October 4, 2021

Ortho Clinical Diagnostics
Ann Quinn
Director, Regulatory Affairs
100 Indigo Creek Drive
Rochester, New York 14626

Re: K201312
Trade/Device Name: VITROS® Immunodiagnostic Products NT-proBNP II Reagent Pack
Regulation Number: 21 CFR 862.1117
Regulation Name: B-Type Natriuretic Peptide Test System
Regulatory Class: Class II
Product Code: NBC
Dated: November 6, 2020
Received: November 9, 2020

Dear Ann Quinn:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database located at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803) for devices or postmarketing safety reporting (21 CFR 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Marianela Perez-Torres, Ph.D.
Deputy Director
Division of Chemistry
and Toxicology Devices
OHT7: Office of In Vitro Diagnostics
and Radiological Health
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 06/30/2023
See PRA Statement below.

510(k) Number *(if known)*
K201312

Device Name
VITROS® Immunodiagnostic Products NT-proBNP II Reagent Pack

Indications for Use *(Describe)*
For in vitro diagnostic use only.

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.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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510(k) Summary

This summary of 510(k) safety and effectiveness information is being submitted in accordance with the requirements of SMDA 1990 and 21 CFR 807.92.

The assigned 510(k) number is: K201312

1. Submitter name, address, contact

Ortho-Clinical Diagnostics, Inc.
100 Indigo Creek Drive
Rochester, NY 14626
P: (585) 453-4152
F: (585) 453-3113
Contact Person: Ann Quinn, Director, Regulatory Affairs

2. Preparation Date

September 23, 2021

3. Device name

Trade or Proprietary Names:
VITROS® Immunodiagnostic Products NT-proBNP II Reagent Pack
Common Name: VITROS NT-proBNP II
Classification: B-Type natriuretic peptide test system (862.1117)
Class II
Product Code: NBC

4. Predicate Device

Roche Elecsys® proBNP II Immunoassay, K072437

5. Device description

The VITROS NT-proBNP II test is performed using the VITROS VITROS NT-proBNP II Reagent Pack and the VITROS NT-proBNP II Calibrators on the VITROS Systems.

The VITROS NT-proBNP II test 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 System. The amount of HRP conjugate bound is directly proportional to the concentration of NT-proBNP present.

6. Device intended use

Rx ONLY
For *in vitro* diagnostic use only.

For the quantitative measurement of N-terminal pro Brain Natriuretic Peptide (NT-proBNP) in human serum and plasma (K_2 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.

7. Comparison to predicate device:

The following tables provide a summary of the key features of the new device assessed against the predicate.

Device Characteristic	**New Device VITROS NT-proBNP II**	**Predicate Device Roche Elecsys proBNP II Immunoassay, K072437**
Intended Use	Rx ONLY For *in vitro* diagnostic use only. For the quantitative measurement of N-terminal pro Brain Natriuretic Peptide (NT-proBNP) in human serum and plasma (K_2 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.	For the quantitative determination of N-terminal pro-Brain natriuretic peptide in human serum and plasma. Elecsys proBNP II assay is used as an aid in the diagnosis of individuals suspected of having congestive heart failure. The test is further indicated for the risk stratification of patients with acute coronary syndrome and congestive heart failure. The test may also serve

<table>
<tr><th>Device Characteristic</th><th>New Device
VITROS NT-proBNP II</th><th>Predicate Device
Roche Elecsys proBNP II Immunoassay, K072437</th></tr>
<tr><td>Intended Use</td><td>See above</td><td>as an aid in the assessment of increased risk of cardiovascular events and mortality in patients at risk for heart failure who have stable coronary artery disease. The electro-chemiluminescense immunoassay “ECLIA” is intended for use on Elecsys and cobas e immunoassay analyzers.</td></tr>
<tr><td>Basic Principle</td><td>Sandwich immunoassay</td><td>Same</td></tr>
<tr><td>Antibody</td><td>Monoclonal anti-NT-proBNP</td><td>Same</td></tr>
<tr><td>Sample Type</td><td>Serum and plasma</td><td>Same</td></tr>
<tr><td>Measuring Range</td><td>20-30,000 pg/mL</td><td>5-35,000 pg/mL</td></tr>
<tr><td>Traceability</td><td>Standardized against the Elecsys proBNP II assay.</td><td>Standardized against the Elecsys proBNP assay.</td></tr>
<tr><td>Result Interpretation</td><td>For patients presenting to the ED settings with acute or worsening dyspnea and clinical suspicion of HF, the VITROS NT-proBNP II test results should be interpreted as indicated in the table below.
<table>
<tr><th>VITROS NT-proBNP II Test Results (pg/mL)</th><th>Age Group (Years)</th><th>Interpretation of Results</th></tr>
<tr><td><300</td><td>All</td><td>Negative: Heart Failure Unlikely</td></tr>
<tr><td></td><td></td><td></td></tr>
<tr><td>≥300 to <450</td><td>22-<50</td><td rowspan="3">Gray Zone: Result Indeterminate - Consider other causes of NT-proBNP elevation</td></tr>
<tr><td>≥300 to <900</td><td>50-<75</td></tr>
<tr><td>≥300 to <1800</td><td>≥75</td></tr>
<tr><td></td><td></td><td></td></tr>
<tr><td>≥450</td><td>22-<50</td><td rowspan="3">Positive: Heart Failure Likely</td></tr>
<tr><td>≥900</td><td>50-<75</td></tr>
<tr><td>≥1800</td><td>≥75</td></tr>
</table></td><td><75 years: 125 pg/mL
≥75 years: 450 pg/mL</td></tr>
</table>

<table>
<tr><th>Device Characteristic</th><th>New Device
VITROS NT-proBNP II</th><th>Predicate Device
Roche Elecsys proBNP II Immunoassay, K072437</th></tr>
<tr><td></td><td>For ambulatory patients presenting to outpatient facilities with clinical suspicion of HF not previously diagnosed and at least one sign, symptom or risk factor for HF, the VITROS NT-proBNP II test results should be interpreted as indicated in the table below.
<table>
<tr><th>VITROS NT-proBNP II Test Results (pg/mL)</th><th>Age Group</th><th>Interpretation of Results</th></tr>
<tr><td><125</td><td>All</td><td>Negative: Heart Failure Unlikely</td></tr>
<tr><td>≥125</td><td>All</td><td>Consider Heart Failure as well as other causes* of NT-proBNP elevation.</td></tr>
</table></td><td></td></tr>
</table>

8. Nonclinical performance

Several nonclinical tests were performed.

Precision

Precision was evaluated consistent with CLSI document EP05-A3, *Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline—Third Edition*. Two replicates of each of eleven fluids, eight human individual or serum pools and three controls, were tested on two separate occasions per day on at least 20 different test days. The experiment was performed using three Master Lots on one VITROS 3600 Immunodiagnostic System. The data presented are a representation of the product performance.

Conc. Units - pg/mL							No. Observations	No. Days
NT-proBNP Conc.	Within-run*		Within-cal**		Within-lab***			
	SD	%CV	SD	%CV	SD	%CV		
33.6	0.52	1.5	1.63	4.9	1.91	5.7	80	20
70.3	1.45	2.1	3.31	4.7	3.87	5.5	80	20
97.8	1.45	1.5	3.90	4.0	4.62	4.7	80	20
214	3.9	1.8	6.4	3.0	7.9	3.7	80	20
365	4.4	1.2	10.9	3.0	14.0	3.8	80	20
815	11.6	1.4	28.7	3.5	30.5	3.7	80	20
937	10.1	1.1	26.6	2.8	29.6	3.2	80	20
1730	19	1.1	86	5.0	84	4.8	80	20

Conc. Units - pg/mL							No. Observations	No. Days
NT-proBNP Conc.	Within-run*		Within-cal**		Within-lab***			
	SD	%CV	SD	%CV	SD	%CV		
5830	80	1.4	155	2.6	159	2.7	80	20
11300	110	1.0	210	1.9	270	2.4	80	20
24600	370	1.5	710	2.9	730	3.0	80	20

* **Within-run (repeatability)**. Between Duplicate precision averaged over all runs.

** **Within-calibration**. Total precision with weighted components of within-run, between-run, and between-day variation.

*** **Within-lab**. A measure of the effect of recalibration on total precision, calculated within reagent lot, using data from at least 4 calibrations.

An additional analysis was conducted to evaluate total imprecision. The data presented are a representation of the product performance.

System	Mean	Between Lot*		Between Day**		Between Runs***		Within Run / Residual****		Total*****		Observations
	pg/mL	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	
3600	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
	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	4.90	1.33	14.4	3.91	240
	823	15.9	1.94	11.6	1.40	20.5	2.49	12.5	1.52	31.0	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	1.50	761	3.08	240
	214	6.78	3.17	3.66	1.71	3.11	1.45	3.50	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

*Between lot: Variability of the test performance from lot to lot.

**Between day: Variability of the test performance from day to day.

***Between run: Variability of the test performance from run to run.

****Within Run / Residual Variability.

*****Total: Variability of the test incorporating factors of Instrument, lot, day and run.

Limit of Detection

Detection studies for the VITROS NT-proBNP II test were evaluated according to CLSI document EP17-A2, *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition.*

The Limit of Detection (LoD) for the VITROS NT-proBNP II test was designed to be less than or equal to 30.0 pg/mL. The observed LoD was determined to be 0.46 pg/mL. The claimed LoD is 0.49 pg/mL. The Limit of Quantitation (LoQ) for the VITROS NT-

proBNP II test was designed to be less than or equal to 30.0 pg/mL at 20% CV. The observed LoQ at 20% CV was determined to be 0.46 pg/mL. The claimed LoQ is set at 20.0 pg/mL to maintain linearity within the measuring range.

Limit of Detection and Limit of Quantitation

LoD	LoQ
pg/mL	pg/mL
0.49	20.0

Linearity

Linearity studies were performed according to CLSI document EP06-A, *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline*. Three Master Lots of VITROS NT-proBNP II test were tested on the VITROS 3600 Immunodiagnostic System. A low and high sample pool was prepared and mixed to give fourteen (14) further pools of intermediate concentrations. The low and high linearity pools and the interim dilutions between the low and high linearity pools were assayed in triplicate. The VITROS NT-proBNP II test was linear over the measuring range, from 20.0 to 30,000 pg/mL for the VITROS 3600 Immunodiagnostic System.

Matrix Comparison

A total of 160 unaltered matched serum, EDTA plasma and lithium heparin plasma samples covering the measuring range of the VITROS NT-proBNP II test were used to assess sample matrix differences between serum, EDTA plasma, and lithium heparin plasma samples.

Samples were collected into each of the following tube types: serum plus clot activator plastic tube (serum), K2-EDTA plasma plastic tube (EDTA), and lithium heparin plasma plastic tube (Li-Hep). Each serum sample was tested in duplicate while the Li-Hep plasma and EDTA plasma samples were assayed in singleton using the VITROS 3600 Immunodiagnostic System.

Overall, the results show EDTA and Li-Hep plasma plastic collection tubes did not show a significant effect on the test results when compared to serum and met the acceptance criteria. All three tube types, serum, EDTA plasma, and Li-Hep plasma are suitable for use with the VITROS NT-proBNP II test.

Analytical Specificity

The VITROS NT-proBNP II test was evaluated for interference according to CLSI guidelines EP07 3rd ed – *Interference Testing in Clinical Chemistry*. Commonly encountered substances were tested on three Master Lots of reagents. Of the compounds tested in the table below, none was found to cause a bias of >10% with the test at the concentrations indicated at nominal NT-proBNP concentrations of 125 pg/mL (14.8 pmol/L) and 2000 pg/mL (236 pmol/L).

Compound	Concentration			Compound	Concentration	
Acetaminophen	156 µg/mL	1030 µmol/L		Ibuprofen	21.9 mg/dL	1.06 mmol/L
Acetylcysteine	15.0 mg/dL	920 µmol/L		Insulin	3.12 µg/dL	5.37 nmol/L
Adrenaline (Epinephrine)	20.0 µg/dL	1.09 µmol/L		Intralipid	2.00 g/dL	NA
Alprazolam	25.8 µg/dL	836 nmol/L		L-dopa (Levodopa)	750 µg/dL	38.0 µmol/L
Amlodipine besylate	10.5 µg/dL	184 nmol/L		Levothyroxine	42.9 µg/dL	552 nmol/L
Amoxicillin	5.40 mg/dL	148 µmol/L		Lidocaine	1.50 mg/dL	64.0 µmol/L
Ascorbic acid	5.25 mg/dL	298 µmol/L		Methyldopa sesquihydrate	2.25 mg/dL	94.4 µmol/L
Atorvastatin calciumtrihydrate	162 µg/dL	1.34 µmol/L		Methylprednisolone	783 µg/dL	20.9 µmol/L
Benazepril HCl	44.0 µg/dL	955 nmol/L		Metoprolol hemitartrate	150 µg/dL	2.19 µmol/L
Bilirubin, conjugated	40.0 mg/dL	474 µmol/L		Metronidazole	12.3 mg/dL	718 µmol/L
Bilirubin, unconjugated	40.0 mg/dL	684 µmol/L		Molsidomine	18.0 µg/dL	743 nmol/L
Biotin	3510 ng/mL	14.4 µmol/L		Naproxen sodium	39.3 mg/dL	1.56 mmol/L
Caffeine	10.8 mg/dL	556 µmol/L		Nicardipine HCL	46.5 µg/dL	901 nmol/L
Carvedilol	43.2 µg/dL	1.06 µmol/L		Nifedipine	58.8 µg/dL	1.70 µmol/L
Ceftriaxone disodium hemi(heptahydrate)	100 mg/dL	1510 µmol/L		Omeprazole	840 µg/dL	24.3 µmol/L
Cholesterol	400 mg/dL	10.3 mmol/L		Oxycodone HCl	32.4 µg/dL	0.92 µmol/L
Clopidogrel hydrogensulfate	2.40 µg/dL	57.2 nmol/L		Phenobarbital	69.0 mg/dL	2.97 mmol/L
Cotinine	240 µg/dL	13.6 µmol/L		Phenprocoumon(Marcumar)	1.50 mg/dL	53.5 µmol/L
Creatinine	15.0 mg/dL	1.33 mmol/L		Propafenone HCL	72.0 µg/dL	1.91 µmol/L
Cyclosporine	180 µg/dL	1.50 µmol/L		Pseudoephedrine HCl	330 µg/dL	16.4 µmol/L
Dextran	2.40 g/dL	600 µmol/L		Rheumatoid Factor	1500 IU/mL	NA
Digitoxin	7.50 µg/dL	98.0 nmol/L		Rifampicin (Rifampin)	4.80 mg/dL	58.3 µmol/L
Digoxin	3.90 µg/dL	49.9 nmol/L		Salicylic acid	2.86 mg/dL	207 µmol/L
Diphenhydramine HCl	77.4 µg/dL	2.65 µmol/L		Salmeterol	1.65 µg/dL	39.7 nmol/L
Dipyrone (as 4-methylaminoantipyrine Hydrochloride)	3.30 mg/dL	130 µmol/L		Sotalol hydrochloride	510 µg/dL	16.5 µmol/L
Dypyridamole	1.00 mg/dL	19.8 µmol/L		Spironolactone	55.5 µg/dL	1.33 µmol/L
Doxycycline hyclate	1.80 mg/dL	35.1 µmol/L		Streptokinase	150,000 U/dL	NA
Enalaprilat dihydrate	81.9 µg/dL	2.13 µmol/L		Theophylline	6.00 mg/dL	333 µmol/L
Ethanol	600 mg/dL	130 mmol/L		Tolbutamide	54.9 mg/dL	2.03 mmol/L
Fibrinogen	1000 mg/dL	NA		Total Protein	15.0 g/dL	NA
Furosemide	1.59 mg/dL	48.1 µmol/L		tPA (Alteplase)	1.20 mg/dL	NA
Gentamicin Sulfate	3.51 mg/dL	61.0 µmol/L		Triglyceride	1500 mg/dL	16.9 mmol/L
Glycerylnitrate (Nitroglycerin)	1.20 µg/dL	52.8 nmol/L		Valproic Acid	31.8 mg/dL	2.21 mmol/L
HAMA (Human Anti-MouseAntibody	800 µg/L	NA		Vancomycin Hydrochloride	12.3 mg/dL	82.8 µmol/L
Hemoglobin	1000 mg/dL	155 µmol/L		Verapamil Hydrochloride	160 µg/dL	3.26 µmol/L
Heparin (Sodium), UFH	330 U/dL	NA		Warfarin	8.00 mg/dL	260 µmol/L

The substances shown to interfere with the VITROS NT-proBNP II test are provided in the table below. These results are representative. The degree of interference at concentrations other than those listed might not be predictable from these results. Other interfering substances may be encountered in the patient population.

Interferent	Interferent Concentration		Measured NT-proBNP Concentration*		% Bias**
			pg/mL	pmol/L	
Cefoxitin sodium	697 mg/dL	15.5 mmol/L	84.3	9.95	-24.1
			922	109	-19.1
	311 mg/dL	6.92 mmol/L	105	12.4	-10.0
	306 mg/dL	6.81 mmol/L	806	95.1	-10.0
Sodium Azide	100 mg/dL	15.4 mmol/L	91.4	10.8	-12.1
	85.9 mg/dL	13.2 mmol/L	93.6	11.0	-10.0

* Average test replicate determinations.

** Estimate of the maximum difference observed as a percentage.

The cross-reactivity of the VITROS NT-proBNP II test was evaluated by adding the following substances to a human serum sample containing no NT-proBNP.

Cross-Reactant	Concentration		Mean Result of Control Sample		Mean Result of Cross-Reactant Sample		% Cross-Reactivity
			pg/mL	pmol/L	pg/mL	pmol/L	
ANP_{28}	3.10 µg/mL	1.01 nmol/L	*	*	*	*	*
proBNP (glycosylated)	3000 pg/mL	N/A	-0.14	-0.02	57.6	6.80	1.9
proBNP (nonglycosylated)	3000 pg/mL	0.249 nmol/L	-0.48	-0.06	863	102	28.8
NT-proANP$_{1–30}$ (preproANP$_{25–55}$)	3.50 µg/mL	0.998 µmol/L	*	*	*	*	*
NT-proANP$_{31–67}$ (preproANP$_{56–92}$)	1.00 ng/mL	0.258 nmol/L	*	*	*	*	*
NT-proANP$_{79–98}$ (preproANP$_{104–123}$)	1.00 ng/mL	0.458 nmol/L	*	*	*	*	*
BNP_{32} (Natrecor®)	3.50 µg/mL	1.01 µmol/L	*	*	*	*	*
CNP_{22}	2.20 µg/mL	1.00 µmol/L	*	*	*	*	*
Adrenomedullin	1.00 ng/mL	0.166 nmol/L	*	*	*	*	*
Aldosterone	0.600 ng/mL	1.66 nmol/L	*	*	*	*	*
Angiotensin I	0.600 ng/mL	0.463 nmol/L	*	*	*	*	*
Angiotensin II	0.600 ng/mL	0.574 nmol/L	*	*	*	*	*
Angiotensin III	1.00 ng/mL	1.07 nmol/L	*	*	*	*	*
Endothelin	20.0 pg/mL	8.03 pmol/L	*	*	*	*	*
Urodilatin	3.50 µg/mL	0.998 µmol/L	*	*	*	*	*
Arg-Vasopressin	1.00 µg/mL	0.922 µmol/L	*	*	*	*	*
Renin	50.0 ng/mL	28.4 nmol/L	*	*	*	*	*

*Not Detectable (ND). Concentration was below the measuring range of the test, 20.0–30,000 pg/mL (2.36–3,540 pmol/L).

The cross-reactivity of the VITROS NT-proBNP II test was evaluated by adding the following substances to a human serum sample containing NT-proBNP at a concentration of 125 pg/mL (14.8 pmol/L).

Cross-Reactant	Concentration		Mean NT-proBNP Result of Control Sample		Mean NT-proBNP Result of Cross-Reactant Sample		% Cross-Reactivity
			pg/mL	pmol/L	pg/mL	pmol/L	
ANP_{28}	3.10 µg/mL	1.01 nmol/L	112	13.2	113	13.3	<1.0
proBNP (glycosylated)	3000 pg/mL	N/A	105	12.4	183	21.6	2.6
proBNP (nonglycosylated)	3000 pg/mL	0.249 nmol/L	118	13.9	1290	152	39.1

NT-proANP$_{1–30}$ (preproANP$_{25–55}$)	3.50 µg/mL	0.998 µmol/L	113	13.3	775	91.5	<1.0
NT-proANP$_{31–67}$ (preproANP$_{56–92}$)	1.00 ng/mL	0.258 nmol/L	112	13.2	113	13.3	<1.0
NT-proANP$_{79–98}$ (preproANP$_{104–123}$)	1.00 ng/mL	0.458 nmol/L	112	13.2	114	13.5	<1.0
BNP$_{32}$ (Natrecor®)	3.50 µg/mL	1.01 µmol/L	112	13.2	115	13.6	<1.0
CNP$_{22}$	2.20 µg/mL	1.00 µmol/L	112	13.2	114	13.5	<1.0
Adrenomedullin	1.00 ng/mL	0.166 nmol/L	112	13.2	114	13.5	<1.0
Aldosterone	0.600 ng/mL	1.66 nmol/L	112	13.2	117	13.8	<1.0
Angiotensin I	0.600 ng/mL	0.463 nmol/L	112	13.2	112	13.2	<1.0
Angiotensin II	0.600 ng/mL	0.574 nmol/L	112	13.2	111	13.1	<1.0
Angiotensin III	1.00 ng/mL	1.07 nmol/L	112	13.2	112	13.2	<1.0
Endothelin	20.0 pg/mL	8.03 pmol/L	113	13.3	113	13.3	<1.0
Urodilatin	3.50 µg/mL	0.998 µmol/L	112	13.2	114	13.5	<1.0
Arg-Vasopressin	1.00 µg/mL	0.922 µmol/L	112	13.2	117	13.8	<1.0
Renin	50.0 ng/mL	28.4 nmol/L	112	13.2	112	13.2	<1.0

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 in percentage terms.

Dilution

Serum or plasma (K_2 EDTA or Lithium Heparin) samples with concentrations greater than the measuring range may be automatically diluted on the system up to 10-fold (1 part sample with 9 parts diluent) by the VITROS 3600 Immunodiagnostic System with the VITROS High Sample Diluent B Reagent Pack prior to testing.

High Dose Hook

The VITROS NT-proBNP II test has no high dose hook effect up to a concentration of 300,000 pg/mL.

Expected Values

It is recommended that each laboratory establish its own expected values for the population it serves. The VITROS NT-proBNP II test Reference Interval (RI) was established for six subgroups, based on age and gender from the serum of 385 female and 374 male healthy donors.

Subjects were excluded if they met any of the following exclusion criteria:

- 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:
  - Troponin $\geq$ 99th percentile ($\geq$ 0.034 ng/mL - VITROS Troponin I ES assay)
  - HbA1c $\geq$ 6.5%
  - Creatinine (eGFR $\leq$ 60 mL/min)

The reference interval was conducted in accordance with the CLSI EP28. Analysis at the 95% confidence level yields the ranges shown in the table.

Age	Gender	n	RI Lower Limit (pg/mL)	RI 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

9. Clinical performance

The clinical performance information should only be used as a guide. It is recommended that each laboratory determine and confirm the diagnostic cutoffs for the population it serves.

Aid in Diagnosis of Heart Failure

Emergency Department Setting

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 test. Subjects 22 years and older presenting to the Emergency Department (ED) with dyspnea (acute or worsening) and clinical suspicion of heart failure (HF) were enrolled into 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. Individuals in the population were African American (36.55%) and Caucasian (59.59%), with the remaining 3.86% represented by other races. Dyspnea was acute in 44.09%, worsening in 55.45% and not specified in 0.45% of subjects.

The VITROS NT-proBNP II test results were determined from 2200 ED subjects, 1016 (46.18%) females and 1184 (53.82%) males, ranging in age from 22 to 106 years. The descriptive statistics for the VITROS NT-proBNP II test results (pg/mL) were determined within and across gender by age group and are summarized in the following tables:

All Subjects

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

Female Subjects

Study Population	Heart Failure				Non-Heart Failure			
Age (years)	**22–<50**	**50–<75**	**≥75**	**All**	**22–<50**	**50–<75**	**≥75**	**All**
N	43	194	217	454	71	306	185	562
Mean	10300	6830	7790	7620	196	648	948	690
SD	32200	10000	10600	13900	356	1800	1280	1540
Median	1920	3620	4710	3850	68.2	157	444	226
Min	20.0	102	296	20.0	20.0	20.0	20.0	20.0
Max	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**
N	71	344	226	641	70	324	149	543
Mean	4720	6330	7830	6680	326	737	1330	846
SD	6780	9170	9730	9180	718	1640	2080	1720
Median	2260	3480	5020	3690	43.6	203	519	275
Min	195	53.7	295	53.7	20.0	20.0	20.0	20.0
Max	39200	70400	86000	86000	4710	17100	13600	17100

The area under the Receiver Operating Characteristic (ROC) curve (AUC) with a 95% confidence interval (CI) for the VITROS NT-proBNP II test within and across age groups within and across gender are presented in the table below. The AUC ranged between 0.904 to 0.954 within and across gender.

Age Group	All Subjects		Female Subjects		Male Subjects	
	AUC	**95% Confidence Interval**	**AUC**	**95% Confidence Interval**	**AUC**	**95% Confidence Interval**
22–<50 years	0.954	0.928–0.979	0.952	0.905–0.999	0.947	0.912–0.983
50–<75 years	0.922	0.907–0.937	0.926	0.904–0.948	0.917	0.896–0.938
≥75 years	0.915	0.895–0.934	0.920	0.895–0.946	0.904	0.872–0.937
Overall	0.920	0.909–0.931	0.925	0.910–0.941	0.914	0.898–0.930

AUC analyses were also performed for relevant clinical subgroups. The AUC with a 95% confidence interval (CI) for the VITROS NT-proBNP II test across gender within the relevant clinical subgroups are presented in the table below. The AUC of the VITROS NT-proBNP II test for each of the relevant clinical subgroups for subjects with or without the condition was greater than or equal to 0.899.

Relevant Clinical Subgroups					
Subjects With the Condition			**Subjects Without the Condition**		
Subgroups	**AUC**	**95% Confidence Interval**	**Subgroups**	**AUC**	**95% Confidence Interval**
History of HF (N=1220)	0.899	0.881–0.918	No History of HF (N=970)	0.932	0.917–0.947
eGFR <60* mL/min/1.73 m^2 (N=1012)	0.900	0.880–0.920	eGFR ≥60 mL/min/1.73 m^2 (N=1166)	0.923	0.908–0.938
BMI ≥30.0 kg/m^2 (N=1160)	0.903	0.885–0.920	BMI <30.0 kg/m^2 (N=1025)	0.945	0.931–0.958
With Comorbidities** (N=1978)	0.915	0.902–0.927	Without Comorbidities*** (N=222)	0.944	0.915–0.974

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

** Subjects with at least one of the following: diabetes, renal insufficiency (eGFR values <60), hypertension (HTN) and/or chronicobstructive pulmonary disease (COPD)

*** Subjects without any of the following: diabetes, renal insufficiency (eGFR values <60), hypertension (HTN) and/or chronic obstructivepulmonary disease (COPD)

Distribution of VITROS NT-proBNP II test results after applying the multiple cutoffs, 300 pg/mL for all age groups and 450pg/mL for 22–<50 years old, 900 pg/mL for 50–<75 years old, 1800 pg/mL for ≥75 years old, versus adjudicated diagnosiswas summarized within and across age groups across gender and is presented in the table below.

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 Result Interpretation	Positive	372	62	434
		Gray Zone	69	153	222
		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 following analyses were conducted to determine clinical performance:

VITROS NT-proBNP II Test Results Classification	Adjudicated Diagnosis		Total
	HF	Non-HF	
Positive: Heart Failure Likely	A	B	A+B
Gray Zone: Result Indeterminate	C	D	C+D
Negative: Heart Failure Unlikely	E	F	E+F
Total	A+C+E	B+D+F	A+B+C+D+E+F

Pretest Probability of HF (prevalence of HF in the study) = (A+C+E)/(A+B+C+D+E+F)

Posttest Probability of HF

Posttest probability of HF for positive test results = A/(A+B)

Posttest probability of HF for Gray zone test results = C/(C+D)

Posttest Probability of non-HF

Posttest probability of non-HF for Gray zone test results = D/(C+D)

Posttest probability of non-HF for negative test results = F/(E+F)

Likelihood ratios (LR) of HF given VITROS NT-proBNP II test result category

LR positive = (A/(A+C+E))/(B/(B+D+F))

LR gray zone = (C/(A+C+E))/(D/(B+D+F))

LR negative = (E/(A+C+E))/(F/(B+D+F))

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 gender 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)	57.0 (443/777)	Positive	**85.7 (372/434)**	82.1–88.7	–	–	4.52	3.60–5.68
		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)	37.7% (43/114)	Positive	**88.6 (39/44)**	76.0–95.0	–	–	12.88	5.50–30.15
		Gray zone	11.1 (1/9)	2.0–43.5	88.9 (8/9)	56.5–98.0	0.21	0.03–1.59
		Negative	–	–	**95.1 (58/61)**	86.5–98.3	0.09	0.03–0.26
50–<75 (N=500)	38.8% (194/500)	Positive	**77.0 (167/217)**	70.9–82.1	–	–	5.27	4.06–6.83
		Gray zone	26.4 (23/87)	18.3–36.6	73.6 (64/87)	63.4–81.7	0.57	0.36–0.88
		Negative	–	–	**98.0 (192/196)**	94.9–99.2	0.03	0.01–0.09
≥75 (N=402)	54.0% (217/402)	Positive	**85.9 (176/205)**	80.4–90.0	–	–	5.17	3.68–7.27
		Gray zone	30.5 (40/131)	23.3–38.9	69.5 (91/131)	61.1–76.7	0.37	0.27–0.51
		Negative	–	–	**98.5 (65/66)**	91.9–99.7	0.01	0.00–0.09

*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)	50.4% (71/141)	Positive	**82.5 (66/80)**	72.7–89.3	–	–	4.65	2.90–7.46
		Gray zone	44.4 (4/9)	18.9–73.3	55.6 (5/9)	26.7–81.1	0.79	0.22–2.82
		Negative	–	–	**98.1 (51/52)**	89.9–99.7	0.02	0.00–0.14
50–<75 (N=668)	51.5% (344/668)	Positive	**82.4 (305/370)**	78.2–86.0	–	–	4.42	3.54–5.51
		Gray zone	31.0 (36/116)	23.3–39.9	69.0 (80/116)	60.1–76.7	0.42	0.29–0.61
		Negative	–	–	**98.4 (179/182)**	95.3–99.4	0.02	0.01–0.05
≥75 (N=375)	60.3% (226/375)	Positive	**85.6 (196/229)**	80.5–89.6	–	–	3.92	2.89–5.31
		Gray zone	31.9 (29/91)	23.2–42.0	68.1 (62/91)	58.0–76.8	0.31	0.21–0.46
		Negative	–	–	**98.2 (54/55)**	90.4–99.7	0.01	0.00–0.09

*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)**	85.4–96.7	–	–	4.69	2.28–9.65
		Gray zone	36.4 (4/11)	15.2–64.6	63.6 (7/11)	35.4–84.8	0.20	0.06–0.65
		Negative	–	–	**81.8 (18/22)**	61.5–92.7	0.08	0.03–0.22
50–<75 (N=642)	63.1% (405/642)	Positive	**87.2 (355/407)**	83.6–90.1	–	–	4.00	3.13–5.09
		Gray zone	38.1 (43/113)	29.6–47.3	61.9 (70/113)	52.7–70.4	0.36	0.25–0.51
		Negative	–	–	**94.3 (115/122)**	88.6–97.2	0.04	0.02–0.08
≥75 (N=460)	71.1% (327/460)	Positive	**90.5 (275/304)**	86.6–93.3	–	–	3.86	2.79–5.34
		Gray zone	41.1 (51/124)	32.9–49.9	58.9 (73/124)	50.1–67.1	0.28	0.21–0.38
		Negative	–	–	**96.9 (31/32)**	83.4–99.4	0.01	0.00–0.10

*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)**	51.0–79.4	–	–	8.15	4.86–13.65
		Gray zone	14.3 (1/7)	2.6–51.3	85.7 (6/7)	48.7–97.4	0.68	0.09–5.41
		Negative	–	–	**100.0 (91/91)**	95.9–100.0	0.00	N/A
50–<75 (N=521)	25.3% (132/521)	Positive	**65.2 (116/178)**	57.9–71.8	–	–	5.51	4.35–6.99
		Gray zone	17.8 (16/90)	11.2–26.9	82.2 (74/90)	73.1–88.8	0.64	0.39–1.05
		Negative	–	–	**100.0 (253/253)**	98.5–100.0	0.00	N/A
≥75 (N=312)	36.9% (115/312)	Positive	**75.0 (96/128)**	66.8–81.7	–	–	5.14	3.70–7.13
		Gray zone	18.8 (18/96)	12.2–27.7	81.3 (78/96)	72.3–87.8	0.40	0.25–0.62
		Negative	–	–	**98.9 (87/88)**	93.8–99.8	0.02	0.00–0.14

*95% Wilson Score Confidence Interval

** Log Method Confidence Interval

N/A: Not applicable

Subjects with Renal Disease (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)	62.3% (43/69)	Positive	**87.2 (41/47)**	74.8–94.0	–	–	4.13	2.04–8.36
		Gray zone	16.7 (1/6)	3.0–56.4	83.3 (5/6)	43.6–97.0	0.12	0.01–0.98
		Negative	–	–	**93.8 (15/16)**	71.7–98.9	0.04	0.01–0.29
50–<75 (N=476)	56.5% (269/476)	Positive	**79.6 (250/314)**	74.8–83.7	–	–	3.01	2.45–3.69
		Gray zone	24.7 (18/73)	16.2–35.6	75.3 (55/73)	64.4–83.8	0.25	0.15–0.42
		Negative	–	–	**98.9 (88/89)**	93.9–99.8	0.01	0.00–0.06
≥75 (N=467)	68.1% (318/467)	Positive	**88.6 (273/308)**	84.6–91.7	–	–	3.65	2.73–4.90
		Gray zone	37.6 (44/117)	29.4–46.6	62.4 (73/117)	53.4–70.6	0.28	0.21–0.39
		Negative	–	–	**97.6 (41/42)**	87.7–99.6	0.01	0.00–0.08

*95% Wilson Score Confidence Interval

** Log Method Confidence Interval

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

Subjects Without Renal Disease (eGFR ≥60 mL/min/1.73 m²)

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)	38.7% (70/181)	Positive	**82.9 (63/76)**	72.9–89.7	–	–	7.68	4.58–12.88
		Gray zone	36.4 (4/11)	15.2–64.6	63.6 (7/11)	35.4–84.8	0.91	0.28–2.98
		Negative	–	–	**96.8 (91/94)**	91.0–98.9	0.05	0.02–0.16
50–<75 (N=679)	38.7% (263/679)	Positive	**81.0 (217/268)**	75.8–85.2	–	–	6.73	5.17–8.76
		Gray zone	31.3 (40/128)	23.9–39.7	68.8 (88/128)	60.3–76.1	0.72	0.51–1.01
		Negative	–	–	**97.9 (277/283)**	95.5–99.0	0.03	0.02–0.08
≥75 (N=306)	40.2% (123/306)	Positive	**78.2 (97/124)**	70.2–84.6	–	–	5.35	3.73–7.66
		Gray zone	24.0 (25/104)	16.8–33.1	76.0 (79/104)	66.9–83.2	0.47	0.32–0.69
		Negative	–	–	**98.7 (77/78)**	93.1–99.8	0.02	0.00–0.14

*95% Wilson Score Confidence Interval

** Log Method Confidence Interval

Subjects With BMI ≥30.0 kg/m²

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)**	82.4–94.8	–	–	9.62	5.15–17.99
		Gray zone	27.8 (5/18)	12.5–50.9	72.2 (13/18)	49.1–87.5	0.40	0.15–1.08
		Negative	–	–	**94.9 (74/78)**	87.5–98.0	0.06	0.02–0.15
50–<75 (N=693)	46.3% (321/693)	Positive	**80.9 (266/329)**	76.3–84.7	–	–	4.89	3.89–6.16
		Gray zone	36.1 (48/133)	28.4–44.5	63.9 (85/133)	55.5–71.6	0.65	0.47–0.90
		Negative	–	–	**97.0 (224/231)**	93.9–98.5	0.04	0.02–0.08
≥75 (N=279)	55.9% (156/279)	Positive	**84.6 (121/143)**	77.8–89.6	–	–	4.34	2.94–6.39
		Gray zone	37.8 (34/90)	28.5–48.1	62.2 (56/90)	51.9–71.5	0.48	0.34–0.68
		Negative	–	–	**97.8 (45/46)**	88.7–99.6	0.02	0.00–0.13

*95% Wilson Score Confidence Interval

** Log Method Confidence Interval

Subjects With BMI <30.0 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)**	51.4–82.0	–	–	4.00	2.34–6.84
		Gray zone	∞ (0/0)	N/A	∞ (0/0)	N/A	∞	N/A
		Negative	–	–	**100.0 (30/30)**	88.6–100.0	0.00	N/A
50–<75 (N=465)	46.5% (216/465)	Positive	**79.8 (206/258)**	74.5–84.3	–	–	4.57	3.58–5.83
		Gray zone	14.7 (10/68)	8.2–25.0	85.3 (58/68)	75.0–91.8	0.20	0.10–0.38
		Negative	–	–	**100.0 (139/139)**	97.3–100.0	0.00	N/A
≥75 (N=498)	57.6% (287/498)	Positive	**86.3 (251/291)**	81.8–89.7	–	–	4.61	3.48–6.12
		Gray zone	26.5 (35/132)	19.7–34.6	73.5 (97/132)	65.4–80.3	0.27	0.19–0.37
		Negative	–	–	**98.7 (74/75)**	92.8–99.8	0.01	0.00–0.07

*95% Wilson Score Confidence Interval

** Log Method Confidence Interval

N/A: Not applicable

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)**	79.0–92.0	–	–	5.36	3.32–8.66
		Gray zone	23.1 (3/13)	8.2–50.3	76.9 (10/13)	49.7–91.8	0.24	0.07–0.86
		Negative	–	–	**94.9 (56/59)**	86.1–98.3	0.04	0.01–0.13
50–<75 (N=1066)	47.3% (504/1066)	Positive	**80.2 (438/546)**	76.7–83.3	–	–	4.52	3.80–5.38
		Gray zone	32.1 (59/184)	25.7–39.1	67.9 (125/184)	60.9–74.3	0.53	0.40–0.70
		Negative	–	–	**97.9 (329/336)**	95.8–99.0	0.02	0.01–0.05
≥75 (N=734)	57.2% (420/734)	Positive	**86.1 (352/409)**	82.4–89.1	–	–	4.62	3.64–5.86
		Gray zone	31.7 (66/208)	25.8–38.3	68.3 (142/208)	61.7–74.2	0.35	0.27–0.45
		Negative	–	–	**98.3 (115/117)**	94.0–99.5	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)**	49.1–87.5	–	–	9.91	4.14–23.71
		Gray zone	40.0 (2/5)	11.8–76.9	60.0 (3/5)	23.1–88.2	2.54	0.46–13.95
		Negative	–	–	**98.1 (53/54)**	90.2–99.7	0.07	0.01–0.48
50–<75 (N=102)	33.3% (34/102)	Positive	**82.9 (34/41)**	68.7–91.5	–	–	9.71	4.82–19.59
		Gray zone	0.0 (0/19)	0.0–16.8	100.0 (19/19)	83.2–100.0	0.00	N/A
		Negative	–	–	**100.0 (42/42)**	91.6–100.0	0.00	N/A
≥75 (N=43)	53.5% (23/43)	Positive	**80.0 (20/25)**	60.9–91.1	–	–	3.48	1.60–7.55
		Gray zone	21.4 (3/14)	7.6–47.6	78.6 (11/14)	52.4–92.4	0.24	0.08–0.73
		Negative	–	–	**100.0 (4/4)**	51.0–100.0	0.00	N/A

*95% Wilson Score Confidence Interval

** Log Method Confidence Interval

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

N/A: Not applicable

Outpatient Setting

The VITROS NT-proBNP II test results were also 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. The prevalence of HF in the population was 6.18% (48/777). Individuals in the population were African American (31.66%) and Caucasian (64.74%), with the remaining 3.60% represented by other races.

The AUC with a 95% CI for the VITROS NT-proBNP II test for subjects presenting to Cardiology Clinics and other outpatient facilities is 0.880 (CI 0.822 to 0.937).

AUC analyses were also performed for relevant clinical subgroups. The AUC with a 95% confidence interval (CI) for the VITROS NT-proBNP II test across gender within the relevant clinical subgroups are presented in the table below. The AUC of the VITROS NT-proBNP II test for each of the relevant clinical subgroups for subjects with or without the condition was greater than or equal to 0.838.

Relevant Clinical Subgroups					
Subjects With the Condition			**Subjects Without the Condition**		
Subgroups	**AUC**	**95% Confidence Interval**	**Subgroups**	**AUC**	**95% Confidence Interval**
≥75 Years old (N=139)	0.838	0.683–0.994	<75 Years old (N=638)	0.894	0.775–1.000
eGFR <60* mL/min/1.73 m^2 (N=81)	0.843	0.655–1.000	eGFR ≥60 mL/min/1.73 m^2 (N=245)	0.940	0.888–0.993
BMI ≥30.0 kg/m^2 (N=430)	0.875	0.739–1.000	BMI <30.0 kg/m^2 (N=346)	0.930	0.858–1.000
With Comorbidities** (N=667)	0.892	0.790–0.993	Without Comorbidities*** (N=110)	0.929	0.799–1.000

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

** 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)

Distribution of VITROS NT-proBNP II test results after applying the rule-out cutoff (125 pg/mL) versus adjudicated diagnosis was summarized for all subjects and is presented in the table below.

VITROS NT-proBNP II Test Results versus Adjudicated Diagnosis

VITROS NT-proBNP II Test Result Interpretation	**Adjudicated Diagnosis**		**Total**
	HF	**Non-HF**	
Positive: Heart Failure Likely	44	239	283
Negative: Heart Failure Unlikely	4	490	494
Total	48	729	777

The clinical performance and the two-tailed 95% CIs of the VITROS NT proBNP II test versus adjudicated diagnosis for subjects presenting to Cardiology Clinics and other outpatient facilities was determined using the rule-out cutoff (125 pg/mL) and is 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

Group	Cutoff (pg/mL)	LR-	95% CI	LR+	95% CI
All Subjects (N=777)	125	0.12	0.05–0.31	2.80	2.44–3.20

The clinical performance and the two-tailed 95% CIs of the VITROS NT proBNP II test versus adjudicated diagnosis for subjects presenting to Cardiology Clinics and other outpatient facilities was also determined using the rule-out cutoff (125 pg/mL) for relevant clinical subgroups and are summarized in the following tables:

Age Group

Relevant Clinical Subgroups	Cutoff (pg/mL)	Sensitivity (%) (n/N)	95% CI* (%)	Specificity (%) (n/N)	95% CI* (%)	NPV (%) (n/N)	95% CI* (%)	PPV (%) (n/N)	95% CI* (%)
≥75 Years old (N=139)	125	93.8 (15/16)	69.8–99.8	36.6 (45/123)	28.1–45.7	97.8 (45/46)	88.5–99.9	16.1 (15/93)	9.3–25.2
<75 Years old (N=638)	125	90.6 (29/32)	75.0–98.0	73.4 (445/606)	69.7–76.9	99.3 (445/448)	98.1–99.9	15.3 (29/190)	10.5–21.2

*95% Exact Confidence Interval

Relevant Clinical Subgroups	Cutoff (pg/mL)	LR-	95% CI	LR+	95% CI
≥75 Years old (N=139)	125	0.17	0.03–1.15	1.48	1.23–1.78
<75 Years old (N=638)	125	0.13	0.04–0.38	3.41	2.87–4.06

Renal Disease

Relevant Clinical Subgroups	Cutoff (pg/mL)	Sensitivity (%) (n/N)	95% CI* (%)	Specificity (%) (n/N)	95% CI* (%)	NPV (%) (n/N)	95% CI* (%)	PPV (%) (n/N)	95% CI* (%)
eGFR <60** mL/min/1.73 m^2 (N=81)	125	84.6 (11/13)	54.6–98.1	45.6 (31/68)	33.5–58.1	93.9 (31/33)	79.8–99.3	22.9 (11/48)	12.0–37.3
eGFR ≥ 60 mL/min/1.73 m^2 (N=245)	125	100.0 (13/13)	75.3–100.0	68.5 (159/232)	62.1–74.5	100.0 (159/159)	97.7–100.0	15.1 (13/86)	8.3–24.5

*95% Exact Confidence Interval

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

Relevant Clinical Subgroups	Cutoff (pg/mL)	LR-	95% CI	LR+	95% CI
eGFR <60* mL/min/1.73 m^2 (N=81)	125	0.34	0.09–1.25	1.56	1.13–2.14
eGFR ≥ 60 mL/min/1.73 m^2 (N=245)	125	0.00	N/A	3.18	2.63–3.84

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

N/A: Not applicable

BMI

Relevant Clinical Subgroups	Cutoff (pg/mL)	Sensitivity (%) (n/N)	95% CI* (%)	Specificity (%) (n/N)	95% CI* (%)	NPV (%) (n/N)	95% CI* (%)	PPV (%) (n/N)	95% CI* (%)
BMI ≥30.0 kg/m² (N=430)	125	82.6 (19/23)	61.2–95.0	76.2 (310/407)	71.7–80.2	98.7 (310/314)	96.8–99.7	16.4 (19/116)	10.2–24.4
BMI <30.0 kg/m² (N=346)	125	100.0 (24/24)	85.8–100.0	55.9 (180/322)	50.3–61.4	100.0 (180/180)	98.0–100.0	14.5 (24/166)	9.5–20.7

*95% Exact Confidence Interval

Relevant Clinical Subgroups	Cutoff (pg/mL)	LR-	95% CI	LR+	95% CI
BMI ≥30.0 kg/m² (N=430)	125	0.23	0.09–0.56	3.47	2.68–4.48
BMI <30.0 kg/m² (N=346)	125	0.00	N/A	2.27	2.01–2.56

Comorbidities

Relevant Clinical Subgroups	Cutoff (pg/mL)	Sensitivity (%) (n/N)	95% CI* (%)	Specificity (%) (n/N)	95% CI* (%)	NPV (%) (n/N)	95% CI* (%)	PPV (%) (n/N)	95% CI* (%)
With comorbidities** (N=667)	125	94.9 (37/39)	82.7–99.4	65.8 (413/628)	61.9–69.5	99.5 (413/415)	98.3–99.9	14.7 (37/252)	10.6–19.7
Without comorbidities*** (N=110)	125	77.8 (7/9)	40.0–97.2	76.2 (77/101)	66.7–84.1	97.5 (77/79)	91.2–99.7	22.6 (7/31)	9.6–41.1

*95% Exact Confidence Interval

** Subjects with at least one of the following: diabetes, renal insufficiency (eGFR values <60), hypertension (HTN) and/or chronicobstructive pulmonary disease (COPD)

*** Subjects without any of the following: diabetes, renal insufficiency (eGFR values <60), hypertension (HTN) and/or chronic obstructivepulmonary disease (COPD)

Relevant Clinical Subgroups	Cutoff (pg/mL)	LR-	95% CI	LR+	95% CI
With comorbidities* (N=667)	125	0.08	0.02–0.31	2.77	2.43–3.16
Without comorbidities** (N=110)	125	0.29	0.09–0.99	3.27	2.00–5.36

*Subjects with at least one of the following: diabetes, renal insufficiency (eGFR values <60), hypertension (HTN) and/or chronicobstructive pulmonary disease (COPD)

** Subjects without any of the following: diabetes, renal insufficiency (eGFR values <60), hypertension (HTN) and/or chronic obstructivepulmonary disease (COPD)

Results of this test should be used in conjunction with clinical presentation, other diagnostic tests, and in accordance withthe appropriate clinical guidelines.

Correlation of the VITROS NT-proBNP II test results with New York Heart Association (NYHA) Functional Classification in patients diagnosed with HF

The VITROS NT-proBNP II test 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 test results (pg/mL) were determined across gender and within gender and are summarized in the following tables:

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 <.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
N/A: Not applicable
Jonckheere-Terpstra test of trend p <.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 <.0001

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. The median VITROS NT-proBNP II test results increase as the NYHA Classification increases from Class II–IV for females and all subjects, and Class I–IV for male subjects.

10. Conclusions

The nonclinical and clinical data presented in the submission provide a reasonable assurance that the VITROS NT-proBNP II test is safe and effective for the stated intended use and is substantially equivalent to the cleared predicate device.