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B-type Natriuretic Peptide (CLIA) PACKAGE INSERT

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INTENDED USE

B-type Natriuretic Peptide (CLIA) is a Chemiluminescence Immunoassay test for the quantitative measurement of B-type natriuretic peptide (BNP) in human anticoagulated whole blood and plasma, which is intended as an aid to diagnosis of heart failure.
The assay can be used for samples over the range of 15pg/mL~5000pg/mL.
The test must be performed on the ACCRE series analyzers (including ACCRE 6, ACCRE 8, ACCRE 90, ACCRE 100, ACCRE 120 and other ACCRE series analyzers).

For *in vitro* diagnostic use only. For professional use only.

SUMMARY

Heart failure (HF) is a clinical syndrome caused by many reasons such as coronary artery disease, hypertension, valvular disease, and myocarditis. Common symptoms of heart failure include shortness of breath, exercise cough, edema of the extremities, and dizziness. More specifically, heart failure refers to the gradual loss of function of the ventricles to pump blood to the lungs and/or limbs. Heart failure can be either systolic or diastolic heart failure, or both. The New York Heart Association of the United States divides it into four grades (NYHA Class I-IV) according to the severity of heart failure.
B-type Natriuretic Peptide (BNP) is a member of the natriuretic peptide family, originally isolated from pig brain tissue (originally named brain natriuretic peptide), but ultimately confirmed that the heart is the main source of BNP. In the event of volume overload or other conditions that cause ventricular stretch, BNP is synthesized and released into the bloodstream, regulating the water and electrolyte balance by interacting with the renin-angiotensin-aldosterone system (RAAS). Pre-BNP (preproBNP, 134 amino acids) is synthesized in cardiomyocytes and converted into a precursor molecule called proBNP (108 amino acids). ProBNP is rapidly decomposed into physiologically active BNP (32 amino acids) and degradation fragment amino terminal brain natriuretic peptide (NT-proBNP, 76 amino acids) by endonuclease. BNP is cleared from the blood circulation by a specific cellular receptor under the action of a neutral endopeptidase with a half-life of approximately 23 minutes. Many studies have shown that BNP can be used for patient diagnosis, prognosis and treatment monitoring. Patients with cardiac insufficiency have higher levels of BNP in their bodies. Plasma BNP levels can provide useful clinical information for the diagnosis and treatment of left ventricular dysfunction and heart failure, and can be used as a supplement to other diagnostic tests (such as electrocardiogram, chest X-ray and echocardiography). BNP levels can be used to assess the severity of heart failure, and there is evidence that BNP levels correlate well with NYHA classification. Left ventricular ejection fraction (LVEF) or exercise tolerance assessments indicate that plasma BNP levels increase with physiological decline in cardiac function reserve. The European Society of Cardiology, the American College of Cardiology, and the American Heart Association all use BNP as an important serum marker for the diagnosis of heart failure.
There is evidence that BNP can be used to stratify patients with heart failure and acute coronary syndrome (ACS). An increase of BNP in patients with heart failure suggests an increase in disease progression and complication rates and mortality. In addition, studies have shown that the probability of heart complications and the mortality after myocardial infarction in ACS patients with elevated BNP values are also correspondingly increased. Preliminary studies have shown that BNP testing can be used to optimize treatment/monitoring in patients with heart failure.

ASSAY PRINCIPLES

The principle of B-type Natriuretic Peptide (CLIA) hereinafter also referred to as the "BNP" is a two-site sandwich method with enzyme catalytic chemiluminescence detection (CLIA). The principle is described as follow: .
Reagents for the assay are ready-to-use and pre-dispensed in the sealed reagent cartridges. All the assay steps are performed automatically by the ACCRE analyzer.
The sample is manually pipetted into the sample well of reagent cartridge, then sample, BNP antibody labeled with alkaline phosphatase and BNP antibody coated magnetic particles are mixed thoroughly together, incubating at 37 °C. The antigen in the sample, labelled antibody and antibody coated magnetic particles are bonded together as an antibody-antigen sandwich immunocomplex. The immunocomplex is then washed in the magnetic field to eliminate the unbound components in the sample. The substrate is added to trigger the chemiluminescent reaction, the result of chemiluminescent reaction is measured by photomultiplier tube (PMT) as relative light units (RLUs). At the end, the results are automatically calculated by the ACCRE analyzer in relation to the calibration curve stored.

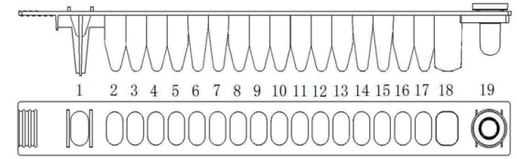
CONTENT OF THE KIT

Package: 60 tests/kit with quality controls, 36 tests/kit with quality controls, 60 tests/kit without quality controls, 36 tests/kit without quality controls
The calibrator in the kit has been traced to Abbott fully automated chemiluminescence immunoassay analyzer and ARCHITECT BNP assay. For calibrator concentration values, see the calibration card for each lot.

Contents	Quantities				Notice
	REF W125A	REF W125C	REF W10A	REF W10C	
BNP Reagents Cartridges	60 strips	36 strips	60 strips	36 strips	Ready to Use
BNP Calibrator 1 (CAL1)	Dissolved to 1.0 mL x 1 vial	Dissolved to 1.0 mL x 1 vial	Dissolved to 1.0 mL x 1 vial	Dissolved to 1.0 mL x 1 vial	Lyophilized powder, Tris buffer, 50mM; ProClin300, 0.48 g/L; BND, 0.2 g/L
BNP Calibrator 2 (CAL2)	Dissolved to 1.0 mL x 1 vial	Dissolved to 1.0 mL x 1 vial	Dissolved to 1.0 mL x 1 vial	Dissolved to 1.0 mL x 1 vial	Lyophilized powder, recombinant BNP, 100 pg/mL
BNP Calibrator3 (CAL3)	Dissolved to 1.0 mL x 1 vial	Dissolved to 1.0 mL x 1 vial	Dissolved to 1.0 mL x 1 vial	Dissolved to 1.0 mL x 1 vial	Lyophilized powder, recombinant BNP, 1000 pg/mL
BNP Quality Control Level 1 (QC1)	Dissolved to 1.0 mL x 1 vial	Dissolved to 1.0 mL x 1 vial	/	/	Lyophilized powder, recombinant BNP, 100 pg/mL Only for the configuration with Quality Controls
BNP Quality Control Level 2(QC2)	Dissolved to 1.0 mL x 1 vial	Dissolved to 1.0 mL x 1 vial	/	/	Lyophilized powder, recombinant BNP, 1000 pg/mL Only for the configuration with Quality Controls
Instruction for Use	1	1	1	1	N/A
Reagent Registration Card	1	1	1	1	N/A
Calibrator Barcode Sheet	1	1	1	1	N/A
Quality Control Sheet	1	1	/	/	Only for the configurations with Quality Controls

Note: Refer to the target value list of the controls from Quality Control Sheet within the kit.

The Reagent Cartridge
The strip consists of 19 wells. The 1st well is the sample well and the last 19th is the reading well for chemiluminescent signal. From the 2nd to the 18th well are all covered with a labeled, aluminum foil seal. The label comprises a barcode which mainly indicates the reagent assay information, reagent lot number and expiration date. All reagents components needed for the assay are ready-to-use and pre-dispensed in the cartridge. And please do not interchange integral component from different reagents or lots.



Description of the reagent cartridge

Well	Component	Content	Volume
3	Sample treatment solution (R3)	Tris buffer, 50mmol/L; ProClin300, 0.48g/L; BND, 0.2g/L	50μL
5	Enzyme Label(R2)	ALP (alkaline phosphatase) labeled anti-BNP antibody (mouse), 3.0mg/L; MES Buffer, 50mmol/L; ProClin300, 0.48g/L; BND, 0.2g/L	50μL
7	Magnetic Particles(R1)	Anti-BNP antibody (mouse) coated magnetic particles, 0.2g/L; Tris buffer, 50mmol/L; ProClin300, 0.48g/L; BND, 0.2g/L	50μL
10, 11, 12	Wash Solutions	Tris buffer, 50mmol/L; ProClin300, 0.48g/L; BND, 0.2g/L	350μL
16	Substrate	Disodium [(4-chlorophenyl) sulfanyl] (10-methyl-9(10H)-acridinylidene) methyl phosphate	200μL

Note: Well 1, 2, 4, 6, 8, 9, 13, 14, 15, 17, 18 are empty.

MATERIALS AND DISPOSABLES REQUIRED BUT NOT PROVIDED

- Sample diluent (manufactured by Tisenc)
- Pipette with disposable tip to dispense 200 μL.
- Powderless and disposable gloves.
- For other specific materials and disposables, please refer to the Instrument User Manual.
- Instruments of the ACCRE series analyzers.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- For professional use only by qualified laboratory personnel.
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious and handled observing the usual safety precautions (do not ingest; do not inhale).
- Do not use reagents after the expiration date indicated on the box label.
- Do not use if the packaging of reagent cartridge is damaged or leaked.
- Do not use the reagents if the quantity is obviously insufficient.
- If any component adheres to inside wall of wells or aluminum film of the reagent cartridge, please gently switch the reagent cartridge to let the component back the wells.
- Do not reverse the reagent cartridge. Otherwise, the result will be unreliable.
- Do not mix reagents (or disposables) from different lots.
- 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.
- Use powderless gloves, as powder has been reported to cause false results for certain enzyme immunoassay tests.

STORAGE

- Store the B-type Natriuretic Peptide (CLIA) kits including reagent cartridges, calibrators and quality controls **upright** at 2~8°C in dark place and the kit can be valid for 18 months from manufactured date.
- After the reagent cartridge is on-board in the ACCRE analyzer, the testing should be finished within 2 hours.
- When opened and re-dissolved, the calibrators and quality controls will be stable for 45 days at -20°C and below -20°C. Calibrators and quality controls can be frozen and thawed 3 times.
- Keep away from sunlight.
- Do not freeze reagent cartridges.

SAMPLES

The sample volume for B-type Natriuretic Peptide (CLIA) assay is 50μL, please note that at least 75μL of sample should be pipetted manually into the reagent cartridge.

Sample Type and Sample Collection
Human plasma/whole blood (EDTA-K_2) are recommended.
Types of tubes:
Plastic tube with clot activator,
- Plastic tube with clot activator and separation gel,
- Plastic tube with EDTA-K_2,

It is recommended to validate collection tubes before using them as some may contain substances which interfere with test results.
Note: Blood sampling tube results may vary from one manufacturer to another depending on the materials and additives used.
It is the responsibility of each laboratory to validate the type of sample tube used and to follow the manufacturer's recommendations for use.

Sample Preparation
The current revision of the WHO/DIL/LAB/99.1 document provides recommendations for the preparation of samples.
For use of the sample tubes, follow the tube manufacturer's instructions for use.
The pre-analytical step, including the preparation of blood samples, is an essential part of medical analyses. In accordance with Good Laboratory Practice, this step is performed under the responsibility of the laboratory manager.
Insufficient clot time can result in the formation of fibrin with micro-clots that are invisible to the naked eye. The presence of fibrin, red blood cells, or suspended particles can lead to erroneous results.
Samples containing suspended fibrin particles should be centrifuged before testing.
If there is a lipid layer over the sample after centrifuging, please transfer the clear sample into a new sample tube without transferring the lipid layer.
Severe hemolysis samples (hemoglobin >500mg/dL) or heat-inactivated samples are not recommended for applying on the reagents.
Preparation of frozen-stored samples: after thawing, these samples must be thoroughly mixed before testing.
Mix using with a vortex-type mixer. If necessary, clarify samples by centrifuging before testing.

Sample stability
Plasma samples can be stored at 15~30°C for up to 4 hours or at 2-8°C for up to 24 hours; if longer storage is required, freeze the plasma at -20 °C or below -20 °C for up to 3 months, without exceeding 2 freeze/thaw cycles.
Whole blood samples can be stored at 15~30°C for up to 4 hours or at 2-8°C for up to 24 hours and not undergone freeze/thaw cycles.

INSTRUCTIONS FOR USE

For complete instructions, see the Instrument User Manual.
When using the assay for the first time. Register the assay using the registration code in the reagent kit before any assay is used in the ACCRE analyzer for the first time. Calibration, QC and sample test are only available after assay was registered successfully.
When opening a new lot of reagents. Each new lot of reagent must be registered by using the registration code in Reagent Registration Card within the reagent kit.

Calibration
Calibration must be performed with the reagents and calibrators from Manufacturer.
Each bottle of lyophilized calibrator should be re-dissolved by purified water to 1.0mL before use.
Calibration is necessary under the following conditions:
(1) New reagent lot number applied;
(2) Same lot of reagents used more than 4 weeks;
(3) Quality control out of control;
(4) When necessary, for example: system maintenance or repairment that may affect the analytical performance.

Test Procedure

1. Remove the kit from storage at 2-8°C and take out the required number of reagent cartridge. Allow the reagent cartridges, specimens, calibrators, and/or controls to equilibrate to room temperature (15-30°C) prior to testing.
2. Use each assay BNP strips for each sample, calibrator or control respectively.
3. Place the reagent cartridge into the reagent rack.
4. Manually pipette the Sample, Calibrator, or Controls into the sample well of each reagent cartridge.

Please note that at least 75μL of sample must be pipetted manually into the reagent cartridge and 50μL sample is needed for each test.

** Before pipetting: Mix the samples using a vortex-type mixer if necessary, and ensure that the samples and sample diluent (if applicable) DO NOT have any bubbles. The same for calibrators and controls.*

5. Put the sample rack into the reagent chamber of ACCRE analyzer.
6. Place the assay tips into the reagent chamber of ACCRE analyzer.
7. Input Sample ID, sample type, dilution etc. into the software as directed by USER MANUAL.
8. B-type Natriuretic Peptide (CLIA) assay will be completed within approximately 15 minutes. Assays will be performed automatically by the ACCRE analyzer.
9. After assay is completed, remove the reagent rack from the ACCRE analyzer Dispose the used reagent cartridge into an appropriate recipient.

Dilution

Samples with concentrations above 5000pg/mL can be diluted with sample diluent manually. The recommended dilution is 1:5. And the concentration after dilution should be more than 1000pg/mL. After manual dilution, multiply the result by the dilution factor. ACCRE analyzer cannot perform auto dilution for samples.

Quality control

Each bottle of lyophilized quality control should be re-dissolved by purified water to 1.0mL before use.

Quality control can be performed in accordance with local regulations or requirements related to accreditation, as well as requirements defined in the laboratory's quality control procedure.

RESULTS AND INTERPRETATION

Calculation of Results

The results are automatically calculated by the ACCRE analyzer using stored calibration curve which generate by 3-point calibration, and calculated according to a predefined mathematical model, the results expressed in pg/mL.

Interpretation of Results

The detection range is from 15pg/mL to 5000pg/mL. For samples with BNP below the upper limit of detection, quantitative determination can be performed; if the sample is lower than the lower limit of detection, the reported result is <15pg/mL; if the sample is higher than the upper limit of detection, the reported result is >5000pg/mL.

Using clinical study data from non-heart failure individuals and heart failure populations in conjunction with literature, we established receiver operating characteristic (ROC) curves (different BNP decisions correspond to clinical sensitivity and specificity). The area under the curve is greater than 0.90. When the test's decision value is 100 pg/mL, it shows good diagnostic performance. Therefore, the BNP project is recommended to diagnose the heart failure threshold of 100 pg/mL.

Control group

Serum samples from 1467 healthy adults (697 male and 770 female) without heart disease in Guangdong were tested and analyzed. The test data were processed by non-parametric method. The data of this test is summarized in the following table:

Control group-Male

Age(years)	all	＜45	45-54	55-64	65-74	≥75
n	697	142	123	125	197	110
The 95th percentile value(pg/mL)	110.0	76.02	45.01	82.02	152.0	126.2
%(＜100pg/mL)	93.69%	95.77%	100%	97.60%	88.32%	89.09%

Control group-Female

Age(years)	all	＜45	45-54	55-64	65-74	≥75
n	770	168	137	142	204	119
The 95th percentile value(pg/mL)	159.7	87.03	117.2	154.1	163.2	291.0
%(＜100pg/mL)	88.44%	95.83%	91.24%	88.73%	85.29%	79.83%

Heart failure group

The plasma samples of 486 patients (including 243 males and 243 females) diagnosed as heart failure in Guangdong were tested and analyzed. The test data were processed by nonparametric method, and the heart failure judgment value of our test method was established by combining 95% reference intervals of non-heart failure individuals. The summary of this data is shown in the table below.

Gender	n	The 95th percentile value(pg/mL)
Male	243	1881
Female	243	1588

In addition, the European Society of Cardiology has included the detection of urinary natriuretic peptides (such as BNP) in the diagnosis or exclusion of heart failure. Clinical studies have shown a good correlation between BNP levels and NYHA classification, which can be used to aid in the diagnosis of heart failure and to assess its severity.

** Please note that results may differ between laboratories due to variations in population and test method. If necessary, each laboratory should establish its own reference range.*

In interpreting the results, patient's clinical condition including symptoms, diseases history, and other relevant data and information should be considered.

LIMITATIONS

1. If the test results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
2. For diagnostic purposes, results should be used in conjunction with other data; e.g., symptoms, results of other tests, clinical impressions, etc.
3. Please note that heterophile antibody in human serum or plasma can interfere the test result.
4. High concentration of HAMA can also cause a false positive or false negative result.

PERFORMANCE CHARACTERISTICS

Detection limits

LOB (Limit of Blank) =10pg/mL
LOD (Limit of Detection) =15pg/mL
Limit of Blank and Limit of Detection were determined according to CLSI® EP17-A2 recommendations.

Measurement Range

The measurement range is the range of values corresponding to the acceptable performance limits (precision and linearity).
The measurement range of the B-type Natriuretic Peptide (CLIA) assay is: 15 pg/mL ~5000 pg/mL.

Linearity

Linearity was evaluated according to CLSI® EP06-A recommendations. The linear B-type Natriuretic Peptide (CLIA) assay is linear between 15 pg/mL to 5000 pg/mL. The absolute linear correlation coefficient (r) should be bigger than 0.9900.

Precision

A precision study was performed according to CLSI® EP05-A3 recommendations.
The within-lot precision CV% is less than 8%.
The between-lot precision CV% is less than 10%.

Accuracy

The sample which is a control with trueness traceability is used for the detection, and the relative deviation between the detection result and the calibration concentration is within ±10%.

Hook effect

No hook effect was found up to BNP concentrations of 100000 pg/mL.

Interference

Potential interference substances were studied according to CLSI ® EP07-A3 recommendations. No significant interference was detected up to the maximum concentrations tested.

Substance	Concentration
Hemoglobin	≤ 500mg/dL
Bilirubin	≤ 20mg/dL
Triglyceride	≤ 3000mg/dL
Total Protein	≤ 5g/dL
Rheumatoid factor	≤ 1500IU/mL

Cross-reactivity

The B-type Natriuretic Peptide (CLIA) assay is specific for B-type natriuretic peptide. The following proteins were tested and found to have an insignificant effect on the measured BNP.

Substance	Concentration	Cross reactivity
Angiotensin I	600pg/mL	≤10pg/mL
Angiotensin II	600pg/mL	≤10pg/mL
Angiotensin III	1000pg/mL	≤10pg/mL
ANP	1000pg/mL	≤10pg/mL
CNP	1000pg/mL	≤10pg/mL
NT-proBNP	1000pg/mL	≤10pg/mL
Epinephrine	400 pg/mL	≤10pg/mL

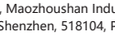
HAMA

Patient samples containing human anti-mouse antibodies (HAMA) may give falsely elevated or decreased values. Although HAMA-neutralizing agents are added, extremely high HAMA plasma concentrations may occasionally influence results[15-17].

WASTE DISPOSAL

Dispose used or unused reagents as well as any other contaminated disposable materials following procedures for infectious or potentially infectious products.
It is the responsibility of each laboratory to handle waste and effluents produced according to their nature and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

INDEX OF SYMBOLS

Symbol	Meaning	Symbol	Meaning	Symbol	Meaning
	Consult instructions for use		Contains sufficient for <n> tests		Date of manufacture
	*In vitro* diagnostic medical device		Use-by date		Do not re-use
	Temperature limit		Batch code		Catalogue number
	Keep away from sunlight		Keep dry		Manufacturer
	Authorized representative in the European Community/ European Union		Do not use if package is damaged and consult instructions for use		Contains biological material of animal origin
	Biological risks		Caution		CE marking

Symbol	Meaning	Symbol	Meaning	Symbol	Meaning
	Upright		Fragile, handle with care		Do not roll
	Control				

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Shenzhen Tisenc Medical Devices Co., Ltd.
Address: 11F, 11G, Kechuang Building, Quanzhi Science and Innovation Park, Maozhoushan Industrial Park, Shajing Community, BaoAn District, Shenzhen, 518104, P.R.China
Phone: +86-0755-23225620
E-mail: globalmarketing@tisenc.com

EC REP

Shanghai International Holding Corp. GmbH (Europe)

Address: Eiffestrasse 80, 20537 Hamburg, Germany
Phone: +49-40-2513175
Fax: +49-40-255726
DIMDI No.: DE/0000040627
E-mail: shholding@hotmail.com

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