510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K232164

B Applicant

Beckman Coulter Inc.

C Proprietary and Established Names

Access NT-proBNP

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 Assay

B Measurand:

N-terminal pro B-type natriuretic peptide (NT-proBNP)

C Type of Test:

Chemiluminescent Assay

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The Access NT-proBNP assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of N-terminal pro B-type natriuretic peptide levels in human serum and plasma using the automated DxI Access Immunoassay Analyzers to aid in the following:

1. diagnosis of patients suspected of having acute heart failure in the Emergency Department

2. assessment of heart failure severity

3. risk stratification of patients with heart failure

4. risk stratification of patients with acute coronary syndrome

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

DxI 9000 Access Immunoassay Analyzer

IV Device/System Characteristics:

A Device Description:

The Access NT-proBNP assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of NT-proBNP levels in human serum and plasma using the automated DxI Access immunoassay.

The Access NT-proBNP reagent pack is ready to use. Each kit contains 2x100 test reagent packs (cartridge). Each cartridge consists of the following reagents:

Well	Contents	Ingredients
R1a	3.37 mL	Dynabeads® paramagnetic particles coated with rabbit anti-human NT-proBNP monoclonal antibody in TRIS buffered saline with surfactant, protein (bovine), < 0.1% sodium azide, and 0.1% ProClin 300.
R1b	12.70 mL	MOPS buffered saline with surfactant, protein (bovine, murine, rabbit), < 0.1% sodium azide, and 0.1% ProClin 300.
R1c	8.75 mL	0.10N Sodium Hydroxide
R1d	3.10 mL	Human-mouse anti-NT-proBNP chimeric monoclonal antibody alkaline phosphatase conjugate in MES buffered saline with

Well	Contents	Ingredients
		surfactant and protein (bovine, murine, recombinant), < 0.1% sodium azide, and 0.1% ProClin 300.
R1e	3.10 mL	Human-mouse anti-NT-proBNP chimeric monoclonal alkaline phosphatase conjugate in MES buffered saline with surfactant and protein (bovine, murine, recombinant), < 0.1% sodium azide, and 0.1% ProClin 300.

Materials needed but not supplied with the reagent kit include Access NT-proBNP calibrators, quality control materials (commercial control material), Lumi-Phos PRO, and UniCel DxI wash buffer II.

B Principle of Operation:

The Access NT-proBNP is a two-site immuno-enzymatic ("sandwich") assay. Paramagnetic particles coated with monoclonal anti-NT-proBNP antibody and monoclonal anti-NT-proBNP antibody conjugated to alkaline phosphatase are added to a reaction vessel along with a surfactant-containing buffer and serum or plasma samples. The human NT-proBNP binds to the anti-NT-proBNP antibody on the solid phase, while the anti-NT-proBNP antibody-alkaline phosphatase conjugate reacts with a different antigenic site on the NT-proBNP molecule.

After incubation, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then the chemiluminescent substrate is added to the vessel and light generated by the reaction is measured with a luminometer. The light production is directly proportional to the concentration of analyte in the sample. Analyte concentration is automatically determined from a stored calibration.

V Substantial Equivalence Information:

A Predicate Device Name(s):
Elecsys®proBNP II Immunoassay

B Predicate 510(k) Number(s):
K072437

C Comparison with Predicate(s):

Device & Predicate Device(s):	K232164	K072437
Device Trade Name	Access NT-proBNP	Elecsys® proBNP II Immunoassay
General Device Characteristic Similarities		
Intended Use/Indications For Use	For the quantitative determination of N-terminal pro B-type natriuretic peptide (NT-	Same

Device & Predicate Device(s):	K232164	K072437
	proBNP) levels in human serum and plasma	
Antibody	Monoclonal anti-NT-proBNP	Same
Sample Type	Serum and Plasma	Same
General Device Characteristic Differences		
High Dose Hook Effect	No high dose hook effect observed up to 400,000 ng/L (pg/mL)	No high dose hook effect observed up to 300,000 pg/mL
Measuring Range	10.0 – 35,000 ng/L (pg/mL)	5 – 35,000 pg/mL

VI Standards/Guidance Documents Referenced:

Clinical & Laboratory Standards Institute (CLSI) EP-09c Measurement Procedure Comparison and Bias Estimation Using Patient Samples

CLSI EP05-A3: Evaluation of Precision of Quantitative Measurement Procedures; 3rd Edition

CLSI EP17-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; 2nd Edition

CLSI EP 07: Interference Testing In Clinical Chemistry; 3rd Edition

CLSI EP25-A: Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline; 2009.

CLSI EP 06: Evaluation of the Linearity of Quantitative Measurement Procedures; 2nd Edition

Class II Special Control Guidance Document for B-Type Natriuretic Peptide Premarket Notifications; Final Guidance for Industry and FDA Reviewers (November 30, 2000).

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

Single – Site Precision Study

A single site precision study was performed following the CLSI guideline EP05-A3. A panel of seven (7) lithium heparin samples, seven (7) serum samples and seven (7) K_2 EDTA plasma samples with concentrations of NT-proBNP spanning the measuring range were used in the study. Samples were assayed for twenty-three (23) days with two runs per day and two replicates per run using one (1) DxI 9000 Access Immunoassay Analyzer. Each sample was tested across three (3) reagent pack lots and one (1) calibrator lot. The overall estimate includes within-run, between-run, between-day and between reagent lot variance components.

Sample	Sample Type	N	Mean (ng/L)	Repeatability (Within Run)		Between Run		Between Day	
				SD	%CV	SD	%CV	SD	%CV
1	K_2 EDTA	258	31	0.7	2.4	0.6	2.0	0.5	1.7
2		252	132	2.8	2.1	6.8	5.2	0.0	0.0
3		272	272	5.3	1.9	15.9	5.8	0.0	0.0
4		251	387	8.7	2.2	25.6	6.6	0.0	0.0
5		257	1912	48.7	2.5	73.5	3.8	52.0	2.7
6		258	12426	233.1	1.9	635.4	5.1	0.0	0.0
7		258	27061	534.1	2.0	1465.7	5.4	0.0	0.0
1	LiHep	254	38	1.0	2.7	1.4	3.8	0.7	1.8
2		252	129	2.8	2.2	2.7	2.1	1.5	1.2
3		252	290	6.0	2.1	6.3	2.2	0.0	0.0
4		257	437	9.6	2.2	9.6	2.2	6.5	1.5
5		258	1705	46.3	2.7	54.7	3.2	21.7	1.3
6		258	10780	178.0	1.7	416	3.9	0.0	0.0
7		258	23014	403.0	1.8	942.5	4.1	0.0	0.0
1	Serum	258	33	0.9	2.8	0.8	2.4	0.7	2.0
2		257	118	2.9	2.5	2.5	2.1	2.5	2.1
03		258	288	6.2	2.2	12.5	4.4	0.0	0
4		252	450	16.0	3.6	13.5	3.0	0.0	0
5		258	1988	49.5	2.5	75.4	3.8	0.0	0
6		257	11497	188.9	1.6	475.5	4.1	0.0	0
7		252	25260	514.9	2.0	393.6	1.6	269.0	1.1

Sample	Sample Type	N	Mean (ng/L)	Between-Reagent Lot		Overall	
				SD	%CV	SD	%CV
1	K_2 EDTA	258	31	0.6	2.0	1.3	4.0
2		252	132	2.3	1.7	7.7	5.8
3		272	272	6.2	2.3	17.9	6.6
4		251	387	9.2	2.4	28.6	7.4
5		257	1912	114.1	6.0	153.3	8.0
6		258	12426	390.2	3.1	781.2	6.3
7		258	27061	880.8	3.3	1791.5	6.6
1	LiHep	254	38	0.5	1.4	2.0	5.2
2		252	129	1.8	1.4	4.5	3.5
3		257	290	6.5	2.3	10.9	3.8
4		258	437	14.1	3.2	20.6	4.7
5		258	1705	90.1	5.3	117.2	6.9
6		258	10780	324.3	3.0	556.7	5.2
7		258	23014	755.6	3.3	1273.4	5.5
1	Serum	258	33	0.5	1.6	1.5	4.5
2		257	118	2.3	1.9	5.1	4.3
3		258	288	3.8	1.3	14.5	5.0
4		252	450	15.4	3.4	26.0	5.8
5		258	1988	110.7	5.6	142.8	7.2
6		257	11497	369.6	3.2	631.2	5.5
7		252	25260	862.3	3.4	1111.7	4.4

Multi – Site Precision Study

The reproducibility performance was established with a multi-site precision evaluation. The study was performed at three sites. Samples for testing were comprised of samples with 7 concentration levels for each sample matrix type with concentrations of NT-proBNP spanning the assay measuring range. Samples were assayed in duplicate per run, two (2) runs per day for five (5) days (n=20 per sample per site per sample type). Two calibrator lots and three reagent lots were used at three (3) sites, with each site receiving one reagent lot. The results are all summarized below.

All Sites Combined Lithium Heparin Reproducibility

Sample	N	Mean (ng/L)	Repeatability		Between Run		Between Day		Between Site/Lot		Reproducibility	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	60	38.24	1.3	3.5	0.7	1.7	0.0	0.0	2.6	6.9	3.0	7.9
2	59	127.31	3.6	2.9	0.0	0.0	0.8	0.6	7.1	5.6	8.0	6.3
3	59	288.19	7.8	2.7	2.0	0.7	4.7	1.6	14.0	4.9	16.8	5.8
4	60	428.00	6.5	1.5	7.3	1.7	0.0	0.0	17.4	4.1	20.0	4.7
5	60	1708.42	33.7	2.0	29.5	1.7	23.2	1.4	5.2	0.3	50.7	3.0
6	60	11096.6	191.6	1.7	135.2	1.2	91.7	0.8	159.7	1.4	298.2	2.7
7	60	23848.6	527.5	2.2	367.5	1.5	0.0	0.0	344.0	1.4	724.4	3.0

All Sites Combined Serum Reproducibility

Sample	N	Mean (ng/L)	Repeatability (Within Run)		Between Run		Between Day		Between Site/Lot		Reproducibility	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	60	32.21	1.1	3.4	0.3	0.9	0.5	1.5	1.7	5.2	2.1	6.5
2	60	113.33	2.7	2.4	2.3	2.0	1.2	1.0	0.0	0.0	3.8	3.3
3	60	278.48	7.7	2.8	4.8	1.7	0.0	0.0	4.7	1.7	10.2	3.7
4	60	440.96	9.8	2.2	7.0	1.6	0.0	0.0	8.5	1.9	14.8	3.3
5	59	1965.83	49.3	2.5	38.1	1.9	0.0	0.0	0.0	0.0	62.4	3.2
6	60	11809.4	196.7	1.7	155.9	1.3	0.0	0.0	66.7	0.6	259.7	2.2
7	60	25997.2	487.6	1.9	221.9	0.9	488.9	1.9	557.0	2.1	914.5	3.5

All Sites Combined K_2 EDTA Reproducibility

Sample	N	Mean (ng/L)	Repeatability		Between Run		Between Day		Between Site/Lot		Reproducibility	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	60	30.30	1.0	3.2	0.0	0.0	0.3	0.8	1.7	5.6	2.0	6.5
2	59	129.15	3.3	2.6	1.1	0.9	1.5	1.1	3.4	2.6	5.1	3.9
3	60	268.19	6.5	2.4	3.1	1.1	4.1	1.5	7.7	2.9	11.3	4.2
4	60	384.61	7.6	2.0	4.7	1.2	5.1	1.3	8.3	2.2	13.3	3.4
5	60	1870.66	64.6	3.5	41.0	2.2	0.0	0.0	25.7	1.4	80.7	4.3
6	60	12905.6	238.5	1.8	145.5	1.1	75.2	0.6	114.4	0.9	311.1	2.4
7	60	28459.3	702.7	2.5	88.8	0.3	133.7	0.5	595.6	2.1	935.1	3.3

2. Linearity:

The linearity performance of the Access NT-proBNP assay run on the DxI 9000 Access Immunoassay Analyzer was established for serum, lithium heparin plasma and K_2EDTA plasma respectively in a study following the recommendations of the CLSI EP06, 2nd Edition guideline. One high sample pool above the measuring range (> 35,000 ng/L) was prepared by pooling high concentration native patient samples for the serum sample. The EDTA and lithium heparin plasma high pools required spiking with additional NT-proBNP antigen to achieve the desired concentration. One low sample pool below the limit of quantification was prepared by pooling low concentration native samples of each respective sample type. The low and high pool samples from each sample type condition were mixed in pre-defined rations to create an additional seven samples for each sample type. Each sample was measured on each of three reagent pack lots and a single calibrator lot on one DxI 9000 Access Immunoassay Analyzer. The low pool from each sample type/panel was run in replicates of eight and all other samples were run in replicates of four. The linearity was analyzed separately for each reagent lot per sample type. Using a weighted linear regression model, the difference between the mean observed value and the value predicted by the weighted linear regression model was derived. At each concentration, the deviation from linearity was less than 10%. The sponsor concluded that the assay yields a linear response over the claimed NT-proBNP measuring range of 10 ng/L to 35,000 ng/L.

3. Analytical Specificity/Interference:

The analytical specificity performance of the Access pro-BNP assay was established by conducting a cross-reactivity study and interference testing for endogenous and exogenous substances following the CLSI EP 07, 3rd Edition guideline.

Endogenous substances

Interference from endogenous substances was assessed using lithium heparin plasma samples with NT-proBNP at approximate concentrations of 125 ng/L and 1800 ng/L. Each of the two samples was divided into two aliquots for a control sample (with no added interferent) and a test sample (with added interferent). Each sample was measured on one DxI 9000 Access Immunoassay Analyzer across 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 control samples, was observed at the following concentrations:

Substance	Highest concentration tested at which no significant interference is observed
Bilirubin, conjugated	19 mg/dL
Bilirubin, unconjugated	15 mg/dL
Cholesterol	400 mg/dL
Creatinine	15 mg/dL
Hemoglobin	1000 mg/dL
Intralipid	1600 mg/dL
Human Serum Albumin	60 g/L

Substance	Highest concentration tested at which no significant interference is observed
Human gamma-globulin	30g/L
Rheumatoid factors	500 IU/mL
HAMA	800 µg/L
Alkaline Phosphatase	2000 U/L

Exogenous substances

Interference from exogenous substances was assessed using lithium heparin plasma samples with NT-proBNP at approximate concentrations of 125 ng/L and 1800 ng/L following the same procedure as for the endogenous substance testing. No significant interference, defined by the sponsor as a difference within ± 10% between the mean for the test sample versus the mean of the control samples, was observed at the following concentrations:

Substance	Highest concentration tested at which no significant interference is observed
(+)-cis-Diltiazem hydrochloride	120 µg/mL
Acetaminophen	1456 µmol/L
Allopurinol	240 µg/mL
Amiodarone	4.2 mg/dL
Amlodipine besylate	4 µg/mL
Ampicillin	200 µg/mL
Ascorbic acid	20 mg/dL
Atenolol	40 µg/mL
Atorvastatin calcium trihydrate	32 µg/mL
Biotin	30,000 ng/mL
Caffeine	10.8 mg/dL
Captopril	40 µg/mL
Carvedilol	74 µmol/L
Chloramphenicol	7.8 mg/dL
Clopidogrel hydrogen sulfate	30 µg/mL
Cyclosporine	40 µg/mL
Diclofenac sodium salt	60 µg/mL
Digitoxin	60 µg/mL
Digoxin	0.0039 mg/dL
Disopyramide	1.68 mg/dL
Dobutamine	100 µg/mL
Dopamine hydrochloride	116 µg/mL
Dipyridamole	30 µg/mL
Enalaprilat dihydrate	16 µg/mL
Erythromycin	13.8 mg/dL
Fenofibrate	45 µg/mL
Fibrinogen	1000 mg/dL
Furosemide	199 µmol/L

Substance	Highest concentration tested at which no significant interference is observed
Heparin	330 units/dL
Hydralazine	20 µg/mL
Hydrochlorothiazide	20 µg/mL
Ibuprofen	2425 µmol/L
Isosorbide dinitrate	0.593 mg/dL
L-Ascorbic Acid	376 µmol/L
Levothyroxine	0.042 mg/dL
Lidocaine	1.5 mg/dL
Lisinopril x $2H_2O$	16 µg/mL
Losartan potassium	130 µmol/L
Lovastatin	0.021mg/dL
Methyldopa sesquihydrate	100 µg/mL
Metoprolol hemitartrate	18.7 µmol/L
Naproxen sodium	2170 µmol/L
Nicotine	1.6 µg/mL
Nicotinic acid	40 µg/mL
Nifedipine	36 µg/mL
Nitrofurantoin	40 µg/mL
Oxazepam	12 µg/mL
Oxytetracycline	100 µg/mL
Phenobarbital	69 mg/dL
Phenytoin	6 mg/dL
Probenecid	600 µg/dL
Propranolol	64 µg/mL
Quinidine	20 µg/mL
Ramipril	14.4 µmol/L
Salicylic acid	200 µg/mL
Simvastatin	32 µg/mL
Spironolactone	600 mg/dL
Sulfamethoxazole	1.7 µmol/L
Theophylline	6 mg/dL
Trasylol/Aprotinin	100 KIE/mL
Trimethoprim	64 µg/mL
Verapamil hydrochloride	96 µg/mL
Warfarin	7.5 mg/dL

Cross-reactivity

A study was conducted to evaluate the performance of the Access pro-BNP assay in the presence of cross reactants. In this study, two lithium heparin plasma samples with NT-proBNP concentrations at 125 ng/L and 1800 ng/L were used. Each sample was then spiked with the potential cross-reactive substance to form a test sample or spiked with solvent to

form a control sample. Each sample was assayed in replicates of 5 on a single DxI 9000 Access Immunoassay Analyzer across three (3) reagent pack lots and one calibrator lot. The % cross-reactivity was calculated as:

% cross-reactivity = 100 x (test conc. – control conc.)/cross-reactant conc.

The results of the cross-reactivity studies are summarized below:

Cross-reactant	Concentration	Percent cross reactivity
ANP28	3.1 µg/mL	0%
		0%
Adrenomedullin	1 ng/mL	0%
		0%
Aldosterone	0.6 ng/mL	0%
		3%
Angiotensin I	0.6 ng/mL	0%
		4%
Angiotensin II	0.6 ng/mL	-1%
		5%
Angiotensin III	1 ng/mL	0%
		-2%
Arg-Vasopressin	1 ng/mL	0%
		1%
BNP 32	3.5 µg/mL	0%
		0%
CNP 22	2.2 µg/mL	0%
		0%
Endothelin	2 ng/mL	0%
		2%
NT-proANP 1-30	3.5 µg/mL	0%
		0%
NT-proANP 31-67	1 ng/mL	0%
		-2%
NT-proANP 79-98	1 ng/mL	0%
		-2%
Renin	50 ng/mL	0%
		0%
Urodilatin	3.5 µg/mL	0%
		0%

High dose hook effect

The Access NT-proBNP Reagent Pack was evaluated for high dose hook effect using lithium heparin plasma samples with 5 concentration levels of NT-proBNP. Each sample was tested in replicates of five (5) using three reagent lots and one calibrator lot on one DxI 9000

Access Immunoassay Analyzer. The results support that the Access NT-proBNP reagent pack does not show a high dose hook effect up to 400,000 ng/L (pg/mL) of NT-proBNP.

4. Assay Reportable Range:

The claimed measuring range is 10.0 to 35,000 ng/L.

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

Metrological traceability

The Access NT-proBNP calibrator is traceable to the manufacturer's working calibrators.

Sample Stability
The sponsor has provided information to support the following claims in their labeling:

- Separated samples are stable for up to 24 hours at room temperature, and for up to 3 days at 2-10°C.

- Separated samples are stable at -15°C for up to 30 days.

The sponsor also provided data to support that samples stored frozen at ≤ -15°C may be subjected to one freeze-thaw cycle.

6. Detection Limit:

The detection capability of the Access NT-proBNP assay run on the Dxl 9000 Access Immunoassay Analyzer was evaluated. The limit of blank (LoB), limit of detection (LoD), and limit of quantification (LoQ) were established following the recommendations in the CLSI EP17-A2 guideline.

Limit of Blank (LoB)

The LoB study was performed on four non-analyte samples using three reagent pack lots and one calibrator lot. Each sample was analyzed across three (3) days with one run per day and five replicates per run per reagent lot on one (1) Dxl 9000 Access Immunoassay Analyzer for a total of 180 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 Access NT-proBNP assay is estimated to be 1.1 ng/L

Limit of Detection (LoD)
The LoD study was performed using five (5) serum, six (6) EDTA, and six (6) lithium heparin plasma native samples containing low levels of NT-proBNP analyte. In the study, LoD was determined separately for each of three reagent pack lots using one calibrator lot on one (1) Dxl 9000 Access Immunoassay Analyzer. With each lot, the samples were each assayed in replicates of five per run, two runs per day over 5 days for a total of 50

measurements per sample per reagent lot. The parametric approach described in 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 Access NT-proBNP assay is estimated to be 4.8 ng/L (serum), 3.2 ng/L (K_2EDTA) and 4.3 ng/dL (LiHep)

Limit of Quantification (LoQ)
The LoQ study was performed using ten (10) serum, nine (9) EDTA and nine (9) Lithium Heparin plasma samples containing low levels of NT-proBNP analyte. In the study, the LoQ was determined separately for each of three reagent pack lots using one calibrator lot on one (1) Dxl 9000 Access Immunoassay Analyzer. With each lot, the samples were each assayed in replicates of five per run, two runs per day over 5 days for a total of 50 measurement per sample per reagent lot. The LoQ was determined as the lowest concentration of analyte which can be measured with a within-laboratory precision of 20% CV, and the highest value obtained from three lots was the reported LoQ for the assay.

- The LoQ for the Access NT-proBNP assay is estimated to be 4.8 ng/L (serum), 3.2 ng/L (K_2 EDTA), and 4.3 ng/L (LiHep).

These studies support the following detection capability claims in the labeling:
Limit of Blank (LoB): 1.1 ng/L
Limit of Detection (LoD): 4.8 ng/L
Limit of Quantitation (LoQ) (≤ 20% within-lab CV): 4.8 ng/L

7. Assay Cut-Off:

   See section D. for Clinical Cut-off information.

B Comparison Studies:

1. Method Comparison with Predicate Device:

   Not Applicable: see clinical studies in Section C.

2. Matrix Comparison:

   The assay is intended for use with serum and plasma (K_2-EDTA and lithium heparin). In addition to precision, linearity and clinical studies, a matrix equivalency study was conducted to support the use of serum and K_2 EDTA samples with the Access NT-proBNP on the DxI 9000 Access Immunoassay Analyzer. In the study, 68 donor matched samples of the aforementioned were collected. For each combination, each sample was tested in three replicates using one DxI 9000 Access Immunoassay Analyzer. The results were analyzed using Passing-Bablok regression analysis comparing the first replicate of the test tube to the mean of the control tube. The results support the use of K_2-EDTA plasma, lithium heparin plasma and serum on the Access NT-proBNP assay.

C Clinical Studies:

1. Clinical Sensitivity:

   See Section VII. C.3.

2. Clinical Specificity:

   See Section VII.C.3.

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

   Clinical Performance Studies in the Emergency Department

   A multi-center prospective study including 17 clinical collection sites and 3 clinical laboratory testing sites across in the United States was conducted to establish the clinical performance characteristics of the Access NT-proBNP assay run on the DxI 9000 Access Immunoassay Analyzer. In this study, subjects 22 years and older presenting with signs and symptoms that raise clinical suspicion of acute heart failure were enrolled in the study. Subjects with stage 4 or 5 chronic kidney disease (CKD), chronic dialysis and subjects with dyspnea clearly not secondary to HF (e.g., primary lung disease or chest trauma) were excluded from the study. For each patient enrollment, a serum, a K_2-EDTA, and a lithium heparin sample were collected for determination of their NT-proBNP concentration. The Access NT pro-BNP assay results were determined on the DxI 9000 Access Immunoassay Analyzer using stored frozen samples collected from 2384 ED subjects consisting of 48.2% (1149/2384) female subjects and 51.8% (1235/2384) male subjects. Individuals in the population were 35.7% Black or African American, 57.8% White or Caucasian, and 6.5% Asian. The sponsor provided information to support the use of the frozen samples in their study.

   The descriptive statistics for the Access NT-proBNP results were determined within and across sex and age groups. The results are summarized below.

Female

	Subjects with Acute Heart Failure*				Subjects without Acute Heart Failure*			
Age (years)	All Ages	<50	50-75	>75	All Ages	<50	50-75	>75
N	450	138	164	148	692	349	209	134
Mean (ng/L)	5264	3786	5597	6272	982	705	948	1758
SD (ng/L)	5795	5257	5753	6076	2341	2287	2325	2349
Median (ng/L)	3342	1835	3420	4178	193	108	193	842
IQR 25^{th} – 75^{th} percentile (ng/L)	1125-7291	840-4976	1243-8463	2028-8691	62-725	47-322	69-572	312-2137
Min(ng/L)	26	26	33	210	<10	<10	<10	26
Max(ng/L)	34973	34973	28037	33866	23996	23996	23968	12651

*only the subjects with numerical values were included in the descriptive statistical analysis.

Male

	Subjects with Acute Heart Failure*				Subjects without Acute Heart Failure*			
Age (years)	All Ages	<50	50-75	>75	All Ages	<50	50-75	>75
N	594	170	276	148	632	220	260	152
Mean (ng/L)	5463	4389	5696	6261	1181	735	1058	2035
SD (ng/L)	5289	4526	5259	5959	2375	2129	2251	2692
Median (ng/L)	4049	3261	4352	4408	263	92	214	981
IQR 25th – 75th percentile (ng/L)	1680-7533	1203-6289	1735-8030	2164-8284	68-1055	26-373	74-850	343-2441
Min(ng/L)	<10	<10	66	188	<10	<10	<10	16
Max(ng/L)	33611	27795	33611	32848	20485	20485	17542	16253

All subjects

	Subjects with Acute Heart Failure*				Subjects without Acute Heart Failure*			
Age (years)	All Ages	<50	50-75	>75	All Ages	<50	50-75	>75
N	1044	308	440	296	1324	569	469	286
Mean (ng/L)	5377	4119	5659	6266	1077	716	1009	1905
SD (ng/L)	5511	4868	5442	6008	2358	2226	2282	2537
Median (ng/L)	3776	2466	4111	4291	218	106	204	926
IQR 25th – 75th percentile (ng/L)	1406-7483	959-5745	1539-8222	2077-8394	63-904	39-347	72-670	335-2325
Min (ng/L)	<10	<10	33	188	<10	<10	<10	16
Max (ng/L)	34973	34973	33611	33866	23996	23966	23968	16253

A diagnosis of acute HF or no acute HF was determined for each subject by an independent central adjudication panel consisting of medical doctors with a specialty in cardiology or heart failure. For the 2384 subjects enrolled in the study, a total of 1059 subjects were adjudicated as subjects with acute HF and 1325 subjects were adjudicated as subjects without acute HF.

	Subject with Acute Heart Failure	Subject without Acute Heart Failure
Age Group		
< 50	35.3% (310/879)	64.7% (569/879)
50-75	48.7% (446/915)	51.2% (469/915)
>75	51.4% (303/590)	48.6% (287/590)
Sex		
Female	39.7% (456/1149)	60.3% (693/1149)
Male	48.8% (603/1235)	51.1% (632/1235)

Access NT-proBNP results versus adjudicated diagnosis

Age (years)	Access NT-proBNP Result	Adjudicated Diagnosis		Total
		HF	No HF	
<50	Positive	279	127	406
	Gray Zone	12	25	37
	Negative	19	417	436
	Total	310	569	879
50-75	Positive	380	105	485
	Gray Zone	47	96	143
	Negative	19	268	287
	Total	446	469	915
>75	Positive	240	94	334
	Gray Zone	59	132	191
	Negative	4	61	65
	Total	303	287	590
All	Positive	899	326	1225
	Gray Zone	118	253	371
	Negative	42	746	788
	Total	1059	1325	2384

The pretest probabilities of HF (prevalence of HF in the clinical study), posttest probabilities, likelihood ratios, and the two-tailed 95% CI of the test results versus adjudicated diagnosis were determined using the age-dependent positive cut-offs (450 ng/L for subjects 22 - < 50 years old; 900 ng/L for subjects 50 - ≤ 75 years old; 1800 ng/L for subjects >75 years old) and age-independent negative (300 ng/mL) cutoff and are summarized in the following tables:

Access NT-proBNP - All subjects (LiHep samples)

Age Group	Prevalence of HF (% (n/N))	Access_ NT-ProBNP results	Posttest Probability of HF		Posttest Probability of non-HF		Likelihood Ratio and CI	
			Estimate % (n/N)	95% CI	Estimate (% (n/N))	95% CI (%)*	LR	95%-CI**
<50	35.3% (310/879)	Positive	**68.7% (279/406)**	(64.1-73.0%)			4.03	(3.44, 4.72)
		Grey Zone	32.4% (12/37)	(19.6-48.5%)	67.6% (25/37)	(51.5-80.4%)	0.88	(0.45,1.73)
		Negative			**95.6% (417/436)**	(93.3-97.2%)	0.08	(0.05, 0.13)
50-75	48.7% (446/915)	Positive	**78.4% (380/485)**	(74.5-81.8%)			3.81	(3.20, 4.52)
		Grey Zone	32.9% (47/143)	(25.7-40.9%)	67.1% (96/143)	(59.1-74.3%)	0.51	(0.37,0.71)
		Negative			**93.4% (268/287)**	(89.9-95.7%)	0.07	(0.05, 0.11)
>75	51.4% (303/590)	Positive	**71.9% (240/334)**	(66.8-76.4%)			2.42	(2.03, 2.88)
		Grey Zone	30.9% (59/191)	(24.8-37.8%)	69.1% (132/191)	(62.2-75.2%)	0.42	(0.33, 0.55)
		Negative			**93.8% (61/65)**	(85.2-97.6%)	0.06	(0.02, 0.16)
All	44.4% (1059/2384)	Positive	73.4% (899/1225)	(70.8-75.8%)			3.45	(3.13, 3.80)
		Grey Zone	31.8% (118/371)	(27.3-36.7%)	68.2% (253/371)	(63.3-72.7%)	0.58	(0.48, 0.71)
		Negative			**94.7% (746/788)**	(92.9-96.0%)	0.07	(0.05, 0.09)

*Wilson Score confidence interval
** log method confidence intervals

Access NT-proBNP - Female Subjects (LiHep Samples)

Age Group	Prevalence of HF (% (n/N))	Access NT-ProBNP results	Posttest Probability of HF		Posttest Probability of non-HF		Likelihood Ratio and CI	
			Estimate % (n/N)	95% CI	Estimate (% (n/N))	95% CI (%)*	LR	95%-CI**
<50	28.5% (139/488)	Positive	**60.9% (123/202)**	(54.0-67.4%)			3.91	(3.19, 4.79)
		Grey Zone	31.3% (5/16)	(14.2-55.6%)	68.8% (11/16)	(44.4-85.8%)	1.14	(0.40, 3.22)
		Negative			**95.9% (259/270)**	(92.9-97.7%)	0.11	(0.06, 0.19)
50-75	44.1% (165/374)	Positive	**76.0% (133/175)**	(69.2-81.7%)			4.01	(3.03, 5.31)
		Grey Zone	30.6% (19/62)	(20.6-43.0%)	69.4% (43/62)	(57.0-79.4%)	0.56	(0.34, 0.92)
		Negative			**90.5% (124/137)**	(84.4-94.4%)	0.13	(0.08, 0.22)
>75	53.0% (152/287)	Positive	**73.9% (119/161)**	(66.6-80.1%)			2.52	(1.93, 3.28)
		Grey Zone	32.6% (30/92)	(23.9-42.7%)	67.4% (62/92)	(57.3-76.1%)	0.43	(0.30, 0.62)
		Negative			**91.2% (31/34)**	(77.0-97.0%)	0.09	(0.03, 0.25)
All	39.7% (456/1149)	Positive	**69.7% (375/538)**	(65.7-73.4%)			3.50	(3.04, 4.03)
		Grey Zone	31.8% (54/170)	(25.2-39.1%)	**68.2% (116/170)**	(60.9-74.8%)	0.71	(0.52, 0.96)
		Negative			**93.9% (414/441)**	(91.2-95.7%)	0.10	(0.07, 0.14)

Access NT-proBNP - Male Subjects (LiHep Samples)

Age Group	Prevalence of HF (% (n/N))	Access NT-ProBNP results	Posttest Probability of HF		Posttest Probability of non-HF		Likelihood Ratio and CI	
			Estimate % (n/N)	95% CI	Estimate (% (n/N))	95% CI *	LR	95%-CI**
<50	43.7% (171/391)	Positive	**76.5% (156/204)**	(70.2-81.8%)			4.18	(3.24, 5.39)
		Grey Zone	33.3% (7/21)	(17.2-54.6%)	66.7% (14/21)	(45.4-82.8%)	0.64	(0.27, 1.56)
		Negative			**95.2% (158/166)**	**(90.8-97.5%)**	0.07	(0.03, 0.13)
50-75	51.9% (281/541)	Positive	**79.7% (247/310)**	(74.8-83.8%)			3.63	(2.91, 4.52)
		Grey Zone	34.6% (28/81)	(25.1-45.4%)	65.4% (53/81)	(54.6-74.9%)	0.49	(0.32, 0.75)
		Negative			**96.0% (144/150)**	(91.5-98.2%)	0.04	(0.02, 0.08)
>75	49.8% (151/303)	Positive	**69.9% (121/173)**	(62.7-76.3%)			2.34	(1.85, 2.96)
		Grey Zone	29.3% (29/99)	(21.2-38.9%)	70.7% (70/99)	(61.1-78.8%)	0.42	(0.29, 0.60)
		Negative			**96.8% (30/31)**	**(83.8-99.4%)**	**0.03**	(0.00, 0.23)
All	48.8% (603/1235)	Positive	**76.3% (524/687)**	(73.0-79.3%)			3.37	(2.94, 3.86)
		Grey Zone	31.8% (64/201)	(25.8-38.6%)	68.2% (137/201)	(61.4-74.2%)	0.49	(0.37, 0.64)
		Negative			**95.7% (332/347)**	**(93.0-97.4%)**	0.05	(0.03, 0.08)

Access NT-proBNP - Subjects with HF history (LiHep)

Age Group	Prevalence of HF (% (n/N))	Access NT-ProBNP results	Posttest Probability of HF		Posttest Probability of non-HF		Likelihood Ratio and CI	
			Estimate % (n/N)	95% CI (%)	Estimate (% (n/N))	95% CI (%)*	LR	95%-CI**
<50	63.4% (208/328)	Positive	**79.0% (188/238)**	**(73.4-83.7%)**			2.17	(1.75, 2.69)
		Grey Zone	43.8% (7/16)	(23.1-66.8%)	56.3% (9/16)	(33.2-76.9%)	0.45	(0.17, 1.17)
		Negative			**82.4% (61/74)**	(72.2-89.4%)	0.12	(0.07, 0.20)
50-75	71.0% (323/455)	Positive	**85.0% (278/327)**	(80.7-88.5%)			2.32	(1.85, 2.91)
		Grey Zone	47.8% (33/69)	(36.5-59.4%)	52.2% (36/69)	(40.6-63.5%)	0.37	(0.24, 0.57)
		Negative			**79.7% (47/59)**	(67.7-88.0%)	0.10	(0.06, 0.17)
>75	66.9% (202/302)	Positive	**78.7% (170/216)**	(72.8-83.6%)			1.83	(1.47, 2.28)
		Grey Zone	41.1% (30/73)	(30.5-52.6%)	58.9% (43/73)	(47.4-69.5%)	0.35	(0.23, 0.52)
		Negative			**84.6% (11/13)**	(57.8-95.7%)	0.09	(0.03, 0.32)
All	67.6% (733/1085)	Positive	**81.4% (636/781)**	(78.6-84.0%)			2.11	(1.85, 2.39)
		Grey Zone	44.3% (70/158)	(36.8-52.1%)	55.7% (88/158)	(47.9-63.2%)	0.38	(0.29, 0.51)
		Negative			**81.5% (119/146)**	(74.4-87.0%)	0.11	(0.08, 0.15)

Access NT-proBNP - Subjects with no HF history (LiHep)

Age Group	Prevalence of HF (% (n/N))	Access NT-ProBNP results	Posttest Probability of HF		Posttest Probability of No non-HF		Likelihood Ratio and CI	
			Estimate % (n/N)	95% CI *	Estimate (% (n/N))	95% CI *	LR	95%-CI**
<50	18.4% (101/550)	Positive	**53.9% (90/167)**	(46.3-61.3%)			5.20	(4.19, 6.44)
		Grey Zone	23.8% (5/21)	(10.6-45.1%)	76.2% (16/21)	(54.9-89.4%)	1.39	(0.52, 3.70)
		Negative			**98.3% (356/362)**	(96.4-99.2%)	0.07	(0.03, 0.16)
50-75	26.8% (123/459)	Positive	**65.0% (102/157)**	(57.2-72.0%)			5.07	(3.93, 6.54)
		Grey Zone	18.9% (14/74)	(11.6-29.3%)	81.1% (60/74)	(70.7-88.4%)	0.64	(0.37, 1.10)
		Negative			**96.9% (221/228)**	**(93.8-98.5%)**	0.09	(0.04, 0.18)
>75	35.0% (100/286)	Positive	**59.0% (69/117)**	(49.9-67.5%)			2.67	(2.03, 3.53)
		Grey Zone	24.8% (29/117)	(17.8-33.3%)	75.2% (88/117)	(66.7-82.2%)	0.61	(0.44, 0.86)
		Negative			**96.2% (50/52)**	(87.0-98.9%)	0.07	(0.02, 0.29)
All	25.0% (324/1295)	Positive	**59.2% (261/441)**	(54.5-63.7%)			4.35	(3.77, 5.01)
		Grey Zone	22.6% (48/212)	(17.5-28.7%)	77.4% (164/212)	(71.3-82.5%)	0.88	(0.65, 1.18)
		Negative			**97.7% (627/642)**	(96.2-98.6%)	0.07	(0.04, 0.12)

Access NT-proBNP - Subjects with eGFR <60 mL/min/1.73 m^2 of BSA (LiHep)

Age Group	Prevalence of HF (% (n/N))	Access NT-ProBNP results	Posttest Probability of HF		Posttest Probability of non-HF		Likelihood Ratio and CI	
			Estimate % (n/N)	95% CI (%)	Estimate (% (n/N))	95% CI (%)*	LR	95%-CI**
<50	38.9% (102/262)	Positive	**68.1% (96/141)**	(60.0-75.2%)			3.35	(2.60, 4.31)
		Grey Zone	40.0% (4/10)	(16.8-68.7%)	60.0% (6/10)	(31.3-83.2%)	1.05	(0.30, 3.62)
		Negative			**98.2% (109/111)**	(93.7-99.5%)	0.03	(0.01, 0.11)
50-75	58.6% (249/425)	Positive	**80.9% (224/277)**	(75.8-85.1%)			2.99	(2.38, 3.76)
		Grey Zone	28.8% (17/59)	(18.8-41.4%)	71.2% (42/59)	(58.6-81.2%)	0.29	(0.17,0.49)
		Negative			**91.0% (81/89)**	(83.3-95.4%)	0.07	(0.04-0.14)
>75	57.9% (205/354)	Positive	**77.9% (169/217)**	(71.9-82.9%)			2.56	(2.01, 3.26)
		Grey Zone	30.8% (33/107)	(22.9-40.1%)	69.2% (74/107)	(59.9-77.1%)	0.32	(0.23, 0.46)
		Negative			**90.0% (27/30)**	(74.4-96.5%)	0.08	(0.03, 0.24)
All	53.4% (556/1041)	Positive	**77.0% (489/635)**	(73.6-80.1%)			2.92	(2.54, 3.36)
		Grey Zone	30.7% (54/176)	(24.3-37.8%)	69.3% (122/176)	(62.2-75.7%)	0.39	(0.29, 0.52)
		Negative			**94.3% (217/230)**	(90.6-96.7%)	0.05	(0.03, 0.09)

Access NT-proBNP - Subjects with eGFR ≥60 mL/min/1.73 m^2 of BSA (LiHep)

Age Group	Prevalence of HF (% (n/N))	Access NT-ProBNP results	Posttest Probability of HF		Posttest Probability of No non-HF		Likelihood Ratio and CI	
			Estimate % (n/N)	95% CI (%)	Estimate (% (n/N))	95% CI (%)*	LR	95%-CI**
<50	33.7% (208/617)	Positive	**69.1% (183/265)**	(63.3-74.3%)			4.39	(3.59, 5.36)
		Grey Zone	29.6% (8/27)	(15.9-48.5%)	70.4% (19/27)	(51.5-84.1%)	0.83	(0.37, 1.86)
		Negative			**94.8% (308/325)**	**(91.8-96.7%)**	**0.11**	(0.07, 0.17)
50-75	40.2% (197/490)	Positive	**75.0% (156/208)**	(68.7-80.4%)			4.46	(3.45, 5.77)
		Grey Zone	35.7% (30/84)	(26.3-46.4%)	64.3% (54/84)	(53.6-73.7%)	0.83	(0.55, 1.24)
		Negative			**94.4% (187/198)**	**(90.3-96.9%)**	0.09	(0.05, 0.15)
>75	41.5% (98/236)	Positive	**60.7% (71/117)**	(51.6-69.1%)			2.17	(1.67, 2.83)
		Grey Zone	31.0% (26/84)	(22.1-41.5%)	69.0% (58/84)	(58.5-77.9%)	0.63	(0.43, 0.93)
		Negative			**97.1% (34/35)**	**(85.5-99.5%)**	0.04	(0.01, 0.28)
All	37.5% (503/1343)	Positive	**69.5% (410/590)**	(65.7-73.1%)			3.80	(3.32-4.36)
		Grey Zone	32.8% (64/195)	(26.6-39.7%)	67.2% (131/195)	(60.3-73.4%)	0.82	(0.62, 1.08)
		Negative			**94.8% (529/558)**	(92.6-96.4%)	0.09	(0.06, 0.13)

Access NT-proBNP - Subjects with BMI ≥30 kg/m^2 (LiHep)

Age Group	Prevalence of HF (% (n/N))	Access NT-ProBNP results	Posttest Probability of HF		Posttest Probability of No non-HF		Likelihood Ratio and CI	
			Estimate % (n/N)	95% CI *	Estimate (% (n/N))	95% CI *	LR	95%-CI**
<50	41.5% (228/549)	Positive	**76.3% (200/262)**	(70.8-81.1%)			4.54	(3.61, 5.71)
		Grey Zone	36.7% (11/30)	(21.9-54.5%)	63.3% (19/30)	(45.5-78.1%)	0.82	(0.40, 1.68)
		Negative			**93.4% (240/257)**	(89.7-95.8%)	0.10	(0.06, 0.16)
50-75	52.3% (266/509)	Positive	**85.0% (210/247)**	(80.0-88.9%)			5.18	(3.83, 7.02)
		Grey Zone	42.4% (39/92)	(32.8-52.6%)	57.6% (53/92)	(47.4-67.2%)	0.67	(0.46, 0.98)
		Negative			**90.0% (153/170)**	(84.6-93.7%)	0.10	(0.06, 0.16)
>75	60.7% (130/214)	Positive	**88.6% (93/105)**	(81.1-93.3%)			5.01	(2.93, 8.55)
		Grey Zone	39.8% (33/83)	(29.9-50.5%)	60.2% (50/83)	(49.5-70.1%)	0.43	(0.30, 0.60)
		Negative			**84.6% (22/26)**	(66.5-93.8%)	0.12	(0.05, 0.29)
All	49.1% (624/1272)	Positive	**81.9% (503/614)**	(78.7-84.8%)			4.71	(3.96, 5.60)
		Grey Zone	40.5% (83/205)	(34.0-47.3%)	59.5% (122/205)	(52.7-66.0%)	0.71	(0.55, 0.91)
		Negative			**91.6% (415/453)**	(88.7-93.8)	0.10	(0.07, 0.13)

Access NT-proBNP - Subjects with BMI < 30 kg/m^2 (LiHep)

Age Group	Prevalence of HF (% (n/N))	Access NT-ProBNP results	Posttest Probability of HF		Posttest Probability of No non-HF		Likelihood Ratio and CI	
			Estimate % (n/N)	95% CI (%)	Estimate (% (n/N))	95% CI (%)*	LR	95%-CI**
<50	24.8% (82/330)	Positive	**54.9% (79/144)**	(46.7-62.8%)			3.68	(2.97, 4.55)
		Grey Zone	14.3% (1/7)	(2.6-51.3%)	85.7% (6/7)	(48.7-97.4%)	0.50	(0.06, 4.13)
		Negative			**98.9% (177/179)**	(96.0-99.7%)	0.03	(0.01, 0.13)
50-75	44.3% (180/406)	Positive	**71.4% (170/238)**	(65.4-76.8%)			3.14	(2.57, 3.84)
		Grey Zone	15.7% (8/51)	(8.2-28.0%)	84.3% (43/51)	(72.0-91.8%)	0.23	(0.11, 0.48)
		Negative			**98.3% (115/117)**	(94.0-99.5%)	0.02	(0.01, 0.09)
>75	46.0% (173/376)	Positive	**64.2% (147/229)**	(57.8-70.1%)			2.10	(1.76, 2.51)
		Grey Zone	24.1% (26/108)	(17.0-32.9%)	75.9% (82/108)	(67.1-83.0%)	0.37	(0.25, 0.55)
		Negative			**100% (39/39)**	(91.0-100.0%)	0.00	(0.00, 0.00)
All	39.1% (435/1112)	Positive	**64.8% (396/611)**	(60.9-68.5%)			2.87	(2.56, 3.21)
		Grey Zone	21.1% (35/166)	(15.6-27.9%)	78.9% (131/166)	(72.1-84.4%)	0.42	(0.29, 0.59)
		Negative			**98.8% (331/335)**	(97.0-99.5%)	0.02	(0.01,0.05)

Access NT-proBNP - Subjects with comorbidities*** (LiHep)

Age Group	Prevalence of HF (% (n/N))	Access NT-ProBNP results	Posttest Probability of HF		Posttest Probability of non-HF		Likelihood Ratio and CI	
			Estimate % (n/N)	95% CI (%)	Estimate (% (n/N))	95% CI *	LR	95%-CI**
<50	46.3% (240/518)	Positive	**75.0% (213/284)**	(69.7-79.7%)			3.48	(2.83, 4.27)
		Grey Zone	37.9% (11/29)	(22.7-56.0%)	62.1% (18/29)	(44.0-77.3%)	0.71	(0.34, 1.47)
		Negative			**92.2% (189/205)**	(87.7-95.1%)	0.10	(0.06, 0.16)
50-75	50.7% (401/791)	Positive	**79.3% (340/429)**	(75.2-82.8%)			3.72	(3.08, 4.48)
		Grey Zone	35.8% (44/123)	(27.9-44.6%)	64.2% (79/123)	(55.4-72.1%)	0.54	(0.39, 0.76)
		Negative			**92.9% (222/239)**	(88.9-95.5%)	0.07	(0.05, 0.12)
>75	51.8% (284/548)	Positive	**71.6% (224/313)**	(66.3-76.3%)			2.34	(1.96, 2.80)
		Grey Zone	32.2% (56/174)	(25.7-39.4%)	67.8% (118/174)	(60.6-74.3%)	0.44	(0.34, 0.58)
		Negative			**93.4% (57/61)**	(84.3-97.4%)	0.07	(0.03, 0.17)
All	49.8% (925/1857)	Positive	**75.7% (777/1026)**	(73.0-78.3%)			3.14	(2.82, 3.51)
		Grey Zone	34.0% (111/326)	(29.1-39.4%)	66.0% (215/326)	(60.6-70.9%)	0.52	(0.42, 0.64)
		Negative			**92.7% (468/505)**	(90.1-94.6%)	0.08	(0.06, 0.11)

*** The comorbidity group was defined as having one or more of the following non-cardiac medical history conditions (within 90 days prior to presentation at the emergency department): Metabolic disorders (e.g., diabetes or nonalcoholic fatty liver disease), renal insufficiency (defined as eGFR <60 to ≥30 mL/min/1.73m^2 BSA), systemic hypertension, and/or pulmonary disease requiring a chronic steroid therapy and/or supplemental home oxygen.

Access NT-proBNP - Subjects without comorbidities (LiHep)

Age Group	Prevalence of HF (% (n/N))	Access NT-ProBNP results	Posttest Probability of HF		Posttest Probability of non-HF		Likelihood Ratio and CI	
			Estimate % (n/N)	95% CI (%)	Estimate (% (n/N))	95% CI (%)*	LR	95%-CI**
<50	19.4% (70/361)	Positive	**54.1% (66/122)**	(45.3-62.7%)			4.90	(3.85, 6.24)
		Grey Zone	12.5% (1/8)	(2.2-47.1%)	87.5% (7/8)	(52.9-97.8%)	0.59	(0.07, 4.75)
		Negative			**98.7% (228/231)**	(96.3, 99.6%)	0.05	(0.02, 0.17)
50-75	36.3% (45/124)	Positive	**71.4% (40/56)**	(58.5-81.6%)			4.39	(2.80, 6.88)
		Grey Zone	15.0% (3/20)	(5.2-36.0%)	85.0% (17/20)	(64.0-94.8%)	0.31	(0.10, 1.00)
		Negative			**95.8% (46/48)**	(86.0-98.8%)	0.08	(0.02, 0.29)
>75	45.2% (19/42)	Positive	**76.2% (16/21)**	(54.9-89.4%)			3.87	(1.74, 8.62)
		Grey Zone	17.6% (3/17)	(6.2-41.0%)	82.4% (14/17)	(59.0-93.8%)	0.26	(0.09, 0.77)
		Negative			**100.0% (4/4)**	(51.0-100.0%)	0.00	(0.00, 0.00)
All	25.4% (134/527)	Positive	**61.3% (122/199)**	(54.4-67.8%)			4.65	(3.78, 5.72)
		Grey Zone	15.6% (7/45)	(7.7-28.8%)	84.4% (38/45)	(71.2-92.3%)	0.54	(0.25, 1.18)
		Negative			**98.2% (278/283)**	(95.9-99.2%)	0.05	(0.02, 0.12)

The sponsor included information in the clinical study section of the labeling regarding the false positives and false negatives in certain subgroups.

Correlation of the Access NT-proBNP assay results with New York Heart Association (NYHA) functional classification in patients diagnosed with HF:

The Assess NT-proBNP results were determined from samples from 1043 subjects with heart failure. The population consisted of 450 females and 593 males. The descriptive statistics for the Access NT-proBNP results (ng/L) were determined across sex and are summarized in the following table:

All Subjects

Statistics	NYHA Functional Classification			
	NYHA Class I	NYHA Class II	NYHA Class III	NYHA Class IV
N	91	271	485	196
Mean	3485	4718	5656	6460
SD	3499	4539	5939	6087
Median	2350	3547	3717	5073
5th Percentile	425	345	382	185
95th Percentile	11643	13291	18446	18290

Jonckheere-Terpstra test of trend p <0.0001

Female

Statistics	NYHA Functional Classification			
	NYHA Class I	NYHA Class II	NYHA Class III	NYHA Class IV
N	37	112	213	88
Mean	2282	4659	5297	7206
SD	1857	4465	5987	7144
Median	1546	3698	3173	4533
5th Percentile	343	294	291	150
95th Percentile	7384	12706	17152	26226

Jonckheere-Terpstra test of trend p =0.0005

Male

Statistics	NYHA Functional Classification			
	NYHA Class I	NYHA Class II	NYHA Class III	NYHA Class IV
N	54	159	272	108
Mean	4309	4761	5938	5852
SD	4093	4604	5896	5020
Median	2948	3422	4173	5149
5th Percentile	425	432	606	379
95th Percentile	12527	13402	20869	14584

Jonckheere-Terpstra test of trend p =0.0033

In the labeling, the sponsor states the following:

The JT test of trending was conducted on the 1043 subjects with an enrollment site NYHA evaluation and an adjudicated acute HF diagnosis resulting in a Z statistic of 4.1991 and p-value < 0.001. These results indicate that there is a statistically significant relationship between Access NT-proBNP results and HF severity as determined by NYHA class.

Risk stratification of patient with heart failure (HF) and risk stratification of acute coronary syndrome (ACS).

The sponsor provided information to support the risk stratification of heart failure and acute coronary syndrome claims based on the following three peer-reviewed literature references:

Reference 1: N-Terminal Pro-Brain Natriuretic Peptide and Other Risk Markers for the Separate Prediction of Mortality and Subsequent Myocardial Infarction in Patients with Unstable Coronary Artery Disease, GUSTO IV Substudy, James, S.K. et al, Circulation 2003, 108(3): 275-281.

Reference 2: N-Terminal Pro-Brain Natriuretic Peptide on Admission for Early Risk Stratification of Patients with Chest Pain and No ST-Segment Elevation, Jernberg, T. et al. Journal of the American College of Cardiology , 2002, 40(3): 437-445.

Reference 3: N-Terminal pro B type natriuretic peptide, but not the new putative cardiac hormone relaxin, predicts prognosis in patients with chronic heart failure. Fisher, C. et al. Heart, 2003, 89 (8):879-881

D Clinical Cut-Off:

For patients in the Emergency Department (ED) settings, the Access NT-proBNP assay results should be interpreted as indicated in the table below.

Age Group (Years)	NT-proBNP Results (ng/L)	Interpretation
All	< 300 ng/L	Heart Failure Unlikely
<50 50-75 >75	≥ 300 - < 450 ≥300 - <900 ≥300 - <1800	Grey Zone: Results Indeterminate-consider other reasons for NT-proBNP elevation
<50 50-75 >75	≥450 ≥900 ≥1800	Heart Failure Likely

E Expected Values/Reference Range:

The expected values of NT-proBNP in healthy adults was established in a study conducted in accordance with CLSI EP28-A3.

In the study, lithium heparin, serum and K_2EDTA levels of NT-proBNP were assayed with the Access NT-proBNP assay on a total of 675 apparently healthy subjects (369 females and 306 males) without HF. The results were subgrouped by sex, with each group of females and males were stratified into three age groups of : < 50 years, 50-75 years, and >75 years. The exclusion criteria used were:

- Disease(s) of /or affecting the cardiovascular system.
- Poorly controlled hypertension (defined as current blood pressure ≥140 mm Hg systolic, or ≥85 mm Hg diastolic).

- Current taking mediation for cardiovascular disease (except medications to control hypertension).
- Body-mass index (BMI) ≥ 30 kg/m^2
- Diabetes
- Chronic kidney disease
- Other serious chronic disease(s) (e.g., cancer, COPD, HIV, lupus erythematosus, etc).
- Acute bacterial or viral infection.

Representative data from lithium heparin samples is summarized in the following tables:

Female ng/L (LiHep)

Age (years)	<50	50-75	>75	All ages
N	135	145	89	369
Mean	63	114	178	111
SD	42	80	111	89
Median	54	91	154	82
IQR (25th -75th percentile)	32-80	54-155	93-216	49-149
95th percentile ULN	152	282	443	294
97.5% percentile ULN	178	317	457	670
%<125 ng/L	91.1%	65.5%	33.7%	67.2%

Male ng/L (LiHep)

Age (years)	<50	50-75	>75	All ages
N	132	111	63	306
Mean	33	68	130	66
SD	38	71	82	72
Media	23	48	115	41
IQR (25th -75th percentile)	13-39	28-77	77-148	20-84
95th percentile ULN	84	188	243	188
97.5% percentile ULN	103	284	434	267
%<125 ng/L	99.2%	87.4%	58.7%	86.6%

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.