NT-proBNP– Alere NT-proBNP

Intended Use

The Alere NT-proBNP for Alinity i assay is a chemiluminescent microparticle immunoassay (CMIA) used for the in vitro quantitative determination of N-terminal pro B-type natriuretic peptide (NT-proBNP) in human serum and plasma on the Alinity i system. In the emergency department, measurements of NT-proBNP are used as an aid in the diagnosis of heart failure (HF) in patients with clinical suspicion of new onset or worsening HF.

Clinical Significance

B-type natriuretic peptide (BNP) is a natriuretic hormone synthesized and secreted into the blood stream by cardiac myocytes in response to volume overload, increased stress on ventricular walls, and ventricular hypertrophy.5 Physiologically active BNP and biologically inert 76 amino acid peptide NT-proBNP are formed through the proteolytic cleavage of the precursor proBNP.6 In patients presenting with dyspnea, the measurement of NT-proBNP is useful to support diagnosis or exclusion of HF.3
In patients with impaired renal function, decreased glomerular filtration rate (GFR) is associated with increased NT-proBNP concentration, since NT-proBNP is cleared by the kidney. BNP/ NT-proBNP levels may also be modified due to biological factors like age, sex, and body mass index.5 Age has the strongest effect, leading to the use of age-dependent positive cutoffs.2, 7 Elevated natriuretic peptide (BNP/NT-proBNP) levels should be interpreted in the context of other clinical information; they should not be used in isolation to diagnose HF.

Methodology

This assay is an automated, two-step immunoassay for the in vitro quantitative determination of NT-proBNP in human serum and plasma using chemiluminescent microparticle immunoassay (CMIA) technology. Sample and anti-NT-proBNP coated paramagnetic microparticles are combined and incubated. The NT-proBNP present in the sample binds to the anti-NT-proBNP coated microparticles. The mixture is washed. Anti-NT-proBNP acridinium-labeled conjugate is added to create a reaction mixture and incubated. Following a wash cycle, Pre-Trigger and Trigger Solutions are added.The resulting chemiluminescent reaction is measured as a relative light unit (RLU). There is a direct relationship between the amount of NT-proBNP in the sample and the RLU detected by the system optics.

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Specimen

Type of Specimen

Specimen Type	Collection Vessel
Serum	Serum Serum separator
Plasma	Dipotassium EDTA Tripotassium EDTA Lithium heparin Lithium heparin separator

Specimen Storage and Stability

1. Tubes of blood are to be kept closed at all times and in a vertical position. It is recommended that the plasma be physically separated from contact with cells within two hours from the time of collection.
2. Do not use: heat-inactivated specimens, pooled specimens, grossly hemolyzed specimens, specimens with obvious microbial contamination, specimens with fungal growth.
3. For accurate results, serum and plasma specimens should be free of fibrin, red blood cells, and other particulate matter.
4. To ensure consistency in results, recentrifuge specimens prior to testing if they contain fibrin, red blood cells, or other particulate matter.
5. **Prepare frozen specimens as follows:** Frozen specimens must be completely thawed before mixing. • Mix thawed specimens thoroughly by low-speed vortex or by inverting 10 times. 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. Recentrifuge specimens.
6. **Recentrifugation of Specimens**: Transfer mixed specimens to a centrifuge tube and centrifuge at a minimum of 50000 g-minutesor 5 minutes with 1000 rcf.
   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.

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Specimen Type	Temperature	Maximum Storage Time	Special Instructions
Serum/ Plasma	Room temperature (20 to 25°C)	48 hours	Remove serum or plasma from the clot, red blood cells, or separator gel if testing will be delayed more than 24 hours.
	2 to 8°C	3 days	
	-20°C or colder	30 days	Remove serum or plasma from the clot, red blood cells, or separator gel.

Avoid more than 3 freeze/thaw cycles.

Sample Dilution Procedures

Serum/Plasma: Samples with a NT-proBNP value exceeding 35 000 ng/L are flagged with the code "> 35 000 ng/L"and may be diluted with either the Automated Dilution Protocol or the Manual Dilution Procedure.

Automated Dilution Protocol

The system performs a 1:2 dilution of the sample and automatically calculates the concentration by multiplying the result by the dilution factor.

Manual Dilution Procedure

Suggested dilution: 1:10. It is recommended that dilutions not exceed 1:10. Add 50 µL of the sample to 450 µL of Alinity i Multi-Assay Manual Diluent.

Reagents

Reagent Handling

- Reagents are shipped on cold packs / wet ice. Upon receipt, gently invert the unopened reagent kit by rotating it over and back for a full 180 degrees, 5 times with green label stripe facing up and then 5 times with green label stripe facing down. This ensures that liquid covers all sides of the bottles within the cartridges.

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- During reagent shipment, microparticles can settle on the reagent septum.
  – Place a check in the square on the reagent kit to indicate to others that the inversions have been completed.
  • After mixing, place reagent cartridges in an upright position for 1 hour before use to allow bubbles that may have formed to dissipate.
- If a reagent cartridge is dropped, place in an upright position for 1 hours before use to allow bubbles that may have formed to dissipate.
- Reagent cartridges cannot be inverted after the septum has been pierced by the system.
- Reagents are susceptible to the formation of foam and bubbles. Bubbles may interfere with the detection of the reagent level in the cartridge and cause insufficient reagent aspiration that may adversely affect results.

Reagent Storage and Stability

	Storage Temperature	**Maximum Storage Time**	**Additional Storage Instructions**
Unopened	2 to 8°C	Until expiration date	Store in upright position. If cartridge does not remain upright, gently invert the cartridge 10 times and place in an upright position for 1 hour before use.
Onboard	System Temperature	30 days	

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Opened	2 to 8°C	Until expiration date	Store in upright position. Do not reuse original reagent caps or replacement caps due to the risk of contamination and the potential to compromise reagent performance.

Calibration

Calibration Required

For instructions on performing a calibration, refer to the Alinity ci-series Operations Manual, Section 5.
Each assay control must be tested to evaluate the assay calibration. Once a calibration is accepted and stored, all subsequent samples may be tested without further calibration unless:
• A reagent kit with a new lot number is used.
• Daily quality control results are outside of statistically-based quality control limits used to monitor and control system performance, as described in the Quality Control Procedures section of this package insert. Recalibration is recommended every 30 days. A calibration may be extended based on acceptable verification of the calibration by the laboratory. This assay may require recalibration after maintenance to critical parts or subsystems or after service procedures have been performed.

Calibration Preparation

Calibration material is the 04S7902 Alere NT-proBNP for Alinity i Calibrators. Calibrators may be thawed at room temperature for 90 to 120 minutes or overnight at 2 to 8°C. Prior to use, mix by gentle inversion 10 times

Calibration Storage and Stability

	Calibrator Storage	Stability Once OPEN
NT-proBNP Cal (CAL A to Cal F)	Unopened: -20 (Until expiration date)	**2 to 8 °C: 30 days**

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Calibration Information

1. Calibrator lots may be configured using the bar code label on the calibrator carton.
2. Verify that the correct calibrator values have been entered into the calibration file.
3. Calibration is performed by running Alinity i STAT High Sensitivity Troponin-I Calibrators
4. For information on configuring calibrator data, refer to the Alinity ci-series Operations Manual, Section 5.

Quality Control

See Policy Chemistry Quality Control Policy

Sample Processing

See Policy RIV-PPP-1199

Reference Range

Test unit= ng/L for all ages and both sexes.

Test	Age Year Low	Age Year High	Ref Low	Ref High
PROBNP	0	1	0	1594
PROBNP	1	19	0	214
PROBNP	19	250	0	299

Analytic Range

AMR Low	AMR High	CRR Low	CRR High
16	35000	16	70000

Author

Melanie Beermann, CLS

Distributions

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