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Immunoassay

REF **CMH0401 / CMH0402 / CMH0403 / CMH0404 / CMH0405**

50 tests*1 / 100 tests*1 / 100 tests*2 / 100 tests*5 / 50 tests*2

NT-proBNP CLIA Microparticles

This assay is based on a chemiluminescent microparticle immunoassay (CLIA Microparticles) for the quantitative determination of NT-proBNP (N-terminal pro B-type natriuretic peptide) in human serum and plasma.

Key to Graphical Symbols Used

LOT	batch code		use by
	manufacturer	Σ	contains sufficient for <n> tests
IVD	*in vitro* diagnostic medical device		temperature limitation
REF	catalogue number		consult instructions for use
EC REP	authorized representative in the European Community		

EC REP

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CE IVD

Introduction

Clinical information and imaging procedures are used to diagnose left ventricular dysfunction.[1]The significance of natriuretic peptides in the control of cardiovascular system function has been demonstrated. Initial studies reveal that natriuretic peptides can be used for diagnostic clinical problems associated with left ventricular dysfunction.[2] The following natriuretic peptides have been described: atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP), and C-type natriuretic peptide (CNP).[3,4]
In subjects with left ventricular dysfunction, serum and plasma concentrations of BNP increase, as does the concentration of the putatively inactive amino-terminal fragment, NT-proBNP. ProBNP, comprising 108 amino acids, is secreted mainly by the ventricle and, in this process, is cleaved into physiologically active BNP (77-108) and the N-terminal fragment NT-proBNP (1-76).[4]
Studies indicate that NT-proBNP can be used in diagnostic and prognostic applications.[5] The concentration of NT-proBNP in serum or plasma correlates with the prognosis of the left ventricular dysfunction.

Measurement Principle

This assay is based upon the two-step sandwich method. The sample and NT-proBNP antibody coated microparticles are combined in the first incubation. After addition of enzyme linked with NT-proBNP antibody, NT-proBNP present in the sample is allowed to react simultaneously with the two antibodies, resulting in the NT-proBNP being sandwiched between the microparticles and enzyme-linked antibodies. After washing, a complex is generated among the microparticles, the NT-proBNP within the sample and enzyme-linked antibodies by immunological reactions. Chemiluminescent Substrate is then added and catalyzed by this complex, resulting in a chemiluminescent reaction. The resulting chemiluminescent reaction is measured as RLUs. The RLU is proportional to the concentration of NT-proBNP in the patient sample.

Materials provided

1. Calibrators

6 vials lyophilized Calibrator A through F. The matrix is Tris-HCl buffer containing BSA. Contains a selection of preservatives.
Reconstitute each lyophilized calibrator with 1.0 mL of distilled water. Allow the reconstituted material to stand for at least 5 minutes. Then invert the calibrator to mix it completely.

2. Reagent pack

Reagent pack provided ready to use.

Σ	50*1	100*1	100*2	100*5	50*2
Microparticles Solution	1.2mL*1	2.3mL*1	2.3mL*2	2.3mL*5	1.2mL*2
Enzyme Conjugate	3.0mL*1	5.5mL*1	5.5mL*2	5.5mL*5	3.0mL*2

- Microparticles Solution

Goat monoclonal NT-proBNP antibody coated microparticles in Tris-NaCl buffer containing BSA. Contains a selection of preservatives.

- Enzyme Conjugate

HRP (horseradish peroxidase) labeled mouse monoclonal NT-proBNP antibody in Tris-NaCl buffer containing BSA (bovine serum albumin). Contains a selection of preservatives.

Assay Analyzers on which the kit can be used

- AutoLumo A2000 Plus
- AutoLumo A2000 Plus B
- AutoLumo A1000

The chemiluminescent microparticle immunoassay (CLIA Microparticles) is intended for use on Assay Analyzers which are AutoLumo A2000 Plus, AutoLumo A2000 Plus B and AutoLumo A1000.

Materials Required but not Provided

1. Assay Analyzer
2. Reaction vessel(s) for sample and reagent reaction
3. Sample tube(s) or cup(s) for sample containing
4. Diluent Universal
5. Chemiluminescent Substrate
6. System Wash for washing the pipetting needle
7. Wash Buffer used in the washing procedure
8. Distilled or deionized water

Metrological Traceability of Calibrators

The analyte in the NT-proBNP calibrators is traceable to the manufacturer's working calibrators. Traceability process is based on EN ISO 17511. The assigned values were established using representative samples from this lot of calibrator and are specific to the assay methodologies of the reagents. Values assigned by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.

Warnings and Precautions

1. For professional use only.
2. Follow the instruction for use carefully. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this instruction for use.
3. Refer to the material safety data sheet and product labeling for any chemical hazards that may be present in this assay.
4. Handle the potentially contaminated materials and wastes safely according to local requirement.
5. This assay contains materials of animal origin. Bovine components originate from countries where BSE has not been reported.
6. Do not smoke, drink, eat or use cosmetics in the working area.
7. Wear protective clothing and disposable gloves when dealing with samples and reagents. Wash hands after operations.
8. Conduct the assay away from bad ambient conditions. e. g. ambient air containing high concentration corrosive gas such as sodium hypochlorite acid, alkaline, acetaldehyde and so on, or containing dust.
9. Do not use reagents beyond the labeled expiry date.
10. Do not mix or use components from kits with different batch codes.
11. When storing the calibrators, be certain the vials are securely sealed.
12. Ensure the microparticles are resuspended before loading it on the analyzer.
13. Avoid foam formation in all reagents and sample types (samples, calibrators and controls).
14. Do not substitute any reagent in this kit from other manufacturers or other lots.
15. When any damage to the protective packaging or any change of analytical performance is observed, do not use the kit.

Storage

1. Store the kit at 2-8℃. Do not freeze. Avoid strong light. When stored as directed, all reagents are stable until the expiration date.
2. Refrigerate the reagent pack at 2-10℃ for a minimum of 2 hours prior to use.
3. Store the unsealed reagents pack upright on the analyzer or 2-10℃ for a maximum of 28 days. After 28 days, the reagent pack must be

discarded. Once they are removed from the analyzer, store them at 2-10℃ in an upright position.

4. Seal and return reconstituted calibrators to 2-8℃ immediately after the experiment, under which conditions the stability will be retained for 14 days, for longer use, store opened calibrators in aliquots and freeze at -20℃. Avoid multiple freeze-thaw cycles, do not freeze-thaw more than 3 cycles.

Sample

1. Collect samples in accordance with correct medical practices.
2. Do not use heat-inactivated samples. Do not use sodium azide preservative in samples.
3. Do not use samples with obvious microbial contamination.
4. Sediments and suspended solids in samples may interfere with the test result which should be removed by centrifugation. Ensure that complete clot formation in samples has taken place prior to centrifugation. Some samples, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the sample is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results. Be sure that the samples are not decayed prior to use.
5. Prior to shipment, it is recommended that samples be removed from the clot or red blood cells.
6. Use caution when handling patient samples to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
7. Insufficient processing of sample or disruption of the sample during transportation may cause depressed results.
8. Avoid grossly hemolytic, lipemic or turbid samples.
9. Cap and store the samples at 18-25℃ for no more than 8 hours, for longer use samples should be capped and stored at 2-8℃ up to 48 hours. Or freeze the samples that need to be stored or transported for more than 48 hours at -20℃. Avoid multiple freeze-thaw cycles. Mix thawed samples thoroughly by low speed vortex or by inverting 10 times. Visually inspect the samples, if layering or stratification is observed, continue mixing until samples are visibly homogeneous. After thawing, bring to room temperature and mix well by gently shaking.
10. Centrifuge the thawed samples containing red blood cells or particulate matter, or which are hazy or cloudy in appearance prior to use to ensure consistency in the results.
11. Note that interfering levels of fibrin may be present in samples that do not have obvious or visible particulate matter.
12. If proper sample collection and preparation cannot be verified, or if samples have been disrupted due to transportation or sample handling, an additional centrifugation step is recommended. Centrifugation conditions should be sufficient to remove particulate matter.
13. For optimal results, inspect all samples for bubbles. Remove bubbles with a tip prior to analysis. Use a new tip for each sample to prevent cross contamination.

Measurement Procedure

1. Check the consumable materials

- Verify adequate volume of consumable materials is present prior to running the test.
- Refer to the Assay Analyzer's operation manual.

2. Load the kit

- Mix contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the analyzer. Avoid foam formation in all reagents. Don't invert the open (punctured) packs. If necessary, shake gently to mix horizontally after the first loading.
- Read the bar code on the reagent pack automatically to obtain the required parameters for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Refer to the Assay Analyzer's operation manual.

3. Order tests

- Place the sample tube(s) or cup(s) on the sample rack. 50 μL of samples for each test. But consider the sample container and 150 μL of system dead volumes, which can be refer to the appropriate Assay Analyzer manuals for the minimum sample volume required.
- Load the sample rack and input the sample information on the system software interface.
- Select "run" to start the test, the analyzer automatically operates tests. It performs the following functions:
  - Moves the sample to the set point
  - Loads a reaction vessel into the process path
  - Aspirates and transfers sample into the reaction vessel.
  - Adds Microparticles Solution to the reaction vessel
  - Mixes, incubates and washes the reaction mixture
  - Adds Enzyme Conjugate to the reaction vessel
  - Mixes, incubates and washes the reaction mixture
  - Adds Chemiluminescent Substrate
  - Measures chemiluminescent emission to determine the quantity of NT-proBNP in the sample
  - Discards the used reaction vessel
  - Calculates the result
- Refer to the Assay Analyzer's operation manual.

4. Calibrate the curve

- Analyzer can read the bar code on the reagent pack automatically to obtain the required parameters for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Transfer the calibrators into the sample tube (s) or cup(s) and place them on the sample rack. Conduct duplicate detection on the system.
- Load the sample rack and input calibrators' information on the system software interface.
- Select "run" to start the test and generate the calibration curve, calibration is required every 28 days.
- Once a calibration curve is accepted and stored, all subsequent samples may be tested without further calibration unless:
  - Controls are out of range after repeated measurements
  - A reagent kit and Chemiluminescent Substrate with new batch code is used
  - Beyond the expiration date of a calibration curve
  - Important parts of the analyzer are replaced or repaired
- Refer to the Assay Analyzer's operation manual.

5. Dilute the sample

Samples with a NT-proBNP value exceeding 32000 pg/mL may be diluted via the program of the analyzer. Diluent Universal is used to dilute the samples. The software takes the dilution into account when reporting the result.

Measurement Results

The sample test results are determined automatically by the system software. The amount of NT-proBNP in the samples is determined from the measured light production by means of the stored calibration data. Refer to the Assay Analyzer's operation manual on reviewing sample

results.

Control Procedure

The recommended control requirement for this assay is to purchase control materials separately and test them together with the samples within the same run. The result is valid if the control values fall within the acceptable ranges. When a control value is out of the specified range, it may indicate deterioration of the reagents or errors in technique. Associated test results may be invalid and may require retesting. Assay recalibration may be necessary. It is recommended that each laboratory establish its accepted range to ensure proper test performance.

Limitations of the Procedure

1. This assay is intended as an aid for the clinical diagnosis. Conduct this assay in conjunction with clinical examination, patient's medical history and other test results.
2. If the results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
3. Samples that detected negative cannot be completely excluded the possibility of heart failure, and should be observed dynamically while performing other tests to aid in the detection.
4. In the 2014, the guidance of *NT-proBNP Clinical Application for Chinese Expert Consensus* mentioned that combining the medical history, symptoms, signs, chest X-ray, Echocardiography and laboratory tests of patients with acute dyspnea, it was found NT-proBNP test is helpful in identifying whether the cause of dyspnea is acute heart failure. Therefore, the results of suspicious samples closed to the reference interval should be combined with other clinical results, electrocardiogram, clinical symptoms and medical history data for comprehensive diagnosis, and it suggests dynamic observation.
5. Whether it is new acute heart failure or acute exacerbation of chronic heart failure, the level of NT-proBNP in the blood increases significantly, the rise is parallel to the severity of heart failure, and falls back after the condition is relieved or effective treatment. Therefore, In the journal of *NT-proBNP Clinical Application for Chinese Expert Consensus (*2014), also recommended a series of NT-proBNP tests at the time of patients visit (before treatment) and after the treatment. If NT-proBNP drops by more than 30% after treatment, the patients' prognosis will be considered good. If there is no pre-treatment NT-proBNP test data, <4000pg/mL can also be used as an indicator of improvement after treatment.
6. NT-proBNP will increase with the increase of age in the test. If the result is larger than the reference interval, it is recommended that the clinical department uses the 'double cutoff' strategy based on the *NT-proBNP Clinical Application for Chinese Expert Consensus 2010*, which defined as:
   If the NT-proBNP is less than 300pg/mL before treatment, the patients is less likely to have acute heart failure ('excluded' the cutoff); if it is higher than the corresponding age level (the cutoff values for 50 years old, 50-75 years old, and above 75 years old, respectively are 450pg/mL, 900pg/mL, and 1,800pg/mL), the patients may have acute heart failure ('diagnosis' cutoff); if the test value is between the above two cutoff values ('gray zone'), it may be a mild degree of acute heart failure, or a slight increase in NT-proBNP caused by non-acute heart failure (such as myocardial ischemia, atrial fibrillation, lung cancer, pulmonary hypertension, pulmonary embolism, etc.), in this case, the diagnosis should be further differentiated in combination with other examination results.
7. Due to the methodological or immunologic specificity, different results may be obtained by testing the same sample with different manufactures' assays. Therefore, the results of different assay tests should not be directly compared with each other to avoid erroneous medical explanation. It is recommended that the laboratory indicate the characteristics of reagents used in the test report sent to the clinician. During a series of tests, if the assay is changed, an additional test should be performed and a parallel comparison with the original reagent results should be done to re-determine the baseline value.
8. Heterophilic antibodies and rheumatoid factors in samples may interfere with test results. 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. Additional information may be required for diagnosis. This kind of samples is not suitable to be tested by this assay.
9. This assay was designed and validated for use with human serum from individual patient and donor samples. Pooled samples must not be used since the accuracy of their test results has not been validated.
10. This test measures concentrations within the range of 30-32000 pg/mL. If the NT-proBNP concentrations are above 32000pg/mL, it is recommended to dilute samples with Diluent Universal, the maximum recommended dilution is 1:4, allowing samples to be quantitated up to approximately 160000 pg/mL.

Biological Reference Interval

A study of 214 normal adult population individuals and 149 heart failure patients was undertaken to determine reference intervals for this assay by ROC method. The reference interval is <114.5 pg/mL. It is recommended that each laboratory establish its own normal range which may be unique to the population it serves depending upon geographical, patient, dietary, or environmental factors.

Performance Characteristics

1. Measurement Precision

3 samples were assayed in duplicate, twice per day across 20 testing days. Data from this study are summarized in the following table.

Sample	n	Mean (pg/mL)	Within-run	Total
			%CV	%CV
1	80	295.68	3.99	5.70
2	80	1485.37	2.95	5.36
3	80	8294.29	3.27	5.35

*Representative data; results in individual laboratories may vary from these data.

2. Sensitivity

Limit of Blank≤5 pg/mL
Limit of Detection=30 pg/mL
Limit of Quantitation: 65 pg/mL with a coefficient of variation of ≤ 20 %.

3. Analytical Specificity

Cross reaction: This assay is designed to have an analytical specificity of less than 100pg/mL cross reactivity with the substances listed below, at the concentration levels listed, in calibrator diluent.

Substances	Concentration (ug/mL)
ANP	3
BNP	3.5
CNP	2.0

Interference: No interference with 125 mg/dL of haemoglobin, 40 mg/dL of Bilirubin, 3000 mg/dL of triglyceride.

4. Measurement Accuracy by Correlation

A study was performed where samples were tested using this assay and a NT-proBNP reference assay. Data were analyzed and are summarized in the following table.

Correlation Method	Number of Samples	Intercept	Slope	Correlation Coefficient
Linear Regression	269	257.84	1.0214	0.9112

Literature References

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