Nano-Check ™ 4 IN 1 Cardiac Marker Test

For in vitro Diagnostic Use

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

1. INTENDED USE

The Nano-Check™ 4 IN 1 Test is a rapid immunoassay for the determination of Cardiac Troponin I (cTnI), Creatine Kinase MB (CK-MB), Myoglobin, and NT-proBNP in human whole blood, serum and plasma specimens at a reference level of 0.5 ng/ml, 5.0 ng/ml, 80 ng/ml, and 125 pg/ml for patients younger than 75 years or 450 pg/ml for patients 75 years and older, as an aid in the diagnosis of cardiac disease. The Nano-Check™ 4 IN 1 Test monitors the rise and fall of cTnI, CK-MB, Myoglobin, and NT-proBNP in conjunction with Nano-Checker 710 reader. Test results should be interpreted by the physician along with other test results and patient clinical symptoms.

2. SUMMARY AND EXPLANATION OF THE TEST

When a myocardial infarction (MI) occurs in the hypo perfused 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 patients by specialized immunoassays. Unfortunately, none of cardiac markers 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 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 Myoglobin, and NT-proBNP are widely used in panel assays intended for the determination of in chest pain patients.

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 (cTnI), troponin T (cTnT), and troponin C (cTnC), and plays a fundamental role in the transmission of intracellular calcium signal actin-myosin interaction. 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. 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. Studies on the release kinetics indicate that cTnI is not early marker of myocardial necrosis. It appears in serum 3-6 hours after symptom onset, similar to the release of CK-MB. However, cTnI remains elevated for 4-9 days post-AMI. 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.

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). 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, 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. CK-MB has been considered the gold standard for the diagnosis of 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.

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. Following injury to any of these muscles, myoglobin appears in the blood more rapidly than any other marker. 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. 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. 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 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.

NT-proBNP

The natriuretic peptides are a family of molecules consisting of several structurally-related hormones including arterial natriuretic peptide (ANP), B-type (or brain) natriuretic peptide (BNP), C-type natriuretic peptide (CNP), and dendroaspis natriuretic peptide (DNP). B-type natriuretic peptides are produced initially as a 134 amino acid pre-pro-peptide, which is cleaved into proBNP as 108 amino acids and this precursor molecule is stored in secretary granules in myocytes. Upon release, proBNP is cleaved by a protease known as furin into N-terminal (NT)-proBNP (a 76 amino acid, biologically-inert portion), and BNP (which is biologically active). In humans, NT-proBNP and BNP are found in largest concentration in the left ventricular (LV) myocardium, but are also detectable in arterial tissue as well as in the myocardium of the right ventricle. A significant body of evidence has developed to demonstrate that NT-proBNP and BNP correlate with diagnosis, clinical status and prognosis in congestive heart failure, and may be useful for the longitudinal management of patients with CHF.

3. PRINCIPLE

The Nano-Check™ 4 IN 1 Test is an immuno-chromatography assay for the quantitative determination of four biochemical markers (cTnI, CK-MB, Myoglobin, and NT-proBNP) simultaneously in human whole blood, serum and plasma specimen. The membrane strip located on left side contains three test lines and one control line, printed with specific antibodies or receptor against each target molecules, monoclonal mouse antibody against CK-MB, monoclonal mouse antibody against Myoglobin, streptavidin for biotinylated cTnI antibody, and rabbit anti-goat antibody for control line. A dye pad containing biotinylated cTnI antibody and gold colloidal particles coupled with CK-MM, cTnI, and Myoglobin antibodies is placed at the end of the membrane. The right membrane strip contains a test line and a control line, printed with streptavidin for biotinylated NT-proBNP antibody and rabbit anti-goat IgG antibody for control line. A dye pad containing biotinylated NT-proBNP antibody and gold colloidal particles coupled with NT-proBNP antibody is placed at the end of the membrane.
When the sample is applied into the sample well, the cardiac makers present in the sample bind to its specific antibody coupled with gold particles on the dried dye pad. These primary immune complexes move along the nitrocellulose membrane through the test lines and bind to their corresponding capture antibodies or receptor molecules immobilized on the test lines. Unbound immune complexes pass through the test lines and are captured by rabbit anti-goat antibody in the control line.
If the concentration of any of these markers in the sample is above the reference level, red bands appear at the corresponding test lines and the control line. If the concentration of the markers in the sample is lower than the reference 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 an analyte, the tested device should be read by Nano-Checker 710 Reader. The reader analyzes the color intensity of the test line and converts it to the concentration of the analyte in the specimen by a predetermined equation.

4. REAGENT

The Nano-Check™ 4 IN 1 Test contains all the reagents necessary for the detection of cTnI, CK-MB, Myoglobin, and NT-proBNP in human whole blood, serum, and plasma. The device contains two membrane strips. The left membrane strip is coated with monoclonal mouse anti-CK-MB, anti- Myoglobin and streptavidin on the test line, and dye pad infused with biotinylated monoclonal mouse anti-cTnI antibody and gold colloidal particles coupled with anti-CK-MM, anti-cTnI and anti-Myoglobin antibodies. The right membrane strip is coated with streptavidin on the test line, and dye pad infused with biotinylated monoclonal mouse anti-NT-proBNP antibodies and gold colloidal particles coupled with anti-NT-proBNP antibodies Stabilizer containing 0.05% sodium azide and BSA protein are deposited on the dye pad in dried form.

5. MATERIALS

Provided

- Nano-Check™ 4 IN 1 Test device containing membrane strips in a sealed pouch with desiccant
- Instructions for Use
- Disposable 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 Reader 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 the 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 pipette 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.
- 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. Do not open pouches until ready to perform the assay.
3. Remove the test device from the sealed pouch immediately before use. Label device with patient or control identification.
4. Using the transfer pipette, deliver contents (80 ul) of sample into each sample well.
5. Read the results at 15 minutes.

10. INTERPRETATION OF RESULTS

The signal intensities of test lines are analyzed by Nano-Checker 710 Reader and reported as concentrations 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. When the value is above the reference range (see the reader screen) but below reference value, the specimen should be retested with the sample collected later. When the reading value is above the reference 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 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. Confirmation of test results should only be made by a physician after all clinical and laboratory findings have been evaluated. Samples containing unusually high titers of certain antibodies such as human anti-mouse IgG or human anti-rabbit IgG antibodies have been known to affect the performance of these devices. However, the effect of such antibodies on the Nano-Check™ 4 IN 1 Test has not been evaluated. 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 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 as if running patient samples. Controls should be run before using each new lot or shipment of Nano-Check™ 4 IN 1 Test and at regular intervals afterwards or any time the validity of the test results is questioned. For the calibration of Nano-Checker 710 Reader, two different levels of reference cassettes are supplied with the reader. The reader should be calibrated periodically with the provided reference cassettes. If the reading value of reference cassette is out of the described range, it should be recalibrated.

13. EXPECTED VALUES

The reference values of the Nano-Check™ 4 IN 1 Test were determined by comparison to the Beckman Coulter quantitative assay, Access AccuTnI™, Access Myoglobin Assay, or Access CK-MB Assay or Roche’s Elecsys Imunoassay. The reference level of each cardiac maker is 0.5 ng/ml for cTnI, 5 ng/ml for CK-MB, 80 ng/ml for Myoglobin and 125 pg/ml for patients younger than 75 years or 450 pg/ml for patients 75 years and older for NT-proBNP. The specimens containing cTnI, CK-MB, Myoglobin, and NT-proBNP at the concentration of equal or above established reference levels will give positive results using the Nano-Check™ 4 IN 1 Test. The reference levels may be different if a quantitative assay system other than Beckman Coulter Access or Roche Elecsys proBNP immunoassay test is used.

14. PERFORMANCE CHARACTERISTICS OF cTnI, CK-MB, AND Myo

1. Assay Cutoff

Patient plasma containing cTnI, CK-MB or Myoglobin were diluted in normal human serum to the concentration at or near the reference levels. Analyte concentrations in the diluted samples were confirmed by Quantitative Assay, Access AccuTnI™, Access Myoglobin Assay, or Access CK-MB Assay of Beckman Coulter. Fifteen devices were tested for each sample at concentrations of 0.31, 0.57 and 1.07 ng/ml for cTnI; 2.4, 4.9, and 10.2 ng/ml for CK-MB; 56.5, 80.4 and 141.3 ng/ml for Myoglobin. The results are shown in following table. The reference concentrations were assigned as 0.5 ng/ml for cTnI, 5.0 ng/ml for CK-MB and 80 ng/ml for Myoglobin.

Analyte	Conc. determined by Access Assay (ng/ml)	Test Result Negative(n)	Positive(n)	Reference level
cTnI	0.31	7	8	0.5ng/ml
	0.57	0	15	
	1.07	0	15	
CK-MB	2.4	15	0	5.0ng/ml
	4.9	0	15	
	10.2	0	15	
Myoglobin	56.5	14	1	80ng/ml
	80.4	0	15	
	141.3	0	15	

2. Recovery Studies

Recovery studies were performed with patient serum diluted in normal human serum. Patient serum containing high levels of either cTnI, CK-MB or Myoglobin were sequentially diluted with normal human serum to yield different concentrations. Each diluted sample was tested in 3 replicates. The data shown in the table below demonstrates recovery rate between observed results and expected results at each concentration of cTnI, CK-MB and Myoglobin.

Analyte	Expected Concentration (ng/ml)	Determined Average Concentration	Agreement % of Expect Values	Total Recovery(%)
cTnI	22.4	24.17	107.8	105.7
	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	
CK-MB	188	199.7	106.2	109.6
	94	101.3	107.8	
	47	42.1	89.6	
	23.5	26.6	113.0	
	11.8	12.5	106.4	
	5.9	7.7	130.5	
	2.9	3.3	113.5	
Myoglobin	716	643.0	89.8	101.5
	358	343.7	96.0	
	179	174.4	97.4	
	89.5	90.8	101.4	
	44.8	61.4	137.2	
	22.4	19.4	86.9	

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
CK-MB	0.8	2.0	2.0
Myoglobin	10	20	20

4. Reportable range for Quantitative assay

Reportable range of each analyte, cTnI, CK-MB 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
CK-MB	2.0-200
Myoglobin	20-1000

5. Analytical Specificity

Potentially interfering substances were spiked into normal serum and patient serum containing either cTnI, CK-MB or Myoglobin at 1.5 times the reference concentration. The substances at the following level did not interfere with the performance of the cTnI, CK-MB and Myoglobin 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 cTnI, CK-MB and Myoglobin Test was tested for interference by potentially cross-reacting endogenous proteins. Potentially cross-reacting proteins, added into normal human serum up

to the following concentrations, did 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
	CKMM	5,000 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, CK-MB and Myoglobin, 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 demonstrated 100% agreement for between run as well within run.

Analyte	Testing Site	Sample 1		Sample 2		Sample 3		% agreement within run
		cTnI 0 CK-MB 0 Myo 0		cTnI 1.03 CK-MB 12.6 Myo 119.9		cTnI 2.05 CK-MB 25.0 Myo 166.3		
		-	+	-	+	-	+	
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 betweenrun	100%		100%		100%		
CK-MB	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 betweenrun	100%		100%		100%		
Myo	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 betweenrun	100%		100%		100%		

7. Precision / Reproducibility Test For Quantitative Assay

Reproducibility of the cTnI, CK-MB and Myoglobin Test 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
CK-MB	Level 1	8.74	1.57	17.99
	Level 2	42.57	4.96	11.66
	Level 3	177.07	27.07	15.29
Myoglobin	Level 1	83.5	13.64	16.33
	Level 2	252.77	35.56	14.07
	Level 3	843.54	153.83	18.24

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 for cTnI; 11-20, 5-10, 3.8-4.4 and <2 ng/ml for CK-MB; 123-248, 85-118, 64-80 and <30 ng/ml for Myoglobin. Ten samples in each group were tested and the results are shown in the table below (lowest levels were all negative in both serum and plasma and not shown):

Analyte	Sample Conc.range samples (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
CK-MB	11-20	10	0	10	0
	5-10	10	0	10	0
	3.8-4.4	8	2	7	3
Myo	123-248	10	0	10	0
	85-118	10	0	10	0
	64-80	8	2	9	1

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 3 IN 1 qualitative test, normal whole blood samples were spiked with clinical specimens containing each analyte, cTnI, CK-MB, Myoglobin to make three different desired levels between negative and 4 times than cutoff values of each analyte. Five samples in each group were tested using Nano-Check™ AMI 3 IN 1 Test, prior to removal of cells and after removal of cells by centrifugation. The test results are shown in the following table:

Analyte	Concentration 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
CK-MB	11.4-15.6	5	0	5	0
	5.0-10.0	5	0	5	0
	2.2-4.6	1	4	1	4
Myo	151-211	5	0	5	0
	83.6-138	5	0	5	0
	63.0-81.5	1	4	1	4

To perform matrix comparison study between plasma and whole blood in Nano-Check™ AMI 3 IN 1 quantitative test, ten different levels of analyte concentrations in reportable range of each analyte were prepared 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™ AMI 3 in 1 device in triplicates. The concentrations in matched sample matrix were analyzed using correlation and regression methods. The values of correlation coefficients (0.83-0.96) suggest a good correlation between plasma and whole blood specimens or between plasma specimens treated with different anticoagulants.

Lithium Heparin Plasma vs. Whole Blood

Analyte	n	Observation Ranges (ng/ml)	Intercept	Slope	Correlation Coefficient
cTn I	30	0.1-30	0.0321	0.9575	0.951
CK-MB	30	2-200	0.7452	0.9702	0.963
Myoglobin	30	20-1000	25.545	0.9117	0.827

EDTA Plasma vs. Whole Blood

Analyte	n	Observation Ranges (ng/ml)	Intercept	Slope	Correlation Coefficient
cTn I	30	0.1-30	-0.0436	0.9413	0.936
CK-MB	30	2-200	0.9751	0.9502	0.951
Myoglobin	30	20-1000	21.545	0.8942	0.863

Lithium Heparin Plasma vs. EDTA Plasma

Analyte	n	Observation Ranges (ng/ml)	Intercept	Slope	Correlation Coefficient
cTn I	30	0.1-30	0.0267	0.9175	0.948
CK-MB	30	2-200	0.5123	0.9502	0.951
Myoglobin	30	20-1000	-16.545	0.9342	0.893

10. Method Comparison Study

Qualitative Test Comparison

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 3 IN 1 Test and the Beckman Coulter Access test system. Results are summarized below.

AMI 3 IN 1 TnI Test Results Compared to Quantitative Access Results (ng/ml)

		Access Accu TnI			
		<0.01-0.29	0.3-0.47	0.52-0.6	0.61->100
Nano-Check™ 3 in1 cTnI	Positive	1	7	6	91
	Negative	143	6	2	0

AMI 3 IN 1 CK-MB Test Results Compared to Quantitative Access Results (ng/ml)

		Access CK-MB			
		0.2-4.0	4.1-4.9	5.0-6.0	6.2->300
Nano-Check™ 3 in1 CK-MB	Positive	1	9	15	99
	Negative	121	10	1	0

AMI 3 IN 1 Myoglobin Test Results Compared to Quantitative Access Results (ng/ml)

		Access Myoglobin			
		10.2-60.9	61-79.1	80.4-90.3	91.9->4000
Nano-Check™ 3 in1 Myo	Positive	0	9	11	120
	Negative	98	16	2	0

Quantitative Test Comparison

A comparison of cTnI, CK-MB and Myoglobin values of heparin plasma samples using Nano-Checker 710 Reader and Beckman Coulter Access system were carried out. The results of cTnI, CK-MB and Myoglobin values obtained by two different methods were analyzed to and the following statistical data were obtained.

Analyte	n	Observation Ranges on Access (ng/ml)	Intercept	Slope	Correlation Coefficient
cTnI	67	0.06-44.80	0.5348	0.9175	0.948
CK-MB	62	0.1-188	0.6855	0.9502	0.951
Myoglobin	55	1.0-1587	85.545	0.7242	0.813

15. PERFORMANCE CHARACTERISTICS OF NT-proBNP

1. Precision Test

Total imprecision of the NT-proBNP Test with Nano-Check 710 Reader was determined in study using plasma based in-house control materials. Samples containing high levels of NT-

proBNP were diluted in normal human plasma to make positive samples of different concentrations and tested over 12 times for 10 days. The within and total standard deviation were calculated by the analysis of variance method.

	Within Run		Total run	
Mean (pg/ml)	SD (pg/ml)	CV(%)	SD (pg/ml)	CV(%)
299.5	26.3	8.8	49.2	16.4
576.9	21.4	3.7	100.4	17.4
1501.0	74.8	5.0	218.6	14.6
3028.3	193.5	6.4	461.1	15.2

2. *Recovery Studies*

Recovery studies were performed with serum containing high levels of NT-proBNP which were sequentially diluted with normal human serum to yield different concentrations. Each diluted sample was tested in 10 replicates. The data shown in the table below demonstrated recovery rate between observed results and expected results at each concentration of NT-proBNP.

Analyte	Expected Concentration (pg/ml)	Determined Concentration	Agreement Expected values (%)	Recovery Rate (%)
NT-proBNP	150	151.3	101	98.2
	300	298.0	99	
	600	529.3	88	
	1500	1465.8	98	
	3000	3056.8	102	
	6000	6043.0	101	

3. *Analytical Sensitivity*

The analytical sensitivity was determined according to the standard CLSI EP17-A. Analytical sensitivity is defined as the concentration at two standard deviations above the blank sample and represents the lowest concentration of NT-proBNP that can be distinguished from zero. For the study, samples, which contained NT-proBNP in low concentration or blank, were tested in 10 replicates. Detection limit of Nano-Check™ NT-proBNP was calculated as 30 pg/ml.

4. *Interference study*

Potentially interfering substances were spiked into normal plasma containing recombinant NT-proBNP concentration of 0 and 400 pg/ml. The substances at the following level did not cause a bias of over 15% with the test at the concentration of NT-proBNP.

Substances	Concentration
Bilirubin	0.1 mg/ml
Hemoglobin	1 mg/ml
Triglycerides	10 mg/ml
BNP	10 µg/ml
ANP	10 µg/ml
CNP	10 µg/ml
Cholesterol	5 mg/ml

5. *Cross- Reactivity*

The cross reactivity of the NT-proBNP Test was evaluated by spiking potential cross-reacting drug compound to the normal human plasma at the concentration of 10 µg/ml. There was no significant interference with the analyte, nor was there any assay cross-reactivity.

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

6. *Matrix comparison study*

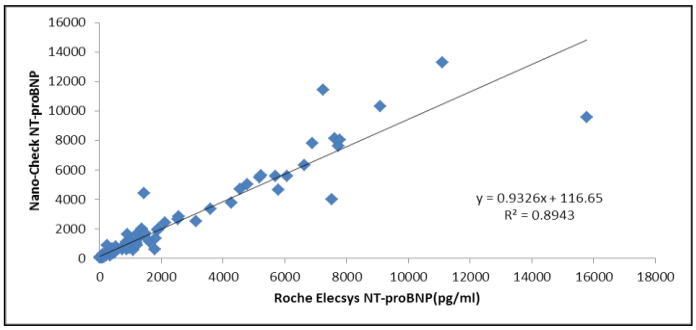
To perform matrix comparison study between plasma and whole blood, 5 different levels of analyte concentrations ranging from 181 pg/ml to 2500 pg/ml were prepared by spiking analyte molecules into normal whole blood pool collected from 10 different healthy volunteers. Corresponding plasma specimens were prepared from each level of whole blood specimens by centrifugation. Each level of whole blood or plasma specimens were run on the same lot of NT-proBNP Test in 4 replicates. The concentrations were measured using the analysis programs for plasma test on Nano-Checker 710N Reader. From the test results, the following correlations were acquired. The formulas prove that the test values of whole blood were correlated to heparinized plasma with 99.9% correlation coefficient.

Matrix	n	Ranges of observation(pg/ml)	Intercept (pg/ml)	Slope	Correlation coefficient
Sodium heparin plasma	20	181-2500	-67.447	1.0226	0.9997

7. *Method Comparison Study*

The method of comparison study was conducted using 127 samples. Clinical samples were collected from patients suspected of having CHF (congestive heart failure) and healthy volunteer. A comparison of NT-proBNP assay on the Nano-Check™ NT-proBNP test and Elecsys NT-proBNP is summarized in the following table and Figure below.

Ranges of Observation (pg/ml)	Intercept (pg/ml)	Slope	Correlation Coefficient
30 – 15,000	116.65	0.9326	0.8943



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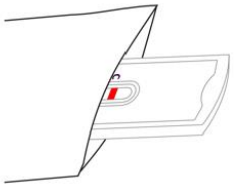
Quick Reference Instruction for Nano-Check ™ 4 IN 1 Test (Option: Nano-Checker ™ 710V analyzer)

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.

Sample preparation

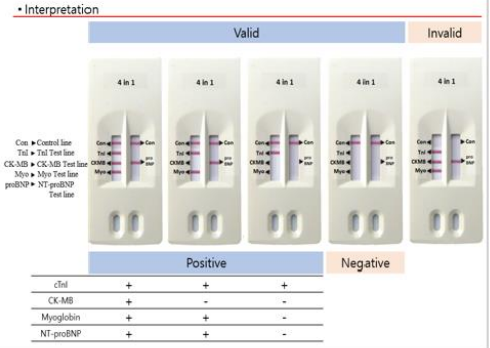
① Collect whole blood, plasma, or serum specimen. Both the test cassette and sample should be brought to room temperature (20℃~30℃) prior to testing.

② Remove the test cassette from the sealed pouch immediately before use.



③ Deliver 80 µl of sample per each sample well (left/right) using sample transfer pipette.

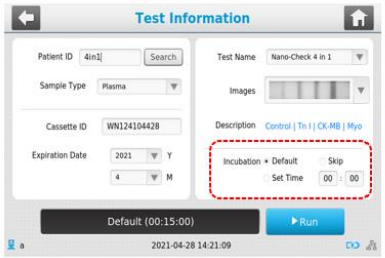
④ Read the results at 15 min.



Using Nano-Checker ™ 710V analyzer to read the cassette for Quantitative Analysis

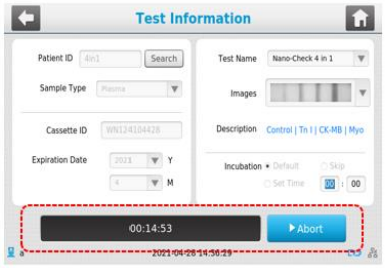
[Default Mode]

※ Set "Default" Mode

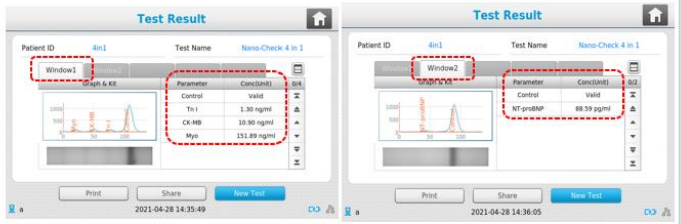


⑤ Insert the cassette immediately and press Run.

⑥ 15-minuate Incubation will automatically start.



⑦ Result will appear on screen in 15min.



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