VITROS Products Immunodiagnostic

INSTRUCTIONS FOR USE NBNP2

VITROS Immunodiagnostic Products NT-proBNP II Reagent Pack

REF 684 4452

VITROS Immunodiagnostic Products NT-proBNP II Calibrators

REF 684 4453

Intended Use

For *in vitro* diagnostic use only.

VITROS Immunodiagnostic Products NT-proBNP II 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 heart failure and for the risk stratification of acute coronary syndrome and heart failure. The test is further indicated as an aid in the assessment of increased risk of cardiovascular events and mortality in patients who have stable coronary artery disease. The test can also be used in the assessment of heart failure severity in patients diagnosed with heart failure.

VITROS Immunodiagnostic Products NT-proBNP II 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

Biochemical Characteristics of Amino-terminal proBNP:

Amino-terminal proBNP (NT-proBNP) and B-type (BNP) natriuretic peptides (NP) are part of the cardiovascular endocrine system. Both are co-released within minutes of their synthesis, in equimolar amounts, following left ventricular (LV) heart wall stress. [1, 2] Induction of the *bnp* gene yields production of $proBNP_{1–108}$ prohormone, which is cleaved by proteases into the biologically active 32-amino acid peptide, BNP, and the biologically inert 76-amino acid peptide, NT-proBNP. All three peptides (proBNP, BNP and NT-proBNP) circulate in humans. [2, 3] BNP elicits natriuresis, diuresis, and vasodilation through activation of cell surface receptor proteins located in kidney, blood vessels and heart. Additionally, BNP opposes activity of the renin–angiotensin–aldosterone system and reduces cardiovascular fibrosis. Overall, these effects work in conjunction to compensate and minimize heart failure (HF) consequences. [4, 5] BNP and NT-proBNP are cleared passively by numerous organs and equally through kidney filtration. [1, 5, 6] In addition, BNP is cleared actively from circulation by receptor binding and enzyme (e.g. neprilysin) degradation [5, 7] ; given the differences in clearance mechanisms, BNP has a considerably shorter half-life (~ 20 min) than NT-proBNP (60-120 min). [8] In healthy adults, circulating concentrations of BNP and NT-proBNP are low, and females tend to have slightly higher NP concentrations than males. In states of acutely decompensated HF (ADHF), NP concentrations typically increase significantly, and sex-dependent differences become less important. [2] BNP and NT-proBNP exhibit parallel changes with age, LV ejection fraction (LVEF), and LV diameter in patients with chronic and symptomatic HF. [9]

Non-heart failure clinical conditions which could modify NP levels:

Although LV dysfunction wall stress is a prime factor responsible for the release of natriuretic peptides, several other conditions may trigger elevation in BNP or NT-proBNP such as valvular abnormalities, arrythmias, pericardial diseases, acute coronary syndrome, heart muscle disease, pulmonary embolism, pulmonary hypertension, sepsis, stroke, cardiotoxic drugs, and renal dysfunction. Obesity, flash pulmonary edema, cardiac tamponade, and pericardial constriction are conditions associated with reduced NP. Therefore, the NT-proBNP (and BNP) values have to be interpreted in the clinical context. [2]

Heart failure definition and terminology:

HF is a clinical syndrome characterized by typical symptoms (e.g. breathlessness, ankle swelling and fatigue) that may be accompanied by signs (e.g. elevated jugular venous pressure, pulmonary crackles and peripheral edema). It is caused by a structural and/or functional cardiac abnormality, resulting in a reduced cardiac output and/or elevated intracardiac pressures at rest or during stress.

Time course and symptoms classify HF into acute (de “novo”), acutely decompensated (when chronic stable HF deteriorates), or chronic stable. [10] Before clinical symptoms become apparent, patients can present with asymptomatic structural or functional cardiac abnormalities (systolic or diastolic LV dysfunction) associated with increased risk for

symptomatic HF and mortality. [10- 12, 13] The prevalence of asymptomatic LV dysfunction ranges from 6%-21% and increases with age. [13- 16]
Heart functionality is assessed by the LV ejection fraction (LVEF); HF comprises patients with similar clinical signs and symptoms and a wide range of LVEF: ≥50% [HF with preserved EF (HFpEF)], 40%–49% [HF with mid-range EF (HFmrEF)], and <40% [HF with reduced EF (HFrEF)]. [10]

Heart Failure severity:
Complementary information regarding the presence and severity of heart failure is provided by the ACCF/AHA stages of HF(A-D) which underline structural changes and by the more subjective but widely used NYHA functional classification (I-IV) which focuses on the severity of symptoms. [14, 17] Rising NT-proBNP levels were shown to correlate with increasing severity of HF. [18, 19]

NT-proBNP Testing for Aid in Diagnosis:
The clinical utility of NT-proBNP testing for aid in diagnosis or exclusion of HF in the acute or non-acute setting was established in numerous studies and led to guideline recommendations for use in clinical practice. [10, 14, 20] The N-terminal Pro-BNP Investigation of Dyspnea in the Emergency Department (PRIDE) study showed that an elevated NT-proBNP value strongly predicted HF diagnosis and was superior to clinical judgement when used in conjunction with clinical assessment for the identification or exclusion of HF. [19] The ICON (International Collaborative on NT-proBNP) and ICON-RELOADED studies further improved diagnostic accuracy by validating age stratification cutoffs for NT-proBNP and concluded that a threshold value <300 pg/mL excludes HF with high negative predictive value (NPV) in the acute setting. [18, 21] In the non-acute setting, NT-proBNP measurement can be useful in determining if new symptoms are likely due to heart failure and in assessing the need for further cardiological investigation in patients with elevated values. [22- 26] A NT-proBNP threshold of 125 pg/mL was found to have high sensitivity and NPV. [23, 24, 25] Hence, the guidelines recommend that, following clinical assessment, NT-proBNP concentrations be used as an initial diagnostic test in patients at risk for HF or with suspected HF, to guide diagnosis and patient management. [10, 20] Patients with NT-proBNP levels below the indicated threshold (300 pg/mL in acute setting and 125 pg/mL in non-acute setting) are unlikely to have HF. [10]

NT-proBNP Testing for Risk Stratification:
Elevated NT-proBNP values, when not associated with heart failure, have been shown to reflect the functional cardiovascular status, thus representing an invaluable tool for risk stratification. [20] Natriuretic peptide prognostic information in acute coronary syndrome (ACS) has been attributed to the association between the extent of myocardial injury, subsequent ventricular dysfunction and increased circulating concentrations of these peptides. Myocardial ischemia may also cause increased production of cardiac natriuretic peptides due to ventricular relaxation abnormalities which could be a stimulus for synthesis and release of NT-proBNP. [27]
Numerous studies have demonstrated that NT-proBNP has cumulative prognostic value to standard approaches of risk assessment, regardless of the variability of the NT-proBNP threshold levels, risk prediction models and lengths of follow-up. [20, 28- 36] Patients with ACS and NT-proBNP levels above certain threshold levels (i.e. 500 pg/mL [28] or higher) have a higher incidence of death and/or cardiovascular events. [28- 36] In the GUSTO IV study, NT-proBNP elevation in patients with non–ST-segment-elevation ACS was a strong predictor of long-term mortality, both independently or when used in conjunction with other biomarkers (creatinine clearance, cardiac troponin): 1 year mortality increased from 0.4% in patients with NT-proBNP levels ≤98 ng/L to 27.1% when levels exceeded 4634 ng/L. [35]
Published studies and current guidelines indicate that measurement of NT-proBNP is also useful for predicting prognosis in patients with HF. [19, 20, 37- 42] A median NT-proBNP value of 2994 pg/mL was predictive of both death and a composite score of death or readmission with worsening HF. [37] In the ICON study, a NT-proBNP threshold of >5180 pg/mL in patients with acute HF, predicted 76-day mortality with odds ratio (OR) of 5.2 (95% CI = 2.2–8.1). [19] NT-proBNP was shown to be a powerful marker of 30-day mortality in patients with decompensated heart failure. Compared with single baseline measurements, serial measurements of NT-proBNP plasma levels within 12 hours after hospital admission may be used to increase the predictive value of NT-proBNP for the early identification of patients who are at high risk of mortality. [38]
In patients with stable coronary artery disease (CAD), data demonstrated that NT-proBNP is a strong and independent prognostic marker associated with incidence of cardiovascular events and death. [43- 53] While the distribution of concentrations varied between studies, baseline NT-proBNP [43- 51, 53] or changes in NT-proBNP at 5 years [52] were significantly higher among individuals with cardiovascular events and/or mortality compared with those without events.

Principles of the Procedure

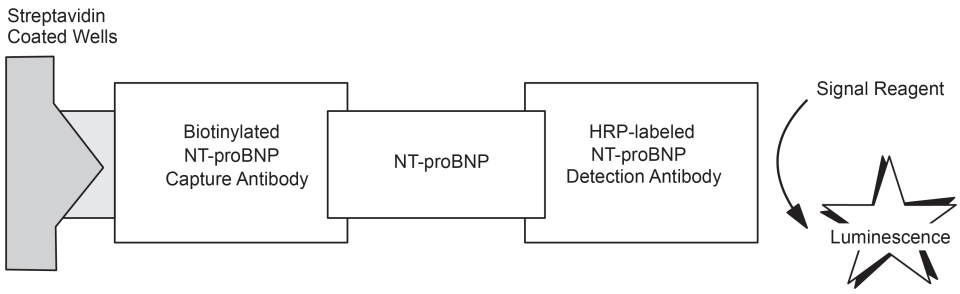
The VITROS NT-proBNP II test utilizes a one-step immunometric bridging design. A well is pushed from the pack and patient sample is dispensed into the antibody coated well. The NT-proBNP II test reagent and the NT-proBNP II HRP reagent are then dispensed into the well with the patient sample. NT-proBNP present in the sample binds with horseradish peroxidase (HRP)-labelled 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.

Test Type	System *	Incubation Time	Time to first result	Test Temperature	Reaction Sample Volume
Immunometric	ECi/ECiQ, 3600, 5600/XT 7600	8 minutes	15 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). [54]

WARNING: ***Contains ProClin 300 (CAS 55965-84-9)*** [55] ***and ProClin 950 (CAS 2682-20-4)***

The VITROS NT-proBNP II Reagent Pack contains 0.5% ProClin 950 and VITROS NT-proBNP II Calibrators contain 1% ProClin 300. H317: May cause an allergic skin reaction. P280: Wear protective gloves. 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 (mouse monoclonal anti-NT-proBNP antibody, 1 µg/mL)
- 8.7 mL test reagent (buffer with sheep serum, bovine gamma globulin, bovine serum albumin, and antimicrobial agent)
- 8.7 mL conjugate reagent (HRP-conjugated sheep monoclonal anti-NT-proBNP antibody, 1 µg/mL in buffer with bovine serum albumin and antimicrobial agent)

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	≤8 weeks
Opened	Refrigerated	2–8 °C (36–46 °F)	≤8 weeks

- The VITROS NT-proBNP II Reagent Pack is suitable for use until the expiration date on the carton when 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.
- Opened reagent packs are moisture/humidity sensitive. Store opened refrigerated reagent packs in a sealed VITROS Immunodiagnostic Products Reagent Pack Storage Box with desiccant.

Calibrator Contents

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

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 set contains sufficient volume for a minimum of 8 calibration events.
- 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 volume 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 II Calibrators are supplied ready for use.
- VITROS NT-proBNP II Calibrators are suitable for use until the expiration date on the carton when stored and handled as specified. Do not use beyond the expiration date.

- The VITROS NT-proBNP II test uses 40 µL of calibrator for each determination. The VITROS NT-proBNP II 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 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
- Plasma (K_2 EDTA, Lithium Heparin)

Specimens Not Recommended

No specimen limitations were identified. Refer to the Limitations of the Procedure section.

Special Precautions

IMPORTANT: *Certain collection devices have been reported to affect other analytes and tests.* [56] *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. [57, 58]
- 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 II 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.
- Follow procedures within your laboratory to avoid cross contamination of patient specimens.
- 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 4 days at 20–25 °C (68–77 °F), 4 days at 2–8 °C (36–46 °F) or 12 months at ≤ -20 °C (-4 °F). [59, 60]
- Avoid repeated freeze-thaw cycles.

Testing Procedure

Materials Provided

- VITROS Immunodiagnostic Products NT-proBNP II Reagent Pack
- VITROS Immunodiagnostic Products NT-proBNP II 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 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 testing. 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 NT-proBNP II. The default short name that will appear on the test selection menus and laboratory reports is NBNP2. 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 70 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 II 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
ECi/ECiQ, 3600, 5600, XT 7600	20.0–30,000 pg/mL (2.36–3,540 pmol/L)

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 II 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
  - If the system is turned off for more than 2 hours
  - After reloading reagent packs that have been removed from the MicroWell Supply and stored for later use
  - 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. [61]

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

Conventional	Alternate
pg/mL (pmol/L× 8.475)	pmol/L (pg/mL× 0.118)

Limitations of the Procedure

Known Interferences

The VITROS NT-proBNP II test was evaluated for interference consistent with CLSI document EP07. [62] Commonly encountered substances were tested on three lots of reagents. The following compound, when tested, caused the bias shown at the concentrations indicated.

Refer to "Specificity" for a list of compounds tested that did not show interference.

Interferent	Interferent Concentration		Analyte Conc*		% Bias**
			pg/mL	pmol/L	
Cefoxitin sodium	697 mg/dL	15.5 mmol/L	77.6	9.16	-25.4
			878	104	-22.3
	281 mg/dL	6.25 mmol/L	107	12.6	-10
			797	94.0	-10
Phenprocoumon (Marcumar)	1.5 mg/dL	53.5 mmol/L	85.7	10.1	-10.6
	1.38 mg/dL	49.2 mmol/L	86.3	10.2	-10
Sodium Azide	100 mg/dL	15.4 mmol/L	92.7	10.9	-12.5
	77.5 mg/dL	11.9 mmol/L	95.4	11.3	-10

* Average test replicate determinations.

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

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

- Heterophilic antibodies in serum or plasma samples may cause interference in immunoassays. [63] These antibodies may be present in blood samples from individuals regularly exposed to animals or who have been treated with animal serum products. Results that are inconsistent with clinical observations indicate the need for additional testing.
- In extremely rare cases (global incidence: <1 in 10 million) patients may show discrepant results when tested with the VITROS NT-proBNP II test (values < Limit of Detection) due to a NT-proBNP genetic variant. [64]
- The VITROS NT-proBNP II test has no high dose hook effect up to a concentration of 300,000 pg/mL (35,400 pmol/L).

Expected Values and Interpretation of Results

Reference Interval

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. 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)	RI Lower Limit (pmol/L)	RI Upper Limit (pmol/L)
22–<50	Female	129	<20.0	95.3	<2.36	11.2
50–<75	Female	127	<20.0	221	<2.36	26.1
≥75	Female	129	<20.0	296	<2.36	34.9
22–<50	Male	131	<20.0	125	<2.36	14.8
50–<75	Male	120	<20.0	299	<2.36	35.3
≥75	Male	123	<20.0	326	<2.36	38.5
Overall		756	<20.0	217	<2.36	25.6

Interpretation of Results

The age-independent and the age-dependent cutoffs at which assay performance was evaluated were previously identified and validated in studies such as PRIDE, ICON, and ICON Reloaded. [18, 19, 21] Results of this test should be used in conjunction with clinical presentation, other diagnostic tests, and in accordance with the appropriate clinical guidelines. The validated cutoffs should be interpreted as indicated in the table below:

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

* Natriuretic peptides values in the gray zones could be caused by several conditions other than heart failure. Clinical conditions such as valvular abnormalities, acute coronary syndrome, heart muscle disease, pulmonary embolism, pulmonary hypertension, sepsis, stroke, cardiotoxic drugs, and renal dysfunction will elevate NT-proBNP levels; obesity, flash pulmonary edema, cardiac tamponade, and pericardial constriction are conditions associated with reduced NT-proBNP. [2, 65, 66]

Performance Characteristics

Clinical Performance Characteristics

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

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 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 HF diagnosis was adjudicated by independent cardiologists or ED physicians all experienced in diagnosing HF.

The VITROS NT-proBNP II test results were determined from 1020 subjects, 487 females and 533 males, ranging in age from 22 to 97 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:

Female Subjects

Study Population	Heart Failure					Non-Heart Failure				
Age (years)	22–<50	50–<75	<75	≥75	All	22–<50	50–<75	<75	≥75	All
N	24	105	129	102	231	36	151	187	69	256
Mean	15800	7910	9380	9930	9620	258	921	794	1130	885
SD	42900	9350	20300	14300	17900	433	2510	2280	1390	2080
Median	2130	4690	3700	5820	4850	58.5	254	209	631	290
Min	20.0	281	20.0	450	20.0	20.0	20.0	20.0	20.0	20.0
Max	167000	49200	167000	99200	167000	2350	23400	23400	6980	23400

Male Subjects

Study Population	Heart Failure					Non-Heart Failure				
Age (years)	22–<50	50–<75	<75	≥75	All	22–<50	50–<75	<75	≥75	All
N	45	162	207	105	312	39	142	181	40	221
Mean	5950	8410	7880	9290	8350	470	1110	972	1230	1020
SD	7690	12200	11400	10000	10900	907	2510	2270	1460	2150
Median	3430	4710	4190	5930	4760	64.4	252	197	595	290
Min	671	188	188	630	188	20.0	20.0	20.0	27.1	20.0
Max	43000	86500	86500	60600	86500	4020	18500	18500	6440	18500

All Subjects

Study Population	Heart Failure					Non-Heart Failure				
Age (years)	22–<50	50–<75	<75	≥75	All	22–<50	50–<75	<75	≥75	All
N	69	267	336	207	543	75	293	368	109	477
Mean	9370	8220	8450	9600	8890	368	1010	882	1170	947
SD	26200	11100	15400	12300	14300	723	2510	2280	1410	2110
Median	3080	4710	3990	5830	4830	63.4	254	206	626	290
Min	20.0	188	20.0	450	20.0	20.0	20.0	20.0	20.0	20.0
Max	167000	86500	167000	99200	167000	4020	23400	23400	6980	23400

Receiver Operating Characteristic (ROC) curves [67] were generated. The diagnostic accuracy of the VITROS NT-proBNP II test, as quantified by areas under the ROC curves (AUC) [68], within and across age group ranged from 0.92 to 0.95. AUC and the two-tailed 95% confidence intervals (CI) [69] by age group is summarized in the following table:

All Subjects

Age Group (years)	N	AUC	95% CI
22–<50	144	0.95	0.91–0.99
50–<75	560	0.92	0.90–0.94
<75	704	0.92	0.90–0.94
≥75	316	0.93	0.90–0.96
Overall	1020	0.93	0.91–0.94

The clinical performance and the two-tailed 95% confidence intervals (CI) of the VITROS NT-proBNP II test versus adjudicated diagnosis was determined within and across gender by age group using the age-dependent rule-in (450 pg/mL for subjects 22–<50 years old or 900 pg/mL for subjects 50–<75 years old or 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)	N	Sensitivity (%) (n/N)	95% CI* (%)	Specificity (%) (n/N)	95% CI* (%)	NPV (%) (n/N)	95% CI* (%)	PPV (%) (n/N)	95% CI* (%)
Rule-in									
22–<50	60	91.67 (22/24)	73.00–98.97	83.33 (30/36)	67.19–93.63	93.75 (30/32)	79.19–99.23	78.57 (22/28)	59.05–91.70
50–<75	256	90.48 (95/105)	83.18–95.34	77.48 (117/151)	69.98–83.87	92.13 (117/127)	86.00–96.16	73.64 (95/129)	65.16–81.01
<75 **	316	90.70 (117/129)	84.31–95.10	78.61 (147/187)	72.03–84.26	92.45 (147/159)	87.19–96.04	74.52 (117/157)	66.96–81.13
≥75	171	88.24 (90/102)	80.35–93.77	79.71 (55/69)	68.31–88.44	82.09 (55/67)	70.80–90.39	86.54 (90/104)	78.45–92.44
Overall ***	487	89.61 (207/231)	84.94–93.23	78.91 (202/256)	73.39–83.74	89.38 (202/226)	84.61–93.08	79.31 (207/261)	73.88–84.06
Rule-out									
All Females	487	98.70 (228/231)	96.25–99.73	50.78 (130/256)	44.48–57.06	97.74 (130/133)	93.55–99.53	64.41 (228/354)	59.17–69.40

* 95% Exact Confidence Interval

** Combining age groups 22–<50 and 50–<75 years old after applying the age-dependent cutoffs

*** Combining all age groups after applying the age-dependent cutoffs

Age Group (Years)	N	LR-	95% CI	LR+	95% CI
Rule-in					
22–<50	60	0.10	0.03–0.38	5.50	2.62–11.53
50–<75	256	0.12	0.07–0.22	4.02	2.97–5.44
<75**	316	0.12	0.07–0.21	4.24	3.20–5.61
≥75	171	0.15	0.09–0.26	4.35	2.71–6.98
Overall***	487	0.13	0.09–0.19	4.25	3.34–5.41
Rule-out					
All Females	487	0.03	0.01–0.09	2.01	1.77–2.27

** Combining age groups 22–<50 and 50–<75 years old after applying the age-dependent cutoffs

*** Combining all age groups after applying the age-dependent cutoffs

Male Subjects

Age Group (Years)	N	Sensitivity (%) (n/N)	95% CI* (%)	Specificity (%) (n/N)	95% CI* (%)	NPV (%) (n/N)	95% CI* (%)	PPV (%) (n/N)	95% CI* (%)
Rule-in									
22–<50	84	100.00 (45/45)	92.13–100.00	71.79 (28/39)	55.13–85.00	100.00 (28/28)	87.66–100.00	80.36 (45/56)	67.57–89.77
50–<75	304	91.36 (148/162)	85.93–95.19	74.65 (106/142)	66.67–81.57	88.33 (106/120)	81.20–93.47	80.43 (148/184)	73.96–85.90
<75 **	388	93.24 (193/207)	88.91–96.25	74.03 (134/181)	67.01–80.25	90.54 (134/148)	84.64–94.73	80.42 (193/240)	74.82–85.24
≥75	145	92.38 (97/105)	85.54–96.65	77.50 (31/40)	61.55–89.16	79.49 (31/39)	63.54–90.70	91.51 (97/106)	84.49–96.04
Overall ***	533	92.95 (290/312)	89.52–95.53	74.66 (165/221)	68.39–80.26	88.24 (165/187)	82.73–92.48	83.82 (290/346)	79.50–87.54
Rule-out									
All Males	533	99.36 (310/312)	97.70–99.92	50.23 (111/221)	43.44–57.00	98.23 (111/113)	93.75–99.78	73.81 (310/420)	69.33–77.95

* 95% Exact Confidence Interval

** Combining age groups 22–<50 and 50–<75 years old after applying the age-dependent cutoffs

*** Combining all age groups after applying the age-dependent cutoffs

Age Group (Years)	N	LR-	95% CI	LR+	95% CI
Rule-in					
22–<50	84	0.00	N/A****	3.55	2.15–5.85
50–<75	304	0.12	0.07–0.20	3.60	2.71–4.80
<75**	388	0.09	0.05–0.15	3.59	2.80–4.60
≥75	145	0.10	0.05–0.20	4.11	2.30–7.32
Overall***	533	0.09	0.06–0.14	3.67	2.92–4.61
Rule-out					
All Males	533	0.01	0.00–0.04	2.00	1.75–2.28

** Combining age groups 22–<50 and 50–<75 years old after applying the age-dependent cutoffs

*** Combining all age groups after applying the age-dependent cutoffs

**** N/A: Not applicable. Sensitivity was 100%

All Subjects

Age Group (Years)	N	Sensitivity (%) (n/N)	95% CI* (%)	Specificity (%) (n/N)	95% CI* (%)	NPV (%) (n/N)	95% CI* (%)	PPV (%) (n/N)	95% CI* (%)
Rule-in									
22–<50	144	97.10 (67/69)	89.92–99.65	77.33 (58/75)	66.21–86.21	96.67 (58/60)	88.47–99.59	79.76 (67/84)	69.59–87.75
50–<75	560	91.01 (243/267)	86.92–94.16	76.11 (223/293)	70.81–80.88	90.28 (223/247)	85.89–93.67	77.64 (243/313)	72.61–82.13
<75 **	704	92.26 (310/336)	88.87–94.88	76.36 (281/368)	71.68–80.61	91.53 (281/307)	87.84–94.39	78.09 (310/397)	73.69–82.06
≥75	316	90.34 (187/207)	85.47–94.00	78.90 (86/109)	70.04–86.13	81.13 (86/106)	72.38–88.08	89.05 (187/210)	84.02–92.93
Overall ***	1020	91.53 (497/543)	88.86–93.73	76.94 (367/477)	72.89–80.65	88.86 (367/413)	85.42–91.73	81.88 (497/607)	78.58–84.86
Rule-out									
All Patients	1020	99.08 (538/543)	97.86–99.70	50.52 (241/477)	45.94–55.10	97.97 (241/246)	95.32–99.34	69.51 (538/774)	66.13–72.74

* 95% Exact Confidence Interval

** Combining age groups 22–<50 and 50–<75 years old after applying the age-dependent cutoffs

*** Combining all age groups after applying the age-dependent cutoffs

Age Group (Years)	N	LR-	95% CI	LR+	95% CI
Rule-in					
22–<50	144	0.04	0.01–0.16	4.28	2.81–6.52
50–<75	560	0.12	0.08–0.18	3.81	3.09–4.69
<75**	704	0.10	0.07–0.15	3.90	3.24–4.70
≥75	316	0.12	0.08–0.18	4.28	2.97–6.17
Overall***	1020	0.11	0.08–0.15	3.97	3.36–4.69
Rule-out					
All Patients	1020	0.02	0.01–0.05	2.00	1.83–2.19

** Combining age groups 22–<50 and 50–<75 years old after applying the age-dependent cutoffs

*** Combining all age groups after applying the age-dependent cutoffs

The impact of the VITROS NT-proBNP II test values between the rule-out cutoff and rule-in cutoffs or “gray zone” (≥300 pg/mL to <450 pg/mL for subjects 22–<50 years old; ≥300 pg/mL to <900 pg/mL for subjects 50–<75 years old; ≥300 pg/mL to <1800 pg/mL for subjects ≥75 years old) for each age group was evaluated.
The prevalence, clinical performance and the two-tailed 95% confidence intervals (CI) of the VITROS NT-proBNP II test result versus adjudicated diagnosis was determined using the age-dependent rule-in (450 pg/mL for subjects 22–<50 years old or 900 pg/mL for subjects 50–<75 years old or 1800 pg/mL for subjects ≥75 years old) and age-independent rule-out (300 pg/mL) cutoffs and are summarized in the following table:

All Subjects

Age Group (Years)	Prevalence of HF (%) (n/N)	VITROS NT-proBNP II Test Result Interpretation	LR	95% CI	Predictive Value of HF (%) (n/N)	95% CI* (%)	Predictive Value of non-HF (%) (n/N)	95% CI* (%)
22–<50	47.92 (69/144)	Positive	4.28	2.81–6.52	79.76 (67/84)	69.59–87.75	20.24 (17/84)	12.25–30.41
		Gray zone	0.00	N/A ****	0.00 (0/6)	0.00–45.93	100.0 (6/6)	54.07–100.0
		Negative	0.04	0.01–0.17	3.70 (2/54)	0.45–12.75	96.30 (52/54)	87.25–99.55
50–<75	47.68 (267/560)	Positive	3.81	3.09–4.69	77.64 (243/313)	72.61–82.13	22.36 (70/313)	17.87–27.39
		Gray zone	0.35	0.22–0.55	24.14 (21/87)	15.60–34.50	75.86 (66/87)	65.50–84.40
		Negative	0.02	0.01–0.06	1.88 (3/160)	0.39–5.38	98.13 (157/160)	94.62–99.61
<75 **	47.73 (336/704)	Positive	3.90	3.24–4.70	78.09 (310/397)	73.69–82.06	21.91 (87/397)	17.94–26.31
		Gray zone	0.32	0.20–0.51	22.58 (21/93)	14.55–32.42	77.42 (72/93)	67.58–85.45
		Negative	0.03	0.01–0.06	2.34 (5/214)	0.76–5.37	97.66 (209/214)	94.63–99.24
≥75	65.51 (207/316)	Positive	4.28	2.97–6.17	89.05 (187/210)	84.02–92.93	10.95 (23/210)	7.07–15.98
		Gray zone	0.20	0.12–0.31	27.03 (20/74)	17.35–38.61	72.97 (54/74)	61.39–82.65
		Negative	0.00	N/A ****	0.00 (0/32)	0.00–10.89	100.0 (32/32)	89.11–100.0
All Patients ***	53.24 (543/1020)	Positive	3.97	3.36–4.69	81.88 (497/607)	78.58–84.86	18.12 (110/607)	15.14–21.42
		Gray zone	0.29	0.21–0.40	24.55 (41/167)	18.23–31.80	75.45 (126/167)	68.20–81.77
		Negative	0.02	0.01–0.04	2.03 (5/246)	0.66–4.68	97.97 (241/246)	95.32–99.34

* 95% Exact Confidence Interval

** Combining age groups 22–<50 and 50–<75 years old after applying the age-dependent cutoffs

*** Combining all age groups after applying the age-dependent cutoffs

**** N/A: Not applicable: There were no samples with VITROS NT-proBNP II test results in this interpretation group

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

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

The clinical performance of the VITROS NT-proBNP II test as an aid in the assessment of HF severity was determined using samples from 3 sites in the United States. Samples from subjects that were diagnosed with HF and presenting to ED or outpatient facilities were acquired. Subjects' NYHA Class (I-IV) was assessed at the time of sample acquisition.
The VITROS NT-proBNP II test results were determined from samples from 515 subjects, 211 females and 304 males, ranging in age from 25 to 97 years. The descriptive statistics for the VITROS NT-proBNP II test results (pg/mL) were determined within and across gender and are summarized in the following tables:

Female Subjects

Statistics	NYHA Functional Classification			
	NYHA Class I	NYHA Class II	NYHA Class III	NYHA Class IV
n	52	72	49	38
Mean	5660	6450	8960	11500
SD	16500	16000	13400	14300
5thPercentile	31.0	78.5	258	453
Median	1480	2110	3380	4560
95thPercentile	26400	22700	36200	49000
% >125 pg/mL	82.7	91.7	95.9	100
% >300 pg/mL	71.2	84.7	91.8	97.4

Jonckheere-Terpstra [70] test of trend p <0.0001

Male Subjects

Statistics	NYHA Functional Classification			
	NYHA Class I	NYHA Class II	NYHA Class III	NYHA Class IV
n	97	64	92	51
Mean	6610	6010	7920	14900
SD	21500	9290	10300	17000
5thPercentile	53.0	42.1	510	1710
Median	1380	2260	3680	9010
95thPercentile	31600	28700	32500	50700
% >125 pg/mL	88.7	85.9	98.9	98.0
% >300 pg/mL	83.5	84.4	97.8	98.0

Jonckheere-Terpstra test of trend p <0.0001

All Subjects

Statistics	NYHA Functional Classification			
	NYHA Class I	NYHA Class II	NYHA Class III	NYHA Class IV
n	149	136	141	89
Mean	6280	6240	8280	13500
SD	19800	13200	11500	15900
5thPercentile	42.3	71.0	398	916
Median	1400	2190	3600	8190
95thPercentile	27300	28700	32500	49000
% >125 pg/mL	86.6	89.0	97.9	98.9
% >300 pg/mL	79.2	84.6	95.7	97.8

Jonckheere-Terpstra test of trend p <0.0001

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

Risk Stratification

A literature review was conducted to demonstrate clinical performance and validity of the VITROS NT-proBNP II test as an aid in the assessment of increased risk of cardiovascular events and mortality in patients who have stable coronary artery disease (CAD) and for the risk stratification of acute coronary syndrome (ACS) and heart failure (HF).
Review of evidence from published studies in patients with stable CAD, ACS and HF demonstrated consistently that increased levels of NT-proBNP are associated with the increased incidence of death, cardiovascular events and composite outcomes of death and cardiovascular events.
The literature references provided are representative of numerous publications evaluated during the literature review. [21, 27- 53]

Limit of Detection

The Limit of Detection (LoD) for the VITROS NT-proBNP II test is 0.49 pg/mL (0.058 pmol/L), determined according to CLSI document EP17.[71] The Limit of Quantitation (LoQ) was determined consistent with CLSI document EP17.[71] The VITROS NT-proBNP II test was designed to have a LoQ less than or equal to 30.0 pg/mL (3.54 pmol/L) at 20% CV. The observed Limit of Quantitation at 20% CV was determined to be 0.56 pg/mL (0.066 pmol/L) and the claimed LoQ was set at 20.0 pg/mL (2.36 pmol/L) to maintain linearity within the measuring range.

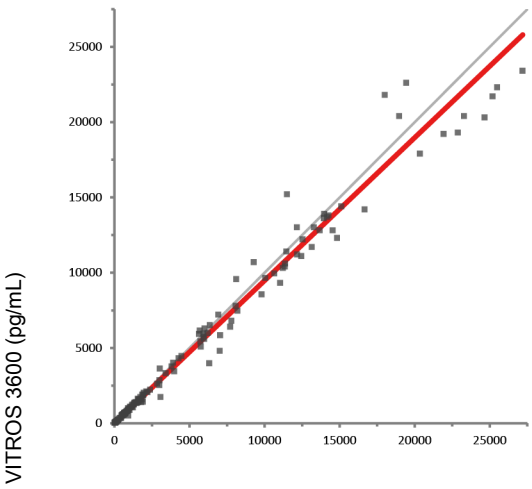
Limit of Detection and Limit of Quantitation

LoD		LoQ	
pg/mL	pmol/L	pg/mL	pmol/L
0.49	0.058	20.0	2.36

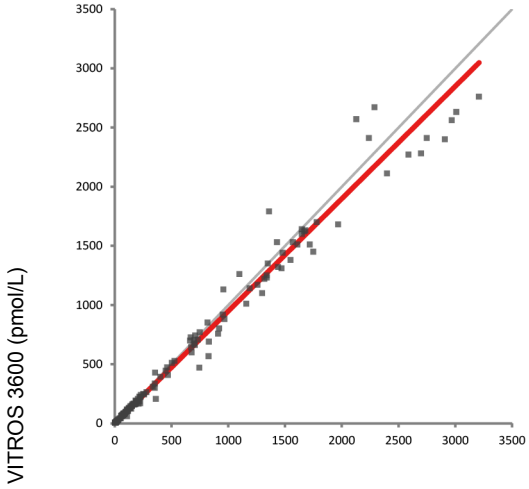
Method Comparison

Accuracy was evaluated consistent with CLSI document EP09.[72] The plots and table show the results of a method comparison study using patient samples analyzed on the VITROS 3600 Immunodiagnostic System compared with the commercially available Elecsys® proBNP II Immunoassay. The relationship between the 2 methods was determined by Passing-Bablok[73] regression and Pearson correlation.

Conventional Units



Alternate Units



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
VITROS 3600* vs. Comparative Method	153	0.95	0.989	24.5–23400	-8.46	2.89–2760	-1.00

* Performance characteristics for the VITROS 3600 Immunodiagnostic System are applicable to the VITROS ECi/ECiQ Immunodiagnostic System and the VITROS 5600/XT 7600 Integrated Systems.

Precision

Precision was evaluated consistent with CLSI document EP05.[74] 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 reagent lots on one VITROS ECi/ECiQ Immunodiagnostic System, one VITROS 3600 Immunodiagnostic System, one VITROS 5600 Integrated System, and one VITROS XT 7600 Integrated System. The data presented are a representation of the product performance.

System	Conc. Units = pg/mL							No. Observ.	No. Days
	NT-proBNP Conc.	Within-run*		Within-calibration**		Within-lab***			
		SD	%CV	SD	%CV	SD	%CV		
ECi/ECiQ	30.9	0.61	2.0	1.21	3.9	1.20	3.9	80	20
	69.2	2.05	3.0	2.41	3.5	2.63	3.8	80	20
	92.6	1.78	1.9	4.11	4.4	4.54	4.9	80	20
	206	4.0	1.9	6.1	3.0	8.0	3.9	80	20
	355	4.5	1.3	13.5	3.8	17.0	4.8	80	20
	776	15.8	2.0	28.1	3.6	33.8	4.3	80	20
	894	18.1	2.0	22.8	2.6	38.1	4.3	80	20
	1660	31	1.9	77	4.6	94	5.7	80	20
	5460	96	1.8	149	2.7	203	3.7	80	20
	10600	180	1.7	300	2.8	400	3.8	80	20
	23600	500	2.1	960	4.0	1220	5.1	80	20
3600	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
	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
5600	32.1	0.42	1.4	1.40	4.4	1.65	5.0	80	20
	70.8	1.06	1.5	2.62	3.8	3.07	4.3	80	20
	100	1.1	1.2	3.6	3.7	4.3	4.2	80	20
	220	2.6	1.2	7.0	3.3	8.7	3.9	80	20
	374	4.3	1.2	17.7	4.8	20.4	5.4	80	20
	814	9.4	1.2	26.1	3.3	32.9	4.0	80	20
	924	11.4	1.3	23.9	2.6	34.7	3.7	80	20
	1730	19	1.1	78	4.5	95	5.4	80	20
	5720	65	1.2	148	2.6	176	3.0	80	20
	11200	140	1.3	200	1.8	240	2.1	80	20
	24300	290	1.2	730	3.1	750	3.0	80	20
XT 7600	35.0	0.67	1.9	1.61	4.7	2.26	6.4	80	20
	72.7	1.90	2.6	3.50	4.9	4.86	6.6	80	20
	102	2.2	2.2	3.6	3.6	5.6	5.4	80	20
	219	3.6	1.7	6.5	3.0	10.0	4.5	80	20
	369	5.3	1.5	14.1	3.9	18.2	4.9	80	20
	801	10.7	1.3	26.6	3.3	29.4	3.6	80	20
	920	12.5	1.4	25.5	2.8	35.7	3.8	80	20
	1730	28	1.6	69	4.0	79	4.5	80	20
	5830	77	1.3	147	2.5	201	3.4	80	20
	11400	160	1.4	250	2.2	340	3.0	80	20
	24700	370	1.5	550	2.2	780	3.1	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.

System	Conc. Units = pmol/L							No. Observ.	No. Days
	NT-proBNP Conc.	Within-run*		Within-calibration**		Within-lab***			
		SD	%CV	SD	%CV	SD	%CV		
ECi/ECiQ	3.65	0.072	2.0	0.143	3.9	0.142	3.9	80	20
	8.17	0.242	3.0	0.284	3.5	0.310	3.8	80	20
	10.9	0.210	1.9	0.485	4.4	0.536	4.9	80	20
	24.3	0.472	1.9	0.720	3.0	0.944	3.9	80	20
	41.9	0.531	1.3	1.59	3.8	2.01	4.8	80	20
	91.6	1.86	2.0	3.32	3.6	3.99	4.3	80	20
	105	2.14	2.0	2.69	2.6	4.50	4.3	80	20
	196	3.66	1.9	9.09	4.6	11.1	5.7	80	20
	644	11.3	1.8	17.6	2.7	24.0	3.7	80	20
	1250	21.2	1.7	35.4	2.8	47.2	3.8	80	20
	2780	59.0	2.1	113	4.0	144	5.1	80	20
3600	3.96	0.061	1.5	0.192	4.9	0.225	5.7	80	20
	8.30	0.171	2.1	0.391	4.7	0.457	5.5	80	20
	11.5	0.171	1.5	0.460	4.0	0.545	4.7	80	20
	25.3	0.460	1.8	0.755	3.0	0.932	3.7	80	20
	43.1	0.519	1.2	1.29	3.0	1.65	3.8	80	20
	96.2	1.37	1.4	3.39	3.5	3.60	3.7	80	20
	111	1.19	1.1	3.14	2.8	3.49	3.2	80	20
	204	2.24	1.1	10.1	5.0	9.91	4.8	80	20
	688	9.44	1.4	18.3	2.6	18.8	2.7	80	20
	1330	13.0	1.0	24.8	1.9	31.9	2.4	80	20
	2900	43.7	1.5	83.8	2.9	86.1	3.0	80	20
5600	3.79	0.050	1.4	0.165	4.4	0.195	5.0	80	20
	8.35	0.125	1.5	0.309	3.8	0.362	4.3	80	20
	11.8	0.130	1.2	0.425	3.7	0.507	4.2	80	20
	26.0	0.307	1.2	0.826	3.3	1.03	3.9	80	20
	44.1	0.507	1.2	2.09	4.8	2.41	5.4	80	20
	96.1	1.11	1.2	3.08	3.3	3.88	4.0	80	20
	109	1.35	1.3	2.82	2.6	4.09	3.7	80	20
	204	2.24	1.1	9.20	4.5	11.2	5.4	80	20
	675	7.67	1.2	17.5	2.6	20.8	3.0	80	20
	1320	16.5	1.3	23.6	1.8	28.3	2.1	80	20
	2870	34.2	1.2	86.1	3.1	88.5	3.0	80	20
XT 7600	4.13	0.079	1.9	0.190	4.7	0.267	6.4	80	20
	8.58	0.224	2.6	0.413	4.9	0.573	6.6	80	20
	12.0	0.260	2.2	0.425	3.6	0.66	5.4	80	20
	25.8	0.425	1.7	0.767	3.0	1.18	4.5	80	20
	43.5	0.625	1.5	1.66	3.9	2.15	4.9	80	20
	94.5	1.26	1.3	3.14	3.3	3.47	3.6	80	20
	109	1.48	1.4	3.01	2.8	4.21	3.8	80	20
	204	3.30	1.6	8.14	4.0	9.32	4.5	80	20
	688	9.09	1.3	17.3	2.5	23.7	3.4	80	20
	1350	18.9	1.4	29.5	2.2	40.1	3.0	80	20
	2910	43.7	1.5	64.9	2.2	92.0	3.1	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.

Specificity

Substances that do not Interfere

The VITROS NT-proBNP II test was evaluated for interference consistent with CLSI document EP07. [62] Of the compounds tested, 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		Insulin	3.12 µg/dL	5.37 nmol/L
Acetylcysteine	15.0 mg/dL	920 µmol/L		Intralipid	2.00 g/dL	NA
Adrenaline (Epinephrine)	20.0 µg/dL	1.09 µmol/L		L-dopa (Levodopa)	750 µg/dL	38.0 µmol/L
Alprazolam	25.8 µg/dL	836 nmol/L		Levothyroxine	42.9 µg/dL	552 nmol/L
Amlodipine besylate	10.5 µg/dL	184 nmol/L		Lidocaine	1.50 mg/dL	64.0 µmol/L
Amoxicillin	5.40 mg/dL	148 µmol/L		Methyldopa sesquihydrate	2.25 mg/dL	94.4 µmol/L
Ascorbic acid	5.25 mg/dL	298 µmol/L		Methylprednisolone	783 µg/dL	20.9 µmol/L
Atorvastatin calcium trihydrate	162 µg/dL	1.34 µmol/L		Metoprolol hemitartrate	150 µg/dL	2.19 µmol/L
Benazepril HCl	44.0 µg/dL	955 nmol/L		Metronidazole	12.3 mg/dL	718 µmol/L
Bilirubin, conjugated	40.0 mg/dL	474 µmol/L		Molsidomine	18.0 µg/dL	743 nmol/L
Bilirubin, unconjugated	40.0 mg/dL	684 µmol/L		Naproxen sodium	39.3 mg/dL	1.56 mmol/L
Biotin	3510 ng/mL	14.4 µmol/L		Nicardipine HCL	46.5 µg/dL	901 nmol/L
Caffeine	10.8 mg/dL	556 µmol/L		Nifedipine	58.8 µg/dL	1.70 µmol/L
Carvedilol	43.2 µg/dL	1.06 µmol/L		Omeprazole	840 µg/dL	24.3 µmol/L
Ceftriaxone disodium hemi(heptahydrate)	100 mg/dL	1510 µmol/L		Oxycodone HCl	32.4 µg/dL	0.92 µmol/L
Cholesterol	400 mg/dL	10.3 mmol/L		Phenobarbital	69.0 mg/dL	2.97 mmol/L
Clopidogrel hydrogen sulfate	2.40 µg/dL	57.2 nmol/L		Propafenone HCL	72.0 µg/dL	1.91 µmol/L
Cotinine	240 µg/dL	13.6 µmol/L		Pseudoephedrine HCl	330 µg/dL	16.4 µmol/L
Creatinine	15.0 mg/dL	1.33 mmol/L		Rheumatoid Factor	1500 IU/mL	NA
Cyclosporine	180 µg/dL	1.50 µmol/L		Rifampicin (Rifampin)	4.80 mg/dL	58.3 µmol/L
Dextran	2.40 g/dL	600 µmol/L		Sacubitril calcium salt	0.915 mg/dL	21.3 µmol/L
Digitoxin	7.50 µg/dL	98.0 nmol/L		Salicylic acid	2.86 mg/dL	207 µmol/L
Digoxin	3.90 µg/dL	49.9 nmol/L		Salmeterol	1.65 µg/dL	39.7 nmol/L
Diphenhydramine HCl	77.4 µg/dL	2.65 µmol/L		Sotalol hydrochloride	510 µg/dL	16.5 µmol/L
Dipyrone (as 4-methylaminoantipyrine Hydrochloride)	3.30 mg/dL	130 µmol/L		Spironolactone	55.5 µg/dL	1.33 µmol/L
Dypyridamole	1.00 mg/dL	19.8 µmol/L		Streptokinase	150,000 U/dL	NA
Doxycycline hyclate	1.80 mg/dL	35.1 µmol/L		Theophylline	6.00 mg/dL	333 µmol/L
Enalaprilat dihydrate	81.9 µg/dL	2.13 µmol/L		Tolbutamide	54.9 mg/dL	2.03 mmol/L
Ethanol	600 mg/dL	130 mmol/L		Total Protein	15.0 g/dL	NA
Fibrinogen	1000 mg/dL	NA		tPA (Alteplase)	1.20 mg/dL	NA
Furosemide	1.59 mg/dL	48.1 µmol/L		Triglyceride	1500 mg/dL	16.9 mmol/L
Gentamicin Sulfate	3.51 mg/dL	61.0 µmol/L		Valproic Acid	31.8 mg/dL	2.21 mmol/L
Glycerylnitrate (Nitroglycerin)	1.20 µg/dL	52.8 nmol/L		Valsartan	1.17 mg/dL	26.9 µmol/L
HAMA (Human Anti-Mouse Antibody	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
Ibuprofen	21.9 mg/dL	1.06 mmol/L				

Cross-Reactivity

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	-0.13	-0.02	891	105	29.7
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	116	13.7	<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	122	14.4	1310	155	39.6
NT-proANP$_{1–30}$ (preproANP$_{25–55}$)	3.50 µg/mL	0.998 µmol/L	105	12.4	774	91.3	<1.0
NT-proANP$_{31–67}$ (preproANP$_{56–92}$)	1.00 ng/mL	0.258 nmol/L	114	13.5	119	14.0	<1.0
NT-proANP$_{79–98}$ (preproANP$_{104–123}$)	1.00 ng/mL	0.458 nmol/L	114	13.5	121	14.3	<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	116	13.7	120	14.2	<1.0
Adrenomedullin	1.00 ng/mL	0.166 nmol/L	114	13.5	117	13.8	<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	114	13.5	120	14.2	1.0
Angiotensin II	0.600 ng/mL	0.574 nmol/L	112	13.2	114	13.5	<1.0
Angiotensin III	1.00 ng/mL	1.07 nmol/L	114	13.5	119	14.0	<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	113	13.3	119	14.0	<1.0
Arg-Vasopressin	1.00 µg/mL	0.922 µmol/L	114	13.5	120	14.2	<1.0
Renin	50.0 ng/mL	28.4 nmol/L	114	13.5	122	14.4	<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.

$$\% \text{ Cross-reactivity} = \frac{(\text{Mean NT-proBNP Result Cross-reactant Sample}) - (\text{Mean NT-proBNP Result Control Sample})}{\text{Concentration of Cross-Reactant}} \times 100$$

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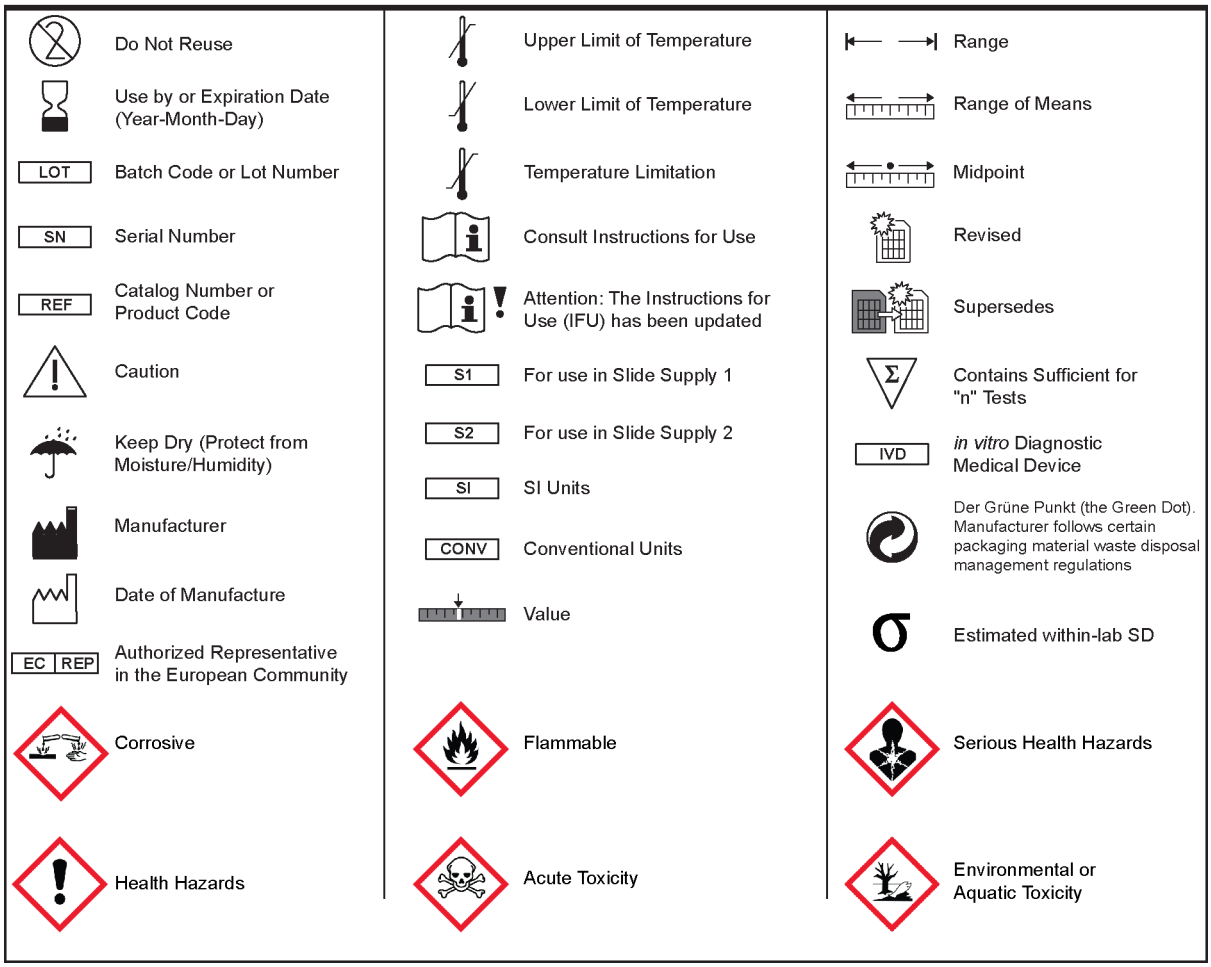
Glossary of Symbols

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



Revision History

Date of Revision	Version	Description of Technical Changes*
2020-08-24	3.0	• Removed statement – Lots 40 and above • Warnings and Precautions: updated P280 statement • Handling and Storage Conditions: changed sample storage times from 2 days and 3 days to 4 days for both conditions • Known Interferences: updated Interferent table • Specificity: removed Phenprocoumon (Marcumar) and Sodium Azide from Substances That Do Not Interfere • References: removed associated references; updated reference section

* 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.

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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

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