# VIDAS® NT-proBNP2 (PBN2)

IVD

VIDAS NT-proBNP2 (PBN2) is an automated quantitative test for use on the instruments of the VIDAS family for the determination of N terminal fragment of Brain-type natriuretic peptide in human serum or plasma (lithium and sodium heparin) using the ELFA technique (Enzyme-Linked Fluorescent Assay). The VIDAS NT-proBNP2 (PBN2) test is used as an aid in the diagnosis of suspected heart failure.

## SUMMARY AND EXPLANATION

Heart failure (HF) is a complex clinical syndrome in which the heart's ability to pump blood is inadequate to meet the metabolic needs of the body. The most common symptoms are shortness of breath, fatigue, tachycardia and edema (1). The natriuretic peptides, ANP (atrial natriuretic peptide), BNP (B for brain-type) and CNP (C-type natriuretic peptide) are neurohormones released from the heart (ANP and BNP) or vascular endothelial cells (CNP) in response to hemodynamic stress. They are involved in the regulation of intravascular homeostasis: vasodilation (antagonism of the renin-angiotensin system), natriuresis (increased renal sodium secretion) and diuresis (2). The BNP gene is activated in cardiomyocytes in response to increased myocardial wall stress due to volume expansion or pressure overload. This results in the production of the precursor molecule proBNP which is further processed to release the biologically active C-terminal fragment BNP and the inactive N-terminal fragment NT-proBNP (2). BNP and NT-proBNP concentrations in blood are well correlated but NT-proBNP levels are higher than BNP due to differences in half-life (120 vs. 60 minutes) (2). The kidneys equally clear both molecules, but BNP is also cleared by natriuretic peptide receptors or neutral endopeptidases (proteolysis) (3). Elevated levels of NT-proBNP are associated with ventricular dysfunction and severity of HF (4). NT-proBNP also detects mild HF (5) and HF with preserved ejection fraction (6).

B-type natriuretic peptides are recommended in professional guidelines for the diagnostic evaluation of patients with acute dyspnea and suspected acute HF, particularly when the clinical diagnosis is uncertain (1, 7). Studies have shown that inclusion of NT-proBNP in diagnostic strategies of emergency department patients with suspected HF led to a reduction in time to discharge and costs (8,9). For diagnosis of acute HF, a single rule-out and multiple age-dependent rule-in cut-off values have been established for NT-proBNP in the ICON Study (10).

A proper differential diagnosis is important, because NT-proBNP is also elevated in other conditions affecting ventricular function such as pulmonary embolism, hypertension, valvular heart disease, muscle heart disease, arrhythmias, critical illness (e.g. sepsis), anemia and stroke (11).

In primary care, NT-proBNP is particularly useful to guide referral of symptomatic chronic HF to specialist care because it excludes suspected left ventricular systolic dysfunction (12). NT-proBNP has independent prognostic value in a variety of clinical settings, including acute dyspnea with or without acute HF (13), chronic HF (14), stable and unstable ischemic heart disease (15). NT-proBNP has incremental prognostic value in acute coronary syndromes with normal troponin (16). NT-proBNP is a useful marker for inpatient monitoring in acute destabilized HF (17) and therapy guidance in chronic HF (18).

The VIDAS NT-proBNP2 assay contains two monoclonal antibodies which recognize epitopes located in the N-terminal part (1-76) of proBNP (1-108).

## PRINCIPLE

The assay principle combines a one-step immunoassay sandwich method with a final fluorescent detection (ELFA).

The Solid Phase Receptacle (SPR®), serves as the solid phase as well as the pipetting device. Reagents for the assay are ready-to-use and pre-dispensed in the strip.

All of the assay steps are performed automatically by the instrument. The sample is transferred into the well containing alkaline phosphatase-labeled anti-NT-proBNP antibody (conjugate). The sample/conjugate mixture is cycled in and out of the SPR several times. This operation enables the antigen to bind with the immunoglobulins fixed to the interior wall of the SPR and to the conjugate to form a sandwich.

Unbound compounds are eliminated during washing steps.

Two detection steps are performed successively. During each step, the substrate (4-Methyl-umbelliferyl phosphate) is cycled in and out of the SPR. The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-Methyl-umbelliferone) the fluorescence of which is measured at 450 nm. The intensity of the fluorescence is proportional to the concentration of antigen present in the sample.

At the end of the assay, results are automatically calculated by the instrument in relation to two calibration curves corresponding to the two detection steps. A fluorescence threshold value determines the calibration curve to be used for each sample. The results are then printed out.

**CONTENT OF THE KIT (60 TESTS):**

| | | |
|---|---|---|
| 60 PBN2 strips | STR | Ready-to-use. |
| 60 PBN2 SPRs<br>2 x 30 | SPR® | Ready-to-use. Interior of SPRs coated with a mouse monoclonal anti-NT-proBNP antibody. |
| PBN2 controls:<br>C1 control<br>1 x 2 mL (liquid)<br>C2 control<br>1 x 2 mL (liquid) | C1<br><br>C2 | Ready-to-use. PBS buffer pH 7.2 + protein and chemical stabilizers + preservatives containing NT-proBNP peptide.<br>MLE data indicate the confidence interval in pg/mL ("Control C1 Dose Value Range") or ("Control C2 Dose Value Range"). |
| PBN2 calibrators:<br>S1 calibrator<br>2 x 2 mL (liquid)<br>S2 calibrator<br>2 x 2 mL (liquid) | S1<br><br>S2 | Ready-to-use. PBS buffer pH 7.2 + protein and chemical stabilizers + preservatives containing NT-proBNP peptide.<br>MLE data indicate the concentration in pg/mL ("Calibrator (S1) Dose Value") or ("Calibrator (S2) Dose Value") and the confidence interval in "Relative Fluorescence Value ("Calibrator (S1) RFV Range) or ("Calibrator (S2) RFV Range"). |
| Sample diluent<br>1 x 2 mL | R1 | Ready-to-use. PBS buffer pH 7.2 + protein and chemical stabilizers + preservatives. |
| Specifications for the factory master data required to calibrate the test:<br>• MLE data (Master Lot Entry) provided in the kit,<br>or<br>• MLE bar code printed on the box label. | | |
| 1 Package Insert provided in the kit or downloadable from www.biomerieux.com/techlib | | |

**The SPR**

The interior of the SPR® is coated during production with a mouse monoclonal anti-NT-proBNP antibody. Each SPR is identified by the "PBN2" code. Only remove the required number of SPRs from the pouch and **carefully reseal the pouch after opening.**

**The reagent strip**

The strip consists of 10 wells covered with a labeled, foil seal. The label comprises a bar code which mainly indicates the assay code, kit lot number and expiration date. The foil of the first well is perforated to facilitate the introduction of the sample. The last well of each strip is a cuvette in which the fluorometric reading is performed. The wells in the center section of the strip contain the various reagents required for the assay.

**Description of the PBN2 strip**

| Wells | Reagents |
|---|---|
| 1 | Sample well. |
| 2 - 3 - 4 | Empty wells. |
| 5 | Conjugate: sheep monoclonal alkaline phosphatase-labeled anti-NT-proBNP antibody + preservative (400 µL). |
| 6 -7 - 8 | TRIS NaCl Tween (pH 7.3) + preservative (600 µL). |
| 9 | Empty well. |
| 10 | Reading cuvette with substrate: 4-Methyl-umbelliferyl phosphate (0.6 mmol/L) + diethanolamine* (DEA*) (0.62 mol/L or 6.6%, pH 9.2) + 1 g/L sodium azide (300 µL). |

* Signal Word: **DANGER**

Hazard statement

H318 : Causes serious eye damage.

Precautionary statement

P280 :Wear protective gloves/protective clothing/eye protection/face protection.
P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

For further information, refer to the Material Safety Data Sheet.

## MATERIALS AND DISPOSABLES REQUIRED BUT NOT PROVIDED

- Pipette with disposable tip to dispense 200 µL.
- Powderless, disposable gloves.
- For other specific materials and disposables, please refer to the Instrument User's Manual.
- Instrument of the VIDAS family.

## WARNINGS AND PRECAUTIONS

- **For *in vitro* diagnostic use only.**
- **For professional use only.**
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious and handled observing the usual safety precautions (do not ingest or inhale).
- Do not use SPRs if the pouch is pierced or if the dot sealing a SPR has come unstuck.
- Do not use visibly deteriorated STRs (damaged foil or plastic).
- Do not mix reagents or disposables from different lots.
- Kit reagents contain 1 g/L sodium azide which can react with lead or copper plumbing to form explosive metal azides. If any liquid containing sodium azide is disposed of in the plumbing system, drains should be flushed with water to avoid build-up.
- Use **powderless** gloves, as powder has been reported to cause false results for certain enzyme immunoassay tests.
- The substrate in well 10 contains an irritant agent (diethanolamine). Refer to the hazard statements "H" and the precautionary statements "P" above.
- Spills should be wiped up thoroughly after treatment with liquid detergent and a solution of household bleach containing at least 0.5% sodium hypochlorite. See the User's Manual for cleaning spills on or in the instrument. Do not autoclave solutions containing bleach.
- The instrument should be regularly cleaned and decontaminated (see the User's Manual).

## STORAGE CONDITIONS

- Store the VIDAS® NT-proBNP2 (PBN2) kit at 2-8°C.
- **Do not freeze reagents.**
- **Store all unused reagents at 2-8°C.**
- After opening the kit, check that the SPR pouch is correctly sealed and undamaged. If not, do not use the SPRs.
- **Carefully reseal the pouch with the desiccant inside after use to maintain stability of the SPRs and return the complete kit to 2-8°C.**
- If stored according to the recommended conditions, all components are stable until the expiration date indicated on the label.

## SPECIMENS

**Specimen type and collection:**

Human serum or plasma.

**Types of tubes validated**:

– Plastic tube with clot activator,
– Plastic tube with clot activator and separation gel,
– Plastic tube with lithium heparin,
– Plastic tube with lithium heparin and separation gel,
– Plastic tube with sodium heparin.

**Note:** Blood sampling tube results may vary from one manufacturer to another depending on the materials and additives used.
It is the responsibility of each laboratory to validate the type of sample tube used and to follow the manufacturer's recommendations for use.

**For patient follow-up, assays must be performed on the same type of sample tube.**
Since EDTA causes a decrease in the values measured, **plasma collected on EDTA should not be used.**
Samples containing suspended fibrin particles or erythrocyte stroma should be centrifuged before testing.

**Sample preparation:**

Follow the tube manufacturer's recommendations for use.

Frozen-stored samples: after thawing, all these samples must be homogenized before testing. Mix using a vortex-type mixer. Clarify the samples before analysis by centrifugation, if necessary.

**Specimen stability**

Samples separated from the clot can be stored at 2-8°C in stoppered tubes for up to 4 days; if longer storage is required, freeze the sera or plasma at -25 ± 6°C. A study performed on frozen samples over a period of 6 months, showed that the quality of results is not affected.
Do not perform more than 4 freeze/thaw cycles.

**Sample-related interference**

Interferences have been studied according to the recommendations of CLSI® document EP7-A2.
None of the following factors have been found to significantly influence this assay:

- albuminemia up to 60 g/L,
- rheumatoid factors up to 590 IU/mL,
- hemolysis (after spiking samples with hemoglobin up to 310 µmol/L or 500 mg/dL) (monomer assay),
- lipemia (after spiking samples with lipids up to 30 g/L equivalent in triglycerides),
- bilirubinemia (after spiking samples with bilirubin up to 510 µmol/L or 30 mg/dL),
- IgG up to 38 mg/mL,
- IgM up to 3.5 mg/mL.

However, it is recommended not to use samples that are clearly hemolyzed, lipemic or icteric and, if possible, to collect a new sample.

## INSTRUCTIONS FOR USE

**For complete instructions, see the User's Manual.**

### Reading VIDAS® Protocole Test Change (PTC) protocol data and MLE data

**When using the assay for the first time:**

With the external instrument barcode reader,

**1.** Scan the PTC barcode(s) at the end of the package insert. or downloadable from www.biomerieux.com/techlib. This reading allows VIDAS® PTC protocol data to be transferred to the instrument software for its update.

**2.** Scan the MLE data on the box label.

**Note: If the MLE data have been read before the VIDAS® PTC protocol, read the MLE data again.**

**When opening a new lot of reagents:**

Enter the specifications (or factory master data) into the instrument using the master lot entry (MLE) data.
If this operation is not performed **before initiating the tests**, the instrument will not be able to print results.

**Note: the master lot data need only be entered once for each lot.**
**It is possible to enter MLE data** manually or automatically **depending on the instrument (refer to the User's Manual).**

### Calibration

Calibration, using the **two calibrators** provided in the kit, must be performed each time a new lot of reagents is opened, after the master lot data have been entered, **and then every 28 days**. This operation provides instrument-specific calibration curves and compensates for possible minor variations in assay signal throughout the shelf-life of the kit.
**The calibrators, identified by S1 and S2**, must be tested **in duplicate** in the same run. The calibration values must be within the set RFV ("Relative Fluorescence Value"). If this is not the case, **recalibrate using S1 and S2**.

### Procedure

1. **Remove the required reagents from the refrigerator. They can be used immediately.**
2. Use one "PBN2" strip and one "PBN2" SPR for each sample, control or calibrator to be tested**. Make sure the storage pouch has been carefully resealed after the required SPRs have been removed.**
3. The test is identified by the "PBN2" code on the instrument. The calibrators must be identified by "S1" and by "S2", and tested **in duplicate**. If the controls need to be tested, they should be identified by C1 and C2 and tested singly.
4. Homogenize the calibrators, controls and samples using a vortex-type mixer (for serum or plasma separated from the pellet).
5. Before pipetting ensure that samples, calibrators, controls and diluent are free of bubbles.
6. **For this test, the calibrators, controls, and samples test portion is 200 µL**.
7. Insert the "PBN2" SPRs and "PBN2" strips into the appropriate position on the instrument. Check to make sure the color labels with the assay code on the SPRs and the Reagent Strips match.
8. Initiate the assay. All the assay steps are performed automatically by the instrument.
9. Reclose the vials and return them to the required temperature after pipetting.
10. The assay will be completed within **approximately 20 minutes.** After the assay is completed, remove the SPRs and strips from the instrument.
11. Dispose of the used SPRs and strips into an appropriate recipient.

## RESULTS AND INTERPRETATION

The results are automatically calculated by the instrument using two calibration curves which are stored by the instrument; the concentrations are expressed in pg/mL or pmol/L.

Conversion factors:

pmol/L x 8.457 = pg/mL
pg/mL x 0.118 = pmol/L

The VIDAS NT-proBNP2 (PBN2) assay is standardized against the Elecsys® proBNPII assay (Roche Diagnostics).
Samples with NT-proBNP concentrations > 25,000 pg/mL should be retested after being diluted 1/4 in the R1 diluent provided.

## QUALITY CONTROL

Two controls C1 and C2 are included in each VIDAS NT-proBNP2 (PBN2) kit. These controls must be performed immediately after opening a new kit to ensure that reagent performance has not been altered. Each calibration must also be checked using these controls. The instrument will only be able to check the control values if they are identified by C1 and C2.
Results cannot be validated if the control values deviate from the expected values. Samples tested in the same run must be reassayed.

### Note

It is the responsibility of the user to perform Quality Control in accordance with any local applicable regulations.

## LIMITATIONS OF THE METHOD

Interference may be encountered with certain sera containing antibodies directed against reagent components.
Assay results should be interpreted taking into consideration the patient's history, and the results of any other tests performed.

## PERFORMANCE

Studies performed using VIDAS® NT-proBNP2 gave the following results:

### Measurement range

The VIDAS NT-proBNP2 measurement range is 15 to 25,000 pg/mL. Values below the lower limit of the measurement range are reported as < 15 pg/mL. Values above the upper limit of the measurement range are reported as > 25,000 pg/mL.

### Detection and quantitation limits

The Limit of Detection (LoD) is the lowest concentration of NT-proBNP that can be detected in a sample (i.e. the concentration is above the upper limit of the blank samples with a probability of 95%). The LoD was determined to be 3.8 pg/mL.

The Limit of Quantitation (LoQ) is the lowest concentration of NT-proBNP that can be quantified with a level of acceptable accuracy and precision. The LoQ was determined to be 4.9 pg/mL.
The study was performed as recommended by CLSI® document EP17-A.

### Functional detection limit

The functional detection limit is defined as the concentration of NT-proBNP measured with an inter-assay coefficient of variation of 20%. During an in-house study, the functional detection limit was determined to be < 21 pg/mL.

### Linearity

The VIDAS NT-proBNP2 assay is linear over its measurement range (**15 to 25,000 pg/mL**), evaluated according to the recommendations of CLSI® document EP6-A.

### Hook effect

No hook effect was found up to NT-proBNP concentrations of 312,000 pg/mL.

### Precision

A study was performed according to the recommendations of CLSI® document EP5-A2. A panel of 7 human samples covering the measurement range was tested as follows: each sample was tested in duplicate in 2 separate runs per day over 20 days, using two reagent lots (10 test days per reagent lot) on 3 instruments (N=240 values per sample). The 3 instruments were located at 3 different sites. Two calibrations were used for each reagent lot (5 test days per calibration for each lot). The repeatability (within-run precision) and reproducibility (within-site within-instrument between-lot) were calculated for each sample using this protocol and are reported in the following table:

| **Sample** | **N** | **Mean concentration (pg/mL)** | **Repeatability** | | **(Within-site within-instrument between-lot) reproducibility** | |
|---|---|---|---|---|---|---|
| | | | Standard deviation (pg/mL) | CV (%) | Standard deviation (pg/mL) | CV (%) |
| **Sample 1** | 240 | 51.2 | 2.14 | 4.2 | 3.87 | 7.6 |
| **Sample 2** | 240 | 118.4 | 5.14 | 4.3 | 7.65 | 6.5 |
| **Sample 3** | 240 | 283.6 | 8.52 | 3.0 | 17.28 | 6.1 |
| **Sample 4** | 240 | 457.2 | 9.39 | 2.1 | 25.36 | 5.5 |
| **Sample 5** | 240 | 964.9 | 16.75 | 1.7 | 41.71 | 4.3 |
| **Sample 6** | 240 | 1,666.1 | 42.23 | 2.5 | 143.25 | 8.6 |
| **Sample 7** | 240 | 14,377.0 | 839.67 | 5.8 | 1,810.98 | 12.6 |

**Analytical specificity**

The VIDAS NT-proBNP2 assay showed no cross-reactivity (< 10%) with the following substances (checked at NT-proBNP concentrations of approximately 125 and 5,000 pg/mL).

| Tested compound | Tested concentration |
|---|---|
| Adrenomedullin | 1 ng/mL |
| Aldosterone | 0.6 ng/mL |
| Angiotensin I | 0.6 ng/mL |
| Angiotensin II | 0.6 ng/mL |
| Angiotensin III | 1 ng/mL |
| $ANP_{28}$, | 3.1 µg/mL |
| Arg-vasopressin | 1 ng/mL |
| $BNP_{32}$ | 3.5 µg/mL |
| $CNP_{22}$ | 2.2 µg/mL |
| Endothelin | 20 pg/mL |
| NT-proANP$_{1-30}$ | 3.5 µg/mL |
| NT-proANP$_{31-67}$ | 1 ng/mL |
| NT-proANP$_{79-98}$ | 1 ng/mL |
| Renin | 50 ng/L |
| Urodilatin | 3.5 µg/mL |
| DNP | 4.2 µg/mL |
| VNP | 2.9 µg/mL |

**Drug interference**

The effect of 24 frequently administered drugs was tested *in vitro*: no other interference was observed (< 10%).

| Drug | Tested concentration | Drug | Tested concentration |
|---|---|---|---|
| Acetaminophen = paracetamol | 1,324 µmol/L | Ibuprofen | 2,425 µmol/L |
| Acetylsalicylic acid | 3.62 mmol/L | Lidocaine | 51.2 µmol/L |
| Ampicillin | 152 µmol/L | Losartan | 30 mg/L |
| Ascorbic acid | 342 µmol/L | Lovastatin / Mevinolin | 48 mg/L |
| Captopril | 23 µmol/L | Metoprolol (+) tartrate | 18.7 µmol/L |
| Carvedilol | 30 mg/L | Methyldopa | 71 µmol/L |
| Chloramphenicol | 155 µmol/L | Nitroglycerin | 120 µg/L |
| Digoxin | 7.8 nmol/L | Prednisolone | 8.31 µmol/L |
| Dopamine | 5.87 µmol/L | Spironolactone | 1.44 µmol/L |
| Enalapril maleate | 0.86 µmol/L | Theophylline | 222 µmol/L |
| Furosemide | 181 µmol/L | Verapamil | 120 mg/L |
| Hydrochlorothiazide | 20.2 µmol/L | Warfarin | 32.5 µmol/L |

**Comparison with the Elecsys® proBNPII method (Roche Diagnostics)**

The comparison of the VIDAS NT-proBNP2 (Y) assay with the Elecsys® proBNPII (X) method gave the following results:
Number of samples analyzed: 333

**Equation for Passing & Bablok regression (19): Y = 0.9900 X –6.8035**

95% confidence interval for slope: [0.9685 ; 1.0116]
95% confidence interval for intercept point: [-9.3068 ;-4.3001]
Coefficient of correlation: 0,9895

The sample concentrations analyzed with VIDAS NT-proBNP2 ranged between 18.9 pg/mL and 23,618.9 pg/mL.

**Clinical data**

**a) Decision thresholds to aid diagnosis**

NT-proBNP concentrations must be interpreted in conjunction with the patient's previous history, physical examination or any other clinical data (e.g.: medical imagery, analyses).

- **Chronic heart failure**

The ESC guidelines recommend 125 pg/mL as single (age-independent) cut-off for exclusion of suspected heart failure in patients with non-acute onset (1).
In the same clinical setting, age-stratified decision limits (125 pg/mL for patients < 75 years old and 450 pg/mL for patients ≥ 75 years old) have been advocated to improve the loss of specificity in the elderly (12).

Both approaches are valid and each laboratory should select the most appropriate decision limits based on local preferences and guidelines.

- **Acute heart failure**

The International Collaborative of NT-proBNP (ICON) study using the Elecsys® proBNP assay established optimal cut-off values for patients with acute heart failure (10).
According to the correlation studies between VIDAS NT-proBNP2 and Elecsys® proBNPII, and between Elecsys® proBNP and Elecsys® proBNPII, the same cut-off values are recommended for VIDAS NT-proBNP2. Refer to the section **Comparison with the Elecsys® proBNPII method (Roche Diagnostics)**.

| **Category** | **Optimal cut-off pg/mL** |
|---|---|
| Rule in cut-off | |
| < 50 years | 450 |
| 50-75 years | 900 |
| > 75 years | 1,800 |
| Rule out cut-off | |
| All patients | 300 |

The ESC guidelines recommend 300 pg/mL as a single age independent rule out cut-off for suspected heart failure patients presenting with acute symptoms (10).

**b) Reference values**

The reference values were determined from 340 individuals without heart failure. This population included healthy individuals (no known previous history, symptoms, cardiac or circulatory disease) and individuals with diabetes, hypertension, pulmonary disease and renal insufficiency. The descriptive statistics for NT-proBNP concentrations in this population are presented in the table below. The values are representative of the results obtained from clinical studies.
These results are given as a guide, it is recommended that each laboratory establish its own reference values from a rigorously selected population.

| **Males and Females** | **18-44 years** | **45 – 54 years** | **55 – 64 years** | **65 – 74 years** | **≥ 75 years** |
|---|---|---|---|---|---|
| **Median** | 35.0 | 38.7 | 37.7 | 64.1 | 112.0 |
| **95$^{th}$ percentile** | 103.9 | 106.3 | 162.7 | 180.8 | 624.0 |
| **% < Cut-off*** | 98.6 | 95.7 | 93.2 | 82.5 | 92.5 |
| **N** | 69 | 69 | 59 | 63 | 80 |

| **Males** | **18-44 years** | **45 – 54 years** | **55 – 64 years** | **65 – 74 years** | **≥ 75 years** |
|---|---|---|---|---|---|
| **Median** | 27.6 | 27.5 | 29.4 | 46.2 | 85.4 |
| **95$^{th}$ percentile** | 70.1 | 61.5 | 133.5 | 166.9 | 696.8 |
| **% < Cut-off*** | 100.0 | 100.0 | 94.1 | 90.0 | 91.1 |
| **N** | 33 | 39 | 34 | 30 | 45 |

| **Females** | **18-44 years** | **45 – 54 years** | **55 – 64 years** | **65 – 74 years** | **≥ 75 years** |
|---|---|---|---|---|---|
| **Median** | 41.5 | 63.0 | 46.8 | 74.7 | 120.4 |
| **95$^{th}$ percentile** | 113.6 | 133.6 | 153.9 | 229.3 | 345.8 |
| **% < Cut-off*** | 97.2 | 90.0 | 92.0 | 75.8 | 94.3 |
| **N** | 36 | 30 | 25 | 33 | 35 |

* Age-stratified cut-offs (125 pg/mL for patients < 75 years old and 450 pg/mL for patients ≥ 75 years old).

**Combined age ranges**

| | Males and females | | Males | | Females | |
|---|---|---|---|---|---|---|
| | **< 75 years** | **≥ 75 years** | **< 75 years** | **≥ 75 years** | **< 75 years** | **≥ 75 years** |
| **Median** | 42.4 | 112.0 | 31.3 | 85.4 | 59.6 | 120.4 |
| **95th percentile** | 142.3 | 624.0 | 103.7 | 696.8 | 153.5 | 345.8 |
| **% < Cut-off** | 92.7 | 92.5 | 96.3 | 91.1 | 88.7 | 94.3 |
| **N** | 260 | 80 | 136 | 45 | 124 | 35 |

**c) Study on patients with heart failure**

Blood samples obtained from 208 patients diagnosed with heart failure (including 74 women and 134 men) were tested for this study. The descriptive statistics for NT-proBNP concentrations in this population, as well as the NYHA Classifications are given in the tables below.

| **Males and Females** | **18-44 years** | **45 – 54 years** | **55 – 64 years** | **65 – 74 years** | **≥ 75 years** |
|---|---|---|---|---|---|
| **Median** | 1,810.3 | 667.3 | 1,172.7 | 1,156.2 | 3,770.8 |
| **95th percentile** | 5,062.7 | 2,815.9 | 7,115.6 | 12,387.6 | > 25,000 |
| **% ≥ Cut-off** | 100.0 | 81.3 | 90.9 | 93.1 | 89.6 |
| **N** | 5 | 16 | 33 | 58 | 96 |

| **Males** | **18-44 years** | **45 – 54 years** | **55 – 64 years** | **65 – 74 years** | **≥ 75 years** |
|---|---|---|---|---|---|
| **Median** | 1,255.0 | 773.3 | 955.9 | 892.6 | 4,150.0 |
| **95th percentile** | 5,178.5 | 2,926.1 | 2,054.2 | 11,883.7 | > 25,000 |
| **% ≥ Cut-off** | 100.0 | 84.6 | 90.0 | 92.7 | 92.9 |
| **N** | 4 | 13 | 20 | 41 | 56 |

| **Females** | **18-44 years** | **45 – 54 years** | **55 – 64 years** | **65 – 74 years** | **≥ 75 years** |
|---|---|---|---|---|---|
| **Median** | 2,221.8 | 221.1 | 3,022.4 | 1,482.5 | 2,492.5 |
| **95th percentile** | 2,221.8 | 2,025.2 | 8,877.9 | 10,162.3 | 22,287.0 |
| **% ≥ Cut-off** | 100.0 | 66.7 | 92.3 | 94.1 | 85.0 |
| **N** | 1 | 3 | 13 | 17 | 40 |

| | Males and females | | Males | | Females | |
|---|---|---|---|---|---|---|
| | **< 75 years** | **≥ 75 years** | **< 75 years** | **≥ 75 years** | **< 75 years** | **≥ 75 years** |
| **Median** | 1,162.0 | 3,770.8 | 919.0 | 4,150.0 | 1,576.5 | 2,492.5 |
| **95th percentile** | 10,236.9 | > 25,000 | 10,371.3 | > 25,000 | 9,135.4 | 22,287.0 |
| **% ≥ Cut-off** | 91.1 | 89.6 | 91.0 | 92.9 | 91.2 | 85.0 |
| **N** | 112 | 96 | 78 | 56 | 34 | 40 |

| **Males and females** according to NYHA classification | **NYHA I** | **NYHA II** | **NYHA III** | **NYHA IV** |
|---|---|---|---|---|
| **Median** | 1,236.0 | 1,435.4 | 2,136.0 | 4,580.2 |
| **5th percentile** | 96.4 | 242.1 | 150.0 | 83.7 |
| **95th percentile** | 23,996.8 | 7,548.3 | 14,675.6 | 21,650.6 |
| **% ≥ Cut-off** | 92.5 | 100.0 | 94.3 | 86.4 |
| **N** | 93 | 21 | 35 | 59 |

| **Males** according to NYHA classification | **NYHA I** | **NYHA II** | **NYHA III** | **NYHA IV** |
|---|---|---|---|---|
| **Median** | 1,172.7 | 1,513.1 | 2,136.0 | 5,215.8 |
| **5th percentile** | 76.5 | 295.6 | 83.4 | 898.8 |
| **95th percentile** | > 25,000 | >25,000 | 19,786.1 | 20,314.8 |
| **% ≥ Cut-off** | 91.3 | 100.0 | 92.6 | 100.0 |
| **N** | 69 | 12 | 27 | 26 |

| **Females** according to NYHA classification | **NYHA I** | **NYHA II** | **NYHA III** | **NYHA IV** |
|---|---|---|---|---|
| **Median** | 1,516.6 | 1,435.4 | 1,926.2 | 2,221.8 |
| **5th percentile** | 240.4 | 231.6 | 748.9 | 61.6 |
| **95th percentile** | 19,214.0 | 5,830.0 | 8,734.5 | 18,683.6 |
| **% ≥ Cut-off** | 95.8 | 100.0 | 100.0 | 75.8 |
| **N** | 24 | 9 | 8 | 33 |

**Sensitivity and specificity**

All the results obtained during the clinical evaluations were processed using both the age-independent and age-dependent cut-offs. The sensitivity and specificity are as follows:

- **Age independent (125 pg/mL cut-off)**

| **Males and females** | **All age groups** |
|---|---|
| Sensitivity %<br>**$CI_{95\%}$** | 93.75<br>[ 89.55 ; 96.63 ] |
| Specificity %<br>**$CI_{95\%}$** | 85.00<br>[ 80.75 ; 88.62 ] |

- **Age dependent (125 pg/mL cut-off for patients < 75 years old and 450 pg/mL cut-off for patients ≥ 75 years old)**

| **Males and females** | **All** | **< 75 years** | **≥ 75 years** |
|---|---|---|---|
| Sensitivity %<br>**$CI_{95\%}$** | 90.38<br>[ 85.54 ; 94.03 ] | 91.07<br>[ 84.19 ; 95.64 ] | 89.58<br>[ 81.68 ; 94.89 ] |
| Specificity %<br>**$CI_{95\%}$** | 92.65<br>[ 89.34 ; 95.19 ] | 92.69<br>[ 88.82 ; 95.54 ] | 92.50<br>[ 84.39 ; 97.20 ] |

$CI_{95\%}$: 95% Confidence Interval

**WASTE DISPOSAL**

Dispose of used or unused reagents as well as any other contaminated disposable materials following procedures for infectious or potentially infectious products.
It is the responsibility of each laboratory to handle waste and effluents produced according to their nature and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

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## INDEX OF SYMBOLS

| Symbol | Meaning |
|---|---|
| REF | Catalog number |
| IVD | *In Vitro* Diagnostic Medical Device |
|  | Manufacturer |
|  | Temperature limit |
|  | Use by date |
| LOT | Batch code |
|  | Consult Instructions for Use |
|  | Contains sufficient for <n> tests |
|  | Date of manufacture |

## WARRANTY

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**REVISION HISTORY**

Change type categories :

| | |
|---|---|
| N/A | Not applicable (First publication) |
| Correction | Correction of documentation anomalies |
| Technical change | Addition, revision and/or removal of information related to the product |
| Administrative | Implementation of non-technical changes noticeable to the user |

***Note:*** *Minor typographical, grammar, and formatting changes are not included in the revision history.*

| Release date | Part Number | Change Type | Change Summary |
|---|---|---|---|
| 2015/01 | 9301622D | Administrative | INDEX OF SYMBOLS<br>REVISION HISTORY |
| | | Technical | CONTENT OF THE KIT (60 TESTS)<br>WARNINGS AND PRECAUTIONS |
| 2015/06 | 9301622E | Technical | CONTENT OF THE KIT (60 TESTS)<br>INSTRUCTIONS FOR USE |



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