Elecsys proBNP II

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REF		Σ	SYSTEM
09744959190	09744959501	100	**cobas e** 402 **cobas e** 801

English

For use in the USA only

System information

Short name	ACN (application code number)	Application
PBNPX	10237	18 minutes
PBNPSTX	10238	9 minutes (STAT = Short Turn Around Time)

Intended use

Immunoassay for the in vitro quantitative determination of N-terminal pro-Brain natriuretic peptide in human serum and plasma. This assay is used as an aid in the diagnosis of individuals suspected of having heart failure. It can be used as an aid in the diagnosis of acute decompensated heart failure (ADHF) in patients presenting with signs and symptoms of ADHF to the emergency department (ED). The test is further indicated for the risk stratification of patients with acute coronary syndrome and heart failure. The test may also serve as an aid in the assessment of increased risk of cardiovascular events and mortality in patients at risk for heart failure who have stable coronary artery disease.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on **cobas e** immunoassay analyzers.

Summary

Left ventricular dysfunction can occur as a part of coronary heart disease, arterial hypertension, valvular disease, and primary myocardial disease. If the left ventricular dysfunction remains untreated and is progressive, the potential for mortality is high, e.g. due to sudden cardiac death.
Chronic cardiac insufficiency is a clinical syndrome caused by impairment of the cardiac pumping function. Based on the symptoms, the severity of cardiac insufficiency is classified in stages (New York Heart Association classification [NYHA] I-IV).[1,2] Clinical information and imaging procedures are used to diagnose left ventricular dysfunction.[3]
The significance of natriuretic peptides in the control of cardiovascular system function has been demonstrated. Studies reveal that natriuretic peptides can be used for diagnostic clinical problems associated with left ventricular dysfunction.[4] The following natriuretic peptides have been described: atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP), and C-type natriuretic peptide (CNP).[5,6]
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.[7,8] In subjects with left ventricular dysfunction, serum and plasma concentrations of BNP increase, as do the concentrations of the biologically inactive prohormone, proBNP.
ProBNP, comprising 108 amino acids, is secreted mainly by the ventricle and, in this process, is cleaved into physiologically active BNP (77-108) and the N-terminal fragment NT-proBNP (1-76).[6]
Studies indicate that NT-proBNP can be used in diagnostic and prognostic applications.[9,10,11] The concentration of NT-proBNP in serum or plasma correlates with the prognosis of the left ventricular dysfunction. Fisher, et al. found that heart failure patients with NT-proBNP values above median had a 1 year mortality rate of 53 % compared to 11 % in patients below median.[12] In the GUSTO IV study which involved more than 6800 patients it was shown that NT-proBNP was the strongest independent predictor of 1 year mortality in patients with acute coronary syndrome.[13]
Three studies involving patients with stable coronary artery disease have shown that elevated levels of NT-proBNP lead to a greater risk of future adverse events. In these studies, NT-proBNP levels above 450 pg/mL conferred approximately a 2- to 6-fold increase in risk for cardiac morbidity and/or mortality.[14,15,16] Furthermore, each of these studies demonstrated that the amount of risk increases somewhat as the NT-proBNP levels approach the above value. Therefore, when a patient with stable coronary artery disease has a NT-proBNP level above 450 pg/mL, and is not shown to have heart failure upon further evaluation, the physician should be aware that the elevated NT-proBNP value may have independent prognostic significance. These patients should receive continuing clinical attention according to established guidelines.[17] NT-proBNP values should be assessed in conjunction with other cardiovascular risk factors and clinical findings.
The test is also useful in assigning symptoms to cardiac or non-cardiac causes, and helps to identify subjects with left ventricular dysfunction. The European Society of Cardiology Task Force for the Diagnosis and Treatment of Chronic Heart Failure recommend in their guidelines that natriuretic peptides including NT-proBNP "may be most useful clinically as a rule out test due to consistent and very high negative predictive values".[3]
When used with the recommended age-independent exclusionary cut-point of 300 pg/mL in patients presenting with signs and symptoms of ADHF to the ED, the NPV ranged from 97.7 % for males to 98.5 % for females (see Expected values: Emergency Department Settings section).
The Elecsys proBNP II assay contains 2 monoclonal antibodies which recognize epitopes located in the N-terminal part (1-76) of proBNP (1-108).

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[a)] 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 onto the surface of the electrode. Unbound substances are then removed with ProCell II 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 **cobas** link.

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

Reagents - working solutions

The **cobas e** pack is labeled as PBNPX.

M Streptavidin-coated microparticles, 1 bottle, 6.4 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-NT-proBNP-Ab~biotin, 1 bottle, 9.0 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~$Ru(bpy)_3^{2+}$, 1 bottle, 8.6 mL:
Monoclonal anti-NT-proBNP antibody (sheep) labeled with ruthenium complex 1.1 µg/mL; Biotin scavenger antibody 1.5 mg/mL; phosphate buffer 40 mmol/L, pH 5.8; preservative.

Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

Elecsys proBNP II

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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 mist or vapours.
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: 1-800-428-2336

Serum concentrations of natriuretic peptides may be elevated in patients with acute myocardial infarction, patients that are candidates for renal dialysis, and patients that have undergone renal dialysis.
The Elecsys proBNP II test, like all laboratory tests, does not provide a definitive diagnosis. As with all in vitro diagnostic tests, the test results should be interpreted by the physician in conjunction with other laboratory test results and patient clinical findings.

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:	
unopened at 2-8 °C	up to the stated expiration date
on the analyzers	16 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 and K_2-EDTA plasma.

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 and calibrators are at 20-25 °C prior to measurement.

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

Sample stability claims were established by experimental data by the manufacturer or based on reference literature and only for the temperatures/time frames as stated in the method sheet. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.

Materials provided

See “Reagents – working solutions” section for reagents.

Materials required (but not provided)

- REF 09315314190, CalSet proBNP II, for 4 x 1.0 mL
- REF 04917049160, PreciControl Cardiac II, for 4 x 2.0 mL
- REF 07299001190, Diluent Universal, 36 mL sample diluent
- General laboratory equipment
- **cobas e** analyzer

Additional materials for the **cobas e** 402 and **cobas e** 801 analyzers:

- REF 06908799190, ProCell II M, 2 x 2 L system solution
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 07485409001, Reservoir Cup, 8 cups to supply ProCell II M and CleanCell M
- REF 06908853190, PreClean II M, 2 x 2 L wash solution
- REF 05694302001, Assay Tip/Assay Cup tray, 6 magazines x 6 magazine stacks x 105 assay tips and 105 assay cups, 3 wasteliners
- REF 07485425001, Liquid Flow Cleaning Cup, 2 adaptor cups to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning Detection Unit
- REF 07485433001, PreWash Liquid Flow Cleaning Cup, 1 adaptor cup to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning PreWash Unit
- REF 11298500160, 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 03121640). This in turn is traceable to pure synthetic NT-proBNP (1-76) 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 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

Elecsys proBNP II

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- after 28 days when using the same **cobas e** pack on the analyzer
- as required: e.g. quality control findings outside the defined limits

Quality control

Use PreciControl Cardiac II or other suitable controls for routine quality control procedures.

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.

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	≤ 3500 ng/mL
Rheumatoid factors	≤ 1500 IU/mL
Albumin	≤ 7 g/dL

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

This assay has no biotin interference in serum concentrations up to 3500 ng/mL. Pharmacokinetic studies have shown that serum concentrations of biotin can reach up to 355 ng/mL within the first hour after biotin ingestion for subjects consuming supplements of 20 mg biotin per day[18] and up to 1160 ng/mL for subjects after a single dose of 300 mg biotin.[19]

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

Drug	Concentration tested mg/L
Carvedilol	37.5
Clopidogrel	75.0
Digoxin	0.25
Epinephrine (Adrenaline)	0.50
Insulin	1.60
Lidocaine	80.0
Lisinopril	10.0
Methylprednisolone	7.50
Metoprolol	150
Nifedipine	30.0
Phenprocoumon (Marcumar)	3.00
Propafenone	300
Reteplase	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 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; reported from Japan), patients may show false low results when tested with the assay kit (values undetectable) due to a NT-proBNP 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

36-35000 pg/mL (defined by the Limit of Quantitation and the maximum of the master curve). Values below the Limit of Quantitation are reported as < 36 pg/mL. Values above the measuring range are reported as > 35000 pg/mL or up to 70000 pg/mL for 2-fold diluted samples.

Lower limits of measurement

Limit of Quantitation

Limit of Quantitation = 36 pg/mL

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

The Limit of Quantitation is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 20 %. It has been determined using low concentration NT-proBNP samples.

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). The concentration of the diluted sample must be ≥ 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 1:2 may entail maximum deviations of 18 % from the theoretical value.

Expected values: Emergency Department Settings

The subjects enrolled in this trial were presenting to the emergency department (ED) with signs and symptoms suggestive of acute decompensated heart failure (ADHF). Therefore these cut-points should be used when assessing patients who may have an acute decompensation in cardiac function. NT-proBNP values above the respective age-specific cut-points (450/900/1800 pg/mL) are denoted as positive test results.

To assess the performance of positive and negative cut-points for ADHF in patients presenting with signs and symptoms of ADHF to the ED, a multicenter trial was performed in the United States. 1485 subjects were enrolled at 17 sites; 744 males and 741 females. Subjects were ≥ 22 years of age with symptoms of suspected ADHF, presenting with dyspnea of a duration no longer than several days. 275 (19 %) were found to have ADHF, based on adjudication by a clinical events committee. As reported in previous studies, the age-based positive cut-points and the negative cut-point for all ages were determined to be:

Elecsys proBNP II

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Age Group	Elecsys proBNP II / Elecsys proBNP II STAT Cut-Points	Interpretation
< 50 years	450 pg/mL	NT-proBNP > 450 pg/mL indicates ADHF is likely
50 to 75 years	900 pg/mL	NT-proBNP > 900 pg/mL indicates ADHF is likely
> 75 years	1800 pg/mL	NT-proBNP > 1800 pg/mL indicates ADHF is likely
All ages	Results within the gray zone for age group	Indeterminate. Further clinical information is needed to determine if ADHF is present.

Elecsys proBNP II / Elecsys proBNP II STAT Cut-Point	Interpretation
300 pg/mL	NT-proBNP < 300 pg/mL indicates ADHF is not likely

Diagnostic category	Median proBNP II STAT	IQR[b)]
Subjects without ADHF	105 pg/mL	37.86-402.80 pg/mL
Subjects with ADHF	3054 pg/mL	1255.5-6759.00 pg/mL

b) IQR = interquartile range

NT-proBNP values above the respective age-specific cut-points (450/900/1800 pg/mL) are denoted as positive test results, and NT-proBNP values below the universal age-independent cut-point (300 pg/mL) are denoted as negative test results. A gray zone exists between the negative and positive cut-points.

Age Range (years)	Result Interpretation	NT-proBNP Concentration (pg/mL)
< 50	Positive	> 450
	Gray	300-450
	Negative	< 300
50 - 75	Positive	> 900
	Gray	300-900
	Negative	< 300
> 75	Positive	> 1800
	Gray	300-1800
	Negative	< 300
All Age Groups	Positive	aggregated
	Gray	aggregated
	Negative	< 300

NYHA classification (ED)

586 subjects had a New York Heart Association (NYHA) functional classification. NT-proBNP values in this group are presented below. NYHA classification is based on clinical presentation and functional observations rather than on measurements with diagnostic tools such as biomarkers or imaging.

NYHA Classes Population-All Subjects

NYHA Functional class					
	All	NYHA I	NYHA II	NYHA III	NYHA IV
Mean	1400	-	1067	1485	1631
SD	3712	-	3087	3044	4973
Median	155	-	111	270	161
5th percentile	36	-	36	36	36
95th percentile	7781	-	4952	8585	7774
% > cut-point	26.3	-	20.8	31.7	24.4
Minimum	36	-	36	36	36
Maximum	35000	-	27776	21402	35000
N	586	-	178	240	168

NYHA Classes Population-Females

NYHA Functional class					
	All	NYHA I	NYHA II	NYHA III	NYHA IV
Mean	1090	-	522	1175	1558
SD	3317	-	1247	2486	5077
Median	115	-	105	121	132
5th percentile	36	-	36	36	36
95th percentile	5160	-	2426	6310	7750
% > cut-point	20.3	-	13.7	27	18.9
Minimum	36	-	36	36	36
Maximum	35000	-	9832	14061	35000
N	301	-	95	111	95

NYHA Classes Population-Males

NYHA Functional class					
	All	NYHA I	NYHA II	NYHA III	NYHA IV
Mean	1727	-	4248	1733	1701
SD	4068	-	115	3394	4779
Median	279	-	36	404	240
5th percentile	36	-	9793	36	36
95th percentile	8852	-	9716	8763	6892
% > cut-point	32.6	-	28.9	35.7	31.5
Minimum	36	-	36	36	36
Maximum	34670	-	28015	21011	34080
N	285	-	83	129	73

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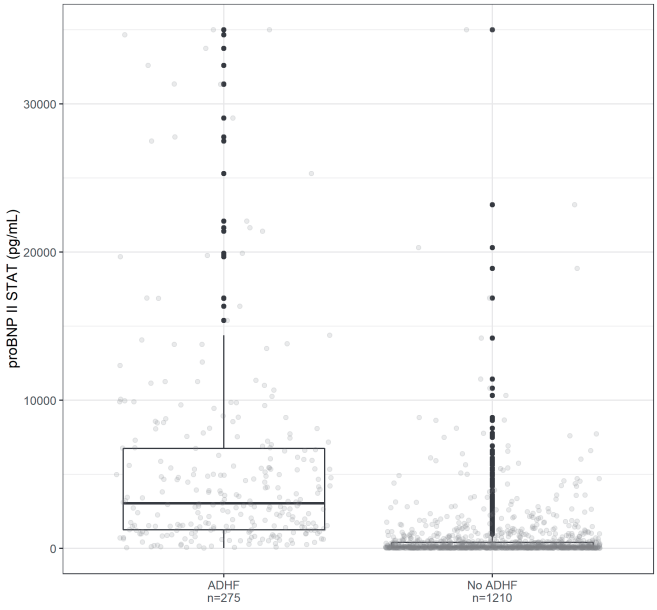
Boxplot of proBNP II STAT values per ADHF group, all subjects (ED)



Censoring into measuring range:
- ADHF: lower limit: 1 subjects; upper limit: 2 subjects.
- No ADHF: lower limit: 291 subjects; upper limit: 2 subjects.

An analysis of the primary endpoint included the gray zone, which is the zone between the age-specific positive cut-points and the negative cut-point for each subject. This gray zone included NT-proBNP levels ≥ 300 pg/mL but less than or equal to the positive cut-point for diagnosis (that is, 450 pg/mL for age < 50 years, 900 pg/mL for age 50-75 years and 1800 pg/mL for age > 75 years).

A total of only 231 subjects (15 %) had NT-proBNP levels with Elecsys proBNP II STAT that fell into the gray zone between cut-points. An analysis (see below) was performed to better understand the performance of the assay for values that fall between the cut-point for positive and the cut-point for negative. In these cases, other causes of cardiac dysfunction should be investigated.

The likelihood ratio (LR) for all 231 subjects (any age) who fell into the gray zone was 0.89 (95 % confidence interval: 0.65-1.23) suggesting no additive diagnostic value. For them, other clinical, imaging or diagnostic information would be necessary for the differential diagnosis of ADHF in patients presenting with acute dyspnea in the emergency department.

The performance of the NT-proBNP-based diagnosis of ADHF through the triple age-specific positive cut-points and the single universal negative cut-point was demonstrated through the calculation of likelihood ratios (positive likelihood ratios (LR+) and negative likelihood ratios (LR-)). Likelihood ratios can provide a quantification of the diagnostic capabilities of the assay for both subjects with reactive Elecsys proBNP II STAT results (LR+) and non-reactive Elecsys proBNP II STAT results (LR-). The likelihood ratio can be used to project the change in the probability of having ADHF from the general disease prevalence (the pre-test probability) to the probability after the test results are interpreted (the post-test probability). Establishing the likelihood ratios for the Elecsys proBNP II and proBNP II STAT assays performed on the **cobas e** 601 analyzer demonstrated the strength of the assays as an aid in diagnosing ADHF and the ability to understand the implications of the diagnosis in patients with signs and symptoms of ADHF to the ED.

Analyses were conducted separately for each sex and age sub-population, as well for all age groups combined and all sexes combined.

Acutely decompensated cut-point performance (ED)

Three-by-Two Contingency Table for the ICON Cut-Points - Elecsys proBNP II STAT - All Evaluable Subjects

Age Group	Test Result Interpretation	ADHF	No ADHF	Total
< 50	Positive	31	27	58
	Gray	0	23	23
	Negative	5	392	397
	Total	36	442	478
50 - 75	Positive	146	114	260
	Gray	25	117	142
	Negative	11	429	440
	Total	182	660	842
> 75	Positive	43	31	74
	Gray	14	52	66
	Negative	0	25	25
	Total	57	108	165
All	Positive	220	172	392
	Gray	39	192	231
	Negative	16	846	862
	Total	275	1210	1485

Pre- and Post-Test Probabilities as well as informative LRs for NT-proBNP Cut-Points for the Diagnosis or Exclusion of ADHF - Elecsys proBNP II STAT - All Evaluable Subjects

Age Group	Prevalence of ADHF (%) (n/N)	Test Result Interpretation	Post-test Probability of ADHF		Post-test Probability of No ADHF		Likelihood Ratio (ADHF)	
			Estimate (%) (n/N)	95%-CI (%) c)	Estimate (%) (n/N)	95%-CI (%) c)	LR d)	95%-CI e)
< 50	7.5 (36/478)	Positive f)	**53.4 (31/58)**	40.0-66.5	--	--	14.10	9.56-20.79
		Gray g)	0.0 (0/23)	0.0-17.8	100.0 (23/23)	82.2-100.0	0.00	N/A h)
		Negative i)	--	--	**98.7 (392/397)**	96.9-99.5	0.16	0.07-0.35
50-75	21.6 (182/842)	Positive	**56.2 (146/260)**	49.9-62.2	--	--	4.64	3.87-5.57
		Gray	17.6 (25/142)	11.9-25.1	82.4 (117/142)	74.9-88.1	0.77	0.52-1.16
		Negative	--	--	**97.5 (429/440)**	95.4-98.7	0.09	0.05-0.17
> 75	34.5 (57/165)	Positive	**58.1 (43/74)**	46.1-69.3	--	--	2.63	1.89-3.66
		Gray	21.2 (14/66)	12.5-33.3	78.8 (52/66)	66.7-87.5	0.51	0.31-0.84
		Negative	--	--	**100.0 (25/25)**	83.4-100.0	0.00	N/A

c) Wilson score confidence intervals with continuity correction

d) LR = likelihood ratios

e) log method confidence intervals

f) Positive: > age-specific cut-point

g) Gray: ≥ 300 pg/mL and ≤ age-specific cut-point

h) N/A = Not applicable

Elecsys proBNP II

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i) Negative: < 300 pg/mL

Three-by-Two Contingency Table for the ICON Cut-Points - Elecsys proBNP II STAT - Females

Age Group	Test Result Interpretation	ADHF	No ADHF	Total
< 50	Positive	13	8	21
	Gray	0	15	15
	Negative	2	229	231
	Total	15	252	267
50 - 75	Positive	58	52	110
	Gray	11	45	56
	Negative	5	229	234
	Total	74	326	400
> 75	Positive	15	17	32
	Gray	6	26	32
	Negative	0	10	10
	Total	21	53	74
All	Positive	86	77	163
	Gray	17	86	103
	Negative	7	468	475
	Total	110	631	741

Pre- and Post-Test Probabilities as well as informative LRs for NT-proBNP Cut-Points for the Diagnosis or Exclusion of ADHF - Elecsys proBNP II STAT - Females

Age Group	Prevalence of ADHF (%) (n/N)	Test Result Interpretation	Post-test Probability of ADHF		Post-test Probability of No ADHF		Likelihood Ratio (ADHF)	
			Estimate (%) (n/N)	95%-CI (%) c)	Estimate (%) (n/N)	95%-CI (%) c)	LR d)	95%-CI e)
< 50	5.6 (15/267)	Positive f)	**61.9 (13/21)**	38.7-81.0	--	--	27.30	13.42-55.54
		Gray g)	0.0 (0/15)	0.0-25.3	100.0 (15/15)	74.7-100.0	0.00	N/A h)
		Negative i)	--	--	**99.1 (229/331)**	96.6-99.8	0.15	0.04-0.53
50-75	18.5 (74/400)	Positive	**52.7 (58/110)**	43.0-62.2	--	--	4.91	3.73-6.48
		Gray	19.6 (11/56)	10.7-32.8	80.4 (45/56)	67.2-89.3	1.08	0.59-1.98
		Negative	--	--	**97.9 (229/234)**	94.8 99.2	0.10	0.04-0.22
> 75	28.4 (21/74)	Positive	**46.9 (15/32)**	29.5-65.0	--	--	2.23	1.38-3.58
		Gray	18.8 (6/32)	7.9-37.0	81.3 (26/32)	63.0-92.1	0.58	0.28-1.21
		Negative	--	--	**100.0 (10/10)**	65.5-100.0	0.00	N/A

Three-by-Two Contingency Table for the ICON Cut-Points - Elecsys proBNP II STAT - Males

Age Group	Test Result Interpretation	ADHF	No ADHF	Total
< 50	Positive	18	19	37
	Gray	0	8	8
	Negative	3	163	166
	Total	21	190	211
50 - 75	Positive	88	62	150
	Gray	14	72	86
	Negative	6	200	206
	Total	108	334	442
> 75	Positive	28	14	42
	Gray	8	26	34
	Negative	0	15	15
	Total	36	55	91
All	Positive	134	95	229
	Gray	22	106	128
	Negative	9	378	387
	Total	165	579	744

Pre- and Post-Test Probabilities as well as informative LRs for NT-proBNP Cut-Points for the Diagnosis or Exclusion of ADHF - Elecsys proBNP II STAT - Males

Age Group	Prevalence of ADHF (%) (n/N)	Test Result Interpretation	Post-test Probability of ADHF		Post-test Probability of No ADHF		Likelihood Ratio (ADHF)	
			Estimate (%) (n/N)	95%-CI (%) c)	Estimate (%) (n/N)	95%-CI (%) c)	LR d)	95%-CI e)
< 50	10.0 (21/211)	Positive f)	**48.6 (18/37)**	32.2-65.3	--	--	8.57	5.41-13.59
		Gray g)	0.0 (0/8)	0.0-40.2	100.0 (8/8)	59.8-100.0	0.00	N/A h)
		Negative i)	--	--	**98.2 (163/166)**	94.4-99.5	0.17	0.06-0.48
50-75	24.4 (108/442)	Positive	**58.7 (88/150)**	50.3-66.6	--	--	4.39	3.45-5.59
		Gray	16.3 (14/86)	9.5-26.1	83.7 (72/86)	73.9-90.5	0.60	0.35-1.02
		Negative	--	--	**97.1 (200/206)**	93.5 98.8	0.09	0.04-0.20
> 75	39.6 (36/91)	Positive	**66.7 (28/42)**	50.4-80.0	--	--	3.06	1.88-4.96
		Gray	23.5 (8/34)	11.4-41.6	76.5 (26/34)	58.4-88.6	0.47	0.24-0.92
		Negative	--	--	**100.0 (15/15)**	74.7-100.0	0.00	N/A

Renal cohort (ED)

Renal disease can alter NT-proBNP values because the peptide is known to be cleared by the kidneys. Below are analyses of performance in patients with and without compromised renal function.

Pre- and Post-Test Probabilities as well as informative LRs for NT-proBNP Cut-Points for the Diagnosis or Exclusion of ADHF - Elecsys proBNP II STAT - Renal Disease (eGFR < 60 mL/min/1.73 m^2)

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Age Group	Prevalence of ADHF (%) (n/N)	Test Result Interpretation	Post-test Probability of ADHF		Post-test Probability of No ADHF		Likelihood Ratio (ADHF)	
			Estimate (%) (n/N)	95%-CI (%) j)	Estimate (%) (n/N)	95%-CI (%) j)	LR k)	95%-CI l)
< 50	40.9 (9/22)	Positive m)	**81.8 (9/11)**	47.8-96.8	--	--	6.50	1.82-23.26
		Gray n)	0.0 (0/4)	0.0-60.4	100.0 (4/4)	39.6-100.0	0.00	N/A o)
		Negative p)	--	--	**100.0 (7/7)**	56.1-100.0	0.00	N/A
50-75	40.5 (85/210)	Positive	**64.2 (77/120)**	54.8-72.6	--	--	2.63	2.05-3.39
		Gray	22.6 (7/31)	10.3-41.5	77.4 (24/31)	58.5-89.7	0.43	0.19-0.95
		Negative	--	--	**98.3 (58/59)**	89.7-99.9	0.03	0.00-0.18
> 75	40.4 (38/94)	Positive	**60.0 (33/55)**	45.9-72.7	--	--	2.21	1.56-3.13
		Gray	16.1 (5/31)	6.1-34.5	83.9 (26/31)	65.5-93.9	0.28	0.12-0.67
		Negative	--	--	**100.0 (8/8)**	59.8-100.0	0.00	N/A

j) Wilson score confidence intervals with continuity correction

k) LR = likelihood ratios

l) log method confidence intervals

m) Positive: > age-specific cut-point

n) Gray: ≥ 300 pg/mL and ≤ age-specific cut-point

o) N/A = Not applicable

p) Negative: < 300 pg/mL

Pre- and Post-Test Probabilities as well as informative LRs for NT-proBNP Cut-Points for the Diagnosis or Exclusion of ADHF - Elecsys proBNP II STAT - No Renal Disease (eGFR ≥ 60 mL/min/1.73 m^2)

Age Group	Prevalence of ADHF (%) (n/N)	Test Result Interpretation	Post-test Probability of ADHF		Post-test Probability of No ADHF		Likelihood Ratio (ADHF)	
			Estimate (%) (n/N)	95%-CI (%) j)	Estimate (%) (n/N)	95%-CI (%) j)	LR k)	95%-CI l)
< 50	6.9 (25/361)	Positive m)	**48.8 (21/43)**	33.6-64.3	--	--	12.83	8.27-19.89
		Gray n)	0.0 (0/16)	0.0-24.1	100.0 (16/16)	75.9-100.0	0.00	N/A o)
		Negative p)	--	--	**98.7 (298/302)**	96.4-99.6	0.18	0.07-0.44
50-75	15.5 (95/592)	Positive	**50.0 (65/130)**	41.5-58.5	--	--	5.43	4.18-7.06
		Gray	16.4 (18/110)	10.2-24.9	83.6 (92/110)	75.1-89.8	1.06	0.68-1.67
		Negative	--	--	**97.4 (343/352)**	95.0-98.7	0.14	0.08-0.27
> 75	26.9 (18/67)	Positive	**50.0 (9/18)**	29.0-71.0	--	--	2.72	1.29-5.76
		Gray	27.3 (9/33)	13.9-45.8	72.7 (24/33)	54.2-86.1	1.02	0.59-1.76
		Negative	--	--	**100.0 (16/16)**	75.9-100.0	0.00	N/A

Of note, the overall positive likelihood ratio (LR+) with the Elecsys proBNP II STAT was found to be lower in patients with renal disease compared to patients without renal disease (LR+ 2.61 and 6.42, respectively). In this population, more false positives are observed. Caution should be used when interpreting NT-proBNP or Elecsys proBNP II STAT results in patients with renal dysfunction.

Body mass index (ED)

Prior studies concluded that the concentrations of BNP[20] and NT-proBNP[21] are lower in obese people, both without and with HF. The most likely biological reason for the lower natriuretic peptide level has been described by the lower release of natriuretic peptides in obesity and also involving pericardial fat, rather than increase in their clearance.[22] The observed natriuretic peptide reduction in obese (vs non-obese) patients was 10-50 %.[22] In line, the overall negative likelihood ratio (LR-) with the Elecsys proBNP II STAT was observed to be higher (i.e., worse) in patients with high BMI compared to patients with low BMI (LR- 0.13 and 0.00, respectively). This is due to the fact that false negatives were characterized by a high BMI. In this study, 15 of the 16 subjects with false negative results had an elevated BMI. In turn, however, patients with low BMI that were tested negative are characterized by a high NPV of 100 % (95 % CI 98.6-100.0).

In the context of obesity, natriuretic peptides should be interpreted with caution. For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Pre- and Post-Test Probabilities as well as informative LRs for NT-proBNP Cut-Points for the Diagnosis or Exclusion of ADHF - Elecsys proBNP II STAT - High BMI (≥ 30 kg/m^2)

Age Group	Prevalence of ADHF (%) (n/N)	Test Result Interpretation	Post-test Probability of ADHF		Post-test Probability of No ADHF		Likelihood Ratio (ADHF)	
			Estimate (%) (n/N)	95%-CI (%) q)	Estimate (%) (n/N)	95%-CI (%) q)	LR r)	95%-CI s)
< 50	11.8 (30/255)	Positive t)	**62.5 (25/40)**	45.8-76.8	--	--	12.50	7.47-20.91
		Gray u)	0.0 (0/11)	0.0-32.1	100.0 (11/11)	67.9-100.0	0.00	N/A v)
		Negative w)	--	--	**97.5 (199/204)**	94.1-99.1	0.19	0.08-0.42
50-75	25.6 (104/407)	Positive	**60.2 (77/128)**	51.1-68.6	--	--	4.40	3.34-5.79
		Gray	25.8 (17/66)	16.1-38.2	74.2 (49/66)	61.8-83.9	1.01	0.61-1.67
		Negative	--	--	**95.3 (203/213)**	91.3-97.6	0.14	0.08-0.26
> 75	32.3 (20/62)	Positive	**55.0 (11/20)**	32.0-76.2	--	--	2.57	1.27-5.18
		Gray	29.0 (9/31)	14.9-48.2	71.0 (22/31)	51.8-85.1	0.86	0.49-1.51
		Negative	--	--	**100.0 (11/11)**	67.9-100.0	0.00	N/A

q) Wilson score confidence intervals with continuity correction

r) LR = likelihood ratios

s) log method confidence intervals

t) Positive: > age-specific cut-point

u) Gray: ≥ 300 pg/mL and ≤ age-specific cut-point

v) N/A = Not applicable

w) Negative: < 300 pg/mL

Pre- and Post-Test Probabilities as well as informative LRs for NT-proBNP Cut-Points for the Diagnosis or Exclusion of ADHF - Elecsys proBNP II STAT - Low BMI (< 30 kg/m^2)

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Age Group	Prevalence of ADHF (%) (n/N)	Test Result Interpretation	Post-test Probability of ADHF		Post-test Probability of No ADHF		Likelihood Ratio (ADHF)	
			Estimate (%) (n/N)	95%-CI (%) q)	Estimate (%) (n/N)	95%-CI (%) q)	LR r)	95%-CI s)
< 50	2.8 (5/177)	Positive t)	**33.3 (5/15)**	13.0-61.3	--	--	17.20	9.43-31.39
		Gray u)	0.0 (0/12)	0.0-30.1	100.0 (12/12)	69.9-100.0	0.00	N/A v)
		Negative w)	--	--	**100.0 (150/150)**	96.9-100.0	0.00	N/A
50-75	18.9 (70/370)	Positive	**51.6 (63/122)**	42.5-60.7	--	--	4.58	3.59-5.83
		Gray	11.1 (7/63)	5.0-22.2	88.9 (56/63)	77.8-95.0	0.54	0.26-1.12
		Negative	--	--	**100.0 (185/185)**	97.5-100.0	0.00	N/A
> 75	36.6 (37/101)	Positive	**59.3 (32/54)**	45.1-72.1	--	--	2.52	1.75-3.61
		Gray	15.2 (5/33)	5.7-32.7	84.8 (28/33)	67.3-94.3	0.31	0.13-0.73
		Negative	--	--	**100.0 (14/14)**	73.2-100.0	0.00	N/A

History of Heart Failure (ED)

Heart failure is a chronic progressive disease. Below are the analyses showing the performance of NT-proBNP in patients with a previous diagnosis of heart failure when presenting to the ED with a suspicion of ADHF.

In addition, 346 subjects in this cohort had a previous diagnosis of heart failure.

Age Group	Test Result Interpretation	ADHF	No ADHF	Total
< 50	Positive	25	12	37
	Gray	0	6	6
	Negative	3	16	19
	Total	28	34	62
50 - 75	Positive	93	42	135
	Gray	13	19	32
	Negative	7	41	48
	Total	113	102	215
> 75	Positive	29	15	44
	Gray	6	16	22
	Negative	0	3	3
	Total	35	34	69
All	Positive	147	69	216
	Gray	19	41	60
	Negative	10	60	70
	Total	176	170	346

Pre- and Post-Test Probabilities as well as informative LRs for NT-proBNP Cut-Points for the Diagnosis or Exclusion of ADHF - Elecsys proBNP II STAT - History of HF

Age Group	Prevalence of ADHF (%) (n/N)	Test Result Interpretation	Post-test Probability of ADHF		Post-test Probability of No ADHF		Likelihood Ratio (ADHF)	
			Estimate (%) (n/N)	95%-CI (%) x)	Estimate (%) (n/N)	95%-CI (%) x)	LR y)	95%-CI z)
< 50	45.2 (28/62)	Positive aa)	**67.6 (25/37)**	50.1-81.4	--	--	2.53	1.58-4.06
		Gray ab)	0.0 (0/6)	0.0-48.3	100.0 (6/6)	51.7-100.0	0.00	N/A ac)
		Negative ad)	--	--	**84.2 (16/19)**	59.5-95.8	0.23	0.07-0.70
50-75	52.6 (113/215)	Positive	**68.9 (93/135)**	60.3-76.4	--	--	2.00	1.56-2.56
		Gray	40.6 (13/32)	24.2-59.2	59.4 (19/32)	40.8-75.8	0.62	0.32-1.19
		Negative	--	--	**85.4 (41/48)**	71.6-93.5	0.15	0.07-0.33
> 75	50.7 (35/69)	Positive	**65.9 (29/44)**	50.0-79.1	--	--	1.88	1.25-2.82
		Gray	27.3 (6/22)	11.6-50.4	72.7 (16/22)	49.6-88.4	0.36	0.16-0.82
		Negative	--	--	**100.0 (3/3)**	31.0-100.0	0.00	N/A

x) Wilson score confidence intervals with continuity correction

y) LR = likelihood ratios

z) log method confidence intervals

aa) Positive: > age-specific cut-point

ab) Gray: ≥ 300 pg/mL and ≤ age-specific cut-point

ac) N/A = Not applicable

ad) Negative: < 300 pg/mL

Pre- and Post-Test Probabilities as well as informative LRs for NT-proBNP Cut-Points for the Diagnosis or Exclusion of ADHF History of HF - Elecsys proBNP II STAT - No History of HF

Age Group	Prevalence of ADHF (%) (n/N)	Test Result Interpretation	Post-test Probability of ADHF		Post-test Probability of No ADHF		Likelihood Ratio (ADHF)	
			Estimate (%) (n/N)	95%-CI (%) x)	Estimate (%) (n/N)	95%-CI (%) x)	LR y)	95%-CI z)
< 50	1.8 (7/396)	Positive aa)	**26.3 (5/19)**	10.1-51.4	--	--	19.85	9.90-39.80
		Gray ab)	0.0 (0/17)	0.0-22.9	100.0 (17/17)	77.1-100.0	0.00	N/A ac)
		Negative ad)	--	--	**99.4 (358/360)**	97.8-99.9	0.31	0.10-1.00
50-75	10.0 (57/569)	Positive	**40.0 (42/105)**	30.7-50.0	--	--	5.99	4.53-7.91
		Gray	12.0 (12/100)	6.6-20.4	88.0 (88/100)	79.6-93.4	1.22	0.72-2.10
		Negative	--	--	**99.2 (361/364)**	97.4-99.8	0.07	0.02-0.22
> 75	20.0 (16/80)	Positive	**45.5 (10/22)**	25.1-67.3	--	--	3.33	1.77-6.29
		Gray	16.7 (6/36)	7.0-33.5	83.3 (30/36)	66.5-93.0	0.80	0.40-1.59
		Negative	--	--	**100.0 (22/22)**	81.5-100.0	0.00	N/A

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Patients with a history of prior HF have a substantially lower performance for positive cut-points compared to the performance in all evaluable subjects of the ICON-Reloaded cohort, which is likely due to a chronic biological elevation of NT-proBNP in these conditions. Higher false positive rates were observed in all subjects and higher false negative rates were seen in those subjects who were less than 50 and between 50-75 years of age. Use caution when interpreting test results in these patients due to a higher false positive and false negative rate.

For the Elecsys proBNP II assay:

The clinical performance information (sensitivity, specificity, negative predictive value, and positive predictive value) in the Reference and Disease groups described below were determined using a previous generation Elecsys proBNP assay (Elecsys proBNP Immunoassay). The performance characteristics of the Elecsys proBNP II assay support that the clinical performance described below is applicable to the Elecsys proBNP II assay.

Expected values: Previous Study

In a study conducted between 1996-2002, NT-proBNP values above the respective age-specific cut-points (125/450 pg/mL) are denoted as positive test results. In this study, subjects were collected from 7 sites in Europe and 9 sites in the US. These sites did not enroll patients with ADHF, but instead patients who were apparently healthy and those with a previous history of cardiac events such as angina, known left ventricular dysfunction, previous myocardial infarction and hypertension, along with those with chronic obstructive pulmonary disease, compromised renal function and diabetes.

NT-proBNP concentrations in the reference group are shown in the following tables. The most appropriate decision threshold apparent from these distributions are 125 pg/mL for patients younger than 75 years and 450 pg/mL for patients 75 years or older.

Elecsys proBNP II STAT test result (pg/mL)	Age Group (years)	Interpretation of Results
< 125	< 75	Negative: HF unlikely
≥ 125	< 75	Consider Heart Failure as well as other causes* of NT-proBNP elevation
< 450	≥ 75	Negative: HF unlikely
≥ 450	≥ 75	Consider Heart Failure as well as other causes* of NT-proBNP elevation

* Natriuretic peptides values elevations could also be caused by several conditions other than heart failure such as acute coronary syndrome, atrial fibrillation, pulmonary embolism, pulmonary hypertension, renal dysfunction, sepsis and stroke.[23,24]

Reference group

The circulating NT-proBNP concentration was determined from 1411 individuals without HF (800 women and 611 men). This population included apparently healthy individuals and individuals with diabetes, hypertension, pulmonary disease, and renal insufficiency. The descriptive statistics for NT-proBNP concentrations in the reference group are shown in the following table. The values (pg/mL) are representative of the values obtained from clinical studies.

Reference group

All

Age (years)	< 45	45-54	55-64	65-74	Total < 75	≥ 75
Mean	67.8	64.6	82.1	111	81.9	243
SD	83.7	96.2	108	95.2	101	211
Median	41.4	39.6	57.7	83.4	55.6	191
95th percentile	167	174	209	319	225	718
% < 125 pg/mL	89.3	89.0	83.3	69.8	82.4	-
% < 450 pg/mL	-	-	-	-	-	88.3
N	56	472	455	308	1291	120

Males

Age (years)	< 45	45-54	55-64	65-74	Total < 75	≥ 75
Mean	47.1	46.1	72.8	83.7	64.2	214
SD	33.6	51.1	140	81.6	98.1	231
Median	34.5	30.6	42.1	67.4	39.6	145
95th percentile	92.6	138	177	229	169	852
% < 125 pg/mL	95.7	93.3	87.8	86.7	90.0	-
% < 450 pg/mL	-	-	-	-	-	88.9
N	23	210	196	128	557	54

Females

Age (years)	< 45	45-54	55-64	65-74	Total < 75	≥ 75
Mean	82.3	79.4	89.2	130	95.4	267
SD	107	119	74.7	99.5	101	192
Median	46.1	56.4	68.1	102	69.3	221
95th percentile	178	192	226	353	252	624
% < 125 pg/mL	84.9	85.5	79.9	57.8	76.7	-
% < 450 pg/mL	-	-	-	-	-	87.9
N	33	262	259	180	734	66

Disease group

HF Population-All

Age (years)	< 45	45-54	55-64	65-74	Total < 75	≥ 75
Mean	1445	1776	2052	2258	1981	2270
SD	2059	2653	3996	3403	3402	2584
Median	632	908	909	987	895	1204
95th percentile	5362	7408	8217	9321	7938	8225
% > 125 pg/mL	82.8	88.5	89.5	92.2	89.3	-
% > 450 pg/mL	-	-	-	-	-	84.7
N	64	148	257	167	636	85

HF Population - Males

Age (years)	< 45	45-54	55-64	65-74	Total < 75	≥ 75
Mean	1590	1840	2261	2215	2080	2003
SD	2225	2789	4423	3318	3605	1901
Median	634	940	916	1074	923	1419
95th percentile	6945	7775	9998	9626	8281	6998
% > 125 pg/mL	81.6	88.2	89.6	91.7	89.0	-
% > 450 pg/mL	-	-	-	-	-	86.5
N	49	127	201	132	509	52

HF Population-Females

Age (years)	< 45	45-54	55-64	65-74	Total < 75	≥ 75
Mean	975	1389	1300	2421	1585	2691
SD	1340	1599	1564	3750	2398	3386
Median	550	788	807	952	770	1127
95th percentile	5362	2755	4822	9321	6788	12188
% > 125 pg/mL	86.7	90.5	89.3	94.3	90.6	-
% > 450 pg/mL	-	-	-	-	-	81.8
N	15	21	56	35	127	33

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Disease group

Blood samples were obtained from 721 patients diagnosed with HF (160 women and 561 men). NYHA Functional Classifications were not available on 20 patients. The descriptive statistics for NT-proBNP concentrations in patients with HF are presented in the table below. These values (pg/mL) are representative of the values obtained from clinical studies. Each laboratory should establish a reference range that represents the patient population that is to be evaluated. In addition, laboratories should be aware of their respective institution's current practice for the evaluation of HF.

HF Population-All

	NYHA Functional class				
	All HF	NYHA I	NYHA II	NYHA III	NYHA IV
Mean	2042	1016	1666	3029	3465
SD	3349	1951	2035	4600	4453
Median	953	342	951	1571	1707
5th percentile	72.0	32.9	103	126	148
95th percentile	7944	3410	6567	10449	12188
% > cutoff	89.4	76.4	93.2	94.4	97.1
Minimum	20.0	20.0	20.0	20.0	97.4
Maximum	40339	13108	10883	40339	20629
N	701	182	250	234	35

HF Population-Males

	NYHA Functional class				
	All HF	NYHA I	NYHA II	NYHA III	NYHA IV
Mean	2083	1072	1795	3116	3015
SD	3504	2090	2175	4869	4812
Median	952	342	985	1683	1369
5th percentile	63.1	31.3	103	126	148
95th percentile	8217	3917	6961	10456	9849
% > cutoff	88.9	75.0	93.8	94.6	95.2
Minimum	20.0	20.0	23.2	20.0	97.4
Maximum	40339	13108	1083	40339	20629
N	552	152	195	184	21

HF Population-Females

	NYHA Functional class				
	All HF	NYHA I	NYHA II	NYHA III	NYHA IV
Mean	1891	731	1208	2709	4140
SD	2704	956	1349	3460	3926
Median	953	338	725	1160	2089
5th percentile	74.1	53.8	74.1	133	691
95th percentile	7944	2455	4211	9321	12188
% > cutoff	91.3	83.3	90.9	94.0	100
Minimum	20.0	53.5	20.0	45.0	691
Maximum	17732	4624	6791	17732	12188
N	149	30	55	50	14

These results (pg/mL) show that there is a relationship between the severity of the clinical signs and symptoms of HF and the median NT-proBNP concentrations, demonstrating that the Elecsys proBNP assay can be used as an aid in the diagnosis of all degrees of HF severity including asymptomatic patients.

Due to the high negative predictive value of the Elecsys proBNP assay, NT-proBNP levels are expected to exceed cut off in any patient with HF. At 1 of 16 sites, in patients with renal insufficiency, the level of NT-proBNP was observed to accumulate to levels that no longer correlate with NYHA classifications (i.e., 25 % of patients with renal insufficiency were classified by NT-proBNP as class IV HF subjects; 14/15 of these had evidence of HF by an alternate assay method). For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Descriptive statistics are provided below for the myocardial infarction and angina cohorts which were not included in the reference cohort.

Myocardial Infarction Cohort

Age (years)	< 45	45-54	55-64	65-74	Total < 75	≥ 75
Mean	107	163	252	304	229	279
SD	96.2	183	386	244	309	191
Median	72.9	87.5	148	231	136	280
95th percentile	273	569	745	759	714	526
% < 125 pg/mL	65.5	61.6	44.9	25.3	47.1	-
% < 450 pg/mL	-	-	-	-	-	75
N	29	112	205	79	425	8

Angina Cohort

Age (years)	< 45	45-54	55-64	65-74	Total < 75	≥ 75
Mean	-	67.7	171	191	156	403
SD	-	47.8	173	158	153	343
Median	-	65.2	73.9	160	94.1	435
95th percentile	-	159	460	568	552	1043
% < 125 pg/mL	-	82.4	55.6	43.8	56.6	-
% < 450 pg/mL	-	-	-	-	-	57.1
N	-	17	27	32	76	7

Interpretation of results

The Receiver Operator Curve (ROC) compares clinical sensitivity and specificity at various cutoffs. The optimum cutoff maximizes the area under the curve (AUC) and represents the highest sensitivity and specificity for the assay. The ROC analysis for the Elecsys proBNP II assay is presented below. The AUC for the Elecsys proBNP II assay is 0.926.

Elecsys proBNP II

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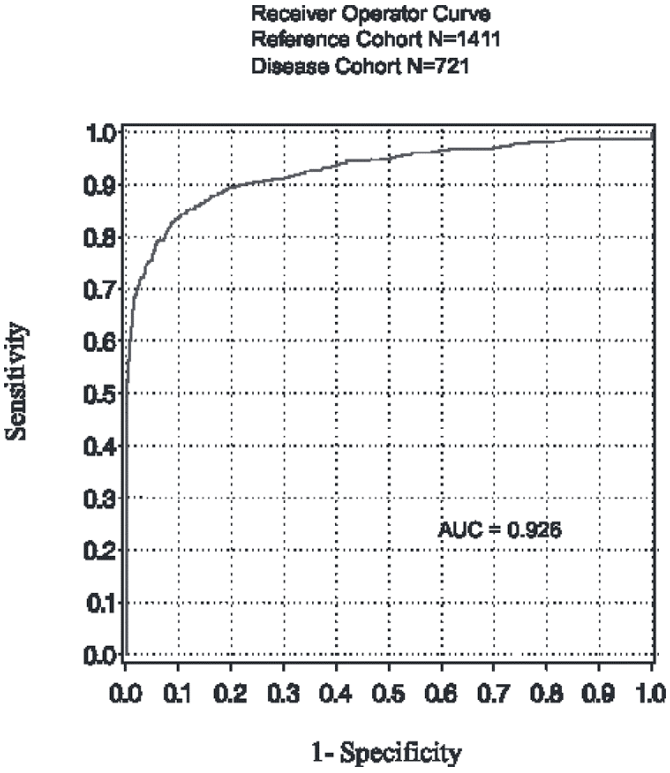
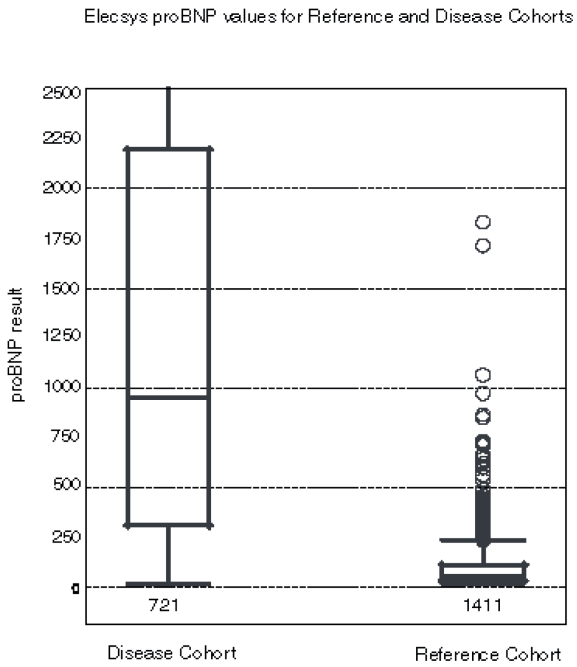


A box and whiskers plot for the clinical study population is also presented below. Recommended clinical thresholds are 125 pg/mL for patients younger than 75 years and 450 pg/mL for patients 75 years and older.



The clinical sensitivity and specificity of the Elecsys proBNP II assay using cutoffs of 125 pg/mL for patients younger than 75 years and 450 pg/mL for patients 75 years or older are presented below.

Sensitivity and specificity vs. age and gender

Males

Age (years)	< 45	45-54	55-64	65-74	Total < 75	≥ 75
% Sensitivity	81.6	88.2	89.6	91.7	89.0	86.5
95 % Confidence interval	68.0-91.2	81.3-93.2	84.5-93.4	85.6-95.8	86.0-91.6	74.2-94.4
% Specificity	95.7	93.3	87.8	86.7	90.0	88.9
95 % Confidence interval	78.1-99.9	89.1-96.3	82.3-92.0	79.6-92.1	87.1-92.3	77.4-95.8
Prevalence	0.7	1.8	6.2	6.8	1.39	9.8
Negative predictive value	100	99.8	99.2	99.3	99.8	98.8

Females

Age (years)	< 45	45-54	55-64	65-74	Total < 75	≥ 75
% Sensitivity	86.7	90.5	89.3	94.3	90.6	81.8
95 % Confidence interval	59.5-98.3	69.6-98.8	78.1-96.0	80.8-99.3	84.1-95.0	64.5-93.0
% Specificity	84.9	85.5	79.9	57.8	76.7	87.9
95 % Confidence interval	68.1-94.9	80.6-89.5	74.5-84.6	50.2-65.1	73.5-79.7	77.5-94.6
Prevalence	0.5	1.3	3.4	6.6	1.16	9.7
Negative predictive value	100	99.9	99.5	99.3	99.9	97.8

Age-matched and incidence-based analysis

An age-matched analysis of the clinical data was performed with the following common age distribution in the groups of individuals with and without HF. Female/male respectively: individuals < 45 years old (7.3/9.5 % of observations), 45-54 years old (9.4/13.0 % of observations), 55-64 years old (16.4/28.6 % of observations), 65-74 years old (27.3/24.0 % of observations), and > 75 years old (39.7/24.9 % of observations). This age distribution reflects the prevalence of HF within the age groups and genders according to data published by the American Heart Association in the 2000 Heart and Stroke Statistical Update, and also reflects the age structure of the United States population, according to the data published by the National Center for Health Statistics in Health, United States, 2000. The resulting area under the ROC curve is 0.926.

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 (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:

cobas e 402 and **cobas e** 801 analyzers (18-minute application)					
		Repeatability		**Intermediate precision**	
Sample	Mean	SD	CV	SD	CV
	pg/mL	pg/mL	%	pg/mL	%
Human serum 1	67.7	1.86	2.8	2.69	4.0
Human serum 2	145	2.84	2.0	4.86	3.4
Human serum 3	342	6.02	1.8	9.20	2.7
Human serum 4	462	9.87	2.1	14.8	3.2
Human serum 5	1014	17.2	1.7	30.9	3.0
Human serum 6	2088	42.8	2.0	73.2	3.5

Elecsys proBNP II

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cobas e 402 and **cobas e** 801 analyzers (18-minute application)					
		Repeatability		**Intermediate precision**	
Sample	Mean	SD	CV	SD	CV
	pg/mL	pg/mL	%	pg/mL	%
Human serum 7	16399	274	1.7	553	3.4
Human serum 8	33786	731	2.2	1153	3.4
PC CARDII[ae])1	140	2.11	1.5	4.07	2.9
PC CARDII2	4846	89.3	1.8	141	2.9

ae) PC CARDII = PreciControl Cardiac II

cobas e 402 and **cobas e** 801 analyzers (9-minute application)					
		Repeatability		**Intermediate precision**	
Sample	Mean	SD	CV	SD	CV
	pg/mL	pg/mL	%	pg/mL	%
Human serum 1	65.1	1.51	2.3	2.09	3.2
Human serum 2	138	2.36	1.7	4.27	3.1
Human serum 3	321	5.30	1.6	8.59	2.7
Human serum 4	443	8.74	2.0	13.0	2.9
Human serum 5	970	17.0	1.7	25.0	2.6
Human serum 6	2012	33.4	1.7	54.6	2.7
Human serum 7	17514	261	1.5	565	3.2
Human serum 8	33353	679	2.0	939	2.8
PC CARDII[ae])1	148	2.20	1.5	3.89	2.6
PC CARDII2	5229	85.4	1.6	137	2.6

Method comparison

a) A comparison of the biotin-remediated Elecsys proBNP II assay (18-minute application), REF 09315284 (y) with the Elecsys proBNP II assay (18-minute application), REF 07027664 (x) on the **cobas e** 801 analyzer gave the following correlations (pg/mL):

Number of samples measured: 161

Passing/Bablok[25]	Linear regression
y =1.02x + 1.89	y = 1.02x - 4.04
τ = 0.991	r = 0.999

The sample concentrations were between 38 and 34019 pg/mL.

Predicted relative bias (%)				
125 pg/mL (95 % CI)	**300 pg/mL (95 % CI)**	**450 pg/mL (95 % CI)**	**900 pg/mL (95 % CI)**	**1800 pg/mL (95 % CI)**
3.1 (2.4, 3.9)	2.2 (1.8, 2.5)	2.0 (1.5, 2.2)	1.8 (1.2, 2.0)	1.7 (1.1, 1.9)

b) A comparison of the biotin-remediated Elecsys proBNP II assay (9-minute application), REF 09315284 (y) with the Elecsys proBNP II assay (9-minute application), REF 07027664 (x) on the **cobas e** 801 analyzer gave the following correlations (pg/mL):

Number of samples measured: 160

Passing/Bablok	Linear regression
y = 1.02x - 8.01	y = 1.01x + 65.6
τ = 0.990	r = 1.00

The sample concentrations were between 39 and 34207 pg/mL.

Predicted relative bias (%)				
125 pg/mL (95 % CI)	**300 pg/mL (95 % CI)**	**450 pg/mL (95 % CI)**	**900 pg/mL (95 % CI)**	**1800 pg/mL (95 % CI)**
-4.3 (-5.6, -2.4)	-0.6 (-0.9, 0.1)	0.3 (0.0, 0.8)	1.2 (0.9, 1.6)	1.6 (1.3, 2.1)

c) A comparison of the Elecsys proBNP II assay (18-minute application), REF 09315284 (y) on the **cobas e** 801 analyzer with the Elecsys proBNP II assay (18-minute application), REF 09315268 (x) on the **cobas e** 601 analyzer gave the following correlations (pg/mL):

Number of samples measured: 160

Passing/Bablok	Linear regression
y =1.03x - 4.24	y = 1.04x - 16.8
τ = 0.985	r = 0.999

The sample concentrations were between 34 and 34019 pg/mL.

Predicted relative bias (%)				
125 pg/mL (95 % CI)	**300 pg/mL (95 % CI)**	**450 pg/mL (95 % CI)**	**900 pg/mL (95 % CI)**	**1800 pg/mL (95 % CI)**
0.0 (-5.2, 2.3)	2.0 (-1.0, 2.9)	2.4 (1.1, 3.1)	2.9 (2.1, 3.4)	3.2 (2.5, 3.7)

Analytical specificity

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):

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

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For further information, please refer to the appropriate user guide or operator's manual for the analyzer concerned, the respective application sheets and the Method Sheets of all necessary components (if available in your country).

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Symbols

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CALIBRATOR	Calibrator
→	Volume for reconstitution
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