**NT-proBNP, Serum/Plasma**

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# Purpose

This procedure provides instructions for performing NT-proBNP on Alinity i analyzers. The Alere NT-proBNP for Alinity i assay is used for the quantitative determination of N-terminal pro B-type natriuretic peptide (NT-proBNP) in human serum and plasma on the Alinity i system.

# Policy Statements

This procedure applies to all personnel responsible for operating the Abbott Alinity i at Children’s Minnesota Laboratory.

# Principle

**Method**: Chemiluminescent microparticle immunoassay (CMIA)

This assay is an automated, two-step immunoassay using 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.1

# Clinical Significance

Heart failure (HF) is a complex clinical syndrome that can result from structural or functional cardiac disorders causing impairment of ventricular filling or ejection of blood from the heart. HF is a clinical diagnosis based upon patient history and physical examination in conjunction with laboratory tests and imaging procedures. Symptoms of HF include dyspnea, ankle swelling, fatigue, and weakness, which may be more pronounced with exertion. The American Heart Association (AHA) / American College of Cardiology (ACC) stages of HF highlight the development and progression of the disease from stage A (at risk of HF, asymptomatic) to stage B (pre-HF, asymptomatic), stage C (symptomatic HF), and stage D (advanced HF). The stages are defined by clinical signs and symptoms, presence of risk factors, and comorbid conditions. Progression from stage A through stage D is associated with increasing levels of cardiac biomarkers (including natriuretic peptides) and echocardiographic findings of structural heart disease and ventricular dysfunction, along with worsening symptoms of HF that interfere with daily life, increased rate of hospitalization, and elevated risk of mortality. For stage C and D HF, the New York Heart Association (NYHA) classification is utilized to categorize patients based on symptoms and functional capacity. 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. Physiologically active BNP and biologically inert 76 amino acid peptide NT-proBNP are formed through the proteolytic cleavage of the precursor proBNP. In patients presenting with dyspnea, the measurement of NT-proBNP is useful to support diagnosis or exclusion of HF. 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. Age has the strongest effect, leading to the use of age-dependent positive cutoffs. 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.1

While prevalence of cardiac disease is lower in children, cardiac involvement in various primary and secondary conditions significantly contributes to pediatric morbidity and mortality. Recent literature suggests clinical value of biomarker measurement in diverse pediatric applications, including heart failure, myocarditis, cardiac surgery, and congenital heart disease. 2

Markedly elevated neonatal concentrations of NT-proBNP have been observed across multiple studies. This neonatal surge is expected to be due to perinatal circulatory changes at birth, including blood redistribution from the placenta to the lungs, leading to increased ventricular volume and pressure load, and stimulation of natriuretic peptide synthesis. Immature renal function and clearance limitations may also contribute. Available studies suggest NT-proBNP concentrations in the post-neonatal period stay constant or decrease slightly until adulthood. In CALIPER cohort, no statistically significant age-specific differences were observed beyond 1 year of age across all cardiac biomarkers examined. No sex-specific association was observed between NT-proBNP concentration and adolescence.2

# Materials

|  |  |  |
| --- | --- | --- |
| *Product Description* | *Product Code* | *Stability* |
| **Alere NT-proBNP Reagent**  (200 tests per box; each box contains 2 reagent sets of 100 tests). Each reagent set consists of microparticles and conjugate.  **Microparticles:** Anti-NT-proBNP (sheep, monoclonal) coated  microparticles in Bis-TRIS buffer with protein (bovine) stabilizer, non-specific binding blocking agents, and surfactant. Minimum concentration: 0.05% solids. Preservative: sodium azide.  **Conjugate**: Anti-NT-proBNP (mouse, monoclonal) acridinium-labeled conjugate in MES buffer with protein (bovine) stabilizer and surfactant. Minimum concentration: 0.12 μg/mL. Preservatives: antimicrobial agents.  Axis-Shield Diagnostics Limited | **MFR**# 04S79-21  **CHC**# TBD | **Store at**: 2-8°C  **Unopened**: Until Expiration  **Opened**: Stable 30 days onboard the analyzer. |
| **Alere NT-proBNP Calibrator**  Axis-Shield Diagnostics Limited | **MFR**# 04S79-02  **CHC**# TBD | **Store at**: -20°C or colder  **Unopened**: Until Expiration  **Opened**: 2-8°C for up to 30 days, not to exceed the expiration date printed on the bottle.  **Preparation**: Calibrators may be thawed at room temperature for 90 to 120 minutes or overnight at 2-8°C. Prior to use, mix by gentle inversion 10 times or by low-speed vortexing. |
| **Alere NT-proBNP Controls**  Levels: Low, Medium, and High  Axis-Shield Diagnostics Limited | **MFR**# 04S79-11  **CHC**# TBD | **Store at**: -20°C or colder  **Unopened**: Until Expiration  **Opened**: 2-8°C for up to 30 days, not to exceed the expiration date printed on the bottle.  **Preparation**: Controls may be thawed at room temperature for 90 to 120 minutes or overnight at 2-8°C. Prior to use, mix by gentle inversion 10 times or by low-speed vortexing. |

# Special Safety Precautions

Reagent microparticles contain Bis-TRIS propane and sodium azide. Contact with skin causes mild skin irritation; if skin irritation occurs get medical advice/attention. Contact with acids liberates a very toxic gas; avoid interactions between reagent and acids.1

Controls and calibrators contain methylisothiazolones and sodium azide which may cause an allergic skin reaction. If on skin, wash with plenty of water. If skin irritation or rash occurs, get medical advice/attention. Contact with acids liberates very toxic gas; avoid interactions between controls/calibrators and acids.1

# Sample

**Sample:** Serum tubes with or without gel barrier are preferred. Alternatively, plasma collected with lithium heparin or EDTA anticoagulants is acceptable. DO NOT use grossly hemolyzed specimens, pooled samples, or specimens with obvious microbial contamination/fungal growth. For accurate results, serum and plasma specimens should be free of fibrin, red blood cells, and other particulate matter.

**Stability:** 48 hours at room temperature (20-25°C), 3 days at 2-8°C, or 30 days at -20°C or colder.

**Rejection Criteria:** Unlabeled tube, gross hemolysis, sample collected in anticoagulant other than lithium heparin or EDTA.

# Test Code

**Sunquest Test Code**: PRBNP

# Analyzer

**Primary Analyzer or Method**: MACI (Minneapolis Alinity i)

**Backup**: Send to MML as MBAT (Mayo test code PBNP, requires 0.5 ml serum minimum)

# Calibration

Calibration Information

|  |  |
| --- | --- |
| Reference Material | Alere NT-proBNP Calibrators; 04S79-02. |
| Calibration Frequency | Calibration is due every 30 days and with each new reagent kit. |
| Calibration Scheme | 6 levels run in duplicate: 4 Parameter  Logistic Curve fit data reduction method (4PLC, Y-weighted) to  generate a calibration and results. |

# Quality Control

**Material**: Alere NT-proBNP controls

**Frequency**: Run 3 levels once per day

**Acceptable Ranges**: Acceptable ranges are set in Unity Real Time software.

# Procedure

For a detailed description of how to run an assay, refer to the Alinity ci-series Operations Manual, Section 5.

* If using primary or aliquot tubes, refer to the Alinity ci-series Operations Manual, Section 4 to ensure sufficient specimen is present.
* Minimum sample cup volume is calculated by the system and printed on the Order List report.
* To minimize the effects of evaporation, verify adequate sample cup volume is present prior to running the test.
* Maximum number of replicates sampled from the same sample cup: 10
  + Priority:
    - Sample volume for first test: 100 µL
    - Sample volume for each additional test from same sample cup: 50 µL
  + ≤3 hours on the reagent and sample manager:
    - Sample volume for first test: 150 µL
    - Sample volume for each additional test from same sample cup: 50 µL
  + >3 hours on the reagent and sample manager:
    - Replace with a fresh aliquot of sample.

# Dilutions

Samples with an NT-proBNP value exceeding 35,000 pg/mL are flagged with the code “>35,000 pg/mL” and may be diluted with either the Automated Dilution Protocol or the Manual Dilution Procedure.

|  |  |
| --- | --- |
| Available Auto Dilutions: | 1:2 |
| Maximum Auto Dilution: | 1:2 |
| Maximum Manual Dilution: | 1:10 |
| Diluent: | Alinity i Multi-Assay Manual Diluent |
| Manual Dilution: | Add 50 µL of the sample to 450 µL of the Alinity i Multi-Assay Manual Diluent.  Follow Abbott [Alinity Operator’s Manual](https://starnet.childrenshc.org/References/labsop/chem/operator/alinity-ci-series-operations-manual.pdf) instructions for programming automated dilutions. The system will automatically calculate the concentration of the sample and report the result |

# Calculations

The Alere NT-proBNP for Alinity i assay utilizes a 4 Parameter Logistic Curve fit data reduction method (4PLC, Y-weighted) to generate a calibration curve and results.

# Interpretation and Resulting

Critical Values: None

Results will be reported to the first decimal (xx.x).

Results between 15.8 pg/mL and 35,000 pg/mL without error messages are released.

Results below 15.8 pg/mL without error messages are reported as <15.8 pg/mL

Results >35,000 pg/mL will be diluted using the onboard automated 1:2 dilution.

Results >70,000 pg/mL will be manually diluted with Alinity i Multi-Assay Diluent. Following dilution, results between 70,000 pg/mL and 350,000 pg/mL are reported as the numerical value.

Results >350,000 pg/mL following manual dilution are reported as >350.000 pg/mL

# Reference Intervals

Pediatric Reference Range2

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Age | Median | 2.5th percentile (pg/mL) | 97.5th percentile (pg/mL) | Reference Interval (pg/mL) |
| 0 to <1 years | 224 | 29.6 | 1594 | <15.8 to 1594 |
| 1 to <18 years | 52 | <15.8 | 214 | <15.8 to 214 |

Reference Range ≥18 years old1

|  |  |  |
| --- | --- | --- |
| Age | Sex | Reference Interval (pg/mL) |
| 18 to <50 years old | Female  Male | <15.8 to 104.8  <15.8 to 180.3 |
| 50 to 75 years old | Female  Male | <15.8 to 334.1  <15.8 to 451.6 |
| >75 years old | Female  Male | <15.8 to 956.1  <15.8 to 683.0 |

# Method Performance Specifications

**Analytical Measuring Range**: 15.8 - 35,0000 pg/mL

**AMR/Calibration verification frequency:** With each calibration

**AMR recommended verification product**: Alere NT-proBNP calibrator

**AMR proximity budget**: 50 pg/mL or 20%

**AMR systematic error budget**: 50%

**Allowable Erro**r: 30% (CLIA, WSLH)

**Precision**: ≤ 10% CV for samples targeted between 100 pg/mL to 35,000 pg/mL.

# Limitations

* Elevated NT-proBNP concentrations may be associated with impaired renal function (estimated glomerular filtration rate [eGFR] < 60 mL/min/1.73 m2), history of HF and other conditions such as acute coronary syndrome, atrial fibrillation, pulmonary embolism, valvular heart disease, myocarditis, pulmonary hypertension, stroke, and sepsis, which may lead to false positive results. Obesity (body mass index [BMI] ≥ 30 kg/m2) and other conditions such as flash pulmonary edema, pericarditis, and cardiac tamponade may lower NT-proBNP concentrations, which may lead to false negative results.
* Results should be used in conjunction with other data; e.g., symptoms, results of other tests, and clinical impressions.
* If the NT-proBNP results are inconsistent with clinical evidence, additional testing is recommended.
* The Alere NT-proBNP for Alinity i assay is susceptible to interference effects from total protein >12.6 g/dL. Total protein at 15.2 g/dL decreased NT-proBNP values at 125 pg/mL by -12.7%.
* 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 such as Alere NT-proBNP for Alinity i that employ mouse monoclonal antibodies. Additional information may be required for diagnosis.

**No significant interference (interference within ± 10%)** was observed at the following concentrations.

* Bilirubin (conjugated or unconjugated), 60 mg/dL
* Biotin, 4250 ng/mL
* Cholesterol, 700 mg/dL
* HAMA, 1500 ng/mL
* Hemoglobin, 1000 mg/dL
* Total protein, 12.6 g/dL
* Intralipid, 3000 mg/dL
* IgG, 6 g/dL
* RF, 600 IU/mL

**Interference beyond ± 10%** was observed at the following concentrations.

* Total protein, 15.2 g/dL. Interference of –9.9% to –12.7%
* RF, 1520 IU/mL. Interference of –8.9% to –11.4%

**Potentially Interfering Drugs**: full list of potentially interfering drugs tested can be found in reagent package insert, page 7.

**Potential Cross Reactants**: full list of potential cross reactants tested can be found in reagent package insert, page 7.

# References

1. Alere NT-proBNP for Alinity i Reagent Package Insert, Abbott Laboratories Diagnostics Division, Abbott Park, IL 60064, January 2025
2. Palm J, Hoffmann G, Klawonn F, Tutarel O, Palm H, Holdenrieder S, et al. Continuous, complete and comparable NT-proBNP reference ranges in healthy children. Clin Chem Lab Med 2020;58:1509-16.

# Appendices

Not applicable

# Training Plan/Competency Assessment

[CH 1.04.T1 Abbott Alinity Training](https://starnet.childrenshc.org/References/labsop/chem/train/ch-1.04.t1-abbott-alinity-training.pdf)

[QP 2.30 Orientation and Training](https://starnet.childrenshc.org/References/labsop/qual/person/qp-2.30-orientation-and-training.pdf)

[QP 2.40 Competency Assessment](https://starnet.childrenshc.org/References/labsop/qual/person/qp-2.40-competency-assessment.pdf)

# Historical Record

|  |  |  |  |
| --- | --- | --- | --- |
| Version | Author | Effective Date | Summary |
| 1 | Abby Rundquist & Matt Johnson | 09/30/2025 | Initial Version |