510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K201312

B Applicant

Ortho Clinical Diagnostics

C Proprietary and Established Names

VITROS® Immunodiagnostic Products NT-proBNP II Reagent Pack

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:

N-terminal pro Brain Natriuretic Peptide (NT-proBNP)

C Type of Test:

Quantitative sandwich immunoassay.

III Intended Use/Indications for Use:

A Intended Use(s):
See Indications for Use below.

B Indication(s) for Use:
For the quantitative measurement of N-terminal pro Brain Natriuretic Peptide (NT-proBNP) in human serum and plasma (K2 EDTA or lithium heparin) using the VITROS 3600 Immunodiagnostic System to aid in the diagnosis of heart failure. The test can also be used in the assessment of heart failure severity in patients diagnosed with heart failure.

C Special Conditions for Use Statement(s):
Rx - For Prescription Use Only

For in vitro diagnostic use only

D Special Instrument Requirements:

VITROS 3600 Immunodiagnostic System

IV Device/System Characteristics:

A Device Description:
The VITROS NT-proBNP II assay (test) is performed using the VITROS® NT-proBNP II Reagent Pack and the VITROS NT-proBNP II Calibrators on the VITROS 3600 Immunodiagnostic System.

VITROS® NT-proBNP II Reagent Pack contains:

- 100 coated wells (mouse monoclonal anti-NT-proBNP antibody, 1 µg/mL).
- 8.7 mL assay reagent (buffer with sheep serum, bovine gamma globulin, bovine serum albumin, and antimicrobial agent).
- 8.7 mL conjugate reagent (HRP-conjugated sheep monoclonal anti-NT-proBNP antibody, 1 µg/mL in buffer with bovine serum albumin and antimicrobial agent).

VITROS Immunodiagnostic Products NT-proBNP II Calibrators Contents:

- 1 set of VITROS NT-proBNP II Calibrators 1, 2 and 3 (Recombinant NT-proBNP in buffer with bovine serum albumin and antimicrobial agent, 2.0 mL; nominal values 0; 450; 20,000 pg/mL.)
- Lot calibration card
- Protocol card
- 24 calibrator bar code labels (8 for each calibrator)

Materials Required but Not Provided with the reagent kit:

- VITROS immunodiagnostic products signal reagent.
- VITROS immunodiagnostic products universal wash reagent.
- VITROS immunodiagnostic products high sample diluent B.
- Quality control materials.
- VITROS immunodiagnostic products reagent pack storage box with desiccant.

B Principle of Operation:

The VITROS® NT-proBNP II Reagent Pack utilizes a one-step immunometric bridging assay design. A well is pushed from the pack and patient sample is dispensed into the antibody coated well. The assay reagent and the conjugate reagent are then dispensed into the well with the patient sample. NT-proBNP present in the sample binds with horseradish peroxidase (HRP)-labeled antibody conjugate which is captured by biotinylated anti-NT-proBNP capture antibody which is bound to streptavidin coated microwells. The well is incubated for 8 minutes, before unbound materials are removed by washing. The bound HRP conjugate is measured by a luminescent reaction. A reagent containing luminogenic substrate (a luminol derivative and a peracid salt) and an electron transfer agent, is added to the wells. The HRP in the bound conjugate catalyzes the oxidation of the luminol derivative, producing light. The electron transfer agent (a substituted acetanilide) increases the level of light produced and prolongs its emission. The light signals are read by the instrument. The amount of HRP conjugate bound and the corresponding signal is directly proportional to the concentration of NT-proBNP present.

V Substantial Equivalence Information:

A Predicate Device Name(s):

Elecsys ProBNP II

B Predicate 510(k) Number(s):

K072437

C Comparison with Predicate(s):

Device & Predicate Device(s):	K201312	K072437
Device Trade Name	VITROS® Immunodiagnostic Products NT-proBNP II Reagent Pack	Elecsys proBNP II
General Device Characteristic Similarities		
Measurand	NT-proBNP	Same
Assay Principle	Sandwich immunoassay	Same
Intended Use/Indications For Use	For the quantitative measurement of N-terminal pro Brain Natriuretic Peptide (NT-proBNP) in human serum and plasma.	Same

Device & Predicate Device(s):	K201312	K072437
General Device Characteristic Differences		
Measuring Range	20-30,000 pg/mL	5-35,000 pg/mL
Result Interpretation	For patients in the ED settings, the VITROS® NT-proBNP II Reagent Pack results should be interpreted as: Negative: Heart failure unlikely for all patients: <300 pg/mL Gray Zone: Indeterminate-consider other causes of NT-proBNP elevation: Age (yrs) NT-proBNP (pg/mL) 22 - <50 ≥300 to <450 50 - <75 ≥300 to <900 ≥75 ≥300 to <1800 Positive: Heart failure likely: Age (yrs) NT-proBNP (pg/mL) 22 - <50 ≥450 50 - <75 ≥900 ≥75 ≥1800 For patients in the outpatient settings, the VITROS® NT-proBNP II Reagent Pack results should be interpreted as: <125 pg/mL: Negative -Heart failure unlikely ≥125 pg/mL: Consider heart failure as well as other sources of NT-proBNP elevation	125 pg/mL for patients <75 years; 450 pg/mL for patients ≥75 years

VI Standards/Guidance Documents Referenced:

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

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

CLSI EP05-A3. Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline—Third Edition; 2014.

CLSI EP17-A2. Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition; 2012.

CLSI EP06-A. Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline; 2003.

CLSI EP07. Interference Testing in Clinical Chemistry. 3rd ed.; 2018.

CLSI EP37. Supplemental Tables for Interferent Testing in Clinical Chemistry – First Edition; 2018.

CLSI EP28-A3c. Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline – Third Edition; 2010.

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

Precision was evaluated consistent with the CLSI guideline EP05-A3. Two (2) replicates of each of 11 samples (eight human individual or serum pools and three controls) were tested on two (2) runs per day on 20 different test days (N=80). The study was performed using three (3) reagent master lots on one VITROS 3600 Immunodiagnostic System for a total of 240 measurements.

Mean	Between Lot		Between Day		Between Run		Within Run		Total*		N
pg/mL	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	
32	2.34	7.32	0.875	2.74	1.28	4.02	0.545	1.71	2.86	8.96	240
69.5†	3.92	5.65	1.89	2.71	1.83	2.63	1.58	2.27	4.98	7.17	240

Mean	Between Lot		Between Day		Between Run		Within Run		Total*		N
pg/mL	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	
97	4.65	4.79	2.65	2.73	1.89	1.95	1.56	1.61	5.89	6.07	240
369	9.14	2.48	6.32	1.71	7.75	2.10	4.9	1.33	14.4	3.91	240
823	15.9	1.94	11.6	1.40	20.5	2.49	12.5	1.52	31	3.77	240
949†	26.6	2.81	17.5	1.84	13.9	1.46	12.2	1.29	36.8	3.88	240
1745	10.8	0.62	44.5	2.55	56.9	3.26	20.3	1.16	75.8	4.34	240
5859†	75	1.28	92.1	1.57	55.4	0.95	80.3	1.37	154	2.62	240
24686	329	1.33	355	1.44	455	1.84	371	1.5	761	3.08	240
214	6.78	3.17	3.66	1.71	3.11	1.45	3.5	1.64	9.02	4.22	240
11392	206	1.81	145	1.28	65.2	0.57	176	1.54	314	2.76	240

* Total: Variability of the test incorporating factors of instrument, lot, day and run.
† Control Samples

2. Linearity:

The claimed measuring range is 20-30,000 pg/mL. Three (3) reagent master lots of VITROS® NT-proBNP II Reagent Packs were tested on one VITROS 3600 Immunodiagnostic System. Low and high serum sample pools were prepared and mixed to produce sample pools with 14 intermediate concentrations. A total of 16 serum samples with NT-proBNP concentrations ranging from 1.6 pg/mL to 39800 pg/mL were tested in triplicate.

The sponsor performed the analysis recommended in CLSI guideline EP06-A and the results support that the assay is linear from 20-30,000 pg/mL.

3. Analytical Specificity/Interference:

The VITROS® NT-proBNP II Reagent Pack was evaluated for potential interference following the CLSI EP07 A3 and CLSI EP37 (1st edition) guidelines. Several endogenous and exogenous compounds were tested with the candidate device in the presence of NT-proBNP at approximate concentrations of 125 pg/mL and 2000 pg/mL using three master reagent lots. No interference was defined as a mean percent recovery of samples containing each interference listed below within 100±10% of control. No interference was observed with the compounds up to the concentrations listed in the table below.

Compound	Concentration	Compound	Concentration
Acetaminophen	156 µg/mL	Ibuprofen	21.9 mg/dL
Acetylcysteine	15.0 mg/dL	Insulin	3.12 µg/dL
Adrenaline (Epinephrine)	20.0 µg/dL	Intralipid	2.00 g/dL

Compound	Concentration	Compound	Concentration
Alprazolam	25.8 µg/dL	L-dopa (Levodopa)	750 µg/dL
Amlodipine besylate	10.5 µg/dL	Levothyroxine	42.9 µg/dL
Amoxicillin	5.40 mg/dL	Lidocaine	1.50 mg/dL
Ascorbic acid	5.25 mg/dL	Methyldopa sesquihydrate	2.25 mg/dL
Atorvastatin calcium trihydrate	162 µg/dL	Methylprednisolone	783 µg/dL
Benazepril HCl	44.0 µg/dL	Metoprolol hemitartrate	150 µg/dL
Bilirubin, conjugated	40.0 mg/dL	Metronidazole	12.3 mg/dL
Bilirubin, unconjugated	40.0 mg/dL	Molsidomine	18.0 µg/dL
Biotin	3510 ng/mL	Naproxen sodium	39.3 mg/dL
Caffeine	10.8 mg/dL	Nicardipine HCL	46.5 µg/dL
Carvedilol	43.2 µg/dL	Nifedipine	58.8 µg/dL
Ceftriaxone disodium hemi(heptahydrate)	100 mg/dL	Omeprazole	840 µg/dL
Cholesterol	400 mg/dL	Oxycodone HCl	32.4 µg/dL
Clopidogrel hydrogen sulfate	2.40 µg/dL	Phenobarbital	69.0 mg/dL
Cotinine	240 µg/dL	Phenprocoumon (Marcumar)	1.50 mg/dL
Creatinine	15.0 mg/dL	Propafenone HCL	72.0 µg/dL
Cyclosporine	180 µg/dL	Pseudoephedrine HCl	330 µg/dL
Dextran	2.40 g/dL	Rheumatoid Factor	1500 IU/mL
Digitoxin	7.50 µg/dL	Rifampicin (Rifampin)	4.80 mg/dL
Digoxin	3.90 µg/dL	Salicylic acid	2.86 mg/dL
Diphenhydramine HCl	77.4 µg/dL	Salmeterol	1.65 µg/dL

Compound	Concentration	Compound	Concentration
Dipyrone (as 4-methylaminoantipyrine Hydrochloride)	3.30 mg/dL	Sotalol hydrochloride	510 µg/dL
Dypyridamole	1.00 mg/dL	Spironolactone	55.5 µg/dL
Doxycycline hyclate	1.80 mg/dL	Streptokinase	150,000 U/dL
Enalaprilat dihydrate	81.9 µg/dL	Theophylline	6.00 mg/dL
Ethanol	600 mg/dL	Tolbutamide	54.9 mg/dL
Fibrinogen	1000 mg/dL	Total Protein	15.0 g/dL
Furosemide	1.59 mg/dL	tPA (Alteplase)	1.20 mg/dL
Gentamicin Sulfate	3.51 mg/dL	Triglyceride	1500 mg/dL
Glycerylnitrate (Nitroglycerin)	1.20 µg/dL	Valproic Acid	31.8 mg/dL
HAMA (Human Anti-Mouse Antibody)	800 µg/L	Vancomycin Hydrochloride	12.3 mg/dL
Hemoglobin	1000 mg/dL	Verapamil Hydrochloride	160 µg/dL
Heparin (Sodium), UFH	330 U/dL	Warfarin	8.00 mg/dL

The interference study demonstrated that cefoxitin sodium and sodium azide interferes with the VITROS® NT-proBNP II assay. Cefoxitin sodium interfered with the candidate device at concentrations above 311 mg/dL, which is above the expected therapeutic concentration of this compound and is therefore not expected to cause significantly interfere with this device. Sodium azide interfered with the candidate device at concentrations above 85.9 mg/dL. Sodium azide is a preservative used in some hospital and laboratory equipment that is not expected to be found in patient samples; therefore, sodium azide is not expected to significantly interfere with this device. The results are shown below.

Interferent	Interferent Concentration mg/dL	In the presence of the average NT-proBNP concentration pg/mL	Mean %Bias
Cefoxitin sodium	697	84.3	-24.1
		922	-19.1
	311	105	-10.0
	306	806	-10.0
Sodium Azide	100	91.4	-12.1
	85.9	93.6	-10.0

The sponsor has the following limitation statements included in the labeling:

- Heterophilic antibodies in serum or plasma samples may cause interference in immunoassays. These antibodies may be present in blood samples from individuals regularly exposed to animals or who have been treated with animal serum products. Results that are inconsistent with clinical observations indicate the need for additional testing.
- The VITROS® NT-proBNP II test has no high dose hook effect up to a concentration of 300,000 pg/mL (35,400 pmol/L).

Cross-reactivity in the absence and presence of NT-proBNP: The cross-reactivity of the VITROS® NT-proBNP II Reagent Pack was evaluated by adding the substances listed in the tables below to a human serum sample that did not contain NT-proBNP and to a sample containing NT-proBNP at a concentration of 125 pg/mL. Percent cross-reactivity was expressed as the mean result obtained for the cross-reactant sample minus the mean result obtained for the control sample divided by the cross-reactant concentration multiplied by 100. The results showed that the cross-reactivity of proBNP (glycosylated) and proBNP (nonglycosylated) was greater than 1.0% when tested in samples in which NT-proBNP was and was not present. Cross-reactivity of NT-proBNP assays to proBNP has been documented previously. The results of the cross-reactivity testing are presented below.

Cross-reactivity in absence of NT-proBNP

Cross-reactant	Concentration	Mean result of control sample pg/mL	Mean result of cross-reactant sample pg/mL	Percent cross-reactivity
ANP28	3.10 µg/mL	*	*	*
proBNP (glycosylated)	3000 pg/mL	-0.14	57.6	1.9
proBNP (nonglycosylated)	3000 pg/mL	-0.48	863	28.8
NT-proANP1–30 (preproANP25–55)	3.50 µg/mL	*	*	*
NT-proANP31–67 (preproANP56–92)	1.00 ng/mL	*	*	*
NT-proANP79–98 (preproANP104–123)	1.00 ng/mL	*	*	*
BNP32 (Natrecor®)	3.50 µg/mL	*	*	*
CNP22	2.20 µg/mL	*	*	*
Adrenomedullin	1.00 ng/mL	*	*	*
Aldosterone	0.600 ng/mL	*	*	*
Angiotensin I	0.600 ng/mL	*	*	*
Angiotensin II	0.600 ng/mL	*	*	*
Angiotensin III	1.00 ng/mL	*	*	*
Endothelin	20.0 pg/mL	*	*	*
Urodilatin	3.50 µg/mL	*	*	*
Arg-Vasopressin	1.00 µg/mL	*	*	*
Renin	50.0 ng/mL	*	*	*

*Not detectable. Concentration was below the measuring range of the test, 20.0–30,000 pg/mL.

Cross-reactivity in the presence of 125 pg/mL NT-proBNP

Cross-reactant	Concentration	Mean NT-proBNP result of control sample pg/mL	Mean NT-proBNP result of cross-reactant sample pg/mL	Percent cross-reactivity
ANP28	3.10 µg/mL	112	113	<1.0
proBNP (glycosylated)	3000 pg/mL	105	183	2.6
proBNP (nonglycosylated)	3000 pg/mL	118	1290	39.1
NT-proANP1–30 (preproANP25–55)	3.50 µg/mL	113	775	<1.0
NT-proANP31–67 (preproANP56–92)	1.00 ng/mL	112	113	<1.0
NT-proANP79–98 (preproANP104–123)	1.00 ng/mL	112	114	<1.0
BNP32 (Natrecor®)	3.50 µg/mL	112	115	<1.0
CNP22	2.20 µg/mL	112	114	<1.0
Adrenomedullin	1.00 ng/mL	112	114	<1.0
Aldosterone	0.600 ng/mL	112	117	<1.0
Angiotensin I	0.600 ng/mL	112	112	<1.0
Angiotensin II	0.600 ng/mL	112	111	<1.0
Angiotensin III	1.00 ng/mL	112	112	<1.0
Endothelin	20.0 pg/mL	113	113	<1.0
Urodilatin	3.50 µg/mL	112	114	<1.0
Arg-Vasopressin	1.00 µg/mL	112	117	<1.0
Renin	50.0 ng/mL	112	112	<1.0

High dose hook effect:
The VITROS® NT-proBNP II Reagent Pack was evaluated for high dose hook effect using nine levels of recombinant NT-BNP II. The results support that the VITROS® NT-proBNP II Reagent Pack does not show a high dose hook effect up to 300,000 pg/mL of NT-proBNP.

4. Assay Reportable Range:

Based on the results from linearity and detection limit studies, the sponsor claims an assay reportable range of 20.0 to 30,000 pg/mL for the VITROS® NT-proBNP II Reagent Pack on the VITROS 3600 Immunodiagnostic System.

Dilution of high samples:
Serum or plasma (K_2 EDTA or lithium heparin) samples with concentrations up to 41,570 pg/mL can be measured after a 10-fold dilution. The percent recovery of diluted samples (up to 41,570 pg/mL) ranged from 91.5% to 109.6%.

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

Traceability:
The product calibrators for the VITROS® NT-proBNP II Reagent Pack are traceable to reference calibrators consisting of recombinant NT-proBNP in citrate buffer that were value assigned using a commercially available assay. The VITROS Immunodiagnostic Products NT-proBNP II Calibrators are value assigned using the reference calibrators on a lot-to-lot basis.

Sample Stability:
The sponsor provided data to support sample stability at different temperatures:

- K_2EDTA plasma, lithium heparin plasma, and serum samples are stable for up to 96 hours stored at 68-77°F (20-25°C).
- K_2EDTA plasma, lithium heparin plasma, and serum samples are stable for up to 26 weeks stored at ≤-4°F (≤-20°C).

The sponsor also provided data to support that samples stored frozen at ≤-4°F (≤-20°C) may be subjected to up to three (3) freeze-thaw cycles.

6. Detection Limit:

The CLSI EP17-A2 guideline was referenced to design the limit of blank (LoB), limit of detection (LoD) and limit of quantitation (LoQ) studies.

Limit of Blank (LoB):
LoB was determined by running 100 replicates for each of four (4) blank samples consisting of human serum/buffer matrix fluid over five days, measuring two runs per day, ten replicates per run, across three lots using the VITROS® NT-proBNP II Reagent Pack on one VITROS 3600 Immunodiagnostic System. A parametric method was used to evaluate the data, and the highest value obtained from three lots was claimed.

- The LoB for the VITROS® NT-proBNP II assay is estimated to be 0.034 pg/mL.

Limit of Detection (LoD):
LoD was assessed using seven (7) samples with low levels of NT-proBNP. These samples were prepared using bulk human serum samples to give approximate target levels in the range of one to six times the estimated LoB. The LoD was calculated by assaying ten replicates of each sample, on two runs per day for five days giving a total of 100 determinations of each sample lot. These determinations were run using three VITROS® NT-proBNP II Reagent Pack master lots on one VITROS 3600 Immunodiagnostic System. The limit of detection (LoD) for the VITROS® NT-proBNP II assay was determined by parametric analysis to be 0.46 pg/mL.

- The LoD claimed for the VITROS® NT-proBNP II assay is 0.49 pg/mL.

Limit of Quantitation (LoQ):
The LoQ was calculated as the lowest concentration of NT-proBNP that can be measured with a coefficient of variation (CV) of at least 20%. The LoQ was calculated using the seven

sample results from the LoD testing. The % CV for each sample was plotted vs. the mean concentration of NT-proBNP measured in each sample and a power function model was fit to the data. The limit of quantitation (LoQ) for the VITROS® NT-proBNP II assay was determined to be 0.18 pg/mL (highest value obtained from three reagent lots tested). Since the calculated LoQ is lower than the claimed LoD, the LoQ is set to be equal to LoD at 0.46 pg/mL.

- The sponsor claims the LoQ as 20.0 pg/mL.

7. Assay Cut-Off:

   See expected values below.

B Comparison Studies:

1. Method Comparison with Predicate Device:

   Not Applicable: see clinical studies in Section C.

2. Matrix Comparison:

   The sponsor conducted a matrix comparison study to support their matrix claims. 160 unaltered matched serum, K_2EDTA plasma and lithium heparin plasma samples containing NT-proBNP concentrations from 21.7 pg/mL to 26550 pg/mL were tested. Each serum sample was tested in duplicate while the lithium heparin plasma and K2-EDTA plasma samples were assayed in singleton using one VITROS 3600 Immunodiagnostic System. The results were analyzed using Passing-Bablok regression and Pearson correlation coefficient (r). The study results support claims for testing serum, K_2 EDTA plasma, and lithium heparin plasma samples with the VITROS® NT-proBNP II Reagent Pack on the VITROS 3600 Immunodiagnostic System.

Items	Serum vs. K_2EDTA plasma	Serum vs. Li-heparin plasma
NT-proBNP II range (pg/mL)	21.7 pg/mL to 26550 pg/mL	
Number of matched samples	160	160
Slope	0.98	1.00
y-intercept	2.24	2.75
Regression Coefficient R	0.999	1.00

C Clinical Studies:

1. Clinical Sensitivity:

   See section 3 below.

2. Clinical Specificity:

   See section 3 below.

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

Aid in Diagnosis of Heart Failure (HF):
Population 1 Emergency Department (ED):
A multi-center prospective study including 20 collection sites across the United States was conducted to establish the performance characteristics of the VITROS® NT-proBNP II Reagent Pack. Subjects 22 years and older presenting to the ED with dyspnea (acute or worsening) and clinical suspicion of heart failure (HF) were enrolled in the study. Subjects with terminal kidney failure on chronic dialysis and subjects with dyspnea clearly not secondary to HF were excluded from the study. The final clinical diagnosis was adjudicated by independent cardiologists or ED physicians experienced in diagnosing HF. The VITROS® NT-proBNP II Reagent Pack results were determined from samples collected from 2200 ED subjects consisting of 1016 (46.18%) female subjects and 1184 (53.82%) male subjects ranging in age from 22 to 106 years. Individuals in the population were African American (36.55%) and Caucasian (59.59%), with the remaining 3.86% represented by other races. Samples were frozen prior to analysis. Data supporting the stability of the samples for the storage time and condition were provided by the sponsor.

The descriptive statistics for the VITROS® NT-proBNP II Reagent Pack results (pg/mL) were determined within and across sex by age group and are summarized in the following tables:

Female subjects:

Study Population	Heart Failure				Non-Heart Failure			
Age (years)	22 - < 50	50 - < 75	≥ 75	All	22 - < 50	50 - <75	≥ 75	All
# of subjects	43	194	217	454	71	306	185	562
Mean (pg/mL)	10300	6830	7790	7620	196	648	948	690
SD (pg/mL)	32200	10000	10600	13900	356	1800	1280	1540
Median (pg/mL)	1920	3620	4710	3850	68.2	157	444	226
Min (pg/mL)	20.0	102	296	20.0	20.0	20.0	20.0	20.0
Max (pg/mL)	178000	86300	94600	178000	2010	23800	7870	23800

Male subjects:

Study Population	Heart Failure				Non-Heart Failure			
Age (years)	22 - < 50	50 - < 75	≥ 75	All	22 - < 50	50 - <75	≥ 75	All
# of subjects	71	344	226	641	70	324	149	543
Mean (pg/mL)	4720	6330	7830	6680	326	737	1330	846

Study Population	Heart Failure				Non-Heart Failure			
Age (years)	22 - < 50	50 - < 75	≥ 75	All	22 - < 50	50 - <75	≥ 75	All
SD (pg/mL)	6780	9170	9730	9180	718	1640	2080	1720
Median (pg/mL)	2260	3480	5020	3690	43.6	203	519	275
Min (pg/mL)	195	53.7	295	53.7	20.0	20.0	20.0	20.0
Max (pg/mL)	39200	70400	86000	86000	4710	17100	13600	17100

All subjects:

Study Population	Heart Failure				Non-Heart Failure			
Age (years)	22 - < 50	50 - < 75	≥ 75	All	22 - < 50	50 - <75	≥ 75	All
# of subjects	114	538	443	1095	141	630	334	1105
Mean (pg/mL)	6840	6510	7810	7070	261	694	1120	767
SD (pg/mL)	20500	9490	10100	11400	568	1720	1690	1630
Median (pg/mL)	2150	3550	4850	3780	58.4	177	473	247
Min (pg/mL)	20.0	53.7	295	20.0	20.0	20.0	20.0	20.0
Max (pg/mL)	178000	86300	94600	178000	4710	23800	13600	23800

VITROS® NT-proBNP II Test Results versus Adjudicated Diagnosis within and across Age Group

Age (Years)	**VITROS® NT-proBNP II Test Result**		**Adjudicated Diagnosis**		**Total**
			HF	**Non-HF**	
22–<50	VITROS® NT-proBNP II Test Result Interpretation	Positive	105	19	124
		Gray Zone	5	13	18
		Negative	4	109	113
		Total	**114**	**141**	**255**
50–<75	VITROS® NT-proBNP II Test Result Interpretation	Positive	472	115	587
		Gray Zone	59	144	203
		Negative	7	371	378
		Total	**538**	**630**	**1168**
≥75	VITROS® NT-proBNP II Test	Positive	372	62	434
		Gray Zone	69	153	222

Age (Years)	VITROS® NT-proBNP II Test Result		Adjudicated Diagnosis		Total
			HF	Non-HF	
	Result Interpretation	Negative	2	119	121
		Total	**443**	**334**	**777**
All Subjects	VITROS® NT-proBNP II Test Result Interpretation	Positive	949	196	1145
		Gray Zone	133	310	443
		Negative	13	599	612
		Total	**1095**	**1105**	**2200**

The pretest probability of HF (prevalence of HF in the study), posttest probabilities, likelihood ratios and the two-tailed 95% CIs of the VITROS® NT-proBNP II test result versus adjudicated diagnosis were determined across and within sex using the age-dependent rule-in (450 pg/mL for subjects 22–<50 years old; 900 pg/mL for subjects 50–<75 years old; 1800 pg/mL for subjects ≥75 years old) and age-independent rule-out (300 pg/mL) cutoffs and are summarized in the following tables:

All Subjects

Age Group (Years)	Pretest Probability of HF (Prevalence of HF in Study) (n/N)	VITROS® NT-proBNP II Test Result Interpretation	Posttest Probability of HF (n/N)		Posttest Probability of non-HF (n/N)		Likelihood Ratio Positive (HF)	95% CI**
			Estimate (%)	95% CI* (%)	Estimate (%)	95% CI* (%)		
22–< 50 (N = 255)	44.7% (114/255)	Positive	**84.7 (105/124)**	77.3–90.0	–	–	6.84	4.48–10.42
		Gray zone	27.8 (5/18)	12.5–50.9	72.2 (13/18)	49.1–87.5	0.48	0.17–1.29
		Negative	–	–	**96.5 (109/113)**	91.3–98.6	0.05	0.02–0.12
50–< 75 (N=1168)	46.1% (538/1168)	Positive	**80.4 (472/587)**	77.0–83.4	–	–	4.81	4.06–5.69
		Gray zone	29.1 (59/203)	23.3–35.7	70.9 (144/203)	64.3–76.7	0.48	0.36–0.63
		Negative	–	–	**98.1 (371/378)**	96.2–99.1	0.02	0.01–0.05
≥75 (N=777)	57% (443/777)	Positive	**85.7 (372/434)**	82.1–88.7	–	–	4.52	3.60–5.68

Age Group (Years)	Pretest Probability of HF (Prevalence of HF in Study) (n/N)	VITROS® NT-proBNP II Test Result Interpretation	Posttest Probability of HF (n/N)		Posttest Probability of non-HF (n/N)		Likelihood Ratio Positive (HF)	95% CI**
			Estimate (%)	95% CI* (%)	Estimate (%)	95% CI* (%)		
		Gray zone	31.1 (69/222)	25.4–37.4	68.9 (153/222)	62.6–74.6	0.34	0.27–0.43
		Negative	–	–	**98.3 (119/121)**	94.2–99.5	0.01	0.00–0.05
All Subjects (N=2200)	49.8% (1095/2200)	Positive	**82.9 (949/1145)**	80.6–85.0	–	–	4.89	4.29–5.56
		Gray zone	30.0 (133/443)	25.9–34.4	70.0 (310/443)	65.6–74.1	0.43	0.36–0.52
		Negative	–	–	**97.9 (599/612)**	96.4–98.8	0.02	0.01–0.04

* 95% Wilson Score Confidence Interval
** Log Method Confidence Interval

The pretest probability of HF (prevalence of HF in the study), posttest probabilities, likelihood ratios and the two-tailed 95% CIs of the VITROS® NT-proBNP II test result versus adjudicated diagnosis were determined for the relevant clinical subgroups using the age-dependent rule-in (450 pg/mL for subjects 22–<50 years old; 900 pg/mL for subjects 50–<75 years old; 1800 pg/mL for subjects ≥75 years old) and age-independent rule-out (300 pg/mL) cutoffs and are summarized in the following tables:

Female Subjects

Age Group (Years)	Pretest Probability of HF (Prevalence of HF in Study) (n/N)	VITROS® NT-proBNP II Test Result Interpretation	Posttest Probability of HF (n/N)		Posttest Probability of non-HF (n/N)		Likelihood Ratio Positive (HF)	95% CI**
			Estimate (%)	95% CI* (%)	Estimate (%)	95% CI* (%)		
22–<50 (N=114)	37.70% (43/114)	Positive	**88.6 (39/44)**	77.3–90.0	–	–	6.84	4.48–10.42
		Gray zone	11.1 (1/9)	12.5–50.9	88.9 (8/9)	49.1–87.5	0.48	0.17–1.29
		Negative	–	–	**95.1 (58/61)**	91.3–98.6	0.05	0.02–0.12
50–<75 (N=500)	38.80% (194/500)	Positive	**77 (167/217)**	77.0–83.4	–	–	4.81	4.06–5.69
		Gray zone	26.4 (23/87)	23.3–35.7	73.6 (64/87)	64.3–76.7	0.48	0.36–0.63
		Negative	–	–	**98 (192/196)**	96.2–99.1	0.02	0.01–0.05
≥75 (N=402)	54% (217/402)	Positive	**85.9 (176/205)**	82.1–88.7	–	–	4.52	3.60–5.68
		Gray zone	30.5 (40/131)	25.4–37.4	69.5 (91/131)	62.6–74.6	0.34	0.27–0.43
		Negative	–	–	**98.5 (65/66)**	94.2–99.5	0.01	0.00–0.05

* 95% Wilson Score Confidence Interval
** Log Method Confidence Interval

Male Subjects

Age Group (Years)	Pretest Probability of HF (Prevalence of HF in Study) (n/N)	VITROS® NT-proBNP II Test Result Interpretation	Posttest Probability of HF (n/N)		Posttest Probability of non-HF (n/N)		Likelihood Ratio Positive (HF)	95% CI**
			Estimate (%)	95% CI* (%)	Estimate (%)	95% CI* (%)		
22–<50 (N=141)	50.40% (71/141)	Positive	**82.5 (66/80)**	77.3–90.0	–	–	6.84	4.48–10.42
		Gray zone	44.4 (4/9)	12.5–50.9	55.6 (5/9)	49.1–87.5	0.48	0.17–1.29

Age Group (Years)	Pretest Probability of HF (Prevalence of HF in Study) (n/N)	VITROS® NT-proBNP II Test Result Interpretation	Posttest Probability of HF (n/N)		Posttest Probability of non-HF (n/N)		Likelihood Ratio Positive (HF)	95% CI**
			Estimate (%)	95% CI* (%)	Estimate (%)	95% CI* (%)		
		Negative	–	–	**98.1 (51/52)**	91.3–98.6	0.05	0.02–0.12
50–<75 (N=668)	51.50% (344/668)	Positive	**82.4 (305/370)**	77.0–83.4	–	–	4.81	4.06–5.69
		Gray zone	31 (36/116)	23.3–35.7	69 (80/116)	64.3–76.7	0.48	0.36–0.63
		Negative	–	–	**98.4 (179/182)**	96.2–99.1	0.02	0.01–0.05
≥75 (N=375)	60.30% (226/375)	Positive	**85.6 (196/229)**	82.1–88.7	–	–	4.52	3.60–5.68
		Gray zone	31.9 (29/91)	25.4–37.4	68.1 (62/91)	62.6–74.6	0.34	0.27–0.43
		Negative	–	–	**98.2 (54/55)**	94.2–99.5	0.01	0.00–0.05

* 95% Wilson Score Confidence Interval
** Log Method Confidence Interval

Subjects with a History of HF

Age Group (Years)	Pretest Probability of HF (Prevalence of HF in Study) (n/N)	VITROS® NT-proBNP II Test Result Interpretation	Posttest Probability of HF (n/N)		Posttest Probability of non-HF (n/N)		Likelihood Ratio Positive (HF)	95% CI**
			Estimate (%)	95% CI* (%)	Estimate (%)	95% CI* (%)		
22–<50 (N=118)	73.7% (87/118)	Positive	**92.9 (79/85)**	77.3–90.0	–	–	6.84	4.48–10.42
		Gray zone	36.4 (4/11)	12.5–50.9	63.6 (7/11)	49.1–87.5	0.48	0.17–1.29
		Negative	–	–	**81.8 (18/22)**	91.3–98.6	0.05	0.02–0.12
50–<75 (N=642)	63.1% (405/642)	Positive	**87.2 (355/407)**	77.0–83.4	–	–	4.81	4.06–5.69
		Gray zone	38.1 (43/113)	23.3–35.7	61.9 (70/113)	64.3–76.7	0.48	0.36–0.63
		Negative	–	–	**94.3 (115/122)**	96.2–99.1	0.02	0.01–0.05
≥75 (N=460)	71.1% (327/460)	Positive	**90.5 (275/304)**	82.1–88.7	–	–	4.52	3.60–5.68
		Gray zone	41.1 (51/124)	25.4–37.4	58.9 (73/124)	62.6–74.6	0.34	0.27–0.43
		Negative	–	–	**96.9 (31/32)**	94.2–99.5	0.01	0.00–0.05

* 95% Wilson Score Confidence Interval
** Log Method Confidence Interval

Subjects with no History of HF

Age Group (Years)	Pretest Probability of HF (Prevalence of HF in Study) (n/N)	VITROS® NT-proBNP II Test Result Interpretation	Posttest Probability of HF (n/N)		Posttest Probability of non-HF (n/N)		Likelihood Ratio Positive (HF)	95% CI**
			Estimate (%)	95% CI* (%)	Estimate (%)	95% CI* (%)		
22–<50 (N=137)	19.7% (27/137)	Positive	**66.7 (26/39)**	77.3–90.0	–	–	6.84	4.48–10.42
		Gray zone	14.3 (1/7)	12.5–50.9	85.7 (6/7)	49.1–87.5	0.48	0.17–1.29
		Negative	–	–	**100 (91/91)**	91.3–98.6	0.05	0.02–0.12
50–<75 (N=521)	25.3% (132/521)	Positive	**65.2 (116/178)**	77.0–83.4	–	–	4.81	4.06–5.69
		Gray zone	17.8 (16/90)	23.3–35.7	82.2 (74/90)	64.3–76.7	0.48	0.36–0.63
		Negative	–	–	**100 (253/253)**	96.2–99.1	0.02	0.01–0.05
≥75 (N=312)	36.9% (115/312)	Positive	**75 (96/128)**	82.1–88.7	–	–	4.52	3.60–5.68
		Gray zone	18.8 (18/96)	25.4–37.4	81.3 (78/96)	62.6–74.6	0.34	0.27–0.43
		Negative	–	–	**98.9 (87/88)**	94.2–99.5	0.01	0.00–0.05

* 95% Wilson Score Confidence Interval
** Log Method Confidence Interval

Subjects with eGFR <60*mL/min/1.73 m^2**

Age Group (Years)	Pretest Probability of HF (Prevalence of HF in Study) (n/N)	VITROS® NT-proBNP II Test Result Interpretation	Posttest Probability of HF (n/N)		Posttest Probability of non-HF (n/N)		Likelihood Ratio Positive (HF)	95% CI**
			Estimate (%)	95% CI* (%)	Estimate (%)	95% CI* (%)		
22–<50 (N=69)	62.30% (43/69)	Positive	**87.2 (41/47)**	77.3–90.0	–	–	6.84	4.48–10.42
		Gray zone	16.7 (1/6)	12.5–50.9	83.3 (5/6)	49.1–87.5	0.48	0.17–1.29
		Negative	–	–	**93.8 (15/16)**	91.3–98.6	0.05	0.02–0.12
50–<75 (N=476)	56.50% (269/476)	Positive	**79.6 (250/314)**	77.0–83.4	–	–	4.81	4.06–5.69
		Gray zone	24.7 (18/73)	23.3–35.7	75.3 (55/73)	64.3–76.7	0.48	0.36–0.63
		Negative	–	–	**98.9 (88/89)**	96.2–99.1	0.02	0.01–0.05
≥75 (N=467)	68.10% (318/467)	Positive	**88.6 (273/308)**	82.1–88.7	–	–	4.52	3.60–5.68
		Gray zone	37.6 (44/117)	25.4–37.4	62.4 (73/117)	62.6–74.6	0.34	0.27–0.43
		Negative	–	–	**97.6 (41/42)**	94.2–99.5	0.01	0.00–0.05

* 95% Wilson Score Confidence Interval
** Log Method Confidence Interval
*** Subjects with renal disease on dialysis were excluded from the study

Subjects with eGFR ≥60*mL/min/1.73 m^2**

Age Group (Years)	Pretest Probability of HF (Prevalence of HF in Study) (n/N)	VITROS® NT-proBNP II Test Result Interpretation	Posttest Probability of HF (n/N)		Posttest Probability of non-HF (n/N)		Likelihood Ratio Positive (HF)	95% CI**
			Estimate (%)	95% CI* (%)	Estimate (%)	95% CI* (%)		
22–<50 (N=181)	38.70% (70/181)	Positive	**82.9 (63/76)**	77.3–90.0	–	–	6.84	4.48–10.42
		Gray zone	36.4 (4/11)	12.5–50.9	63.6 (7/11)	49.1–87.5	0.48	0.17–1.29
		Negative	–	–	**96.8 (91/94)**	91.3–98.6	0.05	0.02–0.12
50–<75 (N=679)	38.70% (263/679)	Positive	**81 (217/268)**	77.0–83.4	–	–	4.81	4.06–5.69
		Gray zone	31.3 (40/128)	23.3–35.7	68.8 (88/128)	64.3–76.7	0.48	0.36–0.63
		Negative	–	–	**97.9 (277/283)**	96.2–99.1	0.02	0.01–0.05
≥75 (N=306)	40.20% (123/306)	Positive	**78.2 (97/124)**	82.1–88.7	–	–	4.52	3.60–5.68
		Gray zone	24 (25/104)	25.4–37.4	76 (79/104)	62.6–74.6	0.34	0.27–0.43
		Negative	–	–	**98.7 (77/78)**	94.2–99.5	0.01	0.00–0.05

* 95% Wilson Score Confidence Interval
** Log Method Confidence Interval
*** Subjects with renal disease on dialysis were excluded from the study

Subjects with BMI ≥30 kg/m^2

Age Group (Years)	Pretest Probability of HF (Prevalence of HF in Study) (n/N)	VITROS® NT-proBNP II Test Result Interpretation	Posttest Probability of HF (n/N)		Posttest Probability of non-HF (n/N)		Likelihood Ratio Positive (HF)	95% CI**
			Estimate (%)	95% CI* (%)	Estimate (%)	95% CI* (%)		
22–<50 (N=188)	48.9% (92/188)	Positive	**90.2 (83/92)**	77.3–90.0	–	–	6.84	4.48–10.42
		Gray zone	27.8 (5/18)	12.5–50.9	72.2 (13/18)	49.1–87.5	0.48	0.17–1.29
		Negative	–	–	**94.9 (74/78)**	91.3–98.6	0.05	0.02–0.12
50–<75 (N=693)	46.3% (321/693)	Positive	**80.9 (266/329)**	77.0–83.4	–	–	4.81	4.06–5.69
		Gray zone	36.1 (48/133)	23.3–35.7	63.9 (85/133)	64.3–76.7	0.48	0.36–0.63
		Negative	–	–	**97 (224/231)**	96.2–99.1	0.02	0.01–0.05
≥75 (N=279)	55.9% (156/279)	Positive	**84.6 (121/143)**	82.1–88.7	–	–	4.52	3.60–5.68
		Gray zone	37.8 (34/90)	25.4–37.4	62.2 (56/90)	62.6–74.6	0.34	0.27–0.43
		Negative	–	–	**97.8 (45/46)**	94.2–99.5	0.01	0.00–0.05

* 95% Wilson Score Confidence Interval
** Log Method Confidence Interval

Subjects with BMI <30 kg/m^2

Age Group (Years)	Pretest Probability of HF (Prevalence of HF in Study) (n/N)	VITROS® NT-proBNP II Test Result Interpretation	Posttest Probability of HF (n/N)		Posttest Probability of non-HF (n/N)		Likelihood Ratio Positive (HF)	95% CI**
			Estimate (%)	95% CI* (%)	Estimate (%)	95% CI* (%)		
22–<50 (N=62)	35.5% (22/62)	Positive	**68.8 (22/32)**	77.3–90.0	–	–	6.84	4.48–10.42
		Gray zone	∞ (0/0)	12.5–50.9	∞ (0/0)	49.1–87.5	0.48	0.17–1.29
		Negative	–	–	**100 (30/30)**	91.3–98.6	0.05	0.02–0.12
50–<75 (N=465)	46.5% (216/465)	Positive	**79.8 (206/258)**	77.0–83.4	–	–	4.81	4.06–5.69
		Gray zone	14.7 (10/68)	23.3–35.7	85.3 (58/68)	64.3–76.7	0.48	0.36–0.63
		Negative	–	–	**100 (139/139)**	96.2–99.1	0.02	0.01–0.05
≥75 (N=498)	57.6% (287/498)	Positive	**86.3 (251/291)**	82.1–88.7	–	–	4.52	3.60–5.68
		Gray zone	26.5 (35/132)	25.4–37.4	73.5 (97/132)	62.6–74.6	0.34	0.27–0.43
		Negative	–	–	**98.7 (74/75)**	94.2–99.5	0.01	0.00–0.05

* 95% Wilson Score Confidence Interval
** Log Method Confidence Interval

Subjects with Comorbidities

Age Group (Years)	Pretest Probability of HF (Prevalence of HF in Study) (n/N)	VITROS® NT-proBNP II Test Result Interpretation	Posttest Probability of HF (n/N)		Posttest Probability of non-HF (n/N)		Likelihood Ratio Positive (HF)	95% CI**
			Estimate (%)	95% CI* (%)	Estimate (%)	95% CI* (%)		
22–<50 (N=178)	55.1% (98/178)	Positive	**86.8 (92/106)**	77.3–90.0	–	–	6.84	4.48–10.42
		Gray zone	23.1 (3/13)	12.5–50.9	76.9 (10/13)	49.1–87.5	0.48	0.17–1.29
		Negative	–	–	**94.9 (56/59)**	91.3–98.6	0.05	0.02–0.12
50–<75 (N=1066)	47.3% (504/1066)	Positive	**80.2 (438/546)**	77.0–83.4	–	–	4.81	4.06–5.69
		Gray zone	32.1 (59/184)	23.3–35.7	67.9 (125/184)	64.3–76.7	0.48	0.36–0.63
		Negative	–	–	**97.9 (329/336)**	96.2–99.1	0.02	0.01–0.05
≥75 (N=734)	57.2% (420/734)	Positive	**86.1 (352/409)**	82.1–88.7	–	–	4.52	3.60–5.68
		Gray zone	31.7 (66/208)	25.4–37.4	68.3 (142/208)	62.6–74.6	0.34	0.27–0.43
		Negative	–	–	**98.3 (115/117)**	94.2–99.5	0.01	0.00–0.05

* 95% Wilson Score Confidence Interval
** Log Method Confidence Interval
*** Subjects with at least one of the following: diabetes, renal insufficiency (eGFR values <60), hypertension (HTN) and/or chronic obstructive pulmonary disease (COPD)

Subjects without Comorbidities

Age Group (Years)	Pretest Probability of HF (Prevalence of HF in Study) (n/N)	VITROS® NT-proBNP II Test Result Interpretation	Posttest Probability of HF (n/N)		Posttest Probability of non-HF (n/N)		Likelihood Ratio Positive (HF)	95% CI**
			Estimate (%)	95% CI* (%)	Estimate (%)	95% CI* (%)		
22–<50 (N=77)	20.8% (16/77)	Positive	**72.2 (13/18)**	77.3–90.0	–	–	6.84	4.48–10.42
		Gray zone	40 (2/5)	12.5–50.9	60 (3/5)	49.1–87.5	0.48	0.17–1.29
		Negative	–	–	**98.1 (53/54)**	91.3–98.6	0.05	0.02–0.12
50–<75 (N=102)	33.3% (34/102)	Positive	**82.9 (34/41)**	77.0–83.4	–	–	4.81	4.06–5.69
		Gray zone	0 (0/19)	23.3–35.7	100 (19/19)	64.3–76.7	0.48	0.36–0.63
		Negative	–	–	**100 (42/42)**	96.2–99.1	0.02	0.01–0.05
≥75 (N=43)	53.5% (23/43)	Positive	**80 (20/25)**	82.1–88.7	–	–	4.52	3.60–5.68
		Gray zone	21.4 (3/14)	25.4–37.4	78.6 (11/14)	62.6–74.6	0.34	0.27–0.43
		Negative	–	–	**100 (4/4)**	94.2–99.5	0.01	0.00–0.05

* 95% Wilson Score Confidence Interval
** Log Method Confidence Interval
*** Subjects with at least one of the following: diabetes, renal insufficiency (eGFR values <60), hypertension (HTN) and/or chronic obstructive pulmonary disease (COPD)

Population 2 – Outpatient Setting:
The VITROS® NT-proBNP II Reagent Pack results were determined from 777 subjects, 391 females and 386 males ranging in age from 23 to 94 years presenting to cardiology clinics and other outpatient facilities with a clinical suspicion of HF (not previously diagnosed) and at least one HF sign, symptom or risk factor at 10 collection sites across the United States. Subjects with terminal kidney failure on chronic dialysis and subjects with dyspnea clearly not secondary to HF were excluded from the study. The final clinical diagnosis was adjudicated by independent cardiologists or ED physicians experienced in diagnosing HF. Individuals in the population were African American (31.66%) and Caucasian (64.74%), with the remaining 3.60% represented by other races. The clinical performance and the two-tailed 95% CIs of the VITROS® NT-proBNP II Reagent Pack results versus adjudicated diagnosis for subjects presenting to cardiology clinics and other outpatient facilities was determined using the rule-out cutoff (125 pg/mL) and are summarized in the following tables:

All subjects:

Group	Cutoff pg/mL	Sensitivity (%) (n/N)	95% CI* (%)	Specificity (%) (n/N)	95% CI* (%)	NPV (%) (n/N)	95% CI* (%)	PPV (%) (n/N)	95% CI* (%)
All Subjects (N=777)	125	91.7 (44/48)	80.0 - 97.7	67.2 (490/729)	63.7 - 70.6	99.2 (490/494)	97.9 - 99.8	15.6 (44/283)	11.5 - 20.3

*95% Exact Confidence Interval

Performance Characteristics of the VITROS® NT-proBNP II Assay Within and Across Sex

Sex	N	Sensitivity (%) (n/N)	95% CI (%)*	Specificity (%) (n/N)	95% CI (%)*	NPV (%) (n/N)	95% CI (%)*	PPV (%) (n/N)	95% CI (%)*
Female	391	90.5 (19/21)	69.6 - 98.8	68.1 (252/370)	63.1 - 72.8	99.2 (252/254)	97.2 - 99.9	13.9 (19/137)	8.6 - 20.8
Male	386	92.6 (25/27)	75.7 - 99.1	66.3 (238/359)	61.2 - 71.2	99.2 (238/240)	97.0 - 99.9	17.1 (25/146)	11.4 - 24.2

*95% Exact Confidence Interval

Subjects stratified by age:

Clinical Subgroups	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	NPV (%) (95% CI)	PPV (%) (95% CI)
≥75 Years old (N=139)	93.8 (69.8 - 99.8)	36.6 (28.1 - 45.8)	97.8 (88.5 - 99.9)	16.1 (9.3 - 25.2)
<75 Years old (N=638)	90.6 (75.0 - 98.0)	73.4 (69.7 - 76.9)	99.3 (98.1 - 99.9)	5.0 (3.5 - 7.0)

Subjects stratified by eGFR

Clinical Subgroups	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	NPV (%) (95% CI)	PPV (%) (95% CI)
eGFR <60* mL/min/1.73 m2 (N=81)	84.6 (54.6 - 98.1)	45.6 (33.5 - 58.1)	93.9 (79.8 - 99.3)	22.9 (12.0 - 37.3)
eGFR ≥ 60 mL/min/1.73 m2 (N=245)	100.0 (75.3 - 100.0)	68.5 (62.1 - 74.5)	100.0 (97.7 - 100.0)	15.1 (8.3 - 24.5)

*Subjects with renal disease on dialysis were excluded from the study

Subjects stratified by BMI

Clinical Subgroups	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	NPV (%) (95% CI)	PPV (%) (95% CI)
BMI ≥ 30.0 kg/m2 (N=430)	82.6 (61.2 - 95.1)	76.2 (71.7 - 80.2)	98.7 (96.8 - 99.7)	16.4 (10.2 - 24.4)
BMI < 30.0 kg/m2 (N=346)	100.0 (85.8 - 100.0)	55.9 (50.3 - 61.4)	100.0 (98.0 - 100.0)	14.5 (9.5 - 20.7)

Subjects stratified by the presence or absence of comorbidities

Clinical Subgroups	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	NPV (%) (95% CI)	PPV (%) (95% CI)
With comorbidities* (N=660)	100.0 (90.8 - 100.0)	6.4 (4.6 - 8.7)	100.0 (91.2 - 100.0)	6.1 (4.4 - 8.3)
Without comorbidities** (N=117)	90.0 (55.5 - 99.8)	4.7 (1.5 - 10.6)	83.3 (35.9 - 99.6)	8.1 (3.8 - 14.8)

*Subjects with at least one of the following: diabetes, renal insufficiency (eGFR values <60), hypertension (HTN) and/or chronic obstructive pulmonary disease (COPD)
**Subjects without any of the following: diabetes, renal insufficiency (eGFR values <60), hypertension (HTN) and/or chronic obstructive pulmonary disease (COPD)

In the labeling, the sponsor states "*the results of this assay should be used in conjunction with clinical presentation, other diagnostic tests, and in accordance with the appropriate clinical guidelines.*"

Correlation of the VITROS® NT-proBNP II Reagent Pack results with New York Heart Association (NYHA) functional classification in patients diagnosed with HF:

The VITROS® NT-proBNP II Reagent Pack results were determined from samples from 1143 subjects with heart failure ranging in age from 22 to 106 years. The population consisted of 475/1143 (41.56%) females and 668/1143 (58.44%) males. The descriptive statistics for the VITROS® NT-proBNP II Reagent results (pg/mL) were determined across gender 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	8	190	567	378
Mean	4190	5010	6680	8170
SD	5910	8410	9770	14100
5th Percentile	156	291	490	701
Median	1480	2220	3660	4520
IQR	6360	4330	5920	7810
95th Percentile	16000	17900	23100	23400

*There were no NYHA Class I female subjects in the study
Jonckheere-Terpstra test of trend p <0.0001

Female Subjects

Statistics	NYHA Functional Classification			
	NYHA Class I*	NYHA Class II	NYHA Class III	NYHA Class IV
N	N/A	85	240	150
Mean	N/A	4560	6650	9970
SD	N/A	6150	9860	20100
5th Percentile	N/A	272	481	559
Median	N/A	2190	3510	4790
IQR	N/A	4250	6310	8580
95th Percentile	N/A	16200	24400	25800

*There were no NYHA Class I female subjects in the study
Jonckheere-Terpstra test of trend p <0.0001

Male Subjects

Statistics	NYHA Functional Classification			
	NYHA Class I	NYHA Class II	NYHA Class III	NYHA Class IV
N	8	105	327	228
Mean	4190	5380	6700	6980
SD	5910	9890	9710	7760
5th Percentile	156	404	516	733
Median	1480	2220	3730	4280
IQR	6360	4380	5700	7140
95th Percentile	16000	17900	20200	21600

Jonckheere-Terpstra test of trend p <0.0001

In the labeling, the sponsor states the following:

The Jonckheere-Terpstra test was used to determine that there is a statistically significant relationship between the median VITROS® NT-proBNP II test results and HF severity for:

- *All Subjects NYHA Class II–IV.*
- *Female Subjects NYHA Class II–IV.*
- *Male Subjects NYHA Class I–IV.*

These results show that there is a relationship between the median VITROS® NT-proBNP II test results and HF severity as determined by NYHA Class.

D Clinical Cut-Off:

Emergency Department Settings

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

VITROS® NT-proBNP II Test Results (pg/mL)	Age Group (Years)	Interpretation of Results
<300	All	Negative: Heart Failure Unlikely
≥300 to <450	22–<50	Gray Zone: Indeterminate – Consider other causes of NT-proBNP elevation*
≥300 to <900	50–<75	
≥300 to <1800	≥75	
≥450	22–<50	Positive: Heart Failure Likely
≥900	50–<75	
≥1800	≥75	

*Natriuretic peptides values in the gray zone could also be caused by several conditions other than heart failure. Clinical conditions such as valvular abnormalities, acute coronary syndrome, heart muscle disease, pulmonary embolism, pulmonary hypertension, sepsis, stroke, cardiotoxic

drugs, and renal dysfunction will elevate NT-proBNP levels; obesity, flash pulmonary edema, cardiac tamponade, and pericardial constriction are conditions associated with reduced NT-proBNP.

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

- *The posttest probability of heart failure in female subjects between the ages of 50 and <75 with a positive result was low (77.0%). In the study, 23% of female subjects between the ages of 50 and <75 with a positive test result did not have an adjudicated diagnosis of heart failure (HF, false positives).*
- *The posttest probability of heart failure in subjects with no history of HF with a positive result was low (69.0%). In the study, 31.0% of subjects with no history of HF who had a positive test result did not have an adjudicated diagnosis of HF (false positives).*
- *The posttest probability of not having heart failure in subjects with no history of HF between the ages of 22 and <50 with a negative result was low (81.8%). In the study, 18.2% of subjects between the ages of 22 and <50 with no history of HF with a negative test result had an adjudicated diagnosis of HF (false negatives).*
- *The posttest probability of heart failure in subjects with eGFR ≥60 mL/min/1.73 m2 ≥75 years of age with a positive result was low (78.2%). In the study, 21.8% of subjects with eGFR ≥60 mL/min/1.73 m2 ≥75 years of age with a positive test result did not have an adjudicated diagnosis of HF (false positives).*
- *The posttest probability of heart failure in subjects with BMI <30 kg/m2 between the ages of 22 and <50 with a positive test result was low (68.8%). In the study, 31.2% of subjects with BMI <30 kg/m2 between the ages of 22 and <50 with a positive test result did not have an adjudicated diagnosis of HF (false positives).*
- *The posttest probability of heart failure in subjects without Comorbidities (diabetes, renal insufficiency (eGFR values <60), hypertension (HTN) and/or chronic obstructive pulmonary disease (COPD)) between the ages of 22 and <50 was low (79.8%). In the study, 20.2% of subjects without comorbidities between the ages of 22 and <50 with a positive test result did not have an adjudicated diagnosis of HF (false positives).*

Outpatient Settings

For patients in the outpatient settings, the VITROS® NT-proBNP II test results should be interpreted as indicated in the table below.

VITROS® NT-proBNP II Test Results (pg/mL)	Age Group	Interpretation of Results
<125	All	Negative: Heart Failure Unlikely.
≥125	All	Consider Heart Failure as well as other causes* of NT-proBNP elevation.

* Natriuretic peptides values elevations could also be caused by several conditions other than heart failure such as valvular abnormalities, acute coronary syndrome, heart muscle disease, pulmonary embolism, pulmonary hypertension, sepsis, stroke, cardiotoxic drugs, and renal dysfunction.

The sponsor included the following statement in their instructions for use concerning the performance of their device in outpatient settings:

This test has very low positive predictive value (PPV) in an outpatient population (<23%). In the study, >77% of patients with a positive result did not have an adjudicated diagnosis of HF (false positives).

E Expected Values/Reference Range:

The reference interval was conducted in accordance with the CLSI EP28 guidance. The VITROS® NT-proBNP II Reagent Pack reference interval (RI) was established for six subgroups, based on age and gender from the serum of 385 female and 374 male healthy donors. The exclusion criteria used to exclude subjects were:

- Current smokers, subjects with cardiac conditions and disease, high blood pressure, kidney disease, diabetes, cancer within the last five years, stroke, and asthma or other lung disease within the last five years.
- Subjects who have reported high cholesterol, high triglycerides, thyroid disease, and females who are pregnant.
- Additional exclusion criteria:
    a. Troponin $\geq$ 99th percentile ($\geq$ 0.034 ng/mL - VITROS Troponin I ES assay)
    b. HbA1c $\geq$ 6.5%
    c. Creatinine (eGFR $\leq$ 60 mL/min)

Analysis at the 95% confidence level yields the reference ranges shown in the table.

Age	Gender	N	Reference interval lower limit (pg/mL)	Reference interval upper limit (pg/mL)
22 – <50	Female	129	<20.0	95.3
50 – <75	Female	127	<20.0	221
≥75	Female	129	<20.0	296
22 – <50	Male	131	<20.0	125
50 – <75	Male	120	<20.0	299
≥75	Male	123	<20.0	326
Overall		756	<20.0	217

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.