Intendend Use

Immunoassay for the in vitro quantitative determination of N-terminal pro B-type natriuretic peptide in human serum and plasma. This assay is indicated as an aid in the diagnosis of individuals suspected of having congestive heart failure and detection of mild forms of cardiac dysfunction.1,2,4,5,6,7,8

The test also aids in the assessment of heart failure severity in patients diagnosed with congestive heart failure.9,10

This assay is further indicated for the risk stratification of patients with acute coronary syndrome11,12,13,14,15 and congestive heart failure, and it can also be used for monitoring the treatment in patients with left ventricular dysfunction.1,2,16,17,18,19,20

The electrochemiluminescence immunoassay ‘ECLA’ is intended for use on the cobas e 801 immunoassay analyzer.

Note: Please note that the catalogue number appearing on the package insert retains only the first 8 digits of the licensed 11-digit Catalogue Number: 07027664190 for the Elecsys proBNP II assay. The last 3 digits -190 have been replaced by -119 for logistic purposes.

Summary

Heart failure is a clinical syndrome characterized by systemic perfusion inadequate to meet the body’s metabolic demands as a result of a structural and/or functional cardiac abnormality, resulting in a reduced cardiac output and/or elevated intracardiac pressures at rest or during stress.1,2 Left ventricular dysfunction can be one of the functional precursors of heart failure.1,2

Heart failure is a progressive disease where in both hospitalized and ambulatory patients, most deaths are due to cardiovascular causes, mainly sudden death and worsening HF.1,2

The typical terminology used to describe HF is based on measurement of the Left Ventricular Ejection Fraction (LVEF). According to latest ESC guidelines, HF comprises a wide range of patients, from those with normal LVEF (typically considered as ≥ 50 %), HF with preserved EF (HFpEF) to those with reduced LVEF (typically considered as ≤ 40 %), HF with reduced EF (HFrEF). Patients with an LVEF in the range of 40-49 % represent a ‘grey area’, which is now defined as HF with midrange EF (HFmrEF).1,2,17 Clinical heart failure and imaging procedures are used to confirm the diagnosis of heart failure.1,2,13

The significance of natriuretic peptides in the control of cardiovascular system function has been demonstrated. The following natriuretic peptides have been described: atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP), and C-type natriuretic peptide (CNP).21,22

ANP and BNP, as antagonists of the renin-angiotensin-aldosterone system, influence by means of their natriuretic and diuretic properties, the electrolyte and fluid balance in an organism.22,24,25 In subjects with left ventricular dysfunction, serum and plasma concentrations of BNP increase, as does the concentration of the putatively inactive amino-terminal fragment, NT-proBNP. ProBNP, comprising 108 amino acids, is secreted mainly by the ventricle and, in this process, is cleaved into physiologically active BNP (77-108) and the N-terminal fragment NT-proBNP.1,7-6

Several studies have demonstrated the significant role of natriuretic peptide testing, including NT-proBNP, in heart failure management from diagnosis to monitoring, leading to the recommendation to use them in clinical practice by major international guidelines with often highest level of evidence and recommendation.1,2

Based on the symptoms, the severity of heart failure is classified in stages (New York Heart Association classification [NYHA] I-IV). When patients are grouped according to their NYHA classification, NT-proBNP levels increase with increasing class numbers and reflect the severity of cardiac impairment.9,10

Heart failure symptoms are often non-specific and do not help to discriminate between heart failure and other conditions, such as (non-cardiogenic) pulmonary edema, chronic obstructive pulmonary disease (COPD), pneumonia or sepsis.1,2

The European Society of Cardiology Heart Failure Guidelines recommends natriuretic peptides, including NT-proBNP, as an initial diagnostic test.1 Patients with NT-proBNP below the recommended NT-proBNP cutoffs for non-acute and acute onsets are unlikely to have HF, and therefore do not require echocardiography – and elevated NT-proBNP help to identify patients who require further cardiac investigation.1 When used with the recommended cutoff, the Elecsys proBNP assay yields negative predictive values ranging from 97 % to 100 % depending on age and gender.10

The test is also useful in the early stages of heart failure, where symptoms may be transient rather than present all the time.3 The high sensitivity of NT-proBNP allows also the detection of mild forms of cardiac dysfunction in asymptomatic patients with structural heart disease.2,5,6,7,8

NT-proBNP can also be used for prognostic applications in patients with acute coronary syndrome. The GUSTO IV study, with more than 6800 patients, showed that NT-proBNP was the strongest independent predictor of one year mortality in patients with acute coronary syndrome.15

In the ICON (International Collaborative of NT-proBNP) study which involved more than 1256 patients presenting acute shortness of breath at their admission in the emergency department, it was shown that evaluating NT-proBNP can increase the specificity and accuracy for diagnosing heart failure in patients presenting acute dyspnea in the emergent setting.33

In patients hospitalized for acute decompensated heart failure, pre-discharge measurement of natriuretic peptides is useful to categorize patient’s risk at discharge.1,16 Changes in NT-proBNP levels during hospitalization demonstrated to be a strong predictor of outcomes.16,17,27,28,29 A decrease in NT-proBNP values of ≥ 30 % has shown to be correlated with favorable outcome, while an increase in NT-proBNP values ≥ 30 % was correlated with 6.5 times higher risk of rehospitalization or death in 6 months.16

In chronic heart failure, serial measurement of NT-proBNP concentration can be used to monitor the disease progression, to predict outcomes and evaluate the success of treatment.1,2,17,18,20,30,31

Elevated NT-proBNP values are strongly predictive of adverse outcomes and rising values identify a risk, while significant lowering of NT-proBNP denotes improved outcomes and better prognosis.1,2,17,32

When NT-proBNP levels change during treatment of chronic heart failure, decrease over the course of the disease correlated with improved clinical outcomes.1,2,18,30 This interpretation of NT-proBNP results remains unchanged when using the new drug class Angiotension receptor–neprilysin inhibitor2-2 (ARNI, e.g. sacubitril-valsartan): In contrast to BNP, NT-proBNP degradation is not inhibited by the drug so that NT-proBNP results are not increased by the mode of action of the drug. 19,33,34 In patients treated with sacubitril-valsartan, rapid and sustained reduction of NT-proBNP levels has been observed, reflecting reduced wall stress33 and benefits of the drug correlating with a lower rate of cardiovascular death and heart failure hospitalization.20

NT-proBNP can be used before non-cardiac surgery to evaluate patients’ perioperative cardiac risk.35

In addition NT-proBNP can be used to identify patients at higher risk of cardiotoxicity which can lead to heart failure and may be helpful in monitoring the use and dosing of cardiotoxic therapies.20,36,37 or interventions causing fluid retention or volume overload (e.g. COX-2 inhibitors, nonsteroidal anti-inflammatory drugs)38,39,40,41,42,43,44,45

In meta-analysis including 95617 patients without history of cardiovascular disease, NT-proBNP concentration strongly predicted first-onset heart failure and augmented chronic heart disease and stroke prediction, suggesting that NT-proBNP could serve as a biomarker in new therapeutic approaches that integrate heart failure into cardiovascular disease primary prevention.46

The Elecsys proBNP II assay contains two monoclonal antibodies which recognize epitopes located in the N-terminal part (1-76) of proBNP (1-108). The Elecsys proBNP II assay was adapted to the Elecsys proBNP assay (first generation, REF 03121640122) with respect to analytical sensitivity, measuring range, standardization and recovery of proBNP in human samples.54
Elecsys ProBNP II

Test principle

Sandwich principle.

Total duration of assay: 18 minutes.

- 1st incubation: Antigen in the sample (9 µL), a biotinylated monoclonal NT-proBNP-specific antibody, and a monoclonal NT-proBNP-specific antibody labeled with a ruthenium complex form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.

Total duration of assay: 9 minutes.

- During a 9 minute incubation, antigen in the sample (9 µL), a biotinylated monoclonal NT-proBNP-specific antibody, a monoclonal NT-proBNP-specific antibody labeled with a ruthenium complex and streptavidin-coated microparticles react to form a sandwich complex, which is bound to the solid phase.

For both assay applications:

- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured on the surface of the electrode. Unbound substances are then removed with ProCell M.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the cobas link.

Reagents – working solutions

The cobas e pack is labeled as PBNP.
M Streptavidin-coated microparticles, 1 bottle, 12.4 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.
R1 Anti-NT-proBNP-Ab – biotin, 1 bottle, 21.0 mL:
Biotinylated monoclonal anti-NT-proBNP antibody (mouse)
1.1 µg/mL; phosphate buffer 40 mmol/L, pH 5.8; preservative.
R2 Anti-NT-proBNP-Ab – Ru(bpy)3²⁺, 1 bottle, 19.7 mL:
Monoclonal anti-NT-proBNP antibody (sheep) labeled with ruthenium complex
1.1 µg/mL; phosphate buffer 40 mmol/L, pH 5.8; preservative.

Precautions and warnings

For in vitro diagnostic use. Exercise the normal precautions required for handling all laboratory reagents. Disposal of all waste material should be in accordance with local guidelines. Safety data sheet available for professional user on request. Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent Handling

The Elecsys proBNP II assay can be used for both the 9-minute application and the 18-minute application. The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated. All information required for correct operation is available via the cobas link.

Storage and stability

Store at 2-8 °C.
Do not freeze.
Store the cobas e pack upright in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:

<table>
<thead>
<tr>
<th>Stability:</th>
<th>up to the stated expiration date</th>
<th>on the cobas e 801 analyzer</th>
</tr>
</thead>
<tbody>
<tr>
<td>unopened at 2-8 °C</td>
<td>16 weeks</td>
<td></td>
</tr>
</tbody>
</table>

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable. Serum collected using standard sampling tubes or tubes containing separating gel.
Li-heparin, K2-EDTA and K3-EDTA plasma.
Peroxidase containing separating gel can be used.
Criterion: Slope 0.9-1.1 + intercept within ±10 pg/mL + coefficient of correlation ≥0.95.

Stable for 3 days at 20-25 °C, 6 days at 2-8 °C, 24 months at -20 C (± 5 °C). Freeze only once.
The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer. Centrifuge samples containing precipitates before performing the assay. Do not use samples and controls stabilized with azide.

Materials provided

See “Reagents – working solutions” section for reagents.

Materials required (but not provided)

- 07360886190, CalSet proBNP II, for 4 x 1.0 mL
- 04917049190, PreciControl Cardiac II, for 2 x 2.0 mL
- 07299001190, Diluent Universal, 45.2 mL sample diluent
- General laboratory equipment
- cobas e 801 analyzer

Accessories for the cobas e 801 analyzer:

- 06908799190, ProCell II M, 2 x 2 L system solution
- 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- 07485409001, Reservoir Cups, 8 cups to supply ProCell II M and CleanCell M
- 06908653190, PreClean II M, 2 x 2 L wash solution
- 05694302001, Assay Tip/Assay Cup tray, 6 magazines x 6 magazine stacks x 105 assay tips and 105 assay cups, 3 wasteliners
- 07486425001, Liquid Flow Cleaning Cup, 2 adaptor cups to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning Detection Unit
- 07485433001, PreWash Liquid Flow Cleaning Cup, 1 adaptor cup to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning PreWash Unit
- 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator’s manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use. Place the cooled (stored at 2-8 °C) cobas e pack on the reagent manager.
Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the cobas e pack.

Calibration

Traceability: This method has been standardized against the Elecsys proBNP assay (REF.03121640122). This in turn is traceable to pure synthetic NT-proBNP (T-76) by weight.
The predefined master curve is adapted to the analyzer using the relevant CalSet.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the cobas e pack was registered on the analyzer).
Calibration interval may be extended based on acceptable verification of calibration by the laboratory.
Renewed calibration is recommended as follows:
- after 12 weeks when using the same reagent lot
- after 28 days when using the same cobas e pack on the analyzer
- as required: e.g. quality control findings outside the defined limits
**Elecsys ProBNP II**

**Quality control**
For quality control, use PreciControl Cardiac II.

In addition, other suitable control material can be used.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per cobas e pack, and following each calibration.

The control intervals and limits should be adapted to each laboratory’s individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

If necessary, repeat the measurement of the samples concerned.

Follow the applicable government regulations and local guidelines for quality control.

**Calculation**
The analyzer automatically calculates the analyte concentration of each sample (either in pmol/L or pg/mL).

Conversion factors:

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

**Limitations - interference**
The effect of the following endogenous substances and pharmaceutical compounds on assay performance was tested. Interferences were tested up to the listed concentrations and no impact on results was observed.

**Endogenous substances**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin</td>
<td>≤ 426 µmol/L or ≤ 25 mg/dL</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>≤ 0.621 mmol/L or ≤ 1000 mg/dL</td>
</tr>
<tr>
<td>Intralipid</td>
<td>≤ 1500 mg/dL</td>
</tr>
<tr>
<td>Biotin</td>
<td>≤ 123 mmol/L or ≤ 30 ng/mL</td>
</tr>
<tr>
<td>Rheumatoid factors</td>
<td>≤ 1500 U/l</td>
</tr>
<tr>
<td>IgG</td>
<td>≤ 6.0 g/dL</td>
</tr>
<tr>
<td>IgA</td>
<td>≤ 1.6 g/dL</td>
</tr>
<tr>
<td>IgM</td>
<td>≤ 1.0 g/dL</td>
</tr>
</tbody>
</table>

Criterion: Recovery of ± 10 ng/mL of initial value ≤ 100 pg/mL and ± 10 % of initial value > 100 pg/mL.

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/dL/day) until at least 8 hours following the last biotin administration.

There are no high-dose hook effect at NT-proBNP concentrations up to 35400 pg/mL (30000 ng/mL).

**Pharmaceutical substances**
In vitro tests were performed on 16 commonly used pharmaceuticals. No interference with the assay was found.

In addition, the following special cardiac drugs were tested. No interference with the assay was found.

**Special cardiac drugs**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration tested</th>
<th>mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carvedilol</td>
<td></td>
<td>37.5</td>
</tr>
<tr>
<td>Clopidogrel</td>
<td></td>
<td>75.0</td>
</tr>
<tr>
<td>Digoxin</td>
<td></td>
<td>0.25</td>
</tr>
<tr>
<td>Epinephrine (Adrenaline)</td>
<td></td>
<td>0.50</td>
</tr>
<tr>
<td>Insulin</td>
<td></td>
<td>1.60</td>
</tr>
<tr>
<td>Lidocaine</td>
<td></td>
<td>80.0</td>
</tr>
<tr>
<td>Lisinopril</td>
<td></td>
<td>10.0</td>
</tr>
<tr>
<td>Methylprednisolone</td>
<td></td>
<td>7.50</td>
</tr>
<tr>
<td>Metoprolol</td>
<td></td>
<td>150</td>
</tr>
</tbody>
</table>

**Drug Concentration tested**

- Nifedipine: 30.0
- Phenprocoumon (Marcumar): 3.00
- Propafenone: 300
- Retelplase: 33.3
- Simvastatin: 30.0
- Spironolactone: 75.0
- Tolbutamide: 1500
- Torasemide: 15.0
- Verapamil: 240

In rare cases, interference due to extremely high titers of antibodies to analyze-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

In extremely rare cases (global incidence: < 1 in 10 million), patients may show discrepant results when tested with the assay kit (values < Limit of Detection) due to a NT-proBNP genetic variant.

For diagnostic purposes, the results should always be assessed in conjunction with the patient’s medical history, clinical examination and other findings.

**Limits and ranges**

**Measuring range**

- 5-35000 pg/mL or 0.6-4130 pmol/L (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 5 pg/mL (< 0.6 pmol/L). Values above the measuring range are reported as > 35000 pg/mL (> 4130 pmol/L) or up to 70000 pg/mL (8260 pg/mL) for 2-fold diluted samples.

**Lower limits of measurement**

- Limit of Blank: 3 pg/mL (0.4 pmol/L)
- Limit of Detection: 5 pg/mL (0.6 pmol/L)
- Limit of Quantitation: 50 pg/mL (5.9 pmol/L)

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95%.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95%).

The Limit of Quantitation is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 20%.

**Dilution**

Samples with NT-proBNP concentrations above the measuring range can be diluted with Diluent Universal. The recommended dilution is 1:2 (either automatically by the analyzer or manually).

After manual dilution, multiply the result by the dilution factor.

After dilution by the analyzer, the software automatically takes the dilution into account when calculating the sample concentration.

Dilutions of up to 1:10 may entail maximum deviations of 25 % from the theoretical value.

**Clinical data**

The following clinical data had been obtained using the Elecsys proBNP assay (first generation, REF 03121640122). All data sets established with the first test generation are marked with an index. The interpretation of NT-proBNP values is different and clinically asymptomatic in its early stages.

With increasing age atherosclerosis and aging processes of the heart (e.g. fibrosis) result in cardiac dysfunction. Development of cardiac dysfunction is individually different and clinically asymptomatic in its early stages.
Elecsys ProBNP II

NT-proBNP levels reflect cardiac function or dysfunction respectively. With increasing age elevated levels of NT-proBNP are more frequently found in apparently healthy individuals, thus reflecting the increasing frequency of cardiac dysfunction.

NT-proBNP values need to be interpreted in conjunction with the medical history, clinical findings and other information (e.g. imaging, laboratory findings, accompanying disorders, treatment effects).

Cutoff values

A number of studies support a decision threshold for NT-proBNP of 125 pg/mL. NT-proBNP values < 125 pg/mL exclude cardiac dysfunction with a high level of certainty in patients with symptoms suggestive of heart failure e.g. dyspnea. NT-proBNP values > 125 pg/mL may indicate cardiac dysfunction and are associated with an increased risk of cardiac complications (myocardial infarction, heart failure, death).

Recommended cutoffs in patients with diagnosed stable chronic heart failure

Patients with stable heart failure (n = 721) were compared to the reference group (n = 2264).

ROC plot analysis at the cutoff value of 125 pg/mL showed a sensitivity of 88 %, a specificity of 92 %, a negative predictive value (NPV), and a positive predictive value (PPV) of 96.7 % and 80.6 %, respectively.

Expected values

NT-proBNP concentrations in the reference group are shown in the following tables. The most appropriate decision threshold apparent from these distributions is 125 pg/mL. Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Reference group

The circulating NT-proBNP concentration was determined in samples from 1981 blood donors aged between 18 and 65 as well as 283 elderly patients aged between 50 and 90, both populations without known cardiac risks, symptoms or medical history.

Furthermore, NT-proBNP concentration was also determined in the pediatric population aged between 1 and 18 with values ranging between 112 and 370 ng/L (97.5th percentile).

The descriptive statistics for NT-proBNP concentrations (pg/mL) in the reference group are shown in the following table:

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Median</th>
<th>95th percentile</th>
<th>97.5th percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-44</td>
<td>1233</td>
<td>35.0</td>
<td>30.2</td>
<td>20.4</td>
<td>97.3</td>
<td>115</td>
</tr>
<tr>
<td>45-54</td>
<td>408</td>
<td>48.3</td>
<td>60.3</td>
<td>30.7</td>
<td>121</td>
<td>172</td>
</tr>
<tr>
<td>55-64</td>
<td>396</td>
<td>70.6</td>
<td>44.4</td>
<td>47.3</td>
<td>198</td>
<td>263</td>
</tr>
<tr>
<td>65-74</td>
<td>102</td>
<td>107</td>
<td>85.0</td>
<td>85.1</td>
<td>285</td>
<td>349</td>
</tr>
<tr>
<td>≥75</td>
<td>33</td>
<td>211</td>
<td>152</td>
<td>174</td>
<td>526</td>
<td>738</td>
</tr>
<tr>
<td>Total</td>
<td>2264</td>
<td>50.3</td>
<td>62.4</td>
<td>27.9</td>
<td>149</td>
<td>196</td>
</tr>
</tbody>
</table>

Specific performance data

Representative performance data on the analyzer is given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, pooled human sera and controls in a protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplicate each for 21 days (n = 84). The following results were obtained:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean</th>
<th>SD</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human serum 1</td>
<td>16.9</td>
<td>2.00</td>
<td>12.4</td>
</tr>
<tr>
<td>Human serum 2</td>
<td>127</td>
<td>15.0</td>
<td>11.9</td>
</tr>
<tr>
<td>Human serum 3</td>
<td>1708</td>
<td>201</td>
<td>1.2</td>
</tr>
<tr>
<td>Human serum 4</td>
<td>19892</td>
<td>2347</td>
<td>34.2</td>
</tr>
<tr>
<td>Human serum 5</td>
<td>32435</td>
<td>3827</td>
<td>1.9</td>
</tr>
</tbody>
</table>

Cobas e 601 analyzer (18-minute application)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean</th>
<th>SD</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human serum 1</td>
<td>16.9</td>
<td>2.00</td>
<td>12.4</td>
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<tr>
<td>Human serum 2</td>
<td>127</td>
<td>15.0</td>
<td>11.9</td>
</tr>
<tr>
<td>Human serum 3</td>
<td>1708</td>
<td>201</td>
<td>1.2</td>
</tr>
<tr>
<td>Human serum 4</td>
<td>19892</td>
<td>2347</td>
<td>34.2</td>
</tr>
<tr>
<td>Human serum 5</td>
<td>32435</td>
<td>3827</td>
<td>1.9</td>
</tr>
</tbody>
</table>

b) PC CARD II = Prec/Control Cardiac II

NYHA functional class

<table>
<thead>
<tr>
<th>NYHA functional class</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Median</th>
<th>95th percentile</th>
<th>97.5th percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>NYHA I</td>
<td>182</td>
<td>1016</td>
<td>205</td>
<td>951</td>
<td>1571</td>
<td>1707</td>
</tr>
<tr>
<td>NYHA II</td>
<td>250</td>
<td>1666</td>
<td>400</td>
<td>932</td>
<td>1263</td>
<td>148</td>
</tr>
<tr>
<td>NYHA III</td>
<td>3029</td>
<td>2035</td>
<td>4600</td>
<td>103</td>
<td>1263</td>
<td>148</td>
</tr>
<tr>
<td>NYHA IV</td>
<td>3465</td>
<td>4453</td>
<td>10449</td>
<td>12188</td>
<td>91</td>
<td></td>
</tr>
</tbody>
</table>

Correlation of NT-proBNP with NYHA classification in patients diagnosed with CHF

NT-proBNP values (pg/mL) for patients with restricted left ventricular ejection fraction (majority under therapy).

- NYHA I
- NYHA II
- NYHA III
- NYHA IV
The Elecsys proBNP II assay does not show any significant cross-reactivity with the following substances, tested with NT-proBNP concentrations of approximately 100 pg/mL and 2500 pg/mL (maximum tested concentration):

<table>
<thead>
<tr>
<th>Cross-reactant</th>
<th>Concentration tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenomedullin</td>
<td>1.0 ng/mL</td>
</tr>
<tr>
<td>Aldosterone</td>
<td>0.6 ng/mL</td>
</tr>
<tr>
<td>Angiotensin I</td>
<td>0.6 ng/mL</td>
</tr>
<tr>
<td>Angiotensin II</td>
<td>0.6 ng/mL</td>
</tr>
<tr>
<td>Angiotensin III</td>
<td>1.0 ng/mL</td>
</tr>
<tr>
<td>ANP28</td>
<td>3.1 μg/mL</td>
</tr>
<tr>
<td>Arg-vasopressin</td>
<td>1.0 ng/mL</td>
</tr>
<tr>
<td>BNP22</td>
<td>3.5 μg/mL</td>
</tr>
<tr>
<td>CNP22</td>
<td>2.2 mg/mL</td>
</tr>
<tr>
<td>Endothelin</td>
<td>20 pg/mL</td>
</tr>
<tr>
<td>NT-proANP1-30</td>
<td>3.5 μg/mL</td>
</tr>
<tr>
<td>NT-proANP1-63</td>
<td>1.0 ng/mL</td>
</tr>
<tr>
<td>NT-proANP79-88</td>
<td>1.0 ng/mL</td>
</tr>
<tr>
<td>Renin</td>
<td>50 ng/mL</td>
</tr>
<tr>
<td>Urotiadolin</td>
<td>3.5 μg/mL</td>
</tr>
</tbody>
</table>

Method comparison

a) A comparison of the Elecsys proBNP II assay (9-minute application), g) 07027664190 (cobas e 801 analyzer; y) with the Elecsys proBNP II STAT assay, g) 05390109190 (cobas e 801 analyzer; x) gave the following correlations (pg/mL):

Number of samples measured: 143

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean</th>
<th>SD</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pg/mL</td>
<td>pmol/L</td>
<td>pmol/L</td>
</tr>
<tr>
<td>Human serum 1</td>
<td>16.8</td>
<td>1.98</td>
<td>0.799</td>
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<tr>
<td>Human serum 2</td>
<td>135</td>
<td>15.9</td>
<td>2.46</td>
</tr>
<tr>
<td>Human serum 3</td>
<td>1907</td>
<td>225</td>
<td>24.8</td>
</tr>
<tr>
<td>Human serum 4</td>
<td>21581</td>
<td>2547</td>
<td>446</td>
</tr>
<tr>
<td>Human serum 5</td>
<td>31916</td>
<td>3766</td>
<td>498</td>
</tr>
<tr>
<td>PC CARDII1</td>
<td>148</td>
<td>17.5</td>
<td>2.51</td>
</tr>
<tr>
<td>PC CARDII2</td>
<td>4903</td>
<td>579</td>
<td>73.0</td>
</tr>
</tbody>
</table>

The sample concentrations were between 6.32 and 31210 pg/mL (0.746 and 3883 pmol/L).

b) A comparison of the Elecsys pro BNP II assay, g) 07027664190 (9-minute application; y) with the Elecsys proBNP II assay, g) 07027664190 (18-minute application; x) on the cobas e 801 analyzer gave the following correlations (pg/mL):

Number of samples measured: 151

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean</th>
<th>SD</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pg/mL</td>
<td>pmol/L</td>
<td>pmol/L</td>
</tr>
<tr>
<td>Human serum 1</td>
<td>16.8</td>
<td>1.98</td>
<td>0.796</td>
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<tr>
<td>Human serum 2</td>
<td>135</td>
<td>15.9</td>
<td>2.57</td>
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<td>1907</td>
<td>225</td>
<td>41.3</td>
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<tr>
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<td>21581</td>
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<td>499</td>
</tr>
<tr>
<td>Human serum 5</td>
<td>31916</td>
<td>3766</td>
<td>927</td>
</tr>
<tr>
<td>PC CARDII1</td>
<td>148</td>
<td>17.5</td>
<td>2.94</td>
</tr>
<tr>
<td>PC CARDII2</td>
<td>4903</td>
<td>579</td>
<td>94.2</td>
</tr>
</tbody>
</table>

The sample concentrations were between 15.7 and 34466 pg/mL (1.85 and 4067 pmol/L).

References


47 Cowie MR, Jourdain P, Maisel A, et al. Clinical applications of B-type...
natriuretic peptide (BNP) testing. Eur Heart J 2003;24,1710-1718.
54 Data established with the Elecsys proBNP assay (first generation, REF 03121640122).

For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets, the product information and the Method Sheets of all necessary components (if available in your country). A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols
Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see https://usdiagnostics.roche.com for definition of symbols used):

<table>
<thead>
<tr>
<th>SYMBOL</th>
<th>MEANING</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTENT</td>
<td>Contents of kit</td>
</tr>
<tr>
<td>SYSTEM</td>
<td>Analyzers/Instruments on which reagents can be used</td>
</tr>
<tr>
<td>REAGENT</td>
<td>Reagent</td>
</tr>
<tr>
<td>CALIBRATOR</td>
<td>Calibrator</td>
</tr>
<tr>
<td>GTIN</td>
<td>Global Trade Item Number</td>
</tr>
</tbody>
</table>

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