NT-proBNP FIA Rapid Test

(Whole Blood/Serum/Plasma)

FIA-PBNP-001

A rapid test for the diagnosis of heart failure to detect NT-proBNP quantitatively in whole blood, serum or plasma with the use of the Biopanda Fluorescence Immunoassay Analyser.
For professional in vitro diagnostic use only.

INTENDED USE

The NT-proBNP FIA Rapid Test is based on Fluorescence Immunoassay for the quantitative determination of human NT-proBNP in whole blood, serum or plasma as an aid in the diagnosis of heart failure.

SUMMARY

The N-terminal of the prohormone brain natriuretic peptide (NT-proBNP) is a 76 amino acid N-terminal inactive protein that is cleaved from proBNP to release brain natriuretic peptide. Both BNP and NT-proBNP levels in the blood are used for screening, diagnosis of acute congestive heart failure (CHF) and may be useful to establish prognosis in heart failure, as both markers are typically higher in patients with worse outcome.[1] The plasma concentrations of both BNP and NT-proBNP are also typically increased in patients with asymptomatic or symptomatic left ventricular dysfunction and is associated with coronary artery disease and myocardial ischemia.[2,3,4]
The NT-proBNP FIA Rapid Test is a simple test that utilizes a combination of anti-NT-proBNP antibody coated particles and capture reagents to quantitatively detect NT-proBNP in whole blood, serum or plasma. The minimum detection level is 0.3 ng/mL.

PRINCIPLE

The NT-proBNP FIA Rapid Test Cassette detects NT-proBNP based on Fluorescence Immunoassay. The sample moves through the strip from sample pad to absorbent pad. If the specimen contains NT-proBNP, it attaches to the fluorescent microspheres-conjugated anti-NT-proBNP antibodies. Then the complex will be captured by the capture antibodies coated on the nitrocellulose membrane (Test line). The concentration of NT-proBNP in the sample correlates linearly with the fluorescence signal intensity captured on the T line. According to the fluorescence intensity of the test and standard curve, the concentration of NT-proBNP in the sample can be calculated by the Biopanda Fluorescence Immunoassay Analyser to show NT-proBNP concentration in specimen.

REAGENTS

The test includes anti-NT-proBNP antibody conjugated fluorophores and capture reagents coated on the membrane.

PRECAUTIONS

1. For professional *in vitro* diagnostic use only.
2. Do not use after the expiration date indicated on the package. Do not use the test if the foil pouch is damaged. Do not reuse.
3. Avoid cross-contamination of specimens by using a new specimen collection container for each specimen obtained.
4. Do not eat, drink or smoke in the area where the specimens and tests are handled. Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout the procedure and follow standard procedures for proper disposal of specimens. Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.
5. Do not interchange or mix reagents from different lots.
6. Humidity and temperature can adversely affect results.
7. Used testing materials should be discarded in accordance with local regulations.
8. Read the entire procedure carefully prior to any testing.
9. The Biopanda NT-proBNP FIA Rapid Test should only be used with the Biopanda Fluorescence Immunoassay Analyser by approved medical professionals.

STORAGE AND STABILITY

1. The kit should be stored at 4-30°C until the expiry date printed on the sealed pouch.
2. The test must remain in the sealed pouch until use.
3. Do not freeze.
4. Care should be taken to protect the components of the kit from contamination. Do not use if there is evidence of microbial contamination or precipitation. Biological contamination of dispensing equipment, containers or reagents can lead to false results.

KIT COMPONENTS

- 10 x foil wrapped NT-proBNP test cassettes
- 10 x Specimen collection tubes with extraction buffer
- 1 x ID card (NT-proBNP)
- Package Insert

MATERIALS REQUIRED BUT NOT PROVIDED

- Timer
- Centrifuge
- Pipette
- Biopanda Fluorescence Immunoassay Analyser

SPECIMEN COLLECTION AND PREPARATION

PREPARATION

1. Before performing the test, please make sure that all components are brought to room temperature (15-30°C). Cold buffer solution or moisture condensation on the membrane can lead to invalid test results.
2. Take a tube with buffer solution out of the kit. Document patients name or ID on it. Open the screw cap.

BLOOD SAMPLE TAKING

1. Collect the specimen according to standard procedures.
2. To collect **Fingerstick Whole blood specimens**:
   Wash the patient's hand with soap and warm water or clean with an alcohol swab. Allow to dry.
   Massage the hand without touching the puncture site by rubbing down the hand towards the fingertip of the middle or ring finger.
   Puncture the skin with a sterile lancet. Wipe away the first sign of blood.
   Gently rub the hand from wrist to palm to finger to form a rounded drop of blood over the puncture site.
   Add the Fingerstick Whole blood specimen to the tube with buffer by using pipette.
3. **For Serum/Plasma:** Separate serum or plasma from blood as soon as possible to avoid hemolysis. Use only clear non-hemolyzed specimens.
4. Testing should be performed immediately after the specimens have been collected. Do not leave the specimens at room temperature for prolonged periods. Serum and plasma specimens may be stored at 2-8°C for up to 2 days. For long term storage, specimens should be kept below -20°C. Whole blood collected by venipuncture should be stored at 2-8°C if the test is to be run within 2 days of collection. Do not freeze whole blood specimens. Whole blood collected by fingerstick should be tested immediately.
5. EDTA K2, Heparin sodium, Citrate sodium and Oxalate potassium can be used as the anticoagulant tube for collecting the blood specimen.
6. Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly.
7. If specimens are to be shipped, they should be packed in compliance with local regulations covering the transportation of etiologic agents.

SAMPLE DILUTION / SAMPLE STABILITY

1. The specimen **(50 ul of whole blood / serum / plasma)** can be added directly with a micro pipette into the buffer tube.
2. Close the tube and shake the sample by hand vigorously for approximately **10 seconds** to mix the sample and dilution buffer.
3. Let the diluted sample homogenize for approximately **1 minute**.
4. It is best to place the diluted sample on an ice pack and leave the sample at room temperature for no more than 8 hours.

DIRECTIONS FOR USE

Refer to the Biopanda Fluorescence Immunoassay Analyser Operation Manual for the complete instructions on use of the Test. The test should be conducted at room temperature.
Allow the test, specimen, buffer and/or controls to reach room temperature (15-30°C) prior to testing.

1. Turn on the Analyser. Then according to the user requirement, select “Standard test” or “Quick test” mode.
2. Take out the ID card and insert it into the Analyser port.
3. Transfer **50 µl of whole blood/serum/plasma** into the buffer tube, mix the specimen and the buffer well.
4. **Add diluted specimen with a Pipette**: Pipette 85 µl of diluted specimen into the sample well of the test cassette. Start the timer at the same time.
5. There are two test modes for the Biopanda Fluorescence Immunoassay Analyser; Standard Test mode and Quick Test mode. Please refer to the user manual of the Biopanda Fluorescence Immunoassay Analyser for details.

“Quick test” mode: Insert the test cassette into the Analyser at 15 minutes after sample application and click "New Test", the Analyser will automatically give the test result after a few seconds.
“Standard test” mode: Insert the test cassette into the Analyser immediately after sample application, click "New test" at the same time, the Analyser will automatically count down the 15 minutes. After the countdown, the Analyser will give the result at once.

INTERPRETATION OF RESULTS

The result of tests for NT-proBNP is calculated by the Biopanda Fluorescence Immunoassay Analyser and displays the result on the screen. For additional information, please refer to the user manual of the Biopanda Fluorescence Immunoassay Analyser.

Linearity range of the Biopanda NT-proBNP FIA Rapid Test is 0.3-22 ng/mL.

QUALITY CONTROL

Each Biopanda NT-proBNP FIA Rapid Test Cassette contains an internal control that satisfies routine quality control requirements. This internal control is performed each time a patient sample is tested. This control indicates that the test device was inserted and read properly by the Biopanda Fluorescence Immunoassay Analyser. An invalid result from the internal control causes an error message on the Biopanda Fluorescence Immunoassay Analyser indicating that the test should be repeated. An invalid result from the internal control causes an “N/A” message on the Biopanda Fluorescence Immunoassay Analyser. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control failure. Review the procedure and repeat the test with a new test. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

LIMITATIONS

1. The Biopanda NT-proBNP FIA Rapid Test Cassette is for professional *in vitro* diagnostic use, and should only be used for the quantitative detection of NT-proBNP.
2. The Biopanda NT-proBNP FIA Rapid Test Cassette will only indicate the presence of NT-proBNP in the specimen and should not be used as the sole criterion for evaluating AMI.
3. As with all diagnostic tests, a confirmed diagnosis should only be made by a physician after all clinical and laboratory findings have been evaluated.
4. High concentrations of NT-proBNP may produce a dose hook effect, resulting in incorrect interpretation of NT-proBNP levels. High dose hook effect has not been observed with this test up to 22 ng/mL of NT-proBNP.
5. The haematocrit level of the whole blood should be between 25% and 65%.
6. The results of the Biopanda NT-proBNP FIA Rapid Tests are based on measuring the levels of NT-proBNP in a specimen. It should not be used as the sole criterion for treatment decisions. If the result is positive, other clinical findings and alternative test methods are recommended to reach proper medical treatments.

EXPECTED RESULTS

Concentrations	Clinical Reference
<0.45 ng/mL	Not indicative of Acute Heart Failure
>0.45 ng/mL	Indicative of Acute Heart Failure

PERFORMANCE CHARACTERISTICS

1. **ACCURACY**: The test deviation is ≤±15%.
2. **SENSITIVITY**: The Biopanda NT-proBNP FIA Rapid Test Cassette can detect levels of NT-proBNP as low as 0.3 ng/mL in whole blood, serum or plasma.
3. **DETECTION RANGE**: 0.3-22 ng/mL
4. **LINEARITY RANGE**: 0.3-22 ng/mL, R≥0.990
5. **PRECISION**

INTRA-LOT PRECISION

Within-run precision has been determined by using 10 replicates of 5 specimens containing 0 ng/ml, 0.45 ng/ml, 2 ng/ml, 10 ng/ml and 20 ng/ml of NT-proBNP. C.V. is ≤ 15%.

INTER-LOT PRECISION

Between-run precision has been determined by using 10 replicates for each of three lots using 5 specimens containing 0 ng/ml, 0.45 ng/ml, 2 ng/ml, 10 ng/ml and 20 ng/ml of NT-proBNP. C.V. is ≤15%.

6**. CROSS-REACTIVITY**

Cross-reactivity studies were carried out with following analytes.
HBsAg, HBsAb, HBeAg, HBeAb, HBcAb, syphilis, anti-HIV, anti-H.pylori, MONO, anti-CMV, anti-Rubella and anti-Toxoplasmosis positive specimens. The results showed no cross-reactivity.

7. **INTERFERING SUBSTANCES**

The following potentially interfering substances were added to NT-proBNP negative and positive specimens, respectively.

Acetaminophen: 20 mg/dL	Caffeine:20 mg/dL
Acetylsalicylic Acid:20 mg/dL	Gentisic Acid:20 mg/dL
Ascorbic Acid:20mg/dL	Albumin:10,500mg/dL
Creatin:200 mg/dL	Hemoglobin:1,000 mg/dL
Bilirubin:1,000mg/dL	Oxalic Acid:600mg/dL
Cholesterol:800mg/dL	Triglycerides:1,600mg/dL

None of the substances at the concentration tested interfered in the assay

REFERENCES

1. Wong SS. Strategic utilization of cardiac markers for diagnosis of acute myocardial infarction. Ann Clin Lab Sci, 26:301-12, 1996.
2. Kagen LJ.NT-proBNP methods and diagnostic uses.CRC Crit.Rev. Clin.Lab.Sci., 2:273,1978.
3. Chapelle JP.et al.Serum NT-proBNP determinations in the assessment of acute myocardial infarction. Eur. Heart Journal, 3:122, 1982.
4. Hamfelt A. et al.Use of biochemical tests for myocardial infarction in the county of Vasternorrland, a clinical chemistry routine for the diagnosis of myocardial infarction. Scand. J. Clin. Lab. Invest. Suppl., 200:20, 1990.

Index of Symbols

	Manufacturer	Σ	Tests per kit	2	Do not reuse test
IVD	*In vitro* diagnostic medical device		Expiration date	REF	Catalogue number
4°C 30°C	Store between 4-30°C	LOT	Lot Number		Consult instructions for use
	Do not use if package is damaged				

Thank you for purchasing Biopanda's NT-proBNP FIA Rapid Test. Please read this manual carefully before operating to ensure proper use.

Biopanda Reagents Ltd.
Unit 14 Carrowreagh Business Park
Carrowreagh Road
Belfast, BT16 1QQ
United Kingdom
Tel: +44 (0) 28 95438774
E-mail: info@biopanda.co.uk
Website: www.biopanda.co.uk

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