Fluoro-Check™ NT-proBNP Test

For in vitro Diagnostic Use

One–step quantitative time resolved fluorescence immuno-chromatographic assay for the detection of NT-proBNP in human Whole blood, serum, or plasma

1. INTENDED USE

The Fluoro-Check™ NT-proBNP Test is a time resolved fluorescence immunoassay for the quantitative determination of NT-proBNP (N-terminal pro-Brain natriuretic peptide) in human whole blood, serum, or plasma specimen at detection limit concentration of 20 pg/mL as an aid in the diagnosis of congestive heart failure (CHF). In conjunction with Fluoro-Checker™ TRF reader, Fluoro-Check ™ NT-proBNP Test can monitor the rise and fall of NT-proBNP. 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

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 acids 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 (76 amino acids, 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 levels 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 Fluoro-Check™ NT-proBNP Test is an immuno-chromatography assay for the quantitative determination of NT-proBNP in whole blood, serum, or plasma. The membrane strip contains one test line and one control line; streptavidin for biotinylated NT-proBNP antibody and goat anti-chicken IgY antibody for the control line. A dye pad is placed at the end of the membrane containing biotinylated NT-proBNP antibody and fluorescence particles coupled with NT-proBNP antibody. When a sample is applied into the sample well, NT-proBNP in sample binds to both NT-proBNP specific fluorescent coupled antibody and biotinylated antibody. These immune complexes move along the nitrocellulose membrane through the test lines and bind to streptavidin immobilized on the test line to produce specific test signal. Unbound immune complexes pass through the test line. IgY coupled with fluorescence particles are captured by goat anti-chicken IgY antibody in the control line, to produce control signal, which indicates assay validity.
To measure the concentration of NT-proBNP, 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 NT-proBNP in the specimen by the predetermined equation.

4. REAGENT

The Fluoro-Check™ NT-proBNP Test contains all the reagents necessary for the detection of NT-proBNP in human whole blood, serum, or plasma. The device contains a membrane strip coated with streptavidin on the test line and dye pad infused with biotinylated monoclonal anti-NT-proBNP antibody and fluorescence particles coupled with anti-NT-proBNP specific antibody. A stabilizer containing 0.05% sodium azide, BSA protein and other chemicals are deposited on the dye pad in dried form.

5. MATERIALS

Provided

- Fluoro-Check™ NT-proBNP Test device containing membrane strip in a sealed pouch with desiccant
- Instructions for Use
- Disposable transfer pipette (if applicable)
- 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, or 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. Do not use whole blood which was not treated with anticoagulant
- 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 2-8°C. If testing cannot be performed

within 24 hours, or for shipment of samples, freeze the samples at -20ºC or below. Do not repeat freezing and thawing cycles, as freezing and thawing may damage NT-proBNP molecule in the sample.

- 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 (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 line and control 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™ NT-proBNP Test kit. The results from this or any other diagnostic test should be used and interpreted only in the context of the overall clinical picture.

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 congestive heart failure (CHF) and requires further confirmation.
- 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 CHF. 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. However, these studies using the Fluoro-Check™ NT-proBNP Test have not been performed.

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™ NT-proBNP 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. REFERENCE RANGE

The cutoff values of the Fluoro-Check™ NT-proBNP Test were estimated by comparison to the Roche's Elecsys® NT-proBNP Immunoassay. The cutoff level of NT-proBNP is 125 pg/mL for patients younger than 75 years and 450 pg/mL for patients 75 years and older. However, each laboratory should establish its own reference range. It has been noted that the cutoff levels are different if a quantitative assay system other than Roche's Elecsys® NT-proBNP Immunoassay is used.

14. PERFORMANCE CHARACTERISTICS

1. Detection limits

Limit of Blank (LoB), Limit of Detection (LoD) and Limit of quantification (LoQ) studies were performed according to CLSI guideline EP17-A. The LoB determination was determined by testing 100 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 18 pg/mL – 30 pg/mL) were tested with 20 replicates per each level. Tentative concentration that is calculated to exceed the LoB was verified in 10 replicates per run across 10 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 (pg/mL)	12 pg/mL	20 pg/mL	20 pg/mL

2. Linearity / assay reportable range

Linearity studies of Fluoro-Check™ NT-proBNP were conducted as instructed from the CLSI guideline, EP6-A. Data set was collected with samples covering dynamic range of Fluoro-Check™ NT-proBNP assay system and it was confirmed that the linear model was capable of interpolating between the experimental points. Fluoro-Check™ NT-proBNP test was demonstrated to be linear from 20 pg/mL to 12800 pg/mL, with 10.8% repeatability within this interval.

3. Interference & specificity test

Potentially interfering substances were spiked into normal plasma and patient plasma containing 600pg/mL NT-proBNP. The substances at the following level do not interfere with the performance of the Fluoro-Check™ NT-proBNP Test.

	Substances	Concentration
Endogenous substances	Human serum albumin	5 g/dL
	Hemoglobin	4 g/dL
	Triglyceride	1 g/dL
	Cholesterol	50 g/dL
	Bilirubin	10 mg/dL

Potentially cross-reacting endogenous proteins	ANP	1 mg/dL
	BNP	1 mg/dL
	CNP	1 mg/dL

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

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 of the Fluoro-Check™ NT-proBNP test with Fluoro-Checker™ reader was determined according to CLSI guideline EP5-A. The study included 3 plasma samples, with concentration of 300 pg/mL, 1500 pg/mL and 5000 pg/mL. Test was conducted with 200 replicates per sample across 10 replicates per run for 20 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 levels of NT-proBNP controls were located between 11.1% and 13.9%.

Analyte	Mean (pg/mL)	Within Run		Total Run	
		SD (pg/mL)	CV (%)	SD (pg/mL)	CV (%)
NT-proBNP	301	30.0	10.0%	33.6	11.1%
	1455	183.6	12.6%	202.4	13.9%
	5191	583.8	11.2%	711.6	13.7%

Assay precision was also investigated using whole blood samples. Whole blood collected from 3 healthy subjects was aliquoted as 3 vials, and each vial was spiked with different amounts of standard NT-proBNP material. Expected NT-proBNP concentration in each vial was 0 pg/mL (negative), 380 pg/mL (positive - low level), or 2000 pg/mL (positive - high level). Each sample was tested on Device, Fluoro-Check™ NT-proBNP, by running 8 replicates for negative sample or each 15 replicates for each positive sample. The coefficient of variation (CV) of within run (within subject) was 9.9% (low level) or 11.2% (high level), and total precision was 10.0% (low level) and 13.5% (high level). The data of whole blood sample was comparable to precision data using plasma sample.

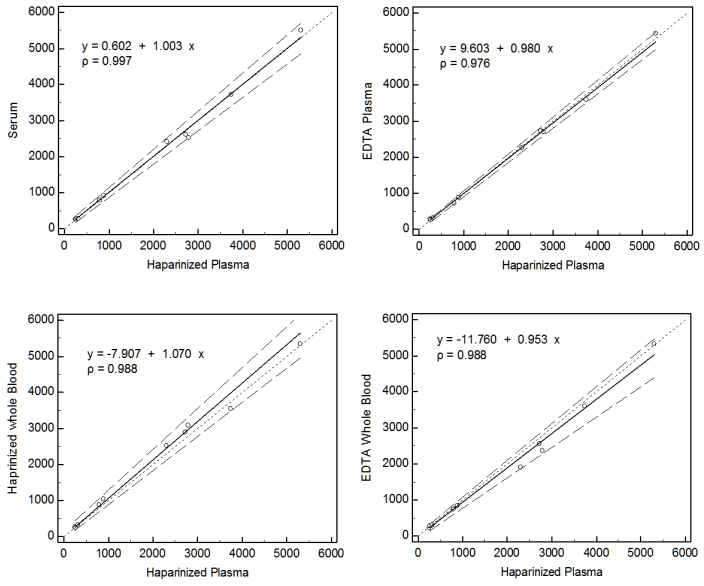
Analyte	Mean (pg/mL)	Within Subject		Total	
		SD (pg/mL)	CV (%)	SD (pg/mL)	CV (%)
NT-proBNP	< 20	N/A	N/A	N/A	N/A
	383	38.1	9.9%	38.4	10.0%
	2060	229.7	11.2%	227.5	13.5%

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 different amount of standard NT-proBNP material. The target concentrations of NT-proBNP were set differently for each specimen, ranges between 250 pg/mL – 5500 pg/mL. Then, each vial of NT-proBNP 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™ NT-proBNP, 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 0.976 to 0.997), which indicated that samples in different matrices, heparinized whole blood, heparinized plasma, EDTA treated whole blood, EDTA treated plasma and serum, perform equivalently for NT-proBNP test on Fluoro-Check™ NT-proBNP Test Device.

		Serum	EDTA treated Plasma	Heparinized Whole Blood	EDTA treated Whole Blood
regression equation*	Intercept A	0.602	9.603	-7.907	-11.760
	Slope B	1.003	0.980	1.070	0.953
ranked correlation*	rho	0.997	0.976	0.988	0.988

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

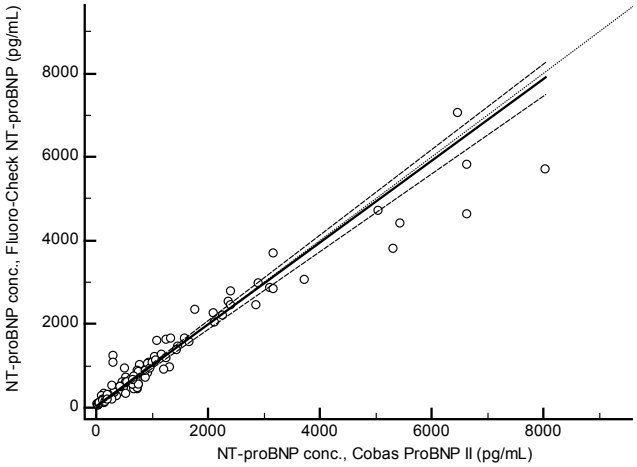


6. Method Comparison Study

Method comparison study was performed for Fluoro-Check™ NT-proBNP in conjunction with Fluoro-Checker™ TRF reader versus Cobas ProBNP II (Roche Diagnostics). Plasma samples were collected from 120 peoples whose NT-proBNP concentration distributes low to high. 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 regression slope of 0.979 and correlation

parameter ρ=0.960 indicating a good correlation between two systems. Therefore, concentration of NT-proBNP measured with Fluoro-Check reader strongly correlated with Cobas ProBNP II.

n	Range of observation (ng/mL)	Intercept (ng/mL)	Slope	Correlation Coefficient
120	21 ~ 8031	28.843	0.979	0.960



15. REFERENCES

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