Snibe Diagnostic

CE 0123 REF 130206516M:100 tests/kit
130606516M: 50 tests/kit
130706516M: 30 tests/kit

MAGLUMI® BNP (CLIA)

INTENDED USE

The kit is an *in vitro* chemiluminescence immunoassay for the quantitative determination of BNP in human plasma using the MAGLUMI series Fully-auto chemiluminescence immunoassay analyzer and Biolumi series Integrated System, and the assay is used for an aid in the diagnosis and assessment of the severity of heart failure.

SUMMARY

Natriuretic peptides (NPs) are a family of structurally similar peptide hormones; the main actions of NPs are implicated in eliciting natriuretic, diuretic, steroidogenic and vasorelaxant effects[1,2]. NPs comprise atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP) and C-type natriuretic peptide (CNP). BNP exists widely in brain, heart, lung, digestive tract and so on. Among them, the heart content is the highest, and the synthesis and secretion of BNP in the heart is mainly in the ventricle. BNP is a 32 amino acid peptide with a central loop containing 17 amino-acids linked by a disulfide bond between 2 cysteine residues[3]. The circular structure of BNP makes it physiologically active, and the half-life of BNP is about 20 min[3, 4]. BNP is derived from a brain natriuretic peptide precursor (proBNP), which cleave into two polypeptides, a N-terminal pro-brain natriuretic peptide comprising 76 amino acids (NT-proBNP) and a C-terminal peptide comprising 32 amino acids (BNP). Both of them are excreted into circulating blood[3,4]. The increased level of BNP is mainly impacted by left ventricle-wall tension and blood load[3,5].

Heart failure (HF) is a clinical syndrome that results from a variety of disorders of the myocardium (eg, idiopathic dilated cardiomyopathy), cardiac valves, pericardium, or vasculature (eg, ischemic heart disease) [6]. BNP measurement is mainly used in the clinical diagnosis, monitoring and prognosis of heart failure[3]. It has already been an important diagnostic criterion. The circulating level of BNP reflects the extent of ventricle volume extension, ventricle overload, and heart damage. BNP circulating levels increase as left ventricular function declines and clinical worsening symptoms of heart failure. Used in conjunction with other clinical information, measurement of B-type natriuretic peptide is useful in establishing or excluding the diagnosis of congestive heart failure in patients with acute dyspnea[7].

TEST PRINCIPLE

Sandwich chemiluminescence immunoassay.

The sample, magnetic microbeads coated with anti-BNP monoclonal antibody, ABEI labeled with another anti-BNP monoclonal antibody are mixed thoroughly, reacting to form sandwich complexes and incubating. After precipitation in a magnetic field, the supernatant is decanted and then a wash cycle is performed. Subsequently, the Starter 1+2 are added to initiate a chemiluminescent reaction. The light signal is measured by a photomultiplier as relative light units (RLUs), which is proportional to the concentration of BNP present in the sample.

REAGENTS

Kit Contents

Component	Description	100 tests/kit	50 tests/kit	30 tests/kit
Magnetic Microbeads	Magnetic microbeads coated with anti-BNP monoclonal antibody (~6.00 μg/mL) in MES buffer, NaN_3 (<0.1%).	2.5 mL	2.0 mL	1.0 mL
Calibrator Low	A low concentration of ProBNP protein in PBS buffer, NaN_3 (<0.1%).	2.0 mL	1.5 mL	1.5 mL
Calibrator High	A high concentration of ProBNP protein in PBS buffer, NaN_3 (<0.1%).	2.0 mL	1.5 mL	1.5 mL
ABEI Label	ABEI labeled with anti-BNP monoclonal antibody (~50.0 ng/mL) in Tris-HCl buffer, NaN_3 (<0.1%).	12.5 mL	7.5 mL	4.8 mL
Diluent	PBS buffer, NaN_3 (<0.1%).	10.0 mL	5.5 mL	3.5 mL
Control 1	A low concentration of ProBNP protein (200 pg/mL) in PBS buffer, NaN_3 (<0.1%).	2.0 mL	1.5 mL	1.5 mL
Control 2	A high concentration of ProBNP protein (1000 pg/mL) in PBS buffer, NaN_3 (<0.1%).	2.0 mL	1.5 mL	1.5 mL
All reagents are provided ready-to-use.				

The control barcode labels are provided.

Warnings and Precautions

- For *in vitro* diagnostic use.
- For professional use only.
- Exercise the normal precautions required for handling all laboratory reagents.
- Personal protective measures should be taken to prevent any part of the human body from contacting samples, reagents, and controls, and should comply with local operating requirements for the assay.
- A skillful technique and strict adherence to the package insert are necessary to obtain reliable results.
- Do not use kit beyond the expiration date indicated on the label.
- Do not interchange reagent components from different reagents or lots.
- Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).
- All waste associated with biological samples, biological reagents and disposable materials used for the assay should be considered potentially infectious and should be disposed of in accordance with local guidelines.
- This product contains sodium azide. Sodium azide may react with lead or copper plumbing to form highly explosive metal azides. Immediately after disposal, flush with a large volume of water to prevent azide build-up. For additional information, see Safety Data Sheets available for professional user on request.

Note: If any serious incident has occurred in relation to the device, please report to Shenzhen New Industries Biomedical Engineering Co., Ltd. (Snibe) or our authorized representative and the competent authority of the Member State in which you are established.

Reagent Handling

- To avoid contamination, wear clean gloves when operating with a reagent kit and sample. When handling reagent kit, replace the gloves that have been in contact with samples, since introduction of samples will result in unreliable results.
- Do not use kit in malfunction conditions; e.g., the kit leaking at the sealing film or elsewhere, obviously turbid or precipitation is found in reagents (except for Magnetic Microbeads) or control value is out of the specified range repeatedly. When kit in malfunction conditions, please contact Snibe or our authorized distributor.
- To avoid evaporation of the liquid in the opened reagent kits in refrigerator, it is recommended that the opened reagent kits to be sealed with reagent seals contained within the packaging. The reagent seals are single use, and if more seals are needed, please contact Snibe or our authorized distributor.
- Over time, residual liquids may dry on the septum surface. These are typically dried salts and have no effect on assay efficacy.
- Use always the same analyzer for an opened reagent integral.
- For magnetic microbeads mixing instructions, refer to the Preparation of the Reagent section of this package insert.
- For further information about the reagent handing during system operation, please refer to Analyzer Operating Instructions.

Storage and Stability

- Do not freeze the integral reagents.
- Store the reagent kit upright to ensure complete availability of the magnetic microbeads.
- Protect from direct sunlight.

Stability of the Reagents	
Unopened at 2-8°C	until the stated expiration date
Opened at 2-8°C	6 weeks
On-board	4 weeks

Stability of Controls	
Unopened at 2-8°C	until the stated expiration date
Opened at 18-25°C	6 hours
Opened at 2-8°C	6 weeks
Frozen at -20°C	3 months
Frozen and thawed cycles	no more than 3 times

SPECIMEN COLLECTION AND PREPARATION

Specimen Types

Only the specimens listed below were tested and found acceptable.

Specimen Types	Collection Tubes
Plasma	K2-EDTA, Na2-EDTA

- Samples should be collected in plastic collection tubes, because the BNP molecule is unstable in glass containers[8].
- The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. Follow tube manufacturers' instructions carefully when using collection tubes.

Specimen Conditions

- Do not use heat-inactivated samples or grossly hemolyzed/hyperlipidaemia specimens and specimens with obvious microbial contamination.
- Samples must be free of fibrin and other particulate matter.
- To prevent cross contamination, use of disposable pipettes or pipette tips is recommended.

Preparation for Analysis

- Inspect all specimens for foam. Remove foam with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.
- Frozen specimens must be completely thawed before mixing. Mix thawed specimens thoroughly by low speed vortexing or by gently inverting. Visually inspect the specimens. If layering or stratification is observed, mix until specimens are visibly homogeneous. If specimens are not mixed thoroughly, inconsistent results may be obtained.
- Specimens should be free of fibrin, red blood cells, or other particulate matter. Such specimens may give reliable results and must be centrifuged prior to testing. Transfer clarified specimen to a sample cup or secondary tube for testing. For centrifuged specimens with a lipid layer, transfer only the clarified specimen and not the lipemic material.
- The sample volume required for a single determination of this assay is 100 μL.

Specimen Storage

Specimens removed from the separator, red blood cells or clot may be stored up to 4 hours at 18-25°C, or 24 hours at 2-8°C, or 6 months frozen at -20°C or colder. Frozen specimens subjected to up to 3 freeze/thaw cycles have been evaluated.

Specimen Shipping

- Package and label specimens in compliance with applicable local regulations covering the transport of clinical specimens and infectious substances.
- Do not exceed the storage limitations listed above.

Specimen Dilution

- Samples, BNP concentrations above the analytical measuring interval, can be diluted with Diluent either the automated dilution protocol or the manual dilution procedure. The recommended dilution ratio is 1:5. The concentration of the diluted sample must be >1000 pg/mL.
- For manual dilution, multiply the result by the dilution factor. For dilution by the analyzers, the analyzer software automatically takes the dilution into account when calculating the sample concentration.

PROCEDURE

Materials Provided

BNP (CLIA) assay. control barcode labels.

Materials Required (But Not Provided)

- General laboratory equipment.
- Fully-auto chemiluminescence immunoassay analyzer Maglumi 600, Maglumi 800, Maglumi 1000, Maglumi 2000, Maglumi 2000 Plus, Maglumi 4000, Maglumi 4000 Plus, MAGLUMI X8, MAGLUMI X3, MAGLUMI X6, or Integrated System Biolumi 8000 and Biolumi CX8.
- Additional accessories of test required for the above analyzers include Reaction Module, Starter 1+2, Wash Concentrate, Light Check, Tip, and Reaction Cup. Specific accessories and accessories' specification for each model refer to corresponding Analyzer Operating Instructions.
- Please use accessories specified by Snibe to ensure the reliability of the test results.

Assay Procedure

Preparation of the Reagent

- Take the reagent kit out of the box and visually inspect the integral vials for leaking at the sealing film or elsewhere. If there is no leakage, please tear off the sealing film carefully.
- Open the reagent area door; hold the reagent handle to get the RFID label close to the RFID reader (for about 2s); the buzzer will beep; one beep sound indicates successful sensing.
- Keeping the reagent straight insert to the bottom along the blank reagent track.
- Observe whether the reagent information is displayed successfully in the software interface, otherwise repeat the above two steps.
- Resuspension of the magnetic microbeads takes place automatically when the kit is loaded successfully, ensuring the magnetic microbeads are totally resuspended homogenous prior to use.

Assay Calibration

- Select the assay to be calibrated and execute calibration operation in reagent area interface. For specific information on ordering calibrations, refer to the calibration section of Analyzer Operating Instructions.
- Execute recalibration according to the calibration interval required in this package insert.

Quality Control

- When new lot used, check or edit the quality control information.
- Scan the control barcode, choose corresponding quality control information and execute testing. For specific information on ordering quality controls, refer to the quality control section of the Analyzer Operating Instructions.

Sample Testing

- After successfully loading the sample, select the sample in interface and edit the assay for the sample to be tested and execute testing. For specific information on ordering patient specimens, refer to the sample ordering section of the Analyzer Operating Instructions.

To ensure proper test performance, strictly adhere to Analyzer Operating Instructions.

Calibration

Traceability: This method has been standardized against the Snibe internal reference standard.

Test of assay specific calibrators allows the detected relative light unit (RLU) values to adjust the master curve.

Recalibration is recommended as follows:

- Whenever a new lot of Reagent or Starter 1+2 is used.
- Every 7 days.
- The analyzer has been serviced.
- Control values lie outside the specified range.

Quality Control

Controls are recommended for the determination of quality control requirements for this assay and should be run in singlicate to monitor the assay performance. Refer to published guidelines for general quality control recommendations, for example Clinical and Laboratory Standards Institute (CLSI) Guideline C24 or other published guidelines [9].

Internal controls are recommended once per day of use, or in accordance with local regulations or accreditation requirements and your laboratory's quality control procedures, quality control could be performed by running the BNP assay:

- Whenever the kit is calibrated.
- Whenever a new lot of Starter 1+2 or Wash Concentrate is used.

Controls are only applicable with MAGLUMI and Biolumi system and only used matching with the same top seven LOT numbers of corresponding reagents. For each target value and range refer to the label.

The performance of other controls should be evaluated for compatibility with this assay before they are used. Appropriate value ranges should be established for all

quality control materials used.
Control values must lie within the specified range, whenever one of the controls lies outside the specified range, calibration should be repeated and controls retested. If control values lie repeatedly outside the predefined ranges after successful calibration, patient results must not be reported and take the following actions:

- Verify that the materials are not expired.
- Verify that required maintenance was performed.
- Verify that the assay was performed according to the package insert.
- If necessary, contact Snibe or our authorized distributors for assistance.

If the controls in kit are not enough for use, please order BNP (CLIA) Controls (REF: 160201425MT) from Snibe or our authorized distributors for more.

RESULTS

Calculation

The analyzer automatically calculates the BNP 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 Analyzer Operating Instructions.
Conversion factor: pg/mL×0.289= pmol/L

Interpretation of Results

Non-Heart Failure Population
The normal reference values of 95% distribution were determined by nonparametric method according to the results of 1559 reference individuals (885 women and 674 men). The data from this study are summarized in the following table:

Group (age)		Number	Median (pg/mL)	95th percentile (pg/mL)
Total	<45 years	315	10.820	32.937
	45-54 years	354	21.510	46.233
	55-64 years	326	32.498	53.389
	65-74 years	275	38.726	71.369
	>75 years	289	63.249	169.576
Males	<45 years	133	8.692	28.350
	45-54 years	136	13.149	32.134
	55-64 years	126	23.764	38.460
	65-74 years	144	32.184	67.369
	>75 years	135	39.526	122.591
Females	<45 years	182	14.590	35.031
	45-54 years	218	25.135	56.855
	55-64 years	200	36.810	75.831
	65-74 years	131	45.336	72.289
	>75 years	154	74.202	170.967

Heart Failure Population
The Heart Failure Population reference values of 95% distribution were determined by nonparametric method according to the results of 321 reference individuals (157 women and 164 men). The data from this study are summarized in the following table:

Group	Number	Median (pg/mL)	95th percentile (pg/mL)
Total	321	188.688	2643.549
Males	164	179.582	2125.268
Females	157	196.072	2857.935

Interpretation of the Results
Based on the above clinical research data, we have established a Receiver Operating Characteristic (ROC). The ROC compares clinical sensitivity and specificity at various decision thresholds. At a decision threshold of 100 pg/mL, the BNP assay demonstrated a clinical sensitivity and specificity of 82.27% and 98.27% respectively, in this study. The area under the curve (AUC) is greater than 0.90. Therefore, it is recommended that the BNP decision thresholds diagnoses heart failure is 100 pg/mL. Results may differ between laboratories due to variations in population and test method. It is recommended that each laboratory establish its own reference interval.

LIMITATIONS

- The assay is mainly used to aid in the diagnosis of individuals with suspected or confirmed heart failure and assessment of the severity of heart failure in patients.
- Results should be used in conjunction with patient's medical history, clinical examination and other findings.
- If the BNP results are inconsistent with clinical evidence, additional testing is needed to confirm the result.
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits which employ mouse monoclonal antibodies[10,11]. Additional information may be required for diagnosis.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or animal serum products can be prone to this interference and anomalous values may be observed[12].
- Bacterial contamination or heat inactivation of the specimens may affect the test results.

SPECIFIC PERFORMANCE CHARACTERISTICS

Representative performance data are provided in this section. Results obtained in individual laboratories may vary.

Precision

Precision was determined using the assay, samples and controls in a protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): duplicates at two independent runs per day for 5 days at three different sites using three lots of reagent kits (n = 180). The following results were obtained:

Sample	Mean (pg/mL) (n=180)	Within-Run		Between-Run		Reproducibility	
		SD (pg/mL)	%CV	SD (pg/mL)	%CV	SD (pg/mL)	%CV
Plasma Pool 1	99.396	3.753	3.78	2.290	2.30	5.064	5.09
Plasma Pool 2	406.022	12.227	3.01	9.343	2.30	17.823	4.39
Plasma Pool 3	2046.964	54.084	2.64	20.238	0.99	84.291	4.12
Control 1	199.293	7.864	3.95	4.170	2.09	10.640	5.34
Control 2	995.828	36.386	3.65	16.862	1.69	51.582	5.18

Linear Range

6.00-5000 pg/mL (defined by the Limit of Quantitation and the maximum of the master curve).

Reportable Interval

4.00-25000 pg/mL (defined by the Limit of Detection and the maximum of the master curve×Recommended Dilution Ratio).

Analytical Sensitivity

Limit of Blank (LoB) =2.00 pg/mL .
Limit of Detection (LoD) =4.00 pg/mL .
Limit of Quantitation (LoQ) =6.00 pg/mL .

Analytical Specificity

Interference

Interference was determined using the assay, three samples containing different concentrations of analyte were spiked with potential endogenous and exogenous interferents in a protocol (EP7-A2) of the CLSI. The measurement deviation of the interference substance is within ±10%. The following results were obtained:

Interference	No interference up to	Interference	No interference up to
Bilirubin	20 mg/dL	Phenobarbital	40 μg/mL
Hemoglobin	500 mg/dL	Phenytoin	40 μg/mL
Intralipid	2000 mg/dL	Adrenaline	1000 pg/mL
ANA	6 (S/CO) strong positive	Simvastatin Capsules	32 μg/mL
Rheumatoid factor	1500 IU/mL	Lovastatin	16 μg/mL
HAMA	30 ng/mL	Amlodipine Besylate	4 μg/mL
Nitroglycerin	0.16 μg/mL		

Cross-Reactivity

Cross-reactivity was determined using the assay, three samples containing different concentrations of analyte were spiked with potential cross-reactants in a protocol (EP7-A2) of the CLSI. The measurement deviation of the interference substance is within ±10%. The following results were obtained:

Cross-reactant	No interference up to	Cross-reactant	No interference up to
ANP	1000 pg/mL	Angiotensin III	1000 pg/mL
Angiotensin I	600 pg/mL	CNP	1000 pg/mL
Angiotensin II	600 pg/mL	NT-proBNP (1-76)	1000 pg/mL

High-Dose Hook

No high-dose hook effect was seen for BNP concentrations up to 100000 pg/mL.

Method Comparison

A comparison of the BNP assay with a commercially available immunoassay, gave the following correlations (pg/mL):
Number of samples measured: 100
Passing-Bablok: y=1.0148x-0.0208, τ=0.971.
The clinical specimen concentrations were between 6.16 and 4857.99 pg/mL.

REFERENCES

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SYMBOLS EXPLANATIONS

	Consult instructions for use		Manufacturer
2°C 8°C	Temperature limit (Store at 2-8°C)		Use-by date
Σ	Contains sufficient for<n> tests		Keep away from sunlight
	This way up	EC REP	Authorized representative in the European Community
IVD	*In vitro* diagnostic medical device	CONTENTS	Kit component
REF	Catalogue number	LOT	Batch code
CE 0123	CE marking with notified body ID number		

Summary of safety and performance is available at Eudamed.

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