INSTRUCTIONS FOR USE
ONE STEP TEST
NT-proBNP Detection
In Whole Blood / Serum / Plasma

TOYO DIAGNOSTICS

Only for professional *in vitro* diagnostic use

Product Code : TNPB01

BACKROUND INFORMATION

Congestive Heart Failure (CHF) is described as a condition in which the heart is unable to maintain adequate circulation of blood in the tissues of the body or to pump out the venous blood returned to it by the venous circulation CHF affects nearly 17 million people worldwide.
NT-proBNP, N-terminal fragment of proBNP, is cleaved from the precursor peptide proBNP. It shows close correlation with the severity of heart failure. NT-proBNP is widely recognized as a definitive marker for the diagnosis of CHF. Some amount of NT-proBNP is available in the serum. However, elevated levels of NT-proBNP indicate the presence of CHF.
The high sensitivity of NT-proBNP allows also the detection of mild forms of cardiac dysfunction in asymptomatic patients with structural heart disease. The European Society of Cardiology Task Force for the Diagnosis and Treatment of Chronic Heart Failure recommend in their guidelines that natriuretic peptides including NT-proBNP may be most useful clinically as a rule out test due to consistent and very high negative predictive values.

INTENDED USE

NT-proBNP Test Device is a rapid immunochromatographic assay for qualitative detection of human NT-proBNP in human whole blood / serum / plasma to aid diagnosis of congestive heart failure (CHF).

REAGENTS

The test device contains anti-NT-proBNP antibodies coated particles and capture reagents immobilized on the nitrocellulose membrane.

METHOD

NT-proBNP Test Device is a rapid, qualitative, immunochromatographic assay for the detection of NT-proBNP in human whole blood / serum / plasma samples. There is capture reagent immobilized to "T" test area of the test. While performing the test; whole blood / serum / plasma sample dropped to the sample well reacts with the particles coated with anti-NT-proBNP antibodies. This complex migrates to the other end of the membrane by capillary action. If there is NT-proBNP in the sample, it binds to capture reagent in the "T" test area and create a visible, colored signal that means the test result is positive. If the sample does not contain NT-proBNP, colored line does not appear in the "T" test area. This means the test result is negative. As a procedural control, a colored line always appears in the "C" control area indicating that proper volume of sample has been introduced and membrane wicking has occurred.

PRECAUTIONS AND LIMITATIONS

1.For professional and *in vitro* diagnostic use only.
2.Do not use test kit beyond expiry date. The test device is single use. Do not reuse.
3.The test device should remain in its original sealed pouch until usage. Do not use the test if the seal is broken or the pouch is damaged.
4.Wear disposable gloves while performing the test.
5.Use a new dropper for each sample.
6.All patient samples should be handled as taking capable of transmitting disease into consideration. Observe established precautions against microbiological hazards throughout all procedures and follow the standard procedures for proper disposal of samples.
7. If the test result is negative and clinical symptoms persist, additional testing using other clinical methods is recommended. A negative result does not at any time preclude the possibility of congestive heart failure.
8.This test will indicate only the presence or absence of NT-proBNP in the sample, and should not be used as the only basis for the diagnosis of congestive heart failure.
As with all diagnostic tests, it should be kept in mind that an identification diagnosis can't be based on a single test result. Diagnosis can only be reached by an expert after the evaluation of all clinical and laboratory findings.

STORAGE

Test device should be kept away from direct sunlight, moisture, heat and radiation sources.
Store at 4 - 30°C (39 - 86°F). Do not freeze.
The test in the original packaging retains stable until expiry date at storage conditions. The test device should be used in maximum one hour after the foil is opened.

SAMPLE COLLECTION AND PREPARATION

The test can be performed using whole blood, serum or plasma. To avoid hemolysis, serum or plasma should be separated from blood as soon as possible.

For Whole Blood Samples: Test should be performed immediately with whole blood samples. Otherwise, whole blood samples should be stored at 2 - 8 °C with anticoagulants (EDTA, heparin, citrate should be used) to avoid coagulation until they are being tested in a period of 2 days after collection.

For Serum Samples: Collect blood into a collection tube without anticoagulant, leave to settle for 30 minutes for blood coagulation and then centrifuge the blood. At the end of centrifuge period remaining supernatant is used as serum.

For Plasma Samples: Collect blood into a collection tube with anticoagulants (EDTA, heparin, citrate should be used) to avoid coagulation of blood sample and then centrifuge the blood. At the end of centrifuge period supernatant is used as plasma.

Do not use turbid, hemolyzed samples. If the sample cannot be tested on the day of collection, store the serum, plasma samples in a refrigerator or freezer. Do not freeze and thaw the serum, plasma samples repeatedly. Do not freeze whole blood sample. Bring the samples to room temperature before testing. Frozen samples must be completely thawed and mixed well prior to testing. Turbid test samples should be centrifuged. Using of frozen and thawed samples should be avoided whenever possible, due to the blocking of the membrane by the debris.

Kit components : Test devices, droppers, diluents (for whole blood samples only) and instructions for use.

Additional materials required but not provided : Sample collection tube, centrifuge and timer, lancet (for only fingerstick whole blood), heparinized dispensing bulbs and capillary tubes (for only fingerstick whole blood).

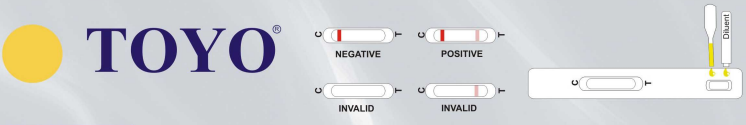
Additional materials recommended but not provided : Micropipettes to deliver mentioned amount of sample in the test procedure, negative and positive control materials.

TEST PROCEDURE

1. Take the test device out of its pouch. Bring the tests and whole blood / serum /plasma samples to room temperature.
2. **For Whole Blood Samples:** Draw whole blood into dropper and put 3 drops (90 µl) into the sample well of the cassette. Immediately after, 1 drop (~40µL) of diluent is added into the sample well and allowed to soak in.
For Serum / Plasma Samples: Draw serum / plasma into dropper and put 4 drops (120 µl) into the sample well of the cassette. Do not use diluent for serum / plasma samples.
Avoid the formation of any air bubbles.
3. Depending on the NT-proBNP concentration in the sample, the test can react even in 5 minutes. Results should be read at 10 minutes as shown below. Results forming after 20 minutes should be regarded as invalid.

INTERPRETATION OF RESULTS

Negative: Only one colored line is visible in "C" area, indicating that NT-proBNP does not exist.
Positive : Two colored lines are visible in "C" and "T" areas, indicating that NT-proBNP exists.
Low concentration of NT-proBNP may cause a faint line in "T" area. Even such a faint line in "T" area should be regarded as "positive".
Invalid : No colored line is visible or only one colored line is visible in "T" area; test should be repeated using a new test device.



QUALITY CONTROL

Tests have built in procedural quality control features. When the test is complete, the user will see a colored line in the "C" area of the test on negative samples and a colored line in the "T" and "C" area on positive samples. The appearance of the control "C" line is considered as an internal procedural control. This line indicates that sufficient volume of sample was added as well as valid test result. It is recommended that a negative control and a positive control be used to verify proper test performance as an external control. Users should follow appropriate federal, state and local guidelines concerning the external quality controls.

PERFORMANCE EVALUATION

Sensitivity and Specificity
The NT-proBNP Test Device has been evaluated with a leading commercial NT-proBNP CLIA test using clinical specimens. The results are as below;

NT-proBNP Test Device - CLIA

Method		CLIA		Total Results
	Results	Positive	Negative	
NT-proBNP Test Device	Positive	55	1	56
	Negative	0	170	170
Total Results		55	171	226

Sensitivity: 100 %
+ Predictive V: 98,2 %
Cut off: 450 pg/mL.
Specificity: 99,4%
- Predictive V: 100 %

Intra-Assay
Within-run precision has been determined by using replicates of 15 tests using NT-proBNP specimen levels at 0 pg/mL, 450 pg/mL, 1.000 pg/mL, 3.000 pg/mL. The specimens were correctly identified >99,9% of the time.

Inter-Assay
Between-run precision has been determined by 3 independent assays on the same four specimens: 0 pg/mL, 450 pg/mL, 1.000 pg/mL, 3.000 pg/mL of NT-proBNP. The NT-proBNP Test Device has been tested using these specimens. The specimens were correctly identified >99,9% of the time.

INTERFERING SUBSTANCES

The NT-proBNP Test Device has been tested and no interference was observed in specimens containing 50 mg/mL human albumin, 350 ug/mL bilirubin, 20 mg/mL hemoglobin, 10 mg/mL cholesterol and 40 mg/mL triglycerides. The following compounds have also been tested using the NT-proBNP Test Device and no interference was observed at a concentration of 100 µg/mL.

Acetaminophen
Acetylsalicylic acid
Anisodamine
Ascorbic Acid
Atenolol
Atorvastatin Calcium
Bisoprolol Fumarate
Caffeine
Captopril
Chloramphanicol
Chlordiazepoxide
Cilazapril
Diclofenac
Digoxin
Erythromycin
Furosemide
Flunarizine Hydrochloride
Hydrochlorothiazide
Isosorbide Mononitrate
Labetalol
Metoprolol Tartrate
Moracizine Hydrochloride
Nifedipine
Oxazepam
Pentoxifyline
Phenobarbital
Quinine
Ramipril
DL-Tyrosine
Trimethoprim
Verapamil

REFERANCES

1.Mueller T, et al. Head-to-head comparison of the diagnostic utility of BNP and NTproBNP in symptomatic and asymptomatic structural heart disease. Clin Chim Acta 2004;341:41-48.
2. Pfister R, et al. Use of NT-proBNP in routine testing and comparison to BNP. Eur J Heart Fail 2004;6(3):289-293.
3. Seino Y, et al. Application of NT-proBNP and BNP measurements in cardiac care: a more discerning marker for the detection and evaluation of heart failure. Eur J Heart Fail 2004; 6(3):295-300.
4. Remme WJ, Swedberg K et al. The European Society of Cardiology Task Force Report: Guidelines for the diagnosis and treatment of chronic heart failure. Eur Heart J 2001;22: 1527-1560.

TÜRKLAB TIBBİ MALZEMELER SAN. TİC. A .Ş.
A.O.S.B 10040 Sok. No:20 Çiğli-İzmir / TURKEY
TEL: +90 232 376 80 81 FAX: +90 232 376 80 40 info@turklab.com.tr www.turklab.com.tr

EC REP **GESAN PRODUCTION s.r.l.**
Via Einaudi, 19 91021 TRE FONTANE - Campobello di Mazara (TP) ITALY

SYMBOLS USED

CE
Manufacturer
EC REP Authorized European Representative
Attention, see instruction for use
Consult instruction for use
IVD *In vitro* diagnostic medical device
Σ n Number of test
For single use only
Storage temperature
LOT Lot number
REF Catalog number
Expiry date