MAGLUMI NT-proBNP (CLIA)

REF 130206004M Σ 100

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CE

FOR PROFESSIONAL USE ONLY
Store at 2...8 °C

COMPLETELY READ THE INSTRUCTIONS BEFORE PROCEEDING

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SYMBOLS EXPLANATIONS

EC REP	Authorized Representative in Europe
	Manufacturer
	Attention. See Instructions For Use
CONT	Contents of kit
IVD	In vitro diagnostic medical device (In vitro diagnostic use)
LOT	Lot number
REF	Catalogue Code
	Expiry date (Use by…)
8°C 2°C	Temperature limitation (store at 2...8 °C)
Σ	Number of tests
	Keep away from sunlight
	Keep upright

INTENDED USE

The kit has been designed for the quantitative determination of N-terminal prohormone b-type natriuretic peptide (NT-proBNP) in human serum.
The method can be used for samples over the range of 0-10000 pg/ml.
The test has to be performed on the MAGLUMI chemiluminescence immunoassay (CLIA) fully auto analyzer (Including MAGLUMI 1000, MAGLUMI 2000, MAGLUMI 2000 Plus and new developed model).

SUMMARY AND EXPLANATION OF THE TEST

Heart failure is an important clinical syndrome which compromises left ventricular systolic or diastolic function or a combination of both. Heart failure occurs when the heart is unable to pump blood at a rate sufficient for metabolic requirements. Its most common causes are coronary artery disease, hypertension, valvular heart diseases and cardiomyopathies. Accurate and early diagnosis is important since effective therapeutic interventions (e.g., angiotensin converting enzyme inhibitors, beta-blockers) are available, which improve both morbidity and mortality. Based on clinical signs and symptoms, the severity of heart failure is classified into four classes of increasing disease progression according to the New York Heart Association classification.
The natriuretic peptide system is a family of structurally similar but genetically distinct peptides, which include atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) of myocardial cell origin and C-type natriuretic peptide (CNP) of endothelial cell origin. These peptides are characterized by a common 17 amino acid ring structure with a disulfide bond between two cysteine residues. The cardiac natriuretic peptides are the naturally occurring antagonists of the rennin-angiotensin-aldosterone system and of the sympathetic nervous system. They promote natriuresis and diuresis, act as vasodilators, and exert antimitogenic effects on cardiovascular tissues. ANP and BNP are secreted by the heart in response to hemodynamic stress. Increased levels of BNP are produced mainly in response to left ventricular wall stretch and volume overload. ANP and BNP are expressed predominantly in the atria and ventricles, respectively, and are important in regulation of blood pressure, electrolyte and volume homeostasis.
The cardiac natriuretic peptide system is activated to its highest degree in ventricular dysfunction and has an important role in maintaining the compensated state of asymptomatic heart failure and delaying disease progression. BNP is synthesized within the cardiomyocyte as a preprohormone (preproBNP) of 134 amino acids, from which a prehormone (proBNP) of 108 amino acids and a signal peptide of 26 amino acids is derived. The proBNP precursor protein is then cleaved into a physiologically active 32 amino acid C-terminal peptide (BNP 77-108; BNP) and a 76 amino acid N-terminal prohormone fragment (NT-pro BNP 1-76). Studies indicate that the pro BNP protein precursor is cleaved either within or on the surface of cardiomyocytes, and that both NT-pro BNP (1-76) and physiologically active C-terminal BNP molecule (77-108) are released into the bloodstream.
Several studies indicate that BNP can be used for a wide range of clinical applications;
Include diagnosis, monitoring and prognosis. The circulating levels of BNP increase with decreasing left ventricular function and increasing clinical severity of heart failure, according to the NYHA classification, which makes it an appropriate test for diagnosis and staging of heart failure. Other studies have demonstrated that an increased level of circulating BNP correlates with higher incidence of cardiac events and mortality in patients with heart failure and Acute Coronary Syndromes, and supports utilization of BNP as a marker for patient prognosis. There are indications that BNP can

be used to provide an index to modulate treatment of patients with heart failure.

It has been reported that patients with acute decompensated heart failure who are candidates for nesiritide (recombinant BNP) infusion should have a baseline BNP measurement taken prior to initiation of therapy. Measurements taken during infusion are reflective of the dose of nesiritide. Because of the short half-life of BNP (20 minutes), measurements taken 2 hours after the cessation of treatment again reflect the level of endogenous BNP. It has also been reported that following infusion, endogenous BNP levels return to baseline by 12 hours and continue to drop at 6 hours to about 80% of preinfusion levels, suggesting a resetting of the neuro-hormonal axis and improvement in ventricular wall tension as a result of treatment. The assay is not approved for nesiritide monitoring.

PRINCIPLE OF THE TEST

Sandwich immunoluminometric assay;

Use an anti-Myoglobin monoclonal antibody to label ABEI, and use another monoclonal antibody to label FITC. Sample, Calibrator or Control with ABEI Label, FITC Label and magnetic microbeads coated with anti-FITC are mixed thoroughly and incubated at 37℃, forming a sandwich; after sediment in a magnetic field, decant the supernatant, then cycle washing it for 1 time. Subsequently, the starter reagents are added and a flash chemiluminescent reaction is initiated. The light signal is measured by a photomultiplier as RLU within 3 seconds and is proportional to the concentration of Myoglobin present in controls or samples.

CONT KIT COMPONENTS

Material Supplies

Reagent Integral for 100 determinations	
Nano magnetic microbeads: TRIS buffer, 1.2%(W/V), 0.2%NaN_3, coated with anti-NT-pro BNP monoclonal antibody	2.5ml
Calibrator Low: bovine serum, 0.2%NaN_3.	3.0ml
Calibrator High: bovine serum, 0.2%NaN_3	3.0ml
ABEI Label: anti-NT pro BNP monoclonal antibody labeled ABEI, contains BSA, 0.2%NaN_3.	12.5ml
All reagents are provided ready-to-use.	

Reagent Vials in kit box	
Internal Quality Control: containing BSA, 0.2%NaN_3. (target value refer to Quality Control Information date sheet)	2.0ml

Accessories Required But Not Provided

MAGLUMI Reaction Module	REF: 630003
MAGLUMI Starter 1+2	REF: 130299004M
MAGLUMI Wash Concentrate	REF: 130299005M
MAGLUMI Light Check	REF: 130299006M

Preparation of the Reagent Integral

Before the sealing is removed, gentle and careful horizontal shaking of the Reagent Integral is essential (avoid foam formation!) Remove the sealing and turn the small wheel of the magnetic microbeads compartment to and fro, until the colour of the suspension has changed into brown. Place the Integral into the reagent area and let it stand there for 30 min. During this time, the magnetic microbeads are automatically agitated and completely resuspended.

Do not interchange integral component from different reagents or lots!

Storage and Stability

- Sealed: Stored at 2-8 ℃ until the expiry date.
- Opened: Stable for 4 weeks. To ensure the best kit performance, it is recommended to place opened kits in the refrigerator if it's not going to be used on board during the next 12 hours.
-  Keep upright for storage.
-  Keep away from direct sunlight.

CALIBRATION AND TRACEABILITY

1) Traceability

Calibrators in the Reagent Kit are from Biodesign

To perform an accurate calibration, we have provided the test calibrators standardized against the SNIBE internal reference substance.

2) 2-Point Recalibration

Via the measurement of calibrators, the predefined master curve is adjusted (recalibrated) to a new, instrument-specific measurement level with each calibration.

3) Frequency of Recalibration

- After each exchange of lot (Reagent Integral or Starter Reagents).
- Every week and/or each time a new Integral is used (recommendation).
- After each servicing of the MAGLUMI Fully Auto analyzer.
- If controls are beyond the expected range.

SPECIMEN COLLECTION AND PREPARATION

Sample material: serum

Collect samples using standard procedures.

Store at 2-8 ℃: 24 hours, for lo
below - 20 ℃

Avoid repeated freezing and thawing cycles, stored samples should be thoroughly mixed prior to use (Vortex mixer).

Please ask local representative of SNIBE for more details if you have any doubt.

Vacuum Tubes

(a) Blank tubes are recommended type for collecting samples.

(b) Please ask SNIBE for advice if special additive must be used in sample collecting.

Specimen Conditions

- Do not use specimens with the following conditions:
  (a) heat-inactivated specimens;
  (b) Cadaver specimens or body fluids other than human serum;
  (c) Obvious microbial contamination.
- Use caution when handling patient specimens to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
- Inspect all samples for bubbles. Remove bubbles with an applicator stick prior to analysis. Use a new applicator stick for each sample to prevent cross contamination.
- Serum specimens should be free of fibrin, red blood cells or other particulate matter.
- Ensure that complete clot formation in serum specimens has taken place prior to centrifugation. Some specimens, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the specimen is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results.

Preparation for Analysis

- Patient specimens with a cloudy or turbid appearance must be centrifuged prior to testing. Following centrifugation, avoid the lipid layer (if present) when pipetting the specimen into a sample cup or secondary tube.
- Specimens must be mixed **thoroughly** after thawing by **low** speed vortexing or by gently inverting, and centrifuged prior to use to remove red blood cells or particulate matter to ensure consistency in the results. Multiple freeze-thaw cycles of specimens should be avoided.
- All samples (patient specimens or controls) should be tested within 3 hours of being placed on board the MAGLUMI System. Refer to the SNIBE service for a more detailed discussion of onboard sample storage constraints.

Storage

- If testing will be delayed for more than 8 hours, remove serum or plasma from the serum or plasma separator, red blood cells or clot. Specimens removed from the separator gel, cells or clot may be stored up to 24 hours at 2-8°C.
- Specimens can be stored up to 30 days frozen at -20°C or colder.

Shipping

- Before shipping specimens, it is recommended that specimens be removed from the serum or plasma separator, red blood cells or clot. When shipped, specimens must be packaged and labeled in compliance with applicable state, federal and international regulations covering the transport of clinical specimens and infectious substances. Specimens must be shipped frozen (dry ice). Do not exceed the storage time limitations identified in this section of the package insert.

WARNING AND PRECAUTIONS FOR USERS

IVD

- For use in *IN-VITRO* diagnostic procedures only.
- Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Safety Precautions

CAUTION: This product requires the handling of human specimens.

- The calibrators in this kit are prepared from bovine serum products. However, because no test method can offer complete assurance that HIV, Hepatitis B Virus or other infectious agents are absent; these reagents should be considered a potential biohazard and handled with the same precautions as applied to any serum or plasma specimen.
- All samples, biological reagents and materials used in the assay must be considered potentially able to transmit infectious agents. They should therefore be disposed of in accordance with the prevailing regulations and guidelines of the agencies holding jurisdiction over the laboratory, and the regulations of each country. Disposable materials must be incinerated; liquid waste must be decontaminated with sodium hypochlorite at a final concentration of 5% for at least half an hour. Any materials to be reused must be autoclaved using an overkill approach (USP 24, 2000, p.2143). A minimum of one hour at 121 ℃ is usually considered adequate, though the users must check the effectiveness of their decontamination cycle by initially validating it and routinely using biological indicators.
- It is recommended that all human sourced materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens13. Biosafety Level 214 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.
- This product contains Sodium Azide; this material and its container must be disposed of in a safe way.
- Safety data sheets are available on request.

Handling Precautions

- Do not use reagent kits beyond the expiration date.
- Do not mix reagents from different reagent kits.
- Prior to loading the Reagent Kit on the system for the first time, the microbeads requires mixing to re-suspend microbeads that have settled during shipment.
- For microbeads mixing instructions, refer to the KIT COMPONENTS, Preparation of the Reagent Integral section of this package insert.
- To avoid contamination, wear clean gloves when operating with a reagent kit and sample.
- Over time, residual liquids may dry on the kit surface, please pay attention the silicon film still exists on the surface of the kit.
- For a detailed discussion of handling precautions during system operation, refer to the SNIBE service information.

TEST PROCEDURE

To ensure proper test performance, strictly adhere to the operating instructions of the MAGLUMI Fully Auto analyzer. Each test parameter is identified via a RFID tag on the Reagent Integral. For further information please refer to the MAGLUMI Chemiluminescence Analyzer Operating Instructions.

100µl +100µl +20µl	Sample, calibrator or controls ABEI Label Nano magnetic microbeads
15 min	Incubation
400µl	Cycle washing
3 s	Measurement

DILUTION

Sample dilution by analyzer is not available in this reagent kit.
Samples with concentrations above the measuring range can be diluted manually. After manual dilution, multiply the result by the dilution factor.
Please choose applicable diluents or ask SNIBE for advice before manual dilution must be processed.

QUALITY CONTROL

- Observe quality control guidelines for medical laboratories.
- Use suitable controls for in-house quality control. Controls should be run at least once every 24 hours when the test is in use, once per reagent kit and after every calibration. The control intervals should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined ranges. Each laboratory should establish guidelines for corrective measures to be taken if values fall outside the range.

LIMITATIONS OF THE PROCEDURE

1) Limitations

A skillful technique and strict adherence to the instructions are necessary to obtain reliable results. Bacterial contamination of samples or repeated freeze-thaw cycles may affect the test results. Assay results should be utilized in conjunction with other clinical and laboratory data to assist the clinician in making individual patient management decisions.

2) Interfering Substances

No interference with test results is seen by concentrations of bilirubin＜0.06mg/ml, haemoglobin＜16mg/dl or triglycerides＜12.5mg/ml.

3) HAMA

Patient samples containing human anti-mouse antibodies (HAMA)

may give falsely elevated or decreased values. Although HAMA-neutralising agents are added, extremely high HAMA serum concentrations may occasionally influence results.

4) High-Dose Hook

High dose hook is a phenomenon whereby very high level specimens may read within the dynamic range of the assay. For the MAGLUMI NT-proBNP assay, no high dose hook effect was observed when samples containing up to 100ng/ml

RESULTS

1) Calculation of Results

- The analyzer automatically calculates the NT-proBNP concentration in each sample by means of a calibration curve which is generated by a 2-point calibration master curve procedure. The results are expressed in pg/ml for further information please refer to the MAGLUMI Chemiluminescence Analyzer Operating Instructions.

2) Interpretation of Results

- Regarding the adults between 18 and 54, the value is 125pg/ml, according to the 95% distribution。

Concentration of NT-proBNP (pg/ml) for adults

Age	18-44	45-54	55-64	65-74	≥75
Number	486	286	275	86	24
Average	34.8	48.9	88.6	117	241
Value of 95% distribution	96.5	123	225	325	610

Concentration of NT-proBNP（pg/ml）for children between 1 to 18：

Age	Number	Value of 75% distribution
1-3	12	234
4-6	23	115
7-9	31	93
10	10	71
11	52	92
12	20	94
13	25	116
14	16	65
15	23	72
16	28	83
17	26	70
18	13	52

- Results may differ between laboratories due to variations in population and test method. If necessary, each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

1) Precision

Intra-assay coefficient of variation was evaluated on 3 different levels of control serum repeatedly measured 20 times in the same run, calculating the coefficient of variation.

Intra-assay precision			
Control	Mean(pg/ml)	SD(pg/ml)	CV%
Level 1	126.55	4.77	3.77%
Level 2	218.26	7.73	3.54%
Level 3	2270.9	83.80	3.69%

Inter-assay coefficient of variation was evaluated on three batches of kits. Repeatedly measured 3 different levels of control serum 21 times, calculating the coefficient of variation.

Inter-assay precision			
Control	Mean(pg/ml)	SD(pg/ml)	CV%
Level 1	131.12	8.25	6.29%
Level 2	223.77	14.41	6.44%
Level 3	2265.8	152.94	6.75%

2) Analytical Sensitivity

The sensitivity is defined as the concentration of NT-proBNP equivalent to the mean RLU of 20 replicates of the zero standard plus two standard deviations corresponding to the concentration from the standard curve. The sensitivity is typically less than 10pg/ml.

3) Specificity

The specificity of the NT-proBNP assay system was assessed by measuring the apparent response of the assay to various potentially cross reactive analytes.

Compound	Concentration	Cross reactivity
Pro-BNP	500 pg/ml	0.2%

4) Recovery

Consider calibrator high of known concentration as a sample, dilute it by 1:2 ratio with diluents, and measure its diluted concentration for 10 times. Then calculate the recovery of measured concentration and expected concentration. The recovery should be within 90% -110%.

Expected	Mean Measuring	Recovery
2578.6 pg/ml	2602.9 pg/ml	101%

5) Linearity

Use NT-proBNP calibrator to prepare the six-point standard curve, measuring all points' RLU except point A, and then do four-parameter linear fitting in double logarithm coordinate, the absolute linear correlation coefficient(r) should be bigger than 0.9800.

Calibrator Point	Concentration pg/ml	Absolute linear correlation coefficient (r)
A	0	
B	200	r=0.9910
C	1000	
D	2000	
E	5000	
F	10000	

6) Method comparison

A comparison of MAGLUMI NT-proBNP (y) with a commercially available NT-proBNP test (x) using clinical samples gave the following correlations (pg/ml):

Linear regression

y = 1.16x-155

r = 0.959

Sy.x =286

Number of samples measured: 155

The sample concentrations were between 45 and 9000 pg/ml.

REFERENCES

1. Richards AM, Doughty R, Nicholls MG et al. Plasma N-terminal pro-brain natriuretic peptide and adrenomedullin: prognostic utility and prediction of benefit from carvedilol in chronic ischemic left ventricular dysfunction. Australia-New Zealand Heart Failure Group.J Am Coll Cardiol 2001;37:1781–7.

2.Nakao K, Ogawa Y, Suga S-I et al. Molecular biology and biochemistry of the natriuretic peptide system. I: natriuretic peptides. J Hypertension 1992;10:907–12.

3. Hobbs FD, Davis RC, Roalfe AK et al. Reliability of N-terminal probrain natriuretic peptide assay in diagnosis of heart failure: cohort study in representative and high risk community populations. BMJ

4. Bhalla V, Willis S, Maisel AS (2004). "B-type natriuretic peptide: the level and the drug--partners in the diagnosis of congestive heart failure". Congest Heart Fail 10 (1 Suppl1): 3–27.

5. Atisha D, Bhalla MA, Morrison LK, Felicio L, Clopton P, Gardetto N, Kazanegra R, Chiu A, Maisel AS (September 2004). "A prospective study in search of an optimal B-natriuretic peptide level to screen patients for cardiac dysfunction". Am. Heart J. 148 (3): 518–23.