510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K241176

B Applicant

Axis-Shield Diagnostics Ltd

C Proprietary and Established Names

Alere NT-proBNP for Alinity i

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
NBC	Class II	21 CFR 862.1117 - B-Type Natriuretic Peptide Test System	CH - Clinical Chemistry

II Submission/Device Overview:

A Purpose for Submission:

New device

B Measurand:

NT-proBNP

C Type of Test:

Quantitative immunoassay

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for 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.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

Alinity i system

IV Device/System Characteristics:

A Device Description:

The Alere NT-proBNP for Alinity i 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.

Reagents:

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.

Materials required but not supplied with the reagent kit include Alere NT-proBNP for Alinity i calibrators and quality controls, Alinity pre-trigger and trigger solution, Alinity-i series concentrated wash buffer and Alinity i multi-assay manual diluent.

B Principle of Operation:

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.

V Substantial Equivalence Information:

A Predicate Device Name(s):
Elecsys ProBNP II Stat Immunoassay

B Predicate 510(k) Number(s):
K092649

C Comparison with Predicate(s):

Device & Predicate Device(s):	K241176	K092649
Device Trade Name	Alere NT-proBNP for Alinity i	Elecsys proBNP II STAT Immunoassay
General Device Characteristic Similarities		
Intended Use/Indications For Use	Immunoassay used for the in vitro quantitative determination of N-terminal pro B-type natriuretic peptide (NT-proBNP) in human serum and plasma. This assay is to be used as an aid in the diagnosis of individuals suspected of having congestive heart failure (CHF).	Same
Specimen Type	Human serum and plasma	Same
Test principle	Sandwich immunoassay	Same
Measurement type	Quantitative	Same
Detection Technology	Chemiluminescence	Same
General Device Characteristic Differences		
Principle of Operation	Chemiluminescent microparticle immunoassay (CMIA)	Electro chemiluminescent microparticle immunoassay (ECLIA)
Results Interpretation	Negative: HF unlikely for all patients: < 300.0 pg/mL Gray Zone: Indeterminate; consider other causes of NT-	125 pg/mL for < 75 years 450 pg/mL for ≥ 75 years

Device & Predicate Device(s):	K241176	K092649
	proBNP elevation: Age (Years) NT-proBNP (pg/mL) 18 to < 50 ≥ 300.0 to < 450.0 50 to 75 ≥ 300.0 to < 900.0 > 75 ≥ 300.0 to < 1800.0 Positive: HF likely: Age (Years) NT-proBNP (pg/mL) 18 to < 50 ≥ 450.0 50 to 75 ≥ 900.0 > 75 ≥ 1800.0	
Measuring Interval	15.8 to 35,000.0 pg/mL	5 to 35,000 pg/mL
Hook Effect	No hook effect up to 372,620 pg/mL	No hook effect up to 300,000 pg/mL

VI Standards/Guidance Documents Referenced:

CLSI EP05-A3: Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition

CLSI EP06: Evaluation of the Linearity of Quantitative Measurement Procedures, 2nd Edition

CLSI EP07: Interference Testing in Clinical Chemistry, 3rd Edition

CLSI EP12: Evaluation of Qualitative, Binary Output Examination Performance, 3rd Edition

CLSI EP17-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedure; Approved Guideline – Second Edition

CLSI EP25: Evaluation of Stability of In Vitro Medical Laboratory Test Reagents, 2nd Edition

CLSI EP28-A3c: Defining Establishing and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline - Third Edition

CLSI EP34: Establishing and Verifying an Extended Measuring Interval Through Specimen Dilution and Spiking, 1st Edition

CLSI EP35: Assessment of Equivalence or Suitability of Specimen Types for Medical Laboratory Measurement Procedures. 1st Edition

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

Within-Laboratory Precision (20-Day)
A study was performed based on guidance from CLSI EP05-A3.

Three controls and 8 human plasma panels (7 native panels and 1 panel supplemented with NT-proBNP analyte) were tested in 2 replicates at 2 separate times per day over 20 days (for a total of 80 measurements per sample). Testing was conducted using 3 lots of the candidate device, 1 lot of the Alere NT-proBNP for Alinity i Calibrators, 1 lot of the Alere NT-proBNP for Alinity i Controls, and 1 Alinity i instrument.

Panels (Panel A to Panel G) were prepared by pooling NT-proBNP K_2EDTA plasma specimens and diluting with human K_2EDTA plasma as necessary to achieve the target concentrations. Panel H was prepared by spiking K_2EDTA plasma with a synthetic NT-proBNP peptide. The sponsor provided evidence to show that the contrived samples mimic patient samples.

Results are summarized in the table below:

Sample	N	Mean (pg/mL)	Repeatability (Within-Run)		Within-Laboratory[a]		Between-Lot		Overall Within-Laboratory[b]	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
Low Control	240	143.0	6.40	4.5	7.63	5.3	4.42	3.1	8.82	6.2
Medium Control	240	503.4	16.26	3.2	18.21	3.6	9.29	1.8	20.44	4.1
High Control	240	5144.2	177.02	3.4	202.18	3.9	21.47	0.4	203.32	4.0
Panel A	240	18.8	1.39	7.4	1.72	9.2	0.75	4.0	1.88	10.0
Panel B	240	55.6	2.64	4.7	3.02	5.4	1.60	2.9	3.41	6.1
Panel C	240	130.4	4.28	3.3	5.44	4.2	4.87	3.7	7.30	5.6
Panel D	240	449.1	13.10	2.9	18.68	4.2	14.02	3.1	23.36	5.2
Panel E	240	1011.4	28.31	2.8	34.49	3.4	21.92	2.2	40.87	4.0
Panel F	240	1938.0	54.02	2.8	65.28	3.4	22.56	1.2	69.07	3.6
Panel G	240	14939.8	417.56	2.8	518.88	3.5	296.61	2.0	597.67	4.0
Panel H	240	31711.0	989.02	3.1	1185.11	3.7	2118.19	6.7	2427.19	7.7

[a]Includes repeatability (within-run), between-run, and between-day variability
[b]Includes repeatability (within-run), between-run, between-day, and between-lot variability

Reproducibility
A study was performed based on guidance from CLSI EP05-A3.

Testing was conducted at 3 sites, using 3 lots of the candidate device, 2 lots of the Alere NT-proBNP for Alinity i Calibrators, 2 lots of the Alere NT-proBNP for Alinity i Controls, and 1 Alinity i instrument.

Three controls and 8 reproducibility panel plasma members were tested in 4 replicates at 2 separate times per day on 5 different days.

The native panel members 1 to 6 were prepared by pooling NT-proBNP K_2EDTA plasma specimens and diluting with human K_2EDTA plasma as necessary to achieve the target concentration. Panel 7 and Panel 8 were prepared by spiking K_2EDTA plasma with a synthetic NT-proBNP peptide. Results are summarized in the table below:

Sample	N	Mean (pg/mL)	Repeatability		Within-Laboratory[a]		Between-Site		Between-Lot		Overall Reproducibility[b]	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Low Control	360	136.6	4.75	3.5	5.75	4.2	2.63	1.9	1.45	1.1	6.49	4.7
Medium Control	360	477.1	12.24	2.6	15.35	3.2	14.20	3.0	8.88	1.9	22.72	4.8
High Control	359	4932.2	201.01	4.1	311.08	6.3	105.17	2.1	0.00	0.0	328.38	6.7
Panel 1	359	14.5	2.10[c]	14.5	2.74[c]	18.9	0.00	0.0	0.00	0.0	2.74c	18.9
Panel 2	360	51.9	2.14	4.1	3.07	5.9	0.49	0.9	0.00	0.0	3.11	6.0
Panel 3	360	127.1	4.67	3.7	5.95	4.7	1.36	1.1	0.78	0.6	6.15	4.8
Panel 4	360	436.9	10.46	2.4	18.67	4.3	3.18	0.7	0.00	0.0	18.94	4.3
Panel 5	360	996.5	21.86	2.2	43.45	4.4	17.12	1.7	0.00	0.0	46.70	4.7
Panel 6	360	1891.1	33.18	1.8	79.86	4.2	35.96	1.9	0.00	0.0	87.58	4.6
Panel 7	359	15664.6	319.91	2.0	974.61	6.2	281.81	1.8	217.28	1.4	1037.54	6.6
Panel 8	360	25630.5	601.42	2.3	1654.86	6.5	220.39	0.9	807.19	3.1	1854.37	7.2

[a] Includes repeatability, between-run, and between-day variance components

[b] Includes repeatability, between-run, between-day, between-site, and between-lot variance components

[c] An outlying run was observed. Based on guidance from CLSI EP05-A3, a replacement run was performed. Without the replacement run, the repeatability SD was 9.53 pg/mL (1.12 pmol/L), within-laboratory SD was 9.64 pg/mL (1.14 pmol/L), and the overall reproducibility SD was 9.64 pg/mL (1.14 pmol/L).

2. Linearity:

A linearity study was performed based on guidance from CLSI EP06, 2nd ed.

Two linearity K_2EDTA plasma sample sets were prepared, each containing 8-9 concentration levels. Each linearity panel was a dilution series prepared by intermixing known volumes of high native sample with analyte-free plasma. The samples were tested in replicates of 10 using 3 reagent lots, 1 calibrator lot, 1 control lot, and 1 Alinity i instrument. All samples within the sample set were tested within a single run.

The linearity was analyzed separately for each reagent lot per sample type. Using a weighted linear regression analysis, the difference and percent difference between the mean observed

concentration and the expected concentration of each sample were calculated. At each concentration, the deviation from linearity was less than 11.3%.

The results of the linearity study support the claimed measuring range of 15.8 to 35,000.0 pg/mL.

Sample Dilution
Plasma samples with NT-proBNP concentrations up to 70,000 pg/mL can be measured after a 1:2 auto dilution, and samples with NT-proBNP concentrations up to 350,000 pg/mL can be measured using a 1:10 manual dilution. The percent recovery of diluted samples ranged from 92.1% to 103.4%.

3. Analytical Specificity/Interference:

Interference studies were performed based on guidance from CLSI EP07, 3rd ed.

Endogenous Substances
Interference from endogenous substances was assessed using K_2EDTA plasma samples at two levels of NT-proBNP analyte (approximately 125 pg/mL and 2000 pg/mL). Each sample was divided into 2 aliquots: one aliquot for the test sample (with added interferent) and one aliquot for the control sample (without added interferent). Each sample was tested in replicates of 30 using one Alinity i Analyzer, three reagent lots, and one calibrator lot.

No significant interference (defined by the sponsor as a difference within ± 10% between the mean for the test sample versus the mean of the reference samples) was observed at the following concentrations:

Potentially Interfering Substance	Highest concentration tested at which no significant interference is observed
Bilirubin (conjugated)	60 mg/dL
Bilirubin (unconjugated)	60 mg/dL
Biotin	4250 ng/mL
Cholesterol	700 mg/dL
Human Anti-Mouse Antibodies (HAMA)	1500 ng/mL
Hemoglobin	1 g/dL
IgG	6 g/dL
Intralipid	3000 mg/dL
Rheumatoid Factor (RF)	600 IU/mL
Total Protein	12.6 g/dL

Interference beyond ± 10.0% (based on 95% Confidence Interval [CI]) was observed at the concentrations shown below for the following substances.

Potentially Interfering Substance	Interferent Level	Analyte Level	% Interference (95% CI)
RF	1520 IU/mL	125 pg/mL	-8.9% (-10.4%, -7.5%)

Potentially Interfering Substance	Interferent Level	Analyte Level	% Interference (95% CI)
	1520 IU/mL	2000 pg/mL	-11.4% (-12.4%, -10.4%)
Total Protein	15.2 g/dL	125 pg/mL	-12.7% (-14.7%, -10.7%)
	15.5 g/dL	2000 pg/mL	-9.9% (-11.4%, -8.5%)

Potentially Interfering Drugs

Interference from exogenous substances was assessed using K_2EDTA plasma samples at two levels of the NT-proBNP analyte (approximately 125 pg/mL and 2000 pg/mL), following the same procedure as for endogenous substances.

No significant interference (defined by the sponsor as a difference within ± 10% between the mean for the test sample versus the mean of the reference samples) was observed at the following concentrations:

Potentially Interfering Drug	Highest concentration tested at which no significant interference is observed
Acetaminophen	15.6 mg/dL
Acetylsalicylic Acid	60 mg/dL
Allopurinol	6.0 mg/dL
Amikacin	15 mg/dL
Amiodarone	4.2 mg/dL
Amlodipine Besylate	0.4 mg/dL
Ampicillin	7.5 mg/dL
Ascorbic acid	5.25 mg/dL
Atenolol	1.0 mg/dL
Atorvastatin	32 mg/dL
Caffeine	10.8 mg/dL
Captopril	5.0 mg/dL
Carbamazepine	4.5 mg/dL
Carvedilol	3.75 mg/dL
Chloramphenicol	7.8 mg/dL
Chlordiazepoxide	1.0 mg/dL
Chlorpromazine	0.33 mg/dL
Cimetidine	3.0 mg/dL
Cinnarizine	3.1 mg/dL
Clopidogrel bisulfate	7.5 mg/dL

Potentially Interfering Drug	Highest concentration tested at which no significant interference is observed
Creatinine	30 mg/dL
Cyclosporine A	0.4 mg/dL
Dextran 40	6000 mg/dL
Diazepam	3.0 mg/dL
Diclofenac	6.0 mg/dL
Digoxin	0.025 mg/dL
Diltiazem	12 mg/dL
Dipyridamole	8.0 mg/dL
Disopyramide	4.0 mg/dL
Dobutamine	10 mg/dL
Dopamine	65 mg/dL
Enalapril Maleate	1.6 mg/dL
Epinephrine	0.05 mg/dL
Erythromycin	13.8 mg/dL
Ethanol	600 mg/dL
Ethosuximide	30 mg/dL
Fenofibrate	4.5 mg/dL
Furosemide	6.0 mg/dL
Gentamicin	12 mg/dL
Heparin	8 U/mL
Hydralazine	2.0 mg/dL
Hydrochlorothiazide	2.0 mg/dL
Insulin	0.16 mg/dL
Ibuprofen	50 mg/dL
Indomethacin	3.6 mg/dL
Isosorbide dinitrate	6.0 mg/dL
Lidocaine	8.0 mg/dL
Lisinopril	1.6 mg/dL
Lithium	4.20 mg/dL
Losartan potassium	5.99 mg/dL
Lovastatin	2.0 mg/dL
L-Thyroxine	0.06 mg/dL
Methyldopa	2.5 mg/dL
Methylprednisolone	0.75 mg/dL
Metoprolol	1.5 mg/dL

Potentially Interfering Drug	Highest concentration tested at which no significant interference is observed
Milrinone	0.183 mg/dL
Naproxen	49.97 mg/dL
Nicotine	0.1 mg/dL
Nicotinic acid	4.0 mg/dL
Nifedipine	6.0 mg/dL
Nitrofurantoin	4.0 mg/dL
Nitroglycerin	0.016 mg/dL
Oxazepam	1.2 mg/dL
Oxytetracycline	10 mg/dL
Penicillin G	25 U/mL
Pentobarbital	12.6 mg/dL
Phenobarbitol	69 mg/dL
Phenprocoumon (Marcumar)	1.53 mg/dL
Phenytoin	6.0 mg/dL
Primidone	5.7 mg/dL
Probenecid	60 mg/dL
Procainamide	4.8 mg/dL
Propafenone	30 mg/dL
Propanolol	0.2 mg/dL
Propoxyphene	0.321 mg/dL
Quinidine	2.0 mg/dL
Ramipril	0.6 mg/dL
Retaplase	3.33 mg/dL
Simvastatin	3.2 mg/dL
Spironolactone	7.5 mg/dL
Sulfamethoxazole	112 mg/dL
Theophylline	6.0 mg/dL
Tolbutamide	150 mg/dL
Torasemide	1.5 mg/dL
Trandolapril	4.0 mg/dL
Trasylol/Aprotinin	501.8 KIE/mL
Trimethoprim	6.4 mg/dL
Uric Acid	23.5 mg/dL
Valproic Acid	50 mg/dL
Verapamil	24 mg/dL

Potentially Interfering Drug	Highest concentration tested at which no significant interference is observed
Warfarin	7.5 mg/dL

Cross-Reactants
A study was conducted to evaluate the performance of the Alere NT-proBNP for Alinity i assay in the presence of potential cross-reactants.

Testing was conducted using K_2EDTA samples at two levels of NT-proBNP (125 pg/mL and 2000 pg/mL). Each sample was divided into 2 aliquots: one aliquot for the test sample (spiked with cross-reactant) and one aliquot for the control sample (spiked with the same diluent used for the preparation of cross-reactant in stock solutions). Each sample was tested in replicates of 30 using 1 reagent lot, 1 calibrator lot, 1 control lot, and 1 Alinity i instrument.

The % Recovery was calculated as:
% Recovery = 100 x (test mean / control mean)

The observed % recovery of NT-proBNP was within 100% ± 10% for all cross-reactants evaluated at each analyte level:

Potential Cross-Reactant	Cross-Reactant Concentration
Adrenomedullin	1000 pg/mL
Aldosterone	600 pg/mL
Angiotensin I	600 pg/mL
Angiotensin II	600 pg/mL
Angiotensin III	1000 pg /mL
ANP 28	3100 ng/mL
Arg-Vasopressin	1000 pg/mL
BNP 32	3500 ng/mL
CNP 22	2200 ng/mL
Endothelin	20 pg/mL
NT-proANP 1-30 (preproANP26-55)	3500 ng/mL
NT-proANP 31-67 (preproANP56-92)	1000 pg/mL
NT-proANP 79-98 (preproANP104-123)	1000 pg/mL
Renin	50 000 pg/mL
Urodilatin	3500 ng/mL

High Dose Hook Effect
The Alere NT-proBNP for Alinity i assay was evaluated for high dose hook effect using K_2EDTA plasma samples with 4 concentration levels of NT-proBNP. No high dose hook effect was observed on samples up to 372,620 pg/mL of NT-proBNP.

4. Assay Reportable Range:

The analytical measuring interval (AMI) for the Alere NT-proBNP for Alinity i assay was determined to be from 15.8 to 35,000.0 pg/mL.

The extended measuring interval (EMI) for the Alere NT-proBNP for Alinity i assay was determined to be from 35,000.0 to 350,000.0 pg/mL.

The reportable interval for the Alere NT-proBNP for Alinity i assay was determined to be from 15.8 to 350,000.0 pg/mL.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

Traceability
The Alere NT-proBNP for Alinity i Calibrators are traceable to internal reference standards at every concentration level that were value assigned on and made traceable to the predicate device by transference, as described by CLSI Document EP32-R.

Specimen Stability

Specimen storage times were validated with serum separator and K_2EDTA plasma tube types. The sponsor has provided information to support the following claims in their labeling:

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	Remove serum or plasma from the clot, red blood cells, or separator gel if testing will be delayed more than 24 hours.
	-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 Onboard Storage
The sponsor provided data to support that samples can be stored on the Alinity i system for up to 3 hours when tested with the Alere NT-proBNP for Alinity i assay.

6. Detection Limit:

The LoB, LoD, and LoQ study was performed based on guidance from CLSI EP17-A2.

Limit of Blank (LoB)
The LoB study was performed on 4 analyte-free K_2EDTA plasma specimens, using 3 reagent lots, 1 calibrator lot, 1 control lot, and 2 Alinity i instruments. Testing occurred over 3 days. Samples were tested in 5 replicates on each day of testing, for a total of 60 replicates. The LoB was determined non-parametrically for each reagent lot separately and the highest value obtained from three lots was taken as the LoB value. The LoB for the Alere NT-proBNP for Alinity i assay was estimated to be 0.1 pg/mL.

Limit of Detection (LoD)
The LoD study was performed on 18 low-level panels (2 samples at each of 9 unique target concentration levels of approximately 3.0, 4.0, 5.0, 7.0, 9.0, 12.0, 16.0, 20.0, and 24.0 pg/mL), using 3 reagent lots, 1 calibrator lot, 1 control lot, and 2 Alinity i instruments. Testing occurred over 3 days. The 18 low-analyte samples were tested in 10 replicates on each day of testing, for a total of 60 measurements per sample per reagent lot. The parametric approach described in CLSI EP17-A2 was followed to determine the LoD. The highest observed LoD of the three lots was the reported LoD for the assay. The LoD for the Alere NT-proBNP for Alinity i assay was estimated to be 3.6 pg/mL.

Limit of Detection (LoQ)
The LoQ study was performed on 18 low-level panels (2 samples at each of 9 unique target concentration levels of approximately 3.0, 4.0, 5.0, 7.0, 9.0, 12.0, 16.0, 20.0, and 24.0 pg/mL), using 3 reagent lots, 1 calibrator lot, 1 control lot, and 2 Alinity i instruments. Testing occurred over 3 days. The 18 low-analyte samples were tested in 10 replicates on each day of testing, for a total of 60 measurements per sample per reagent lot. The LoQ was determined as the lowest concentration of analyte at which the maximum allowable precision of 20% CV was met. The highest value obtained from three lots was the reported LoQ for the assay. The LoQ for the Alere NT-proBNP for Alinity i assay was estimated to be 15.8 pg/mL.

7. Assay Cut-Off:

See Section VII.D for Clinical Cut-off information.

B Comparison Studies:

1. Method Comparison with Predicate Device:

Not applicable.

2. Matrix Comparison:

A study was conducted to evaluate equivalence between different blood collection tubes. The study was performed based on guidance from the CLSI document EP35 1st ed.

In the study, samples were collected from 63 subjects, representative of the intended use population. A primary tube (control condition, K_2EDTA) and a minimum of 1 candidate tube (test condition) were collected from each enrolled subject. Testing of specimens collected in different tube types was initiated within 2 hours of draw. Two replicates from each specimen tube type were tested. Testing was conducted using 2 Alinity i instruments, 2 reagent lots, 3 calibrator lots, and 3 control lots.

The results were analyzed using Passing-Bablok regression analysis comparing the first replicate of the evaluation tube to the first replicate of the control tube (K_2EDTA).

Tube Type	N	Concentration Range (pg/mL)	Slope	Intercept	r
K_3EDTA	60	20.2-29670.1	0.98	1.1	0.999
Serum	56	20.2-19929.3	1.02	1.6	0.993
Serum Separator Tube	57	21.8-29670.1	1.00	1.0	0.999
Lithium Heparin	61	20.2-29670.1	1.00	0.1	0.998
Lithium Heparin (Plasma Separator)	57	20.2-29670.1	0.99	-1.2	1.000

The study results demonstrate equivalence between K_2EDTA plasma, K_3EDTA plasma, lithium heparin plasma, and serum samples.

C Clinical Studies:

1. Clinical Sensitivity:

   Not applicable.

2. Clinical Specificity:

   Not applicable.

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

   A multi-center prospective study including 17 collection sites across the US was conducted to establish the performance characteristics of the Alere NT-proBNP for Alinity i assay. Subjects 18 years and older presenting to the emergency department (ED) with signs and symptoms consistent with a clinical suspicion of new onset or acute exacerbation of HF were included in the study. Subjects with renal insufficiency requiring dialysis or known estimated glomerular filtration rate (eGFR) < 15 mL/min/1.73 m^2 and subjects with dyspnea after chest trauma were excluded from the study. For each patient enrollment, whole blood samples

were collected in K_2EDTA tubes, processed to plasma, and stored frozen until testing. Data supporting the stability of the samples for the storage time and condition were provided by the sponsor.

The results were determined from 2127 ED subjects. Of the 2127 ED subjects, 1030 (48.4%) were female and 1097 (51.6%) were male, ranging in age from 19 to 97 years. Individuals in the population were White (53.1%), Black or African American (39.45%), Asian (1.7%), American Indian or Alaska Native (0.8%), Native Hawaiian or Other Pacific Islander (0.3%), or represented by other races (0.9%), with the remaining 3.6% of Unknown / Not Reported race. A total of 1934 (90.9%) ED subjects were not Hispanic or Latino, and 168 (7.9%) ED subjects were Hispanic or Latino, with the remaining 25 (1.2%) ED subjects of Unknown / Not Reported ethnicity.

The clinical study specimen testing was performed with clinical specimens that were randomly distributed across 3 clinical testing sites using 3 lots of the Alere NT-proBNP for Alinity i Reagents, 2 lots of the Alere NT-proBNP for Alinity i Calibrators, 2 lots of the Alere NT-proBNP for Alinity i Controls, and 1 Alinity i instrument at each clinical site. One replicate of each specimen was tested with the investigational device.

Adjudication was performed by a panel of 10 board-certified cardiologists. A determination of HF (acute exacerbation or new onset) or non-HF was rendered for each subject. The adjudicators were blinded to the diagnosis and HF severity of the local attending physician in the ED, and the determination of other members of the adjudication panel. For subjects determined to have HF, severity was assessed based on New York Heart Association (NYHA) classification (I, II, III, IV) and a determination of new or worsening HF.

The descriptive statistics for the Alere NT-proBNP for Alinity i test results (pg/mL) were determined within and across sex by age group and are summarized in the following table:

Alere NT-proBNP for Alinity i – All Subjects

Adjudicated Diagnosis	**HF**				**Non-HF**			
Age Group (Years)	<50	50 to 75	>75	All	<50	50 to 75	>75	All
N	160	553	167	880	300	799	148	1247
Mean	4354.5	6092.9	6891.3	5928.3	437.9	760.4	1738.8	798.9
SD	5454.61	8144.69	6860.32	7521.45	1686.57	1833.48	3494.96	2100.93
Median	2664.9	3297.2	4787.6	3420.7	70.1	198.9	565.9	181.8
Minimum	22.8	19.8	158.9	19.8	0.0	0.0	4.2	0.0
Maximum	38880.9	60716.9	38938.4	60716.9	24737.1	17988.9	31154.0	31154.0

Alere NT-proBNP for Alinity i – Male Subjects

Adjudicated Diagnosis	**HF**				**Non-HF**			
Age Group (Years)	<50	50 to 75	>75	All	<50	50 to 75	>75	All
N	105	317	82	504	135	392	66	593

Mean	4522.0	6631.0	6971.2	6247.0	638.8	911.6	2258.0	999.4
SD	4977.78	8410.52	6708.84	7590.10	2369.85	2084.06	4464.35	2557.26
Median	2780.1	4066.5	4773.9	3904.3	64.1	221.0	701.8	203.2
Min	58.3	19.8	186.9	19.8	0.0	0.0	4.2	0.0
Max	26986.3	60716.9	32286.4	60716.9	24737.1	17988.9	31154.0	31154.0

Alere NT-proBNP for Alinity i – Female Subjects

Adjudicated Diagnosis	**HF**				**Non-HF**			
Age Group (Years)	<50	50 to 75	>75	All	<50	50 to 75	>75	All
N	55	236	85	376	165	407	82	654
Mean	4034.7	5370.1	6814.2	5501.2	273.5	614.8	1320.8	617.2
SD	6302.82	7732.10	7042.32	7417.01	732.64	1543.13	2403.63	1556.92
Median	1851.5	2541.7	4787.6	2786.4	73.1	174.9	499.7	161.2
Min	22.8	24.9	158.9	22.8	0.0	0.9	25.7	0.0
Max	38880.9	47385.1	38938.4	47385.1	5633.9	14849.5	18827.7	18827.7

For the 2127 subjects enrolled in the study, a total of 880 subjects were adjudicated as subjects with HF and 1247 subjects were adjudicated as subjects without HF.

	Subject with Heart Failure	**Subject without Heart Failure**
Age Group		
< 50	34.8% (160/460)	65.2% (300/460)
50-75	40.9% (553/1352)	59.1% (799/1352)
>75	53.0% (167/315)	47.0% (148/315)
Sex		
Female	36.5% (376/1030)	63.5% (654/1030)
Male	45.9% (504/1097)	54.1% (593/1097)

The pretest probability of adjudicated HF (prevalence of adjudicated HF in the study), post-test probabilities, and likelihood ratios of the Alere NT-proBNP for Alinity i result vs. adjudicated diagnosis (along with the 95% CIs) were determined based on guidance from CLSI EP12, 3rd ed., for all subjects and by sex using the age-dependent positive cutoffs (450 pg/mL for subjects 18 to < 50 years of age, 900 pg/mL for subjects 50 to 75 years of age, and 1800 pg/mL for subjects > 75 years of age) and age-independent negative cutoff (300 pg/mL).

The results for all subjects are presented in the following tables:

Clinical Performance – All Subjects:

Category	NT-proBNP Test Result	Adjudicated Diagnosis		Total	Posttest Probability of HF % (n / N) (95% CI)	Posttest Probability of Non-HF % (n / N) (95% CI)	Likelihood Ratio (HF) (95% CI)
		HF	Non-HF				
Age Group (years): 18 - <50 Pretest Probability of HF (Prevalence of HF in study): 34.78% (160/460)	Positive	140	48	**188**	**74.5 (140 / 188) (67.8, 80.2)**	-	5.47 (4.19, 7.13)
	Gray Zone	7	13	**20**	35.0 (7 / 20) (18.1, 56.7)	65.0 (13 / 20) (43.3, 81.9)	1.01 (0.41, 2.48)
	Negative	13	239	**252**	-	**94.8 (239 / 252) (91.4, 97.0)**	0.10 (0.06, 0.17)
	Total	**160**	**300**	**460**			
Age Group (years): 50 - 75 Pretest Probability of HF (Prevalence of HF in study): 40.90% (553/1352)	Positive	437	148	**585**	**74.7 (437 / 585) (71.0, 78.1)**	-	4.27 (3.67, 4.96)
	Gray Zone	80	147	**227**	35.2 (80 / 227) (29.3, 41.7)	64.8 (147 / 227) (58.3, 70.7)	0.79 (0.61, 1.01)
	Negative	36	504	**540**	-	**93.3 (504 / 540) (90.9, 95.1)**	0.10 (0.07, 0.14)
	Total	**553**	**799**	**1352**			
Age Group (years): >75 Pretest Probability of HF (Prevalence of HF in study): 53.02% (167/315)	Positive	131	38	**169**	**77.5 (131 / 169) (70.6, 83.2)**	-	3.06 (2.30, 4.06)
	Gray Zone	34	59	**93**	36.6 (34 / 93) (27.5, 46.7)	63.4 (59 / 93) (53.3, 72.5)	0.51 (0.36, 0.73)
	Negative	2	51	**53**	-	**96.2 (51 / 53) (87.2, 99.0)**	0.03 (0.01, 0.14)
	Total	**167**	**148**	**315**			
All Subjects Pretest Probability of HF (Prevalence of HF in study): 41.37% (880/2127)	Positive	708	234	**942**	**75.2 (708 / 942) (72.3, 77.8)**	-	4.29 (3.80, 4.83)
	Gray Zone	121	219	**340**	35.6 (121 / 340) (30.7, 40.8)	64.4 (219 / 340) (59.2, 69.3)	0.78 (0.64, 0.96)
	Negative	51	794	**845**	-	**94.0 (794 / 845) (92.2, 95.4)**	0.09 (0.07, 0.12)
	Total	**880**	**1247**	**2127**			

Clinical Performance – Female Subjects:

Category	NT-proBNP Test Result	Adjudicated Diagnosis		Total	Posttest Probability of HF % (n / N) (95% CI)	Posttest Probability of Non-HF % (n / N) (95% CI)	Likelihood Ratio (HF) (95% CI)
		HF	Non-HF				
Age Group (years): 18 - <50 Pretest Probability of HF (Prevalence of HF in study): 25.00% (55/220)	Positive	45	20	**65**	**69.2 (45 / 65) (57.2, 79.1)**	-	6.75 (4.39, 10.37)
	Gray Zone	3	7	**10**	30.0 (3 / 10) (10.8, 60.3)	70.0 (7 / 10) (39.7, 89.2)	1.29 (0.34, 4.80)
	Negative	7	138	**145**	-	**95.2 (138 / 145) (90.4, 97.6)**	0.15 (0.08, 0.31)
	Total	**55**	**165**	**220**			
Age Group (years): 50 - 75 Pretest Probability of HF (Prevalence of HF in study): 36.70% (236/643)	Positive	173	56	**229**	**75.5 (173 / 229) (69.6, 80.7)**	-	5.33 (4.13, 6.88)
	Gray Zone	35	76	**111**	31.5 (35 / 111) (23.6, 40.7)	68.5 (76 / 111) (59.3, 76.4)	0.79 (0.55, 1.15)
	Negative	28	275	**303**	-	**90.8 (275 / 303) (87.0, 93.5)**	0.18 (0.12, 0.25)
	Total	**236**	**407**	**643**			
Age Group (years): >75 Pretest Probability of HF (Prevalence of HF in study): 50.90% (85/167)	Positive	64	19	**83**	**77.1 (64 / 83) (67.0, 84.8)**	-	3.25 (2.15, 4.91)
	Gray Zone	20	34	**54**	37.0 (20 / 54) (25.4, 50.4)	63.0 (34 / 54) (49.6, 74.6)	0.57 (0.36, 0.90)
	Negative	1	29	**30**	-	**96.7 (29 / 30) (83.3, 99.4)**	0.03 (0.00, 0.24)
	Total	**85**	**82**	**167**			
All Female Subjects Pretest Probability of HF (Prevalence of HF in study): 36.50% (376/1030)	Positive	282	95	**377**	**74.8 (282 / 377) (70.2, 78.9)**	-	5.16 (4.25, 6.27)
	Gray Zone	58	117	**175**	33.1 (58 / 175) (26.6, 40.4)	66.9 (117 / 175) (59.6, 73.4)	0.86 (0.65, 1.15)
	Negative	36	442	**478**	-	**92.5 (442 / 478) (89.7, 94.5)**	0.14 (0.10, 0.19)
	Total	**376**	**654**	**1030**			

Clinical Performance – Male Subjects:

Category	NT-proBNP Test Result	Adjudicated Diagnosis		Total	Posttest Probability of HF % (n / N) (95% CI)	Posttest Probability of Non-HF % (n / N) (95% CI)	Likelihood Ratio (HF) (95% CI)
		HF	Non-HF				
Age Group (years): 18 - <50 Pretest Probability of HF (Prevalence of HF in study): 43.75% (105/240)	Positive	95	28	**123**	**77.2 (95 / 123) (69.1, 83.8)**	-	4.36 (3.12, 6.10)
	Gray Zone	4	6	**10**	40.0 (4 / 10) (16.8, 68.7)	60.0 (6 / 10) (31.3, 83.2)	0.86 (0.25, 2.96)
	Negative	6	101	**107**	-	**94.4 (101 / 107) (88.3, 97.4)**	0.08 (0.03, 0.17)
	Total	**105**	**135**	**240**			
Age Group (years): 50 - 75 Pretest Probability of HF (Prevalence of HF in study): 44.71% (317/709)	Positive	264	92	**356**	**74.2 (264 / 356) (69.4, 78.4)**	-	3.55 (2.95, 4.27)
	Gray Zone	45	71	**116**	38.8 (45 / 116) (30.4, 47.9)	61.2 (71 / 116) (52.1, 69.6)	0.78 (0.56, 1.10)
	Negative	8	229	**237**	-	**96.6 (229 / 237) (93.5, 98.3)**	0.04 (0.02, 0.09)
	Total	**317**	**392**	**709**			
Age Group (years): >75 Pretest Probability of HF (Prevalence of HF in study): 55.41% (82/148)	Positive	67	19	**86**	**77.9 (67 / 86) (68.1, 85.4)**	-	2.84 (1.92, 4.20)
	Gray Zone	14	25	**39**	35.9 (14 / 39) (22.7, 51.6)	64.1 (25 / 39) (48.4, 77.3)	0.45 (0.26, 0.80)
	Negative	1	22	**23**	-	**95.7 (22 / 23) (79.0, 99.2)**	0.04 (0.01, 0.26)
	Total	**82**	**66**	**148**			
All Male Subjects Pretest Probability of HF (Prevalence of HF in study): 45.94% (504/1097)	Positive	426	139	**565**	**75.4 (426 / 565) (71.7, 78.8)**	-	3.61 (3.10, 4.19)
	Gray Zone	63	102	**165**	38.2 (63 / 165) (31.1, 45.8)	61.8 (102 / 165) (54.2, 68.9)	0.73 (0.54, 0.97)
	Negative	15	352	**367**	-	**95.9 (352 / 367) (93.4, 97.5)**	0.05 (0.03, 0.08)
	Total	**504**	**593**	**1097**			

The pretest probability of adjudicated HF (prevalence of adjudicated HF in the study), posttest probabilities, and likelihood ratios of the Alere NT-proBNP for Alinity i result vs adjudicated diagnosis (along with the 95% CIs) were also determined for relevant clinical subgroups using the age-dependent positive cutoffs and age-independent negative cutoff.

Clinical Performance – Subjects with History of Heart Failure:

Category	NT-proBNP Test Result	Adjudicated Diagnosis		Total	Posttest Probability of HF % (n / N) (95% CI)	Posttest Probability of Non-HF % (n / N) (95% CI)	Likelihood Ratio (HF) (95% CI)
		HF	Non-HF				
Age Group (years): 18 - <50 Pretest Probability of HF (Prevalence of HF in study): 71.70% (114/159\)	Positive	103	17	**120**	**85.8 (103 / 120) (78.5, 91.0)**	-	2.39 (1.64, 3.50)
	Gray Zone	5	3	**8**	62.5 (5 / 8) (30.6, 86.3)	37.5 (3 / 8) (13.7, 69.4)	0.66 (0.16, 2.64)
	Negative	6	25	**31**	-	**80.6 (25 / 31) (63.7, 90.8)**	0.09 (0.04, 0.22)
	Total	**114**	**45**	**159**			
Age Group (years): 50 - 75 Pretest Probability of HF (Prevalence of HF in study): 65.09% (427/656)	Positive	344	82	**426**	**80.8 (344 / 426) (76.7, 84.2)**	-	2.25 (1.88, 2.69)
	Gray Zone	58	45	**103**	56.3 (58 / 103) (46.7, 65.5)	43.7 (45 / 103) (34.5, 53.3)	0.69 (0.48, 0.99)
	Negative	11	402	**413**	-	**80.3 (102 / 127) (72.6, 86.3)**	0.13 (0.09, 0.20)
	Total	**427**	**229**	**656**			
Age Group (years): >75 Pretest Probability of HF (Prevalence of HF in study): 69.31% (131/189)	Positive	107	22	**129**	**82.9 (107 / 129) (75.5, 88.5)**	-	2.15 (1.53, 3.02)
	Gray Zone	24	21	**45**	53.3 (24 / 45) (39.1, 67.1)	46.7 (21 / 45) (32.9, 60.9)	0.51 (0.31, 0.83)
	Negative	2	36	**38**	-	**100.0 (15 / 15) (79.6, 100.0)**	0.01 (0.00, 0.24)
	Total	**131**	**58**	**189**			
All Subjects with History of HF Pretest Probability of	Positive	554	121	**675**	**82.1 (554 / 675) (79.0, 84.8)**	-	2.26 (1.95, 2.62)
	Gray Zone	87	69	**156**	55.8 (87 / 156) (47.9, 63.3)	44.2 (69 / 156) (36.7, 52.1)	0.62 (0.47, 0.83)

Category	NT-proBNP Test Result	Adjudicated Diagnosis		Total	Posttest Probability of HF % (n / N) (95% CI)	Posttest Probability of Non-HF % (n / N) (95% CI)	Likelihood Ratio (HF) (95% CI)
		HF	Non-HF				
HF (Prevalence of HF in study): 66.93% (672/1004)	Negative	31	142	**173**	-	**82.1 (142 / 173) (75.8, 87.1)**	0.11 (0.07, 0.16)
	Total	**672**	**332**	**1004**			

Clinical Performance – Subjects without a History of Heart Failure:

Category	NT-proBNP Test Result	Adjudicated Diagnosis		Total	Posttest Probability of HF % (n / N) (95% CI)	Posttest Probability of Non-HF % (n / N) (95% CI)	Likelihood Ratio (HF) (95% CI)
		HF	Non-HF				
Age Group (years): 18 - <50 Pretest Probability of HF (Prevalence of HF in study): 15.28% (46/301)	Positive	37	31	**68**	**54.4 (37 / 68) (42.7, 65.7)**	-	6.62 (4.62, 9.48)
	Gray Zone	2	10	**12**	16.7 (2 / 12) (4.7, 44.8)	83.3 (10 / 12) (55.2, 95.3)	1.11 (0.25, 4.90)
	Negative	7	214	**221**	-	**96.8 (214 / 221) (93.6, 98.5)**	0.18 (0.09, 0.36)
	Total	**46**	**255**	**301**			
Age Group (years): 50 - 75 Pretest Probability of HF (Prevalence of HF in study): 18.10% (126/696)	Positive	93	66	**159**	**58.5 (93 / 159) (50.7, 65.9)**	-	6.37 (4.97, 8.18)
	Gray Zone	22	102	**124**	17.7 (22 / 124) (12.0, 25.4)	82.3 (102 / 124) (74.6, 88.0)	0.98 (0.64, 1.48)
	Negative	11	402	**413**	-	**97.3 (402 / 413) (95.3, 98.5)**	0.12 (0.07, 0.22)
	Total	**126**	**570**	**696**			
Age Group (years): >75 Pretest Probability of HF (Prevalence of HF in study): 28.57% (36/126)	Positive	24	16	**40**	**60.0 (24 / 40) (44.6, 73.7)**	-	3.75 (2.27, 6.19)
	Gray Zone	10	38	**48**	20.8 (10 / 48) (11.7, 34.3)	79.2 (38 / 48) (65.7, 88.3)	0.66 (0.37, 1.17)
	Negative	2	36	**38**	-	**94.7 (36 / 38) (82.7, 98.5)**	0.14 (0.04, 0.55)
	Total	**36**	**90**	**126**			

Category	NT-proBNP Test Result	Adjudicated Diagnosis		Total	Posttest Probability of HF % (n / N) (95% CI)	Posttest Probability of Non-HF % (n / N) (95% CI)	Likelihood Ratio (HF) (95% CI)
		HF	Non-HF				
All Subjects without History of HF Pretest Probability of HF (Prevalence of HF in study): 18.52% (208/1123)	Positive	154	113	**267**	**57.7 (154 / 267) (51.7, 63.5)**	-	6.00 (4.96, 7.25)
	Gray Zone	34	150	**184**	18.5 (34 / 184) (13.5, 24.7)	81.5 (150 / 184) (75.3, 86.5)	1.00 (0.71, 1.40)
	Negative	20	652	**672**	-	**97.0 (652 / 672) (95.4, 98.1)**	0.13 (0.09, 0.21)
	Total	**208**	**915**	**1123**			

Clinical Performance – Subjects with eGFR < 60 mL/min/1.73 m^2:

Category	NT-proBNP Test Result	Adjudicated Diagnosis		Total	Posttest Probability of HF % (n / N) (95% CI)	Posttest Probability of Non-HF % (n / N) (95% CI)	Likelihood Ratio (HF) (95% CI)
		HF	Non-HF				
Age Group (years): 18 - <50 Pretest Probability of HF (Prevalence of HF in study): 72.22% (39/54)	Positive	37	5	**42**	**88.1 (37 / 42) (75.0, 94.8)**	-	2.85 (1.39, 5.84)
	Gray Zone	1	0	**1**	100.0 (1 / 1) (20.7, 100.0)	0.0 (0 / 1) (0.0, 79.3)	1.18 (0.05, 27.37)
	Negative	1	10	**11**	-	**90.9 (10 / 11) (62.3, 98.4)**	0.04 (0.01, 0.28)
	Total	**39**	**15**	**54**			
Age Group (years): 50 - 75 Pretest Probability of HF (Prevalence of HF in study): 57.11% (237/415)	Positive	201	58	**259**	**77.6 (201 / 259) (72.1, 82.3)**	-	2.60 (2.09, 3.24)
	Gray Zone	27	50	**77**	35.1 (27 / 77) (25.3, 46.2)	64.9 (50 / 77) (53.8, 74.7)	0.41 (0.26, 0.62)
	Negative	9	70	**79**	-	**88.6 (70 / 79) (79.7, 93.9)**	0.10 (0.05, 0.19)
	Total	**237**	**178**	**415**			
Age Group (years): >75 Pretest Probability of	Positive	78	22	**100**	**78.0 (78 / 100) (68.9, 85.0)**	-	2.45 (1.72, 3.49)
	Gray Zone	16	22	**38**	42.1 (16 / 38)	57.9 (22 / 38)	0.50 (0.29, 0.88)

Category	NT-proBNP Test Result	Adjudicated Diagnosis		Total	Posttest Probability of HF % (n / N) (95% CI)	Posttest Probability of Non-HF % (n / N) (95% CI)	Likelihood Ratio (HF) (95% CI)
		HF	Non-HF				
HF (Prevalence of HF in study): 59.12% (94/159)					(27.9, 57.8)	(42.2, 72.1)	
	Negative	0	21	**21**	-	**100.0 (21 / 21) (84.5, 100.0)**	0.02 (0.00, 0.26)
	Total	**94**	**65**	**159**			
Subjects with eGFR<60 mL/min/1.73m² Pretest Probability of HF (Prevalence of HF in study): 58.92% (370/628)	Positive	316	85	**401**	**78.8 (316 / 401) (74.5, 82.5)**	-	2.59 (2.17, 3.10)
	Gray Zone	44	72	**116**	37.9 (44 / 116) (29.6, 47.0)	62.1 (72 / 116) (53.0, 70.4)	0.43 (0.30, 0.60)
	Negative	10	101	**111**	-	**91.0 (101 / 111) (84.2, 95.0)**	0.07 (0.04, 0.13)
	Total	**370**	**258**	**628**			

Clinical Performance – Subjects with eGFR ≥ 60 mL/min/1.73 m²:

Category	NT-proBNP Test Result	Adjudicated Diagnosis		Total	Posttest Probability of HF % (n / N) (95% CI)	Posttest Probability of Non-HF % (n / N) (95% CI)	Likelihood Ratio (HF) (95% CI)
		HF	Non-HF				
Age Group (years): 18 - <50 Pretest Probability of HF (Prevalence of HF in study): 30.25% (121/400)	Positive	103	43	**146**	**70.5 (103 / 146) (62.7, 77.3)**	-	5.52 (4.15, 7.34)
	Gray Zone	6	13	**19**	31.6 (6 / 19) (15.4, 54.0)	68.4 (13 / 19) (46.0, 84.6)	1.06 (0.41, 2.73)
	Negative	12	223	**235**	-	**94.9 (223 / 235) (91.3, 97.1)**	0.12 (0.07, 0.21)
	Total	**121**	**279**	**400**			
Age Group (years): 50 - 75 Pretest Probability of HF (Prevalence of HF in study): 33.51% (312/931)	Positive	232	90	**322**	**72.0 (232 / 322) (66.9, 76.7)**	-	5.11 (4.18, 6.26)
	Gray Zone	53	97	**150**	35.3 (53 / 150) (28.1, 43.3)	64.7 (97 / 150) (56.7, 71.9)	1.08 (0.80, 1.47)
	Negative	27	432	**459**	-	**94.1 (432 / 459) (91.6, 95.9)**	0.12 (0.09, 0.18)

Category	NT-proBNP Test Result	Adjudicated Diagnosis		Total	Posttest Probability of HF % (n / N) (95% CI)	Posttest Probability of Non-HF % (n / N) (95% CI)	Likelihood Ratio (HF) (95% CI)
		HF	Non-HF				
	Total	**312**	**619**	**931**			
Age Group (years): >75 Pretest Probability of HF (Prevalence of HF in study): 46.45% (72/155)	Positive	52	16	**68**	**76.5 (52 / 68) (65.1, 85.0)**	-	3.75 (2.36, 5.95)
	Gray Zone	18	37	**55**	32.7 (18 / 55) (21.8, 45.9)	67.3 (37 / 55) (54.1, 78.2)	0.56 (0.35, 0.89)
	Negative	2	30	**32**	-	**93.8 (30 / 32) (79.9, 98.3)**	0.08 (0.02, 0.31)
	Total	**72**	**83**	**155**			
All Subjects with eGFR≥60 mL/min/1.73m^2 Pretest Probability of HF (Prevalence of HF in study): 33.98% (505/1486)	Positive	387	149	**536**	**72.2 (387 / 536) (68.3, 75.8)**	-	5.05 (4.32, 5.89)
	Gray Zone	77	147	**224**	34.4 (77 / 224) (28.5, 40.8)	65.6 (147 / 224) (59.2, 71.5)	1.02 (0.79, 1.31)
	Negative	41	685	**726**	-	**94.4 (685 / 726) (92.4, 95.8)**	0.12 (0.09, 0.16)
	Total	**505**	**981**	**1486**			

Clinical Performance – Subjects with BMI ≥ 30 kg/m^2:

Category	NT-proBNP Test Result	Adjudicated Diagnosis		Total	Posttest Probability of HF % (n / N) (95% CI)	Posttest Probability of Non-HF % (n / N) (95% CI)	Likelihood Ratio (HF) (95% CI)
		HF	Non-HF				
Age Group (years): 18 - <50 Pretest Probability of HF (Prevalence of HF in study): 40.75% (108/265)	Positive	90	26	**116**	**77.6 (90 / 116) (69.2, 84.2)**	-	5.03 (3.51, 7.22)
	Gray Zone	7	8	**15**	46.7 (7 / 15) (24.8, 69.9)	53.3 (8 / 15) (30.1, 75.2)	1.27 (0.48, 3.40)
	Negative	11	123	**134**	-	**91.8 (123 / 134) (85.9, 95.4)**	0.13 (0.07, 0.23)
	Total	**108**	**157**	**265**			
Age Group (years): 50 - 75	Positive	231	54	**285**	**81.1 (231 / 285) (76.1, 85.2)**	-	4.84 (3.74, 6.25)

Category	NT-proBNP Test Result	Adjudicated Diagnosis		Total	Posttest Probability of HF % (n / N) (95% CI)	Posttest Probability of Non-HF % (n / N) (95% CI)	Likelihood Ratio (HF) (95% CI)
		HF	Non-HF				
Pretest Probability of HF (Prevalence of HF in study): 46.92% (328/699)	Gray Zone	67	64	**131**	51.1 (67 / 131) (42.7, 59.5)	48.9 (64 / 131) (40.5, 57.3)	1.18 (0.87, 1.61)
	Negative	30	253	**283**	-	**89.4 (253 / 283) (85.3, 92.5)**	0.13 (0.09, 0.19)
	Total	**328**	**371**	**699**			
Age Group (years): >75 Pretest Probability of HF (Prevalence of HF in study): 59.81% (64/107)	Positive	48	5	**53**	**90.6 (48 / 53) (79.7, 95.9)**	-	6.45 (2.80, 14.88)
	Gray Zone	16	19	**35**	45.7 (16 / 35) (30.5, 61.8)	54.3 (19 / 35) (38.2, 69.5)	0.57 (0.33, 0.97)
	Negative	0	19	**19**	-	**100.0 (19 / 19) (83.2, 100.0)**	0.02 (0.00, 0.28)
	Total	**64**	**43**	**107**			
All Subjects with BMI ≥ 30 kg/m^2 Pretest Probability of HF (Prevalence of HF in study): 46.69% (500/1071)	Positive	369	85	**454**	**81.3 (369 / 454) (77.4, 84.6)**	-	4.96 (4.05, 6.07)
	Gray Zone	90	91	**181**	49.7 (90 / 181) (42.5, 56.9)	50.3 (91 / 181) (43.1, 57.5)	1.13 (0.87, 1.47)
	Negative	41	395	**436**	-	**90.6 (395 / 436) (87.5, 93.0)**	0.12 (0.09, 0.16)
	Total	**500**	**571**	**1071**			

Clinical Performance – Subjects with BMI < 30 kg/m^2:

Category	NT-proBNP Test Result	Adjudicated Diagnosis		Total	Posttest Probability of HF % (n / N) (95% CI)	Posttest Probability of Non-HF % (n / N) (95% CI)	Likelihood Ratio (HF) (95% CI)
		HF	Non-HF				
Age Group (years): 18 - <50 Pretest Probability of HF (Prevalence of HF in study): 30.41% (45/148)	Positive	44	16	**60**	**73.3 (44 / 60) (61.0, 82.9)**	-	6.29 (4.00, 9.90)
	Gray Zone	0	4	**4**	0.0 (0 / 4) (0.0, 49.0)	100.0 (4 / 4) (51.0, 100.0)	0.25 (0.01, 4.60)
	Negative	1	83	**84**	-	**98.8 (83 / 84)**	0.03 (0.00, 0.19)

Category	NT-proBNP Test Result	Adjudicated Diagnosis		Total	Posttest Probability of HF % (n / N) (95% CI)	Posttest Probability of Non-HF % (n / N) (95% CI)	Likelihood Ratio (HF) (95% CI)
		HF	Non-HF				
						(93.6, 99.8)	
	Total	**45**	**103**	**148**			
Age Group (years): 50 - 75 Pretest Probability of HF (Prevalence of HF in study): 36.46% (198/543)	Positive	185	80	**265**	**69.8 (185 / 265) (64.0, 75.0)**	-	4.03 (3.31, 4.90)
	Gray Zone	9	69	**78**	11.5 (9 / 78) (6.2, 20.5)	88.5 (69 / 78) (79.5, 93.8)	0.23 (0.12, 0.45)
	Negative	4	196	**200**	-	**98.0 (196 / 200) (95.0, 99.2)**	0.04 (0.01, 0.09)
	Total	**198**	**345**	**543**			
Age Group (years): >75 Pretest Probability of HF (Prevalence of HF in study): 51.08% (95/186)	Positive	76	31	**107**	**71.0 (76 / 107) (61.8, 78.8)**	-	2.35 (1.73, 3.18)
	Gray Zone	17	32	**49**	34.7 (17 / 49) (22.9, 48.7)	65.3 (32 / 49) (51.3, 77.1)	0.51 (0.30, 0.85)
	Negative	2	28	**30**	-	**93.3 (28 / 30) (78.7, 98.2)**	0.07 (0.02, 0.28)
	Total	**95**	**91**	**186**			
All Subjects with BMI < 30 kg/m^2 Pretest Probability of HF (Prevalence of HF in study): 38.54% (338/877)	Positive	305	127	**432**	**70.6 (305 / 432) (66.1, 74.7)**	-	3.83 (3.28, 4.48)
	Gray Zone	26	105	**131**	19.8 (26 / 131) (13.9, 27.5)	80.2 (105 / 131) (72.5, 86.1)	0.39 (0.26, 0.59)
	Negative	7	307	**314**	-	**97.8 (307 / 314) (95.5, 98.9)**	0.04 (0.02, 0.08)
	Total	**338**	**539**	**877**			

Clinical Performance – Subjects with Comorbidities:

Comorbidities include at least one of the following: diabetes, renal dysfunction (eGFR less than 60 mL/min/1.73 m2), hypertension, and/or chronic obstructive pulmonary disease (COPD). The sponsor described in the labeling that patients with these comorbidities had a higher rate of false positives compared to patients without these comorbidities.

Category	NT-proBNP Test Result	Adjudicated Diagnosis		Total	Posttest Probability of HF % (n / N) (95% CI)	Posttest Probability of Non-HF % (n / N) (95% CI)	Likelihood Ratio (HF) (95% CI)
		HF	Non-HF				
Age Group (years): 18 - <50 Pretest Probability of HF (Prevalence of HF in study): 43.67% (131/300)	Positive	115	34	**149**	**77.2 (115 / 149) (69.8, 83.2)**	-	4.36 (3.21, 5.93)
	Gray Zone	7	10	**17**	41.2 (7 / 17) (21.6, 64.0)	58.8 (10 / 17) (36.0, 78.4)	0.90 (0.35, 2.31)
	Negative	9	125	**134**	-	**93.3 (125 / 134) (87.7, 96.4)**	0.09 (0.05, 0.18)
	Total	**131**	**169**	**300**			
Age Group (years): 50 - 75 Pretest Probability of HF (Prevalence of HF in study): 43.23% (527/1219)	Positive	415	136	**551**	**75.3 (415 / 551) (71.6, 78.7)**	-	4.01 (3.42, 4.69)
	Gray Zone	78	133	**211**	37.0 (78 / 211) (30.7, 43.7)	63.0 (133 / 211) (56.3, 69.3)	0.77 (0.60, 0.99)
	Negative	34	423	**457**	-	**92.6 (423 / 457) (89.8, 94.6)**	0.11 (0.08, 0.15)
	Total	**527**	**692**	**1219**			
Age Group (years): >75 Pretest Probability of HF (Prevalence of HF in study): 55.25% (163/295)	Positive	128	36	**164**	**78.0 (128 / 164) (71.1, 83.7)**	-	2.88 (2.15, 3.85)
	Gray Zone	33	48	**81**	40.7 (33 / 81) (30.7, 51.6)	59.3 (48 / 81) (48.4, 69.3)	0.56 (0.38, 0.81)
	Negative	2	48	**50**	-	**96.0 (48 / 50) (86.5, 98.9)**	0.03 (0.01, 0.14)
	Total	**163**	**132**	**295**			
Subjects with Comorbidities Pretest Probability of HF (Prevalence of HF in study): 45.26% (821/1814)	Positive	658	206	**864**	**76.2 (658 / 864) (73.2, 78.9)**	-	3.86 (3.41, 4.38)
	Gray Zone	118	191	**309**	38.2 (118 / 309) (32.9, 43.7)	61.8 (191 / 309) (56.3, 67.1)	0.75 (0.61, 0.92)
	Negative	45	596	**641**	-	**93.0 (596 / 641) (90.7, 94.7)**	0.09 (0.07, 0.12)
	Total	**821**	**993**	**1814**			

Clinical Performance – Subjects without Comorbidities:

Category	NT-proBNP Test Result	Adjudicated Diagnosis		Total	Posttest Probability of HF % (n / N) (95% CI)	Posttest Probability of Non-HF % (n / N) (95% CI)	Likelihood Ratio (HF) (95% CI)
		HF	Non-HF				
Age Group (years): 18 - <-50 Pretest Probability of HF (Prevalence of HF in study): 18.13% (29/160)	Positive	25	14	**39**	**64.1 (25 / 39) (48.4, 77.3)**	-	8.07 (4.81, 13.51)
	Gray Zone	0	3	**3**	0.0 (0 / 3) (0.0, 56.1)	100.0 (3 / 3) (43.9, 100.0)	0.64 (0.03, 12.00)
	Negative	4	114	**118**	-	**96.6 (114 / 118) (91.6, 98.7)**	0.16 (0.06, 0.39)
	Total	**29**	**131**	**160**			
Age Group (years): 50 - 75 Pretest Probability of HF (Prevalence of HF in study): 19.55% (26/133)	Positive	22	12	**34**	**64.7 (22 / 34) (47.9, 78.5)**	-	7.54 (4.32, 13.18)
	Gray Zone	2	14	**16**	12.5 (2 / 16) (3.5, 36.0)	87.5 (14 / 16) (64.0, 96.5)	0.59 (0.14, 2.43)
	Negative	2	81	**83**	-	**97.6 (81 / 83) (91.6, 99.3)**	0.10 (0.03, 0.39)
	Total	**26**	**107**	**133**			
Age Group (years): >75 Pretest Probability of HF (Prevalence of HF in study): 20.00% (4/20)	Positive	3	2	**5**	**60.0 (3 / 5) (23.1, 88.2)**	-	6.00 (1.46, 24.69)
	Gray Zone	1	11	**12**	8.3 (1 / 12) (1.5, 35.4)	91.7 (11 / 12) (64.6, 98.5)	0.36 (0.06, 2.05)
	Negative	0	3	**3**	-	**100.0 (3 / 3) (43.9, 100.0)**	0.52 (0.03, 8.39)
	Total	**4**	**16**	**20**			
Subjects without Comorbidities Pretest Probability of HF (Prevalence of HF in study): 18.85% (59/313)	Positive	50	28	**78**	**64.1 (50 / 78) (53.0, 73.9)**	-	7.69 (5.33, 11.08)
	Gray Zone	3	28	**31**	9.7 (3 / 31) (3.3, 24.9)	90.3 (28 / 31) (75.1, 96.7)	0.46 (0.15, 1.47)
	Negative	6	198	**204**	-	**97.1 (198 / 204) (93.7, 98.6)**	0.13 (0.06, 0.28)
	Total	**59**	**254**	**313**			

The sponsor included the following statements in their instructions for use concerning the performance of their device in ED settings in certain clinical subgroups:

- Patients with a history of HF had a higher rate of false positives compared to patients without a history of HF. Of the 332 total patients with a history of HF and adjudicated as non-HF, 121 (36.4%) had NT-proBNP concentrations greater than or equal to age-dependent cutoffs. Of the 915 total patients with no history of HF and adjudicated as non-HF, 113 (12.3%) had NT-proBNP concentrations greater than or equal to age-dependent cutoffs. This difference is likely due to long-standing chronic elevation of NT-proBNP in patients with a previous diagnosis of HF. Results should always be assessed in conjunction with patient's medical history, clinical examination, and other findings.

- Patients with an eGFR less than 60 mL/min/1.73 m^2 had a higher rate of false positives compared to patients with an eGFR greater than or equal to 60 mL/min/1.73 m^2. Of the 258 total patients with an eGFR less than 60 mL/min/1.73 m^2 and adjudicated as non-HF, 85 (32.9%) had NT-proBNP concentrations greater than or equal to age-dependent cutoffs. Of the 981 total patients with an eGFR greater than or equal to 60 mL/min/1.73 m^2 and adjudicated as non-HF, 149 (15.2%) had NT-proBNP concentrations greater than or equal to age-dependent cutoffs. Eight patients adjudicated as non-HF (0.6%) had an unknown eGFR. Patients with an eGFR less than 60 mL/min/1.73 m^2 had a higher rate of false negatives (9.0% [10/111]) compared to patients with an eGFR greater than or equal to 60 mL/min/1.73 m^2 (5.6% [41/726]). All 10 of the false negatives in the eGFR less than 60 mL/min/1.73 m^2 group were from patients with a BMI greater than or equal to 30 kg/m^2. In patients with eGFR less than 60 mL/min/1.73 m^2, caution should be used when interpreting NT-proBNP results. Results should always be assessed in conjunction with the patient's medical history, clinical examination, and other findings.

- Patients with a BMI greater than or equal to 30 kg/m^2 had a higher rate of false negatives compared to patients with a BMI less than 30 kg/m^2. Of the total observed false negatives (51/880), 41 (80.4%) were from patients with a BMI greater than or equal to 30 kg/m^2, 7 (13.7%) were from patients with a BMI less than 30 kg/m^2, and 3 (5.9%) were from patients with an unknown BMI. In patients with a BMI less than 30 kg/m^2, the age greater than 75 years group had a higher rate of false negatives (6.7% [2/30]) than the other age groups. Results should always be assessed in conjunction with the patient's medical history, clinical examination, and other findings. In patients with obesity, natriuretic peptides should be interpreted with caution.

The sponsor also included the following limitation in their labeling:

- 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/m^2), and other conditions such as flash pulmonary edema, pericarditis, and cardiac

tamponade may lower NT-proBNP concentrations, which may lead to false negative results.

New York Heart Association (NYHA) Functional Classification
865 subjects had a NYHA functional classification. Descriptive statistics by the NYHA classification for the 865 subjects adjudicated to have HF for all subjects and for male and female subjects are presented in the table below:

Statistics	NYHA Functional Classification		
	Class II	Class III	Class IV
All Subjects			
N	12	369	499
Mean	5535.8	5187.5	6485.6
SD	6834.18	7056.92	7829.43
Min	89.5	27.1	19.8
5th Percentile	89.5	244.0	303.0
25th Percentile	1319.5	989.4	1492.8
Median	2846.4	2817.5	3928.2
75th Percentile	7399.7	6230.9	8349.3
95th Percentile	23734.0	20653.1	22086.6
Max	23734.0	60716.9	59178.1
Male Subjects			
N	8	206	290
Mean	5469.9	5955.9	6475.2
SD	7566.03	7674.86	7547.84
Min	89.5	166.7	19.8
5th Percentile	89.5	365.8	375.5
25th Percentile	1954.8	1221.8	1599.4
Median	2846.4	3702.6	4094.2
75th Percentile	5415.8	7202.5	8515.5
95th Percentile	23734.0	21410.7	21598.1
Max	23734.0	60716.9	59178.1
Female Subjects			
N	4	163	209
Mean	5667.6	4216.4	6500.1
SD	6135.91	6073.57	8222.56
Min	264.5	27.1	22.8
5th Percentile	264.5	149.2	159.4
25th Percentile	492.6	749.2	1266.5
Median	4542.9	2172.1	3713.5
75th Percentile	11967.5	5022.1	8159.8
95th Percentile	13320.3	19295.7	24566.4
Max	13320.3	38880.9	47385.1

D Clinical Cut-Off:

The sponsor describes the following cut-offs for patients presenting to ED settings where new onset or exacerbation of HF is suspected:

Age Group (Years)	NT-proBNP (pg/mL)	Interpretation
All	< 300.0	Negative: HF unlikely
18 to < 50	≥ 300.0 to < 450.0	Grayzone: Indeterminate Consider other causes of NT-proBNP elevation.
50 to 75	≥ 300.0 to < 900.0	
> 75	≥ 300.0 to < 1800.0	
18 to < 50	≥ 450.0	Positive: HF likely
50 to 75	≥ 900.0	
> 75	≥ 1800.0	

E Expected Values/Reference Range:

A reference interval study was performed based on guidance from CLSI EP28-A3c.

K_2EDTA plasma specimens were obtained from a total of 861 apparently healthy individuals (≥ 18 years old, 442 females and 419 males) from 3 external clinical sites located in the US. The age-range distribution and sex of the individuals are described in the table below:

The exclusion criteria used to exclude subjects were:

- current smoker
- known cardiac condition and/or cardiac disease
- high blood pressure (hypertension)
- kidney disease (eGFR < 60 mL/min/1.73m^2)
- diabetes (HbA1c ≥ 6.5%)
- active cancer or diagnosis of cancer within the last five years
- previous stroke
- lung disease within the last five years
- thyroid disease
- recently reported high cholesterol or triglycerides
- reported abnormal troponin (>99th percentile or cutoff for normal)
- female known to be pregnant
- previous enrollment in this study

The specimens were tested with the Alere NT-proBNP for Alinity i assay on the Alinity i system. Based on the results, the 95% reference interval of an apparently healthy population for each sex and age range was determined to be as follows:

Age Range	Sex	n	Median (pg/mL)	Reference Interval (2.5th to 97.5th Percentile) (pg/mL)
18 to < 50 years old	Female	181	30.7	< 15.8 to 104.8
	Male	137	<15.8	< 15.8 to 180.3

Age Range	Sex	n	Median (pg/mL)	Reference Interval (2.5th to 97.5th Percentile) (pg/mL)
50 to 75 years old	Female	134	51.7	< 15.8 to 334.1
	Male	146	39.9	< 15.8 to 451.6
> 75 years old	Female	127	81.4	< 15.8 to 956.1
	Male	136	65.1	< 15.8 to 683.0

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.