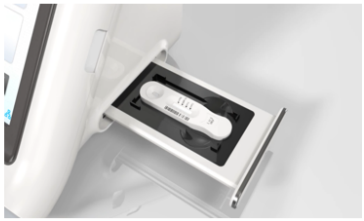


Using Fluoro-Checker ™ TRF analyzer to read the cassette

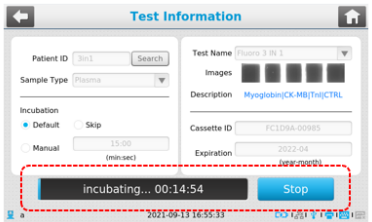
Select "Sample Type"
Set "Default" Mode for Incubation



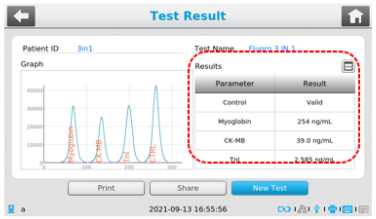
⑤ Insert the cassette immediately and press Run.



⑥ 15-minute Incubation will automatically start.



⑦ Result will appear on screen in 15 min.



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