VITROS Products Immunodiagnostic

INSTRUCTIONS FOR USE NTBNP

VITROS Immunodiagnostic Products NT-proBNP Reagent Pack	REF	680 2156
VITROS Immunodiagnostic Products NT-proBNP Calibrators	REF	680 2157

Intended Use

For *in vitro* diagnostic use only.

VITROS Immunodiagnostic Products NT-proBNP Reagent Pack

For the quantitative measurement of N-terminal pro Brain Natriuretic Peptide (NT-proBNP) in human serum and plasma (EDTA or heparin) using the VITROS ECi/ECiQ/3600 Immunodiagnostic Systems and the VITROS 5600/XT 7600 Integrated Systems to aid in the diagnosis of congestive heart failure and for the risk stratification of acute coronary syndrome and congestive heart failure. The test is further indicated 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 test can also be used in the assessment of heart failure severity in patients diagnosed with congestive heart failure.

VITROS Immunodiagnostic Products NT-proBNP Calibrators

For use in the calibration of the VITROS ECi/ECiQ/3600 Immunodiagnostic Systems and the VITROS 5600/XT 7600 Integrated Systems for the quantitative measurement of N-terminal pro Brain Natriuretic Peptide (NT-proBNP) in human serum and plasma (EDTA or heparin).

Summary and Explanation of the Test

Left ventricular dysfunction can occur as part of coronary heart disease, arterial hypertension, valvular disease and primary myocardial disease. If the left ventricular dysfunction remains untreated and is progressive, the potential for mortality is high, e.g., due to sudden cardiac death. Chronic cardiac insufficiency is a clinical syndrome caused by impairment of the cardiac pumping function. Based on the symptoms, the severity of cardiac insufficiency is classified in stages (New York Heart Association classification [NYHA] I-IV). Clinical tests and imaging procedures are used to diagnose left ventricular dysfunction. [1] The significance of natriuretic peptides in the control of cardiovascular system function has been demonstrated. Initial studies reveal that natriuretic peptides can be used for diagnostic clinical problems associated with left ventricular dysfunction. [2] The following natriuretic peptides have been described: atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP). [3, 4] ANP and BNP, by means of their natriuretic and diuretic properties, and as antagonists of the renin-angiotensin-aldosterone system, influence the electrolyte and fluid balance in an organism. [5, 6]

In subjects with left ventricular dysfunction, serum and plasma concentrations of BNP increase, as do the concentrations of the biologically inactive prohormone, proBNP. ProBNP, comprising 108 amino acids, is secreted mainly by the left ventricle of the heart and, in this process, is cleaved into physiologically active BNP (77-108) and the N-terminal fragment NT-proBNP (1-76). [4] Studies indicate that NT-proBNP can be used in the following diagnostic and prognostic applications. [7- 11] The concentration of NT-proBNP in serum and plasma indicates the prognosis for left ventricular dysfunction. It is also useful in assigning symptoms to cardiac or non-cardiac causes. NT-proBNP determination helps to identify subjects with left ventricular dysfunction. Changes in NT-proBNP concentration can be used to evaluate the success of treatment in patients with left ventricular dysfunction. There are indications that NT-proBNP, due to its functions, is suitable for use in assessing vascular remodeling, and therefore contributes to the establishment of individualized rehabilitation procedures. [12, 13] Fisher et al. found that congestive heart failure (CHF) patients with elevated NT- proBNP values had one year mortality rate of 53% compared to 11% in patients with lower values. [10] In the GUSTO IV study, which involved more than 6800 patients, it was shown that NT-proBNP was the strongest independent predictor of one year mortality in patients with acute coronary syndrome. [11]

Three studies involving patients with stable coronary artery disease have shown that elevated levels of NT-proBNP lead to a greater risk of future adverse events. In these studies, NT-proBNP levels above 450 pg/mL conferred approximately a two to six fold increase in risk for cardiac morbidity and/or mortality. [14- 17] Furthermore, each of these studies demonstrated that the amount of risk increases somewhat as the NT-proBNP levels approach the above value. Therefore, when a patient with stable coronary artery disease has a NT-proBNP level above 450 pg/mL, and is not shown to have congestive heart failure upon further evaluation, the physician should be aware that the elevated NT-proBNP level may have independent prognostic significance. These patients should receive continuing clinical attention according to established guidelines. [18]

The European Society of Cardiology Task Force for the Diagnosis and Treatment of Chronic Heart Failure recommend in their guidelines that natriuretic peptides including NT-proBNP “may be most useful clinically as a rule out test due to consistent and very high negative predictive values”. [19]

NT-proBNP levels are increased in patients with unstable angina and following myocardial infarction. [11, 20, 21] Several studies indicate NT-proBNP measurements, although not diagnostic for these conditions, provide prognostic information for the short- and long- term risk stratification of patients with unstable angina or myocardial infarction. [21- 23]

The VITROS Immunodiagnostic Products NT-proBNP Reagent Pack contains polyclonal antibodies, which recognize epitopes located in the N-terminal part (1-76) of proBNP (1-108).

Principles of the Procedure

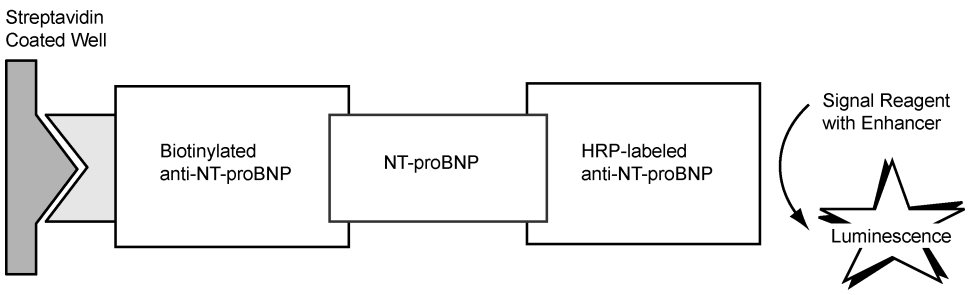
An immunometric immunoassay technique is used, which involves the simultaneous reaction of NT-proBNP present in the sample with a biotinylated antibody (sheep anti-NT-proBNP) and a horseradish peroxidase (HRP)-labeled antibody conjugate (sheep anti-NT-proBNP). The antigen-antibody complex is captured by streptavidin on the wells. Unbound materials are removed by washing.

The bound HRP conjugate is measured by a luminescent reaction. [24] A reagent containing luminogenic substrates (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.

Test Type	System *	Incubation Time	Time to first result	Test Temperature	Reaction Sample Volume
Immunometric	ECi/ECiQ, 3600, 5600, XT 7600	8 minutes	16 minutes	37 °C	40 µL

* Not all products and systems are available in all countries.

Reaction Scheme



Warnings and Precautions

WARNING: Potentially Infectious Material

Use caution when handling material of human origin. Consider all samples potentially infectious. No test method can offer complete assurance that hepatitis B virus, hepatitis C virus (HCV), human immunodeficiency virus (HIV 1+2) or other infectious agents are absent. Handle, use, store and dispose of solid and liquid waste from samples and test components, in accordance with procedures defined by appropriate national biohazard safety guideline or regulation (e.g. CLSI document M29). [25, 26]

WARNING: Contains ProClin 300 (CAS 55965-84-9) [27]

The VITROS NT-proBNP Reagent Pack and VITROS NT-proBNP Calibrators contain 1% ProClin 300. **H317**: May cause an allergic skin reaction. **P280**: Wear protective gloves/protective clothing/eye protection/face protection. **P302** + **P352**: IF ON SKIN: Wash with plenty of soap and water. **P333** + **P313**: If skin irritation or rash occurs: Get medical advice/attention. **P363**: Wash contaminated clothing before reuse.

Refer to www.Orthoclinicaldiagnostics.com for the Safety Data Sheets and for Ortho contact information.

WARNING

Reagents

Reagent Pack Contents

1 reagent pack containing:

- 100 coated wells (streptavidin, bacterial; binds ≥3 ng biotin/well)
- 8.2 mL conjugate reagent [HRP-sheep polyclonal anti-NT-proBNP, binds ≥37,000 pg NT-proBNP/mL (≥4366pmol NT-proBNP/L)] in buffer with bovine serum albumin and antimicrobial agent
- 8.2 mL biotinylated antibody reagent [biotin-sheep polyclonal anti-NT-proBNP, binds ≥37,000 pg NT-proBNP/mL (≥4366 pmol NT-proBNP/L)] in buffer with sheep serum, bovine serum albumin, bovine gamma globulin and antimicrobial agent

Reagent Pack Handling

- The reagent pack is supplied ready for use.
- The reagent pack contains homogeneous liquid reagents that do not require shaking or mixing prior to loading onto the system.
- Handle the reagent pack with care. Avoid the following:
  - allowing condensation to form on the pack
  - causing reagents to foam
  - agitation of the pack

Reagent Pack Storage and Preparation

Reagent	Storage Condition		Stability
Unopened	Refrigerated	2–8 °C (36–46 °F)	expiration date
Opened	On system	System turned on	≤12 weeks
Opened	Refrigerated	2–8 °C (36–46 °F)	≤12 weeks

- The VITROS NT-proBNP Reagent Pack is suitable for use until the expiration date on the carton when they are stored and handled as specified. Do not use beyond the expiration date.
- Do not freeze unopened reagent packs.
- Load reagent packs directly from refrigerated storage to minimize condensation.
- Store opened refrigerated reagent packs in a sealed reagent pack storage box that contains dry desiccant.

Calibrator Contents

- 1 set of VITROS NT-proBNP Calibrators 1, 2 and 3 (synthetic NT-proBNP in buffer with bovine serum albumin and antimicrobial agent, 2mL); nominal values 0; 150 and 12,500 pg/mL (0; 17.7 and 1475 pmol/L)
- Lot calibration card
- Protocol card
- 24 calibrator bar code labels (8 for each calibrator)

Calibrator Handling

- Use only with reagent packs of the same lot number. Mix thoroughly by inversion and bring to 15–30 °C (59–86 °F) before use. Each pack contains sufficient for a minimum of 6 determinations of each calibrator.
- Handle calibrators in stoppered containers to avoid contamination and evaporation. To avoid evaporation, limit the amount of time calibrators are on the system. Refer to the operating instructions for your system. Return to 2–8 °C (36–46 °F) as soon as possible after use, or load only sufficient for a single determination.

Calibrator Storage and Preparation

Calibrator	Storage Condition		Stability
Unopened	Refrigerated	2–8 °C (36–46 °F)	expiration date
Opened	Refrigerated	2–8 °C (36–46 °F)	≤13 weeks

- VITROS NT-proBNP Calibrators are supplied ready for use.
- The VITROS NT-proBNP Calibrators are suitable for use until the expiration date on the carton when they are stored and handled as specified. Do not use beyond the expiration date.

- The VITROS NT-proBNP test uses 40 µL of calibrator for each determination. The VITROS NT-proBNP Calibrators may be used directly on the VITROS Immunodiagnostic and VITROS Integrated Systems. Alternatively, transfer an aliquot of each calibrator into a sample container (taking account of the minimum fill volume of the container), which may be bar coded with the labels provided. For details on minimum fill volume of sample cups or containers, refer to the operating instructions for your system.

Specimen Collection, Preparation and Storage

Patient Preparation

No special patient preparation is necessary.

Specimens Recommended

- Serum
- Heparin plasma
- EDTA plasma

Specimens Not Recommended

- Do not use turbid specimens. Turbidity in specimens may affect test results.
- Do not use hemolyzed specimens as hemolysis may affect test results.

Special Precautions

IMPORTANT:	Certain collection devices have been reported to affect other analytes and tests.[28] Owing to the variety of specimen collection devices available, Ortho Clinical Diagnostics is unable to provide a definitive statement on the performance of its products with these devices. Confirm that your collection devices are compatible with this test.

Specimen Collection and Preparation

- Collect specimens using standard procedures.[29, 30]
- Samples should be thoroughly separated from all cellular material. Failure to do so may lead to an erroneous result.
- Thoroughly mix samples by inversion and bring to 15–30 °C (59–86 °F) before use.
- The VITROS NT-proBNP test uses 40 µL of sample for each determination. This does not take account of the minimum fill volume of the chosen sample container. For details on minimum fill volume of sample cups or containers, refer to the operating instructions for your system.

Handling and Storage Conditions

- Handle samples in stoppered containers to avoid contamination and evaporation.
- The amount of time samples are on the system prior to analysis should be limited to avoid evaporation. Refer to the operating instructions for your system.
- Return to 2–8 °C (36–46 °F) as soon as possible after use, or load sufficient volume for a single determination.
- Serum and plasma samples may be stored for up to 3 days at 20–25 °C (68–77 °F), 3 days at 2–8 °C (36–46 °F) or 12 months at -20 °C (-4 °F).[31]
- Avoid repeated freeze-thaw cycles.

Testing Procedure

Materials Provided

- VITROS Immunodiagnostic Products NT-proBNP Reagent Pack
- VITROS Immunodiagnostic Products NT-proBNP Calibrators

Materials Required but Not Provided

- 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 (optional) with desiccant

Operating Instructions

Check the inventory regularly to aid the management of reagents and ensure that sufficient VITROS Signal Reagent, VITROS Universal Wash Reagent and calibrated reagent lots are available for the work planned. When performing panels of tests on a single sample, ensure that the sample volume is sufficient for the tests ordered.

For detailed information refer to the operating instructions for your system.

Note:	Do not use visibly damaged product.

Sample Dilution

Serum or plasma (EDTA or 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 Immunodiagnostic and VITROS Integrated Systems with the VITROS High Sample Diluent B Reagent Pack prior to test. Refer to the VITROS High Sample Diluent B Reagent Pack instructions for use.

Default Test Name

The default test name which will appear on patient reports is NTBNP. The default short name that will appear on the test selection menus and laboratory reports is NTBNP. These defaults may be reconfigured, if required. For detailed information refer to the operating instructions for your system.

Calibration

Calibration Procedure

- Calibration is lot specific; reagent packs and calibrators are linked by lot number. Reagent packs from the same lot may use the same calibration.
- A Master Calibration (a dose response curve covering the full calibration range) is established for each new reagent lot. Concentrations for the linked lot of calibrators are determined from the Master Calibration.
- Ensure that the Master Calibration for each new reagent lot is available on your system.
- Process calibrators in the same manner as samples. Calibration need not be programmed if bar code labels are used; load the calibrators in any order, calibration will be initiated automatically.
- When the calibrators are processed the signal expected for each calibrator is compared against the actual signal obtained. The Master Calibration is then rescaled to reflect the differences between the actual and expected signals. The validity of this calibration curve is assessed against a range of quality parameters, and if acceptable, it is stored for use with any reagent pack of that lot.
- The quality of calibration cannot be completely described by a single parameter. The calibration report should be used in conjunction with acceptable control values to determine the validity of the calibration.
- Recalibration is required after a pre-determined calibration interval, or when a different reagent lot is loaded.
- Calibration results are assessed against a range of quality parameters. Failure to meet any of the defined quality parameter ranges will be coded in the calibration report. For actions to be taken following a failed calibration refer to the operating instructions for your system.

Refer to the operating instructions for your system for detailed instructions on the calibration process.

When to Calibrate

- Calibrate when the reagent pack and calibrator lot changes.
- Calibrate every 28 days.
- After specified service procedures have been performed.
- If quality control results are consistently outside of your acceptable range.

For additional information on when to calibrate, refer to the operating instructions for your system.

Traceability of Calibration

Calibration of the VITROS NT-proBNP test is traceable to in-house reference calibrators, which have been value-assigned to correlate to another commercially available test.

Calibration Model

A modified four-parameter logistic curve fit function is used to construct the Master Calibration. The calibration process rescales the Master Calibration to establish a valid stored curve for the VITROS Immunodiagnostic and VITROS Integrated Systems.

Measuring (Reportable) Range

System	Measuring (Reportable) Range
3600 5600 XT 7600 ECi/ECiQ	11.1*–35,000 pg/mL (1.31–4130 pmol/L)

* lower limit of measuring range reported by the system software is based on the Limit of Quantitation.

The lower limit reported by the system can be reconfigured if desired. For details on how to reconfigure the lower limit refer to the operating instructions for your system.

Quality Control

Quality Control Material Selection

Controls containing suitable levels of NT-proBNP are recommended for use with the VITROS Immunodiagnostic and VITROS Integrated Systems. The performance of commercial control fluids should be evaluated for compatibility with this test before they are used for quality control.

Control materials may show a difference when compared with other NT-proBNP methods if they contain high concentrations of preservatives, stabilizers, or other nonphysiological additives, or otherwise depart from a true human sample matrix.

Appropriate quality control value ranges must be established for all quality control materials used with the VITROS NT-proBNP test.

Quality Control Procedure Recommendations

- Good laboratory practice requires that controls be processed to verify the performance of the test.
- Choose control levels that check the clinically relevant concentrations.
- To verify system performance, analyze control materials:
  - After calibration
  - According to local regulations or at least once each day that the test is being performed
  - After specified service procedures are performed

If quality control procedures within your laboratory require more frequent use of controls, follow those procedures.

- Analyze quality control materials in the same manner as patient specimens.
- If control results fall outside your acceptable range, investigate the cause before deciding whether to report patient results.
- Refer to published guidelines for general quality control recommendations. [32]

For more detailed information, refer to the operating instructions for your system.

Quality Control Material Preparation and Storage

Refer to the manufacturer’s product literature for preparation, storage, and stability information.

Results

Results are automatically calculated by the VITROS Immunodiagnostic and VITROS Integrated Systems.

Reporting Units and Unit Conversion

Analyte results are quoted in units of pg/mL or pmol/L. To configure the units, refer to the operating instructions for your system.

International	Alternate
pg/mL (pmol/L× 8.457)	pmol/L (pg/mL× 0.118)

Limitations of the Procedure

Known Interferences

The VITROS NT-proBNP test was evaluated for interference consistent with CLSI document EP7. [33] Commonly encountered substances were tested on 3 lots of reagents. Of the compounds tested, hemoglobin may interfere with the VITROS NT-proBNP test. At an NT-proBNP level 88.4 pg/mL (10.4 pmol/L), hemoglobin at 400 and 500 mg/dL caused a positive bias of 11.4 and 15.8 pg/mL respectively.

Refer to “Specificity” for a list of other compounds tested that did not show interference.

Interferent	Interferent Concentration		Units = pg/mL (pmol/L)	
			Analyte Conc*	Bias**
Hemoglobin	0.248 mmol/L	400 mg/dL	88.4 (10.4)	11.4 (1.34)
Hemoglobin	0.310 mmol/L	500 mg/dL	88.4 (10.4)	15.8 (1.86)

* Average test concentration of replicate determinations using 3 different lots of reagent.

** Estimate of the average difference observed.

Note: 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.

Other Limitations

- The results from this or any other diagnostic test should be used and interpreted only in the context of the overall clinical picture.
- Serum concentrations of natriuretic peptides may be elevated in patients with acute myocardial infarction [11, 20, 21] and renal insufficiency. [34] Factors such as these should be considered when interpreting results from any NT-proBNP or BNP method.
- Certain drugs and clinical conditions are known to alter NT-proBNP concentrations *in vivo*. For additional information, refer to one of the published summaries. [35- 37]
- Heterophilic antibodies in the serum or plasma of certain individuals are known to cause interference with immunoassays. [38] These antibodies may be present in the blood samples from individuals regularly exposed to animals or who have been treated with animal serum products. Results which are inconsistent with clinical observation indicate the need for additional testing.
- The VITROS NT-proBNP test has no high dose hook effect up to 500,000 pg/mL (59,000 pmol/L).
- Biotin levels in serum remain elevated for up to 24 hours after oral or intravenous biotin administration. [39]

Expected Values and Interpretation of Results

It is recommended that each laboratory establish its own expected values for the population it serves.
NT-proBNP concentrations in the Reference Cohort are shown in the following tables. The decision thresholds are 125 pg/mL (14.8 pmol/L) for patients younger than 75 years and 450 pg/mL (53.1 pmol/L) for patients 75 years and older.

Reference Cohort

The circulating NT-proBNP concentration was determined from 242 individuals without CHF (95 men and 147 women). This population included apparently healthy individuals and individuals with diabetes, hypertension, pulmonary disease, and mild renal insufficiency. The descriptive statistics for NT-proBNP concentrations in the reference cohort are shown in the following tables.

	All					
Age (yrs)	<45	45–54	55–64	65–74	<75	≥75
Mean (pg/mL)	64.0	61.5	102	120	90.2	249
SD	74.4	53.1	132	95.0	104	170
Median (pg/mL)	43.8	40.2	63.6	98.3	60.6	206
95th Percentile	251	158	288	288	271	598
% <125 pg/mL	90.2	86.0	75.6	65.9	78.6	-
% <450 pg/mL	-	-	-	-	-	90.6
N	41	43	82	44	210	32

Males						
Age (yrs)	<45	45–54	55–64	65–74	<75	≥75
Mean (pg/mL)	61.4	36.5	96.3	146	92.3	187
SD	89.0	34.0	173	127	138	128
Median (pg/mL)	32.6	22.4	48.0	107	52.2	145
95th Percentile	266	87.7	351	346	310	391
% <125 pg/mL	87.5	100.0	88.6	63.2	84.1	-
% <450 pg/mL	-	-	-	-	-	100.0
N	16	12	35	19	82	13

Females						
Age (yrs)	<45	45–54	55–64	65–74	<75	≥75
Mean (pg/mL)	65.6	71.2	106	101	88.9	292
SD	65.3	56.4	91.6	55.5	74.2	185
Median (pg/mL)	55.7	47.0	73.4	87.3	66.7	254
95th Percentile	168	162	288	213	228	615
% <125 pg/mL	92.0	80.6	66.0	68.0	75.0	-
% <450 pg/mL	-	-	-	-	-	84.2
N	25	31	47	25	128	19

Disease Cohort

The circulating NT-proBNP concentration was determined from 242 individuals with CHF (135 men and 107 women). This population included individuals with diabetes, hypertension, pulmonary disease, and mild and moderate renal insufficiency. The descriptive statistics for NT-proBNP concentrations in the disease cohort are shown in the following tables.

CHF Population - All						
Age (yrs)	<45	45–54	55–64	65–74	<75	≥75
Mean (pg/mL)	2440	919	1056	1713	1429	3147
SD	2052	786	1657	2360	2014	5945
Median (pg/mL)	2510	735	522	1180	857	1920
95th Percentile	5068	2422	3310	4956	3727	8751
% >125 pg/mL	83.3	84.2	89.1	95.9	91.7	-
% >450 pg/mL	-	-	-	-	-	82.7
N	6	19	46	73	144	98

CHF Population - Males						
Age (yrs)	<45	45–54	55–64	65–74	<75	≥75
Mean (pg/mL)	3847	1025	1352	2121	1809	2661
SD	1604	919	2047	2883	2478	2387
Median (pg/mL)	3650	749	731	1360	984	2010
95th Percentile	5351	2499	5468	7804	6694	7518
% >125 pg/mL	100.0	70.0	85.2	95.6	89.4	-
% >450 pg/mL	-	-	-	-	-	86.0
N	3	10	27	45	85	50

CHF Population - Females						
Age (yrs)	<45	45–54	55–64	65–74	<75	≥75
Mean (pg/mL)	1034	801	634	1058	881	3654
SD	1421	641	700	765	767	8154
Median (pg/mL)	327	581	380	860	577	1500
95th Percentile	2436	1848	2338	2105	2401	10,303
% >125 pg/mL	66.7	100.0	94.7	96.4	94.9	-
% >450 pg/mL	-	-	-	-	-	79.2
N	3	9	19	28	59	48

Disease Cohort (by NYHA Classification)

Each laboratory should establish a reference range that represents the patient population that is to be evaluated. In addition, laboratories should be aware of their respective institution's current practice for the evaluation of CHF. Blood samples were obtained from 242 patients diagnosed with CHF (135 men and 107 women). The descriptive statistics for NT-proBNP concentrations in patients with CHF and NYHA Classifications I–IV are presented in the tables below.

CHF Population - All					
NYHA Functional Class					
	ALL	NYHA I	NYHA II	NYHA III	NYHA IV
Mean (pg/mL)	2124	1299	1467	3390	2956
SD	4165	1730	1638	7089	3179
Median (pg/mL)	1210	741	917	1870	1665
5th Percentile	109	137	85.3	191	260
95th Percentile	7193	2923	4514	10,584	8993
% > cutoff	88.0	86.0	83.8	91.0	100.0
N	242	50	99	67	26
Minimum	15.3	78.1	15.3	48.0	139
Maximum	55,600	9220	7900	55,600	10,800

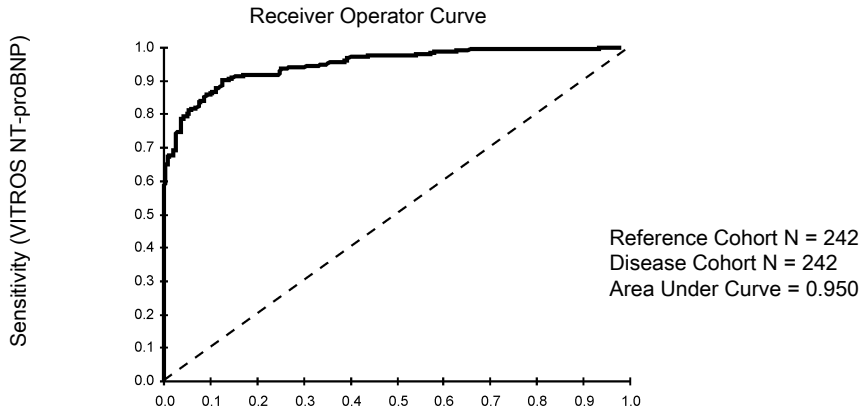
CHF Population- Males					
NYHA Functional Class					
	ALL	NYHA I	NYHA II	NYHA III	NYHA IV
Mean (pg/mL)	2124	1338	1674	2905	3598
SD	2471	1761	1791	3232	3066
Median (pg/mL)	1490	802	960	2305	2115
5th Percentile	98.0	125	83.1	385	318
95th Percentile	7305	2941	5664	8044	8758
% > cutoff	88.1	85.7	83.1	93.8	100.0
N	135	28	59	32	16
Minimum	48.0	78.1	50.6	48.0	253
Maximum	16,000	9220	7900	16,000	9110

CHF Population - Females					
NYHA Functional Class					
	ALL	NYHA I	NYHA II	NYHA III	NYHA IV
Mean (pg/mL)	2125	1249	1161	3832	1929
SD	5632	1730	1344	9360	3241
Median (pg/mL)	862	658	735	1550	701
5th Percentile	138	301	103	259	202
95th Percentile	6957	2397	3100	9896	7348
% > cutoff	87.9	86.4	85.0	88.6	100.0
N	107	22	40	35	10
Minimum	15.3	133	15.3	56.3	139
Maximum	55,600	8380	7200	55,600	10,800

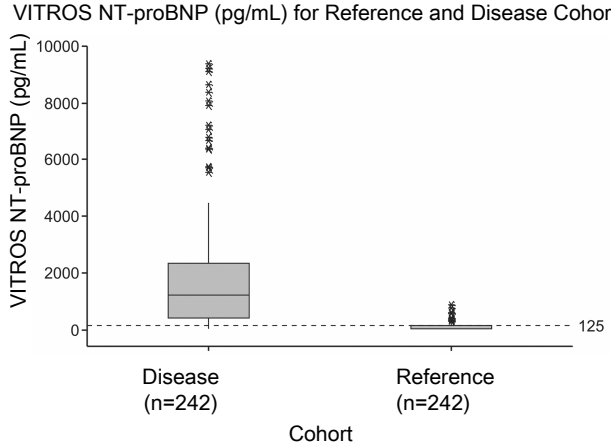
These results together with other published data [8, 40- 45] show that there is a relationship between the severity of the clinical signs and symptoms of CHF and the NT-proBNP concentrations.

Interpretation of Results

The Receiver Operator Curve (ROC) compares clinical sensitivity and specificity at various decision thresholds. The optimum decision threshold maximizes the area under the curve (AUC) and represents the highest sensitivity and specificity for the test. The ROC analysis for the VITROS NT-proBNP test is presented below. The AUC for the VITROS NT-proBNP test is 0.950 with a 95% Confidence Interval of 0.931 to 0.968.



A box and whisker plot for the clinical study population is presented below. Recommended clinical thresholds are 125 pg/mL (14.8 pmol/L) for patients younger than 75 years and 450 pg/mL (53.1 pmol/L) for patients 75 years and older.



Sensitivity and Specificity by Age and Gender

The clinical sensitivity and specificity of the VITROS NT-proBNP immunoassay using decision thresholds of 125 pg/mL (14.8 pmol/L) for patients younger than 75 years and 450 pg/mL (53.1 pmol/L) for patients 75 years and older are presented below. Due to the high negative predictive value of the VITROS test, NT-proBNP levels are expected to exceed decision thresholds in patients with CHF.

Males						
Age (yrs)	<45	45–54	55–64	65–74	<75	≥75
% Sensitivity	100.0	70.0	85.2	95.6	89.5	86.0
95% Confidence Interval	29.2–100.0	34.8–93.3	66.3–95.8	84.9–99.5	80.8–95.0	73.3–94.9
% Specificity	87.5	100.0	88.6	63.2	84.1	100.0
95% Confidence Interval	61.7–98.4	73.5–100.0	73.3–96.8	38.4–83.7	74.4–91.3	75.3–100.0
Prevalence	0.7	1.8	6.2	6.8	1.4	9.8
Negative Predictive Value	100.0	99.5	98.9	99.5	99.8	98.5

Females						
Age (yrs)	<45	45–54	55–64	65–74	<75	≥75
% Sensitivity	66.7	100.0	94.7	96.4	94.9	79.2
95% Confidence Interval	9.43 - 99.2	66.4–100.0	74.0–99.9	81.7–99.9	85.9–98.9	65.0–91.4
% Specificity	92.0	80.6	66.0	68.0	75.0	84.2
95% Confidence Interval	74.0–99.0	62.5–92.5	50.7–79.1	46.5–85.1	66.6–82.2	60.4–97.1
Prevalence	0.5	1.3	3.4	6.6	1.2	9.7
Negative Predictive Value	99.8	100.0	99.7	99.6	99.9	97.4

Age-matched and Incidence-based Analysis

An age-matched analysis of the clinical data was performed with the following common age distribution in the groups of individuals with and without CHF.

Males					
Age (yrs)	<45	45–54	55–64	65–74	≥75
Study Population (%)	8.26	9.57	26.96	27.83	27.39
US Population (%)	7.77	13.84	30.02	23.48	24.88

Females					
Age (yrs)	<45	45–54	55–64	65–74	≥75
Study Population (%)	11.02	15.75	25.98	20.87	26.38
US Population (%)	5.64	9.87	17.00	27.16	40.31

This age distribution reflects the prevalence of CHF within the age groups and genders according to data published in the American Heart Association's Heart Disease and Stroke Statistics - 2004 Update [46] and also reflects the age structure of the United States population, according to data published by the National Center for Health Statistics in Health, United States, 2000.

Clinical Agreement with a Comparative Method

The comparison of the above mentioned study population against a comparative method (Roche Elecsys® proBNP) gave a 98.3% overall clinical agreement for Disease and Reference Cohorts. The AUC for the same population in the Roche Elecsys proBNP test was 0.949 with the 95% Confidence Interval of 0.931 to 0.967.

Performance Characteristics

Limit of Detection

The Limit of Detection (LoD) for VITROS NT-proBNP is 11.1 pg/mL (1.31 pmol/L), determined according to CLSI document EP17 [47] and with proportions of false positives (α) less than 5% and false negatives (β) less than 5%; based on 1200 determinations with 320 blank and 880 low-level replicates. The Limit of Blank (LoB) is 6.81 pg/mL (0.804 pmol/L). The Limit of Quantitation (LoQ) was set at 11.1 pg/mL (1.31 pmol/L) to ensure acceptable precision within the lower range of the test. The data presented are representative of the product performance.

Limit of Blank, Limit of Detection and Limit of Quantitation

LoB*		LoD		LoQ	
pg/mL	pmol/L	pg/mL	pmol/L	pg/mL	pmol/L
6.81	0.804	11.1	1.31	11.1	1.31

* Limit of Blank, or the highest value likely to be observed with a sample containing no analyte, replaces the term "analytical sensitivity."

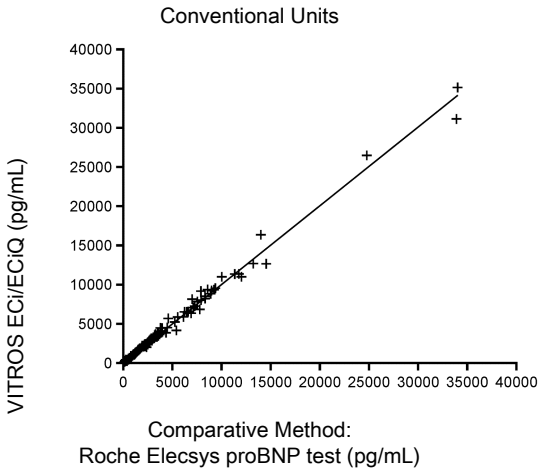
Functional Sensitivity

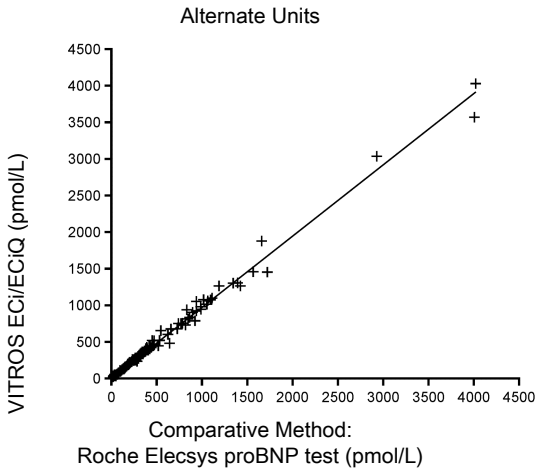
The functional sensitivity of this test is less than 10.0 pg/mL (1.18 pmol/L). It is defined as the lowest concentration, which corresponds to the 20% between-test coefficient of variation from the precision dose profile. [48] The functional sensitivity of the VITROS NT-proBNP test is calculated across a single 28 day calibration interval, using a human plasma matrix.

Accuracy (Method Comparison)

Accuracy was evaluated consistent with NCCLS document EP9. [49] The plots and table shows the results of a method comparison study using patient samples from a variety of clinical categories analyzed on the VITROS ECi/ECiQ Immunodiagnostic System compared with those analyzed using the Roche Elecsys proBNP test. The relationship between the 2 methods was determined by Deming regression. [50]

The table also shows the results of method comparison studies [49] using patient serum and plasma samples analyzed on the VITROS ECi/ECiQ Immunodiagnostic System compared with those analyzed using the VITROS 3600 Immunodiagnostic System and the VITROS 5600 Integrated System. The relationship between the 2 methods was determined by Deming regression. [50]





System	n	Slope	Correlation Coefficient	Conventional Units (pg/mL) Range of Samples	Conventional Units (pg/mL) Intercept	Alternate Units (pmol/L) Range of Samples	Alternate Units (pmol/L) Intercept
ECi/ECiQ vs. Comparative Method	595	0.9839	0.9966	12.5–34,600	19.7	1.48–4083	2.32
3600 vs. ECi/ECiQ	105	1.002	0.9988	16.6–31,360	57.0	1.96–3700	6.73
5600* vs. ECi/ECiQ	109	1.004	0.9993	16.6–31,360	102	1.96–3700	12.0

* Performance characteristics for the VITROS 5600 System are applicable to the VITROS XT 7600 System.

Precision

VITROS ECi/ECiQ Immunodiagnostic System

Precision was evaluated consistent with NCCLS document EP5. [51] Two replicates of each of 3 frozen control samples and 4 frozen human samples were tested on 2 separate occasions per day on at least 20 different days. The experiment was performed using 3 reagent lots on 3 different VITROS Immunodiagnostic Systems. The data presented are a representation of the product performance.

VITROS 3600 Immunodiagnostic System and VITROS 5600 Integrated System

Precision was evaluated consistent with NCCLS document EP5. [51] Two replicates of each of 3 control samples were tested on 2 separate occasions per day on at least 20 different days. The experiment was performed using 1 reagent lot on each system. The data presented are a representation of the product performance.

System	Units = pg/mL								
	Mean NT-proBNP Conc.	Within-run*		Within-calibration**		Within-lab***		No. Observ.	No. Days
		SD	CV (%)	SD	CV (%)	SD	CV (%)		
ECi/ECiQ system 1	68.4	0.746	1.1	3.08	4.6	3.48	5.0	84	28
	112	0.825	0.7	6.66	6.0	6.26	5.5	84	28
	395	3.97	1.0	18.0	4.6	16.8	4.2	84	28
	26,383	289	1.1	533	2.0	549	2.1	84	28
	212	2.03	1.0	5.24	2.5	5.67	2.6	84	28
	680	5.17	0.8	11.4	1.7	12.3	1.8	84	28
	7640	88.0	1.2	146	1.9	157	2.0	84	28
ECi/ECiQ system 2	67.6	1.18	1.7	3.19	4.7	3.03	4.5	84	28
	111	1.27	1.1	6.20	5.6	6.31	5.7	84	28
	394	4.83	1.2	16.5	4.2	16.9	4.3	84	28
	26,723	357	1.3	889	3.3	1053	4.0	84	28
	212	1.74	0.8	4.51	2.1	4.37	2.1	84	28
	692	6.74	1.0	11.7	1.7	12.6	1.8	84	28
	7826	63.2	0.8	122	1.6	155	2.0	84	28
ECi/ECiQ system 3	71.2	0.817	1.2	3.11	4.4	3.04	4.2	84	28
	116	1.01	0.9	6.90	6.0	6.06	5.2	84	28
	406	3.48	0.9	20.1	5.0	16.8	4.1	84	28
	27,449	342	1.3	831	3.1	705	2.5	84	28
	214	1.54	0.7	4.98	2.4	5.78	2.7	84	28
	685	4.48	0.7	13.4	2.0	14.2	2.0	84	28
	7702	79.3	1.0	170	2.3	179	2.3	84	28
3600	154	1.67	1.1	2.49	1.6	3.67	2.4	84	21
	547	5.80	1.1	7.83	1.4	11.5	2.1	84	21
	5145	56.0	1.1	76.5	1.5	118	2.3	84	21
5600****	148	1.50	1.0	2.76	1.9	3.04	2.0	88	22
	533	5.64	1.1	7.82	1.5	9.51	1.8	88	22
	5071	40.2	0.8	65.1	1.3	86.8	1.7	88	22

* 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

**** Performance characteristics for the VITROS 5600 System are applicable to the VITROS XT 7600 System.

System	Units = pmol/L								
	Mean NT-proBNP Conc.	Within-run*		Within-calibration**		Within-lab***		No. Observ.	No. Days
		SD	CV (%)	SD	CV (%)	SD	CV (%)		
ECi/ECiQ system 1	8.08	0.088	1.1	0.364	4.6	0.411	5.0	84	28
	13.3	0.097	0.7	0.785	6.0	0.739	5.5	84	28
	46.6	0.469	1.0	2.13	4.6	1.98	4.2	84	28
	3113	34.1	1.1	62.9	2.0	64.7	2.1	84	28
	25.0	0.239	1.0	0.619	2.5	0.669	2.6	84	28
	80.2	0.610	0.8	1.35	1.7	1.45	1.8	84	28
	901	10.4	1.2	17.3	1.9	18.6	2.0	84	28

System	Units = pmol/L							No. Observ.	No. Days
	Mean NT-proBNP Conc.	Within-run*		Within-calibration**		Within-lab***			
		SD	CV (%)	SD	CV (%)	SD	CV (%)		
ECi/ECiQ system 2	7.98	0.139	1.7	0.377	4.7	0.357	4.5	84	28
	13.1	0.150	1.1	0.732	5.6	0.745	5.7	84	28
	46.4	0.569	1.2	1.95	4.2	1.99	4.3	84	28
	3153	42.2	1.3	105	3.3	124	4.0	84	28
	25.1	0.206	0.8	0.532	2.1	0.516	2.1	84	28
	81.7	0.795	1.0	1.38	1.7	1.49	1.8	84	28
	923	7.46	0.8	14.4	1.6	18.3	2.0	84	28
ECi/ECiQ system 3	8.40	0.096	1.2	0.367	4.4	0.358	4.2	84	28
	13.6	0.120	0.9	0.814	6.0	0.715	5.2	84	28
	47.9	0.411	0.9	2.37	5.0	1.99	4.1	84	28
	3239	40.3	1.3	98.1	3.1	83.2	2.5	84	28
	25.2	0.181	0.7	0.587	2.4	0.682	2.7	84	28
	80.9	0.529	0.7	1.59	2.0	1.67	2.0	84	28
	909	9.35	1.0	20.1	2.3	21.1	2.3	84	28
3600	18.2	0.197	1.1	0.294	1.6	0.433	2.4	84	21
	64.5	0.684	1.1	0.924	1.4	1.36	2.1	84	21
	607	6.61	1.1	9.03	1.5	13.9	2.3	84	21
5600****	17.5	0.177	1.0	0.326	1.9	0.359	2.0	88	22
	62.9	0.666	1.1	0.923	1.5	1.12	1.8	88	22
	598	4.74	0.8	7.68	1.3	10.2	1.7	88	22

* 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

**** Performance characteristics for the VITROS 5600 System are applicable to the VITROS XT 7600 System.

Specificity

Substances that do not Interfere

The VITROS NT-proBNP test was evaluated for interference consistent with CLSI document EP7. [33] Of the compounds tested, none was found to cause a bias of >10% with the test at the concentrations indicated at NT-proBNP concentrations of approximately 125 pg/mL (14.8 pmol/L).

Compound	Concentration	
Acetaminophen	20 mg/dL	1.32 mmol/L
N-Acetyl-L-cysteine	271 mg/dL	16.6 mmol/L
Acetylsalicylic acid	100 mg/dL	5.55 mmol/L
Adrenalin (Epinephrine HCl)	0.037 mg/dL	1.68 µmol/L
Ampicillin	100 mg/dL	2.69 mmol/L
Ascorbic acid	30 mg/dL	1.70 mmol/L
Bilirubin	20 mg/dL	0.342 mmol/L
Biotin	0.002 mg/dL	81.9 nmol/L
Bisoprolol Hemifumarate	1 mg/dL	0.026 mmol/L
Captopril	15 mg/dL	0.690 mmol/L
Carvedilol	5 mg/dL	0.123 mmol/L
Cefoxitin Sodium	250 mg/dL	5.56 mmol/L
Cyclosporin A	0.500 mg/dL	0.004 mmol/L
Clopidogrel Hydrogensulfate	7.5 mg/dL	0.179 mmol/L

Compound	Concentration	
Lisinopril	4 mg/dL	0.099 mmol/L
Lovastatin (Mevinolin)	8 mg/dL	0.198 mmol/L
Marcumar (Warfarin)	0.600 mg/dL	0.019 mmol/L
Methyl Dopa	2 mg/dL	0.084 mmol/L
6-alpha-Methylprednisolone	8 mg/dL	0.214 mmol/L
Metoprolol +/- Tartrate	1.5 mg/dL	0.022 mmol/L
Metronidazole	20 mg/dL	1.17 mmol/L
Molsidomine	2.4 mg/dL	0.099 mmol/L
Nicardipine HCl	9 mg/dL	0.174 mmol/L
Nifedipine	6 mg/dL	0.173 mmol/L
Phenylbutazone	40 mg/dL	1.30 mmol/L
Pravastatin	4 mg/dL	0.090 mmol/L
Propafenone HCl	90 mg/dL	2.38 mmol/L
Propranolol HCl	228 mg/dL	7.71 mmol/L

Compound	Concentration		Compound	Concentration	
Digitoxin	0.030 mg/dL	0.392 µmol/L	Retavase	0.112 mg/dL	na
Digoxin	0.050 mg/dL	0.640 µmol/L	Rifampicin	6 mg/dL	0.073 mmol/L
Dipyrone	1000 mg/dL	30 mmol/L	Simvastatin	4 mg/dL	0.096 mmol/L
Doxycycline Hyclate	6.9 mg/dL	0.067 mmol/L	Sodium Azide	200 mg/dL	30.8 mmol/L
Enalapril Maleate	4 mg/dL	0.081 mmol/L	Sotalol HCl	32 mg/dL	1.04 mmol/L
Gentamicin sulfate	0.050 mg/dL	0.001 mmol/L	Spironolactone	40 mg/dL	0.960 mmol/L
Glycerylnitrate (Nitroglycerin)	19.2 mg/dL	0.845 mmol/L	Streptokinase	na	300 IE
Heparin (Sodium)	na	5000 U/L	Theophylline	100 mg/dL	5.54 mmol/L
Ibuprofen	50 mg/dL	2.42 mmol/L	Tolbutamide	64 mg/dL	2.37 mmol/L
Insulin	0.084 mg/dL	0.145 µmol/L	Torsemide	20 mg/dL	0.574 mmol/L
Intralipid	1 mg/dL	na	Triolein	3000 mg/dL	33.9 mmol/L
Levodopa	2 mg/dL	0.101 mmol/L	Urokinase	na	600 IE
Lidocaine	10 mg/dL	0.427 mmol/L	Verapamil	12 mg/dL	0.244 mmol/L

Cross-Reactivity

The cross-reactivity of the VITROS NT-proBNP test was evaluated by adding the following substances to samples containing 0 and approximately 125 pg/mL (14.8 pmol/L) of analyte.

Cross-Reactant Tested	Concentration		% Cross-reactivity
ANP_{28}	3.1 µg/mL	1.01 µmol/L	<0.1
NT-proANP $_{1-30}$ (preproANP $_{26-55}$)	3.5 µg/mL	0.998 µmol/L	<0.1
NT-proANP $_{31-67}$ (preproANP $_{56-92}$)	1.0 ng/mL	0.258 nmol/L	<0.1
NT-proANP $_{79-98}$ (preproANP $_{104-123}$)	1.0 ng/mL	0.458 nmol/L	<0.1
BNP_{32}(Natrecor®)	3.5 µg/mL	1.01 µmol/L	<0.1
CNP_{22}	2.2 µg/mL	1.00 µmol/L	<0.1
Adrenomedullin	1.0 ng/mL	0.166 nmol/L	<0.1
Aldosterone	0.6 ng/mL	1.66 nmol/L	<0.1
Angiotensin I	0.6 ng/mL	0.463 nmol/L	<0.1
Angiotensin II	0.6 ng/mL	0.574 nmol/L	<0.1
Angiotensin III	1.0 ng/mL	1.29 nmol/L	<0.1
Endothelin	20 pg/mL	8.45 pmol/L	<0.1
Urodilatin	3.5 µg/mL	0.998 µmol/L	<0.1
Arg-Vasopressin	1.0 ng/mL	0.922 nmol/L	<0.1
Renin	50 ng/mL	21.9 nmol/L	<0.1

Cross-reactivity was expressed as the mean result obtained for the cross-reactant pool divided by the cross-reactant concentration in percentage term.

$$\% \text{ Cross-reactivity} = \frac{\text{Mean Result for the Cross-Reactant Pool (ng/mL)}}{\text{Concentration of Cross-Reactant (ng/mL)}} \times 100$$

References

1. Cleland JGF, Erdmann E, Ferrari R, Hess OM, Poole-Wilson PA, Remme W, et al. Guidelines for the Diagnosis of Heart Failure. The Task Force on Heart Failure of the European Society of Cardiology. *Eur Heart J* 1995;16:741-751.
2. Richards AM, Nicholls MG, Yandle TG, Frampton C, Espiner EA, Turner JG, et al. Plasma N-Terminal Pro-Brain Natriuretic Peptide and Adrenomedullin: New Neurohormonal Predictors of Left Ventricular Function and Prognosis After Myocardial Infarction. *Circulation* 1998; 97:1921-1929.
3. de Bold AJ. Atrial Natriuretic Factor: A Hormone Produced by the Heart. *Science* 1985; 230:767-770.
4. Valli N, Gobinet A, Bordenave L. Review of 10 Years of the Clinical Use of Brain Natriuretic Peptide in Cardiology. J Lab Clin Med 1999; 134: 437-444.

5. de Bold AJ, Borenstein HB, Veress AT, Sonnenberg H. A Rapid and Potent Natriuretic Response to Intravenous Injection of Atrial Myocardial Extract in Rats. *Life Sci* 1981; 28: 89-94.
6. Epstein M, Loutzenhiser R, Friedland E, Aceto RM, Camargo MJF, Atlas SA. Relationship of Increased Plasma Atrial Natriuretic Factor and Renal Sodium Handling During Immersion-induced Central Hypervolemia in Normal Humans. J *Clin Invest* 1987; 79: 738-745.
7. Struthers AD. How to Use Natriuretic Peptide Levels for Diagnosis and Prognosis. The European Society of Cardiology. *Eur Heart J* 1999; 20:1374-1375.
8. Hunt PJ, Richards AM, Nicholls MG, Yandle TG, Doughty RN, Espiner EA. Immunoreactive Amino-Terminal Pro-Brain Natriuretic Peptide (NT-proBNP): a New Marker of Cardiac Impairment. *Clin Endocrinol* 1997; 47: 287-296.
9. Talwar S, Squire IB, Davies JE, Barnett DB, Ng LL. Plasma N-Terminal Pro-Brain Natriuretic Peptide and the ECG in the Assessment of Left-Ventricular Systolic Dysfunction in a High Risk Population. *Eur Heart J* 1999; 20: 1736-1744.
10. Fisher C, et al. NT-proBNP Predicts Prognosis in Patients with Chronic Heart Failure. *Heart* 2003; 89: 879-881.
11. James SK, et al. NT-proBNP and Other Risk Markers for the Separate Prediction of Mortality and Subsequent Myocardial Infarction in Patients with Unstable Coronary Artery Disease. (GUSTO)-IV Substudy. *Circulation* 2003; 108: 275-281.
12. Darbar D, Davidson NC, Gillespie N, Choy A-MJ, Lang CC, Shyr Y, et al. Diagnostic Value of B-Type Natriuretic Peptide Concentrations in Patients with Acute Myocardial Infarction. *Am J Cardiol* 1996; 78: 284-287.
13. McDonagh TA, Robb SD, Murdoch DR, Morton JJ, Ford I, Morrison CE, et al. Biochemical Detection of Left-Ventricular Systolic Dysfunction. *Lancet* 1998; 351:9-13.
14. Schnabel R, Rupprecht HJ, Lackner KJ, Lubos E, Bickel C, Meyer J, et al. Analysis of N-Terminal-Pro-Brain Natriuretic Peptide and C-Reactive Protein for Risk Stratification in Stable and Unstable Coronary Artery Disease: results from the AtheroGene study. *Eur Heart J* 2005;26:241-249.
15. Kragelund C, Gronning B, Kober L, Hildebrandt P, Steffensen R. N-terminal Pro-B-Type Natriuretic Peptide and Long-Term Mortality in Stable Coronary Heart Disease. *N Engl J Med* 2005; 352:666-675.
16. Ndrepepa G, Braun S, Niemoller K, Mehilli J, von Beckerath N, von Beckerath O, et al. Prognostic Value of N-terminal Pro-Brain Natriuretic Peptide in Patients with Chronic Stable Angina. *Circulation* 2005; 112:2102-2107.
17. Tarnow L, Hildebrandt P, Hansen BV, Borch-Johnsen K, Parving HH. Plasma N-Terminal Pro-Brain Natriuretic Peptide as an Independent Predictor of Mortality in Diabetic Nephropathy. *Diabetologia* 2005; 48: 149-155.
18. Hunt SA, Abraham WT, Chin MH, Feldman AM, Francis GS, et al. ACC/AHA 2005 Guideline Update for the Diagnosis and Management of Chronic Heart Failure in the Adult: A Report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Writing Committee to Update the 2001 Guidelines for the Evaluation and Management of Heart Failure). American College of Cardiology Web Site. Available at: http://www.Acc.org/clinical/guidelines/failure/index.pdf
19. Remme WJ, Swedberg K, et al. Guidelines for the Diagnosis and Treatment of Chronic Heart Failure. Task Force for the Diagnosis and Treatment of Chronic Heart Failure, European Society of Cardiology. *Eur Heart J* 2001; 22:1527-1560.
20. Talwar S, Squire IB, Downie PF, Davies JE, Ng LL. Plasma N-Terminal Pro-Brain Natriuretic Peptide and Cardiotrophin 1 are Raised in Unstable Angina. *Heart* 2000; 84:421-424.
21. Jernberg T, Stridsberg M, Venge P, Lindahl B. N-terminal Pro-Brain Natriuretic Peptide on Admission for Early Risk Stratification of Patients with Chest Pain and No ST-Segment Elevation. *J Am Coll Cardiol* 2002; 40:437-445.
22. Zeller M, Cottin Y, Laurent Y, Danchin N, L'Huillier I, et al. N-terminal Pro-Brain Natriuretic Peptide Levels in Patients with Non-ST-Elevation Myocardial Infarction. *Cardiology* 2004; 102:37-40.
23. Richards AM, Nicholls MG, Espiner EA, Lainchbury JG, et al. B-Type Natriuretic Peptides and Ejection Fraction for Prognosis After Myocardial Infarction. *Circulation* 2003; 107:2786-2792.
24. Summers M et al. Luminogenic Reagent Using 3-Chloro 4-Hydroxy Acetanilide to Enhance Peroxidase/Luminol Chemiluminescence. *Clin Chem*. 41:S73; 1995.
25. CLSI. *Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline– Fourth Edition*. CLSI. document M29-A4. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.
26. CDC-NIH. *Biosafety in Microbiological and Biomedical Laboratories-3rd edition*. HHS Publication No. CDO93-8395. U.S Governnent Printing Office, Washington, D.C. 1993.
27. Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006.
28. Calam RR. Specimen Processing Separator Gels: An Update. *J Clin Immunoassay*. 11:86–90; 1988.
29. CLSI. *Collection of Diagnostic Venous Blood Specimens. 7th ed.* CLSI standard GP41. Wayne, PA: Clinical and Laboratory Standards Institute; 2017.
30. NCCLS. *Procedures and Devices for the Collection of Diagnostic Capillary Blood Specimens; Approved Standard – Fifth Edition*. NCCLS document H4-A5 [ISBN 1-56238-538-0]. NCCLS, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 2004.
31. Elecsys® proBNP Assay Package Insert Sheet, Roche Diagnostics GmbH, January 2006.
32. CLSI. *Statistical Quality Control for Quantitative Measurements: Principles and Definitions; Approved Guideline - Third Edition*. CLSI document C24-A3 [ISBN 1-56238-613-1]. CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 2006.
33. NCCLS. *Interference Testing in Clinical Chemistry; Approved Guideline*. NCCLS document EP7-A (ISBN 1-56238-480-5). NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898, USA 2002.

34. Anwaruddin S, Lloyd-Jones DM, Baggish A, Chen A, Krauser D, Tung R, Chae C, Januzzi JL. Renal Function, Congestive Heart Failure, and Amino-Terminal Pro-Brain Natriuretic Peptide Measurement. Results from the ProBNP Investigation of Dyspnea in the Emergency Department (PRIDE) Study. *Journal of the American College of Cardiology* 2006; 47:91-97.
35. Young DS. *Effects of Drugs on Clinical Laboratory Tests*. ed. 4. Washington, D.C.: AACC Press; 1995.
36. Friedman RB, Young DS. *Effects of Disease on Clinical Laboratory Tests*. ed. 3. Washington, D.C.: AACC Press; 1997.
37. Tryding N, Tufvesson C, Sonntag O (eds). *Drug Effects in Clinical Chemistry*. ed. 7. Stockholm: The National Corporation of Swedish Pharmacies, Pharmasoft AB, Swedish Society for Clinical Chemistry; 1996.
38. Levinson SS. The Nature of Heterophilic Antibodies and Their Role in Immunoassay Interference. *J Clin Immunoassay.* 15:108–115; 1992.
39. Scientific Committee on Food. Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Biotin. European Commission, SCF/CS/NUT/UPPLEV/55 Final, Brussels, 2001.
40. Rademaker MT, Richards AM. Cardiac Natriuretic Peptides for Cardiac Health. *Clinical Science* 2005; 108:23-36.
41. O'Donoghue M, Chen A, Baggish AL, Anwaruddin S, Krauser DG, Tung R, Januzzi JL. The Effects of Ejection Fraction on N-Terminal ProBNP and BNP Levels in Patients with Acute CHF: Analysis from the ProBNP Investigation of Dyspnea in the Emergency Department (PRIDE) Study. *Journal of Cardiac Failure* 2005; 11:S9-S14.
42. Elin RJ, Winter WE. Laboratory and Clinical Aspects of B-Type Natriuretic Peptides. *Archives of Pathology and Laboratory Medicine 2004*; 128:697-699.
43. Williams SG, Ng LL, O'Brien RJ, Taylor S, Wright DJ, Li YF, Tan LB. Complementary Roles of Simple Variables, NYHA and N-BNP, in Indicating Aerobic Capacity and Severity of Heart Failure International Journal of *Cardiology* 2005; 102:279-286.
44. Januzzi JL, van Kimmenade R, Lainchbury J, Bayes-Genis A, Ordonez-Llanos J, Santalo-Bel M, Pinto YM, Richards M. NT-proBNP Testing for Diagnosis and Short-Term Prognosis in Acute Destabilized Heart Failure: An International Pooled Analysis of 1256 Patients. The International Collaborative of NT-proBNP Study. *European Heart Journal* 2006; 27:330-337.
45. Angermann CE, Ertl G. Natriuretic Peptides—New Diagnostic Markers in Heart Disease. Herz 2004; 29:609-617.
46. American Heart Association. *Heart Disease and Stroke Statistics–2004 Update*. Dallas, TX.: American Heart Association; 2003. ©2003, American Heart Association.
47. CLSI. *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition*. CLSI document EP17-A2. Wayne, Pennsylvania: Clinical and Laboratory Standards Institute; 2012.
48. Spencer CA, Takeuchi M, Kazarosyan M, MacKenzie F, Beckett GJ, and Wilkinson E. Interlaboratory/Intermethod Differences in Functional Sensitivity of Immunometric Assays of Thyrotropin (TSH) and Impact on Reliability of Measurement of Subnormal Concentrations of TSH. *Clin Chem* 1995; 41: 367-374.
49. NCCLS. *Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline - Second Edition*. NCCLS document EP9-A2 (ISBN 1-56238-472-4). NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2002.
50. Deming *WE, Statistical Adjustment of Data. New York, NY: John Wiley and Sons; 1943.*
51. NCCLS. *Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-Second Edition*. NCCLS document EP5-A2 (ISBN 1-56238-542-9). NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898, USA 2004.

Glossary of Symbols

The following symbols may have been used in the labeling of this product.

Do Not Reuse	Upper Limit of Temperature	Range
Use by or Expiration Date (Year-Month-Day)	Lower Limit of Temperature	Range of Means
LOT Batch Code or Lot Number	Temperature Limitation	Midpoint
SN Serial Number	Consult Instructions for Use	Revised
REF Catalog Number or Product Code	Attention: The Instructions for Use (IFU) has been updated	Supersedes
Caution	S1 For use in Slide Supply 1	Σ Contains Sufficient for "n" Tests
Keep Dry (Protect from Moisture/Humidity)	S2 For use in Slide Supply 2	IVD *in vitro* Diagnostic Medical Device
Manufacturer	SI SI Units	Der Grüne Punkt (the Green Dot). Manufacturer follows certain packaging material waste disposal management regulations
Date of Manufacture	CONV Conventional Units	σ Estimated within-lab SD
EC REP Authorized Representative in the European Community	Value	
Corrosive	Flammable	Serious Health Hazards
Health Hazards	Acute Toxicity	Environmental or Aquatic Toxicity

Revision History

Date of Revision	Version	Description of Technical Changes*
2019-09-06	10.1	• Glossary of Symbols: updated • Added EC Representative address
2018-01-10	10.0	• Added information for the VITROS XT 7600 Integrated System • Minor formatting and wording updates • References: updated • Glossary of Symbols: updated

* The change bars indicate the position of a technical amendment to the text with respect to the previous version of the document.

When this Instructions For Use is replaced, sign and date below and retain as specified by local regulations or laboratory policies, as appropriate.

Signature

Obsolete Date

Conditions of supply: all supplies are made subject to the standard terms and conditions of Ortho Clinical Diagnostics or its distributors. Copies of these are available on request.

CE

EC REP

Ortho-Clinical Diagnostics
1500 Boulevard Sébastien Brant
B.P. 30335
67411 Illkirch
CEDEX, France

Ortho-Clinical Diagnostics
Felindre Meadows
Pencoed
Bridgend
CF35 5PZ
United Kingdom

Manufactured under license from Roche Diagnostics US 5,786,163

Ortho Clinical Diagnostics