Elecsys proBNP II STAT

cobas®

		Σ	SYSTEM
05390109119	05390109500	100	**cobas e** 601 **cobas e** 602

English

System information

For **cobas e** 601 and **cobas e** 602 analyzers: Application Code Number 173

Intended 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.[1,2,3,4,5,6,7,8]

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 syndrome[11,12,13,14,15] 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 **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on the **cobas e** 601 and **cobas e** 602 immunoassay analyzers.

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: 05390109190 for the Elecsys proBNP II STAT 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 The last 3 digits -190 have been replaced by -119 for logistic purposes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.[1,2,3] 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).[1,2,3] Clinical information and imaging procedures are used to confirm the diagnosis of heart failure.[1,2,3]

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.[23,24,25] 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 (1-76).[22,23]

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.[4,5,6,7,8]

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 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.[16,26,27,28,29] 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 6.6 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.[1,2,18,20] This interpretation of NT-proBNP results remains unchanged when using the new drug class Angiotensin receptor–neprilysin inhibitor[1,2] (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 stress[33] 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 tumor drugs[1,36,37] 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 STAT 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.[59]

Test principle

Sandwich principle. Total duration of assay: 9 minutes.

- During a 9 minutes incubation, antigen in the sample (15 µL), a biotinylated monoclonal NT-proBNP-specific antibody, a monoclonal NT-proBNP-specific antibody labeled with a ruthenium complex[a)] and streptavidin-coated microparticles react to form a sandwich complex, which is bound to the solid phase.

- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode or e-barcode.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Reagents - working solutions

The reagent rackpack is labeled as PROBNPST.

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL:

Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-NT-proBNP-Ab~biotin (gray cap), 1 bottle, 9 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~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 9 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.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H317 May cause an allergic skin reaction.

Prevention:

P261 Avoid breathing dust/fume/gas/mist/vapours/spray.

P272 Contaminated work clothing should not be allowed out of the workplace.

P280 Wear protective gloves.

Response:

P333 + P313 If skin irritation or rash occurs: Get medical advice/attention.

P362 + P364 Take off contaminated clothing and wash it before reuse.

Disposal:

P501 Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent handling

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 read in from the respective reagent barcodes.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the Elecsys reagent kit **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	12 weeks
on the analyzers	8 weeks

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, K_2-EDTA and K_3-EDTA plasma.

Plasma tubes 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.

Ensure the samples, calibrators and controls are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples, calibrators and controls on the analyzers should be analyzed/measured within 2 hours.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- REF 05390117190, proBNP II STAT CalSet, for 4 x 1.0 mL
- REF 04917049190, PreciControl Cardiac II, for 4 x 2.0 mL
- REF 11732277122, Diluent Universal, 2 x 16 mL sample diluent or
  REF 03183971122, Diluent Universal, 2 x 36 mL sample diluent
- General laboratory equipment
- **cobas e** analyzer

Additional materials for **cobas e** 601 and **cobas e** 602 analyzers:

- REF 04880340190, ProCell M, 2 x 2 L system buffer
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- REF 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- REF 03004899190, PreClean M, 5 x 600 mL detection cleaning solution
- REF 12102137001, AssayTip/AssayCup, 48 magazines x 84 reaction cups or pipette tips, waste bags
- REF 03023150001, WasteLiner, waste bags
- REF 03027651001, SysClean Adapter M
- REF 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

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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. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers.

PreClean M solution is necessary.

Bring the cooled reagents to approximately 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the bottles.

Calibration

Traceability: This method has been standardized against the Elecsys proBNP II assay (REF 04842464190).

Every Elecsys reagent set has a barcoded label containing specific information for calibration of the particular reagent lot. 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 reagent kit 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 7 days when using the same reagent kit on the analyzer
- as required: e.g. quality control findings outside the defined limits

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 reagent kit, 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

Compound	Concentration tested
Bilirubin	≤ 428 µmol/L or ≤ 25 mg/dL
Hemoglobin	≤ 0.621 mmol/L or ≤ 1000 mg/dL
Intralipid	≤ 1500 mg/dL
Biotin	≤ 123 nmol/L or ≤ 30 ng/mL
Rheumatoid factors	≤ 1500 IU/mL
IgG	≤ 6.0 g/dL
IgA	≤ 1.6 g/dL
IgM	≤ 1.0 g/dL

Criterion: Recovery of ± 20 % 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/day) until at least 8 hours following the last biotin administration.

There is no high-dose hook effect at NT-proBNP concentrations up to 35400 pmol/L (300000 pg/mL).

In vitro tests were performed on 51 commonly used pharmaceuticals. No interference with the assay was found.

In rare cases, interference due to extremely high titers of antibodies to analyte-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.

cobas e 601 and **cobas e** 602 modules:

Note: Only required if the Elecsys proBNP II STAT assay runs on the same analyzer module as the Elecsys Troponin I/ Troponin I STAT assay.

Make sure that in the Special Wash List (Screen → Utility → Special Wash → Immune) the Elecsys proBNP II STAT assay is combined with all assays performed on the analyzer.

From test	Step	To test	Step 0	Step 1	Step 2
Elecsys proBNP II STAT	1	all items	X	X	X

The described additions to the Special Wash List have to be entered manually. Please refer to the operator's manual.

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 pmol/L) for 2-fold diluted samples.

Lower limits of measurement

Limit of Detection

Limit of Detection: 5 pg/mL (0.6 pmol/L)

The Limit of Detection was determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A requirements.

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 %).

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 analyzers or manually). The concentration of the diluted sample must be > 1770 pmol/L or > 15000 pg/mL.

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

After dilution by the analyzers, 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

Some of the following clinical data was obtained using the Elecsys proBNP assay (first generation, REF 03121640122). All data sets established with the first test generation are marked with an index [59].

Interpretation of NT-proBNP values

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.[47,48] 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).

Expected values

NT-proBNP concentrations in the reference group are shown in the following tables.

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 4266 subjects aged between 35 and 74 years, enrolled into the Gutenberg Health Study in Germany. [49] These individuals had no prevalent cardiovascular diseases such as former history of stroke, myocardial infarction, coronary artery disease, peripheral artery disease, chronic heart failure or atrial fibrillation. The descriptive statistics for NT-proBNP (pg/mL) in the reference group are shown in the following table:

Age (years)	**Men**				**Women**			
	Median	95th percentile	97.5th percentile	99th percentile	Median	95th percentile	97.5th percentile	99th percentile
35-44	18.9	90.8	115	137	59.9	202	237	311
45-54	23.5	121	173	273	63.8	226	284	395
55-64	47.4	262	386	920	81.8	284	352	417
65-74	89.3	486	879	2346	133	470	623	784
All	35.6	238	344	703	78.6	304	389	509

The circulating NT-proBNP concentration was also determined in samples from 2812 subjects aged between 20 and above 70 years, enrolled in a cardiovascular health screening program at a tertiary medical center in Taipei, Taiwan.[50] These individuals had no known cardiovascular or systemic co-morbidities, and no structural heart diseases. The descriptive statistics for NT-proBNP (pg/mL) in the reference group are shown in the following table:

Age (years)	**Men (N=1836)**				**Women (N=976)**			
	N	Median	25th percentile	75th percentile	N	Median	25th percentile	75th percentile
20-29	48	9	5	19.7	33	30.1	10.3	41.9
30-39	369	13.5	5	29.7	153	34.9	20.8	60.4
40-49	708	17	7.8	32.4	346	40.1	18.9	62.5
50-59	558	22.8	11.6	42.6	310	44.4	27.3	64.7
60-69	130	31.5	16.6	59.1	112	61.7	30.8	85.2
≥70	23	66.1	34.2	106.6	22	77.5	46.3	123.0

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).[51,59]

Recommended cutoffs in patients for diagnosis of chronic heart failure in non-acute onset[59]

A number of studies and ESC guidelines support a decision threshold for NT-proBNP of 125 pg/mL in non-acute onset for the diagnosis of heart failure.[1,3,52,53,54,55,56] 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). At the cut-off value, ESC Guidelines state that natriuretic peptides have a very high negative predictive value (NPV) comprised between 94 % and 98.0 % and a positive predictive value (PPV) comprised between 44 % and 57 %.[1]

Patients with stable heart failure (n = 721) including patients with asymptomatic left ventricular dysfunction (n = 176) and patients with congestive heart failure (n = 545) were compared to a reference group (n = 2264).

ROC plot analysis at the cutoff value of 125 pg/mL showed a sensitivity of 90.01 % and a specificity of 93.11 %.

Correlation of NT-proBNP with NYHA classification in patients diagnosed with chronic heart failure[59]

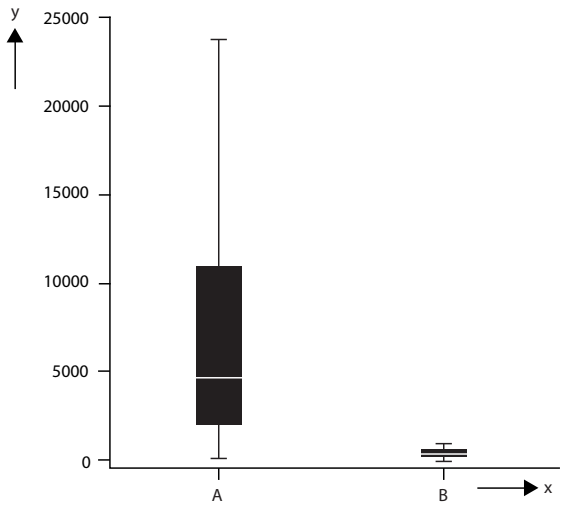
NT-proBNP values (pg/mL) for patients with restricted left ventricular ejection fraction (majority under therapy).

NYHA functional class				
	NYHA I	NYHA II	NYHA III	NYHA IV
N	182	250	234	35
Mean	1016	1666	3029	3465
SD	1951	2035	4600	4453
Median	342	951	1571	1707
5th percentile	32.9	103	126	148
95th percentile	3410	6567	10449	12188
% > 125 pg/mL	78.6	94.0	95.3	97.1

Recommended cutoffs in patients for diagnosis of chronic heart failure in acute onset

ICON (International Collaborative of NT-proBNP) study[10,59]

NT-proBNP concentrations were determined in samples from 1256 patients presenting with acute shortness of breath to emergency departments at four hospitals. This population included patients with a prior history of hypertension, coronary artery disease, myocardial infarction, heart failure, or pulmonary disease. 720 subjects were found to be suffering from acute exacerbation of heart failure, while the remainder were determined to present dyspnea due to other causes. The descriptive statistics for NT-proBNP concentrations (pg/mL) for both groups are shown in the following figure adapted from the ICON study:[10]



X --> A: Acute CHF (n = 720); B: Not acute CHF (n = 536)

Y --> NT-proBNP (pg/mL)

Diagnostic category	Median (IQR) NT-proBNP, pg/mL
Acute CHF	4639 (1882–10818)
Not Acute CHF	108 (37-381)

By using the optimal cutoffs established by the ICON study group and shown in the table below, physicians can increase the specificity and accuracy for diagnosing heart failure in patients presenting acute dyspnea in the emergent setting.

Category	Optimal cut-point pg/mL	Sensitivity %	Specificity %	PPV %	NPV %	Accuracy %
Rule in cut-point						
< 50 years (n = 184)	450	97	93	76	99	94
50-75 years (n = 537)	900	90	82	83	88	85
> 75 years (n = 535)	1800	85	73	92	55	83
Rule out cut-point						
All patients (n=1256)	300	99	60	77	98	83

Performance of NT-proBNP for diagnosis of acute heart failure in an Asian compared with a Western setting[57]

NT-proBNP concentrations were determined in samples from patients presenting with acute shortness of breath to emergency departments in Singapore (n = 606) and in New Zealand (n = 500). This population included patients with a prior history of hypertension, hyperlipidemia, coronary artery disease, myocardial infarction, heart failure, or pulmonary disease. NT-proBNP concentration in patients with final adjudicated diagnosis of acute heart failure was 4234 [2008-9799] pg/mL in Singapore (median [25-75th percentile], n = 148) and 4429 [2123-9479] pg/mL in New Zealand (n = 180).

The diagnostic performance of NT-proBNP at the cutoffs established in the ICON Study[10] are shown in the table below for both populations:

Category	Optimal cut-point pg/mL	Sensitivity %	Specificity %	PPV %	NPV %	Accuracy %
Rule in cut-point						
< 50 years						
Singapore (n=196)	450	100	91	58	100	92
New Zealand (n=41)		86	76	43	96	78
50-75 years						
Singapore (n=350)	900	88	83	68	95	85
New Zealand (n=236)		91	75	58	96	80
>75 years						
Singapore (n=60)	1800	79	81	73	85	80
New Zealand (n=223)		87	63	69	84	75
Rule out cut-point						
All patients						
Singapore (n=606)	300	97	73	54	99	79
New Zealand (n=500)		97	42	49	96	62

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, pooled human sera and controls in a protocol (EP5-A2) 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:

cobas e 601 and **cobas e** 602 analyzers	**Repeatability**				
Sample	Mean		SD		CV
	pg/mL	pmol/L	pg/mL	pmol/L	%
Human serum 1	59.3	7.00	2.10	0.248	3.5
Human serum 2	142	16.8	2.88	0.340	2.0
Human serum 3	422	49.8	7.57	0.893	1.8
Human serum 4	935	110	17.57	2.08	1.9
Human serum 5	6552	773	133	15.7	2.0
PC CARDII[b)]1	130	15.34	3.09	3.32	2.4
PC CARDII2	4942	583	108	12.7	2.2

b) PC CARDII = PreciControl Cardiac II

cobas e 601 and **cobas e** 602 analyzers	**Intermediate precision**				
Sample	Mean		SD		CV
	pg/mL	pmol/L	pg/mL	pmol/L	%
Human serum 1	59.3	7.00	2.10	0.248	3.5
Human serum 2	142	16.8	3.57	0.421	2.5
Human serum 3	422	49.8	8.56	1.01	2.0
Human serum 4	935	110	23.0	2.71	2.5
Human serum 5	6552	773	153	18.0	2.3
PC CARDII1	130	15.34	3.21	0.379	2.5
PC CARDII2	4942	583	126	14.9	2.6

Method comparison

A comparison of the Elecsys proBNP II STAT assay, REF 05390109190 (y) with the Elecsys proBNP II assay REF 04842464190 (x) using clinical samples gave the following correlations (pg/mL):

Number of samples measured: 132

Passing/Bablok[58]	Linear regression
y = 0.957x - 8.03	y = 0.968x - 13.97
τ = 0.989	r = 0.999

The sample concentrations were between approximately 6 and 32800 pg/mL (approximately 0.7 and 3870 pmol/L).

Analytical specificity

The Elecsys proBNP II STAT assay does not show any significant cross reactions with the following substances, tested with NT-proBNP concentrations of approximately 230 pg/mL and 2300 pg/mL (max. tested concentration):

Cross-reactant	**Concentration tested**
Adrenomedullin	1.0 ng/mL
Aldosterone	0.6 ng/mL
Angiotensin I	0.6 ng/mL
Angiotensin II	0.6 ng/mL
Angiotensin III	1.0 ng/mL
ANP_{28}	3.1 µg/mL
Arg-vasopressin	1.0 ng/mL
BNP_{32}	3.5 µg/mL
CNP_{22}	2.2 µg/mL
Endothelin	20 pg/mL
NT-proANP$_{1-30}$ (preproANP$_{26-55}$)	3.5 µg/mL
NT-proANP$_{31-67}$ (preproANP$_{56-92}$)	1.0 ng/mL
NT-proANP$_{79-98}$ (preproANP$_{104-123}$)	1.0 ng/mL
Renin	50 ng/mL
Urodilatin	3.5 µg/mL

Functional sensitivity

50 pg/mL (5.9 pmol/L)

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

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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 dialog.roche.com for definition of symbols used):

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume for reconstitution
GTIN	Global Trade Item Number

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