09315276501V1.0

Elecsys proBNP II STAT

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REF		Σ	SYSTEM
09315276160	09315276501	100	**cobas e** 601 **cobas e** 602

English

For use in the USA only

System information

For **cobas e** 601 and **cobas e** 602 analyzers: Application Code Number 575

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. 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 **electrochemiluminescence immunoassay** "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).[9]

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 one 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 one 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 cut-offs, the Elecsys proBNP II STAT assay yields negative predictive values ranging from 97.8 % to 100 % depending on age and gender.

The Elecsys proBNP II STAT 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: 9 minutes.

- During a 9 minute 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 PBNPSTX.

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; 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.

For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

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:

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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: 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 STAT 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 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 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, 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.

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 09315306190, proBNP II STAT CalSet, for 4 x 1.0 mL
- REF 04917049160, PreciControl Cardiac II, for 4 x 2.0 mL
- REF 05192943190, Diluent Universal 2, 2 x 36 mL sample diluent
- General laboratory equipment
- **cobas e** 601 or **cobas e** 602 analyzer

Additional materials for the **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 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. 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 assay (REF 03121640). This in turn was standardized against reference standards by weighing pure synthetic NT-proBNP (1-76) into an equine serum matrix.

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.

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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	≤ 3500 ng/mL
Rheumatoid factors	≤ 1500 IU/mL
Albumin	≤ 7g/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 300000 pg/mL.

Pharmaceutical substances

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; reported from Japan), patients may show false-low results when tested with the assay kit (values < LoD) due to a NT-proBNP 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

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 limit 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 2. The recommended dilution is 1:2 (either automatically by the analyzers 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.

For the Elecsys proBNP II STAT:

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 STAT support that the clinical performance described below is applicable to the Elecsys proBNP II STAT.

Expected values

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.

Reference group

The circulating NT-proBNP concentration was determined from 1411 individuals without CHF (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.

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

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Age (years)	< 45	45-54	55-64	65-74	Total < 75	≥ 75
% < 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

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. 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	2063	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 immunoassay 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 immunoassay, NT-proBNP levels are expected to exceed cut-off in any patient with HF. At one 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).

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

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Myocardial Infarction Cohort

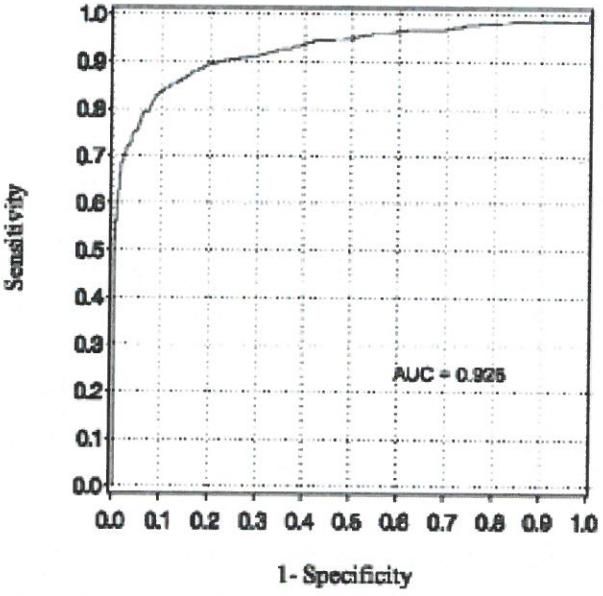
Age (years)	< 45	45-54	55-64	65-74	Total < 75	≥ 75
Mean	107	163	252	304	229	279
SD	96.2	183	386	244	309	191
Median	72.9	87.5	148	231	136	280
95th percentile	273	569	745	759	714	526
% < 125 pg/mL	65.5	61.6	44.9	25.3	47.1	-
% < 450 pg/mL	-	-	-	-	-	75
N	29	112	205	79	425	8

Angina Cohort

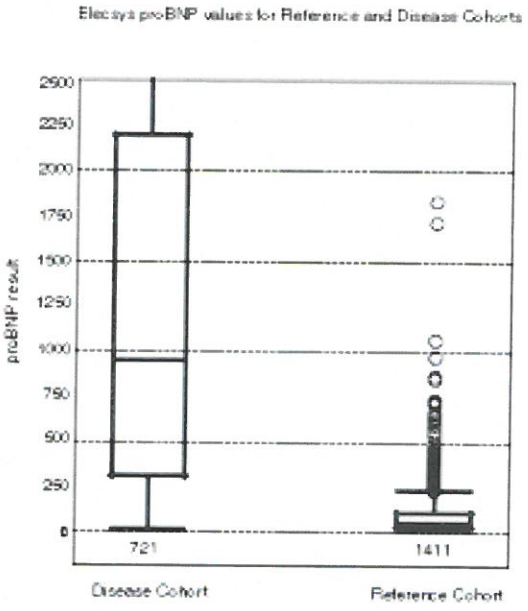
Age (years)	< 45	45-54	55-64	65-74	Total < 75	≥ 75
Mean	-	67.7	171	191	156	403
SD	-	47.8	173	158	153	343
Median	-	65.2	73.9	160	94.1	435
95th percentile	-	159	460	568	552	1043
% < 125 pg/mL	-	82.4	55.6	43.8	56.6	-
% < 450 pg/mL	-	-	-	-	-	57.1
N	-	17	27	32	76	7

Interpretation of results

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



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 immunoassay using cut-offs 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

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distribution reflects the prevalence of CHF 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 proBNP II STAT immunoassay 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:

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:

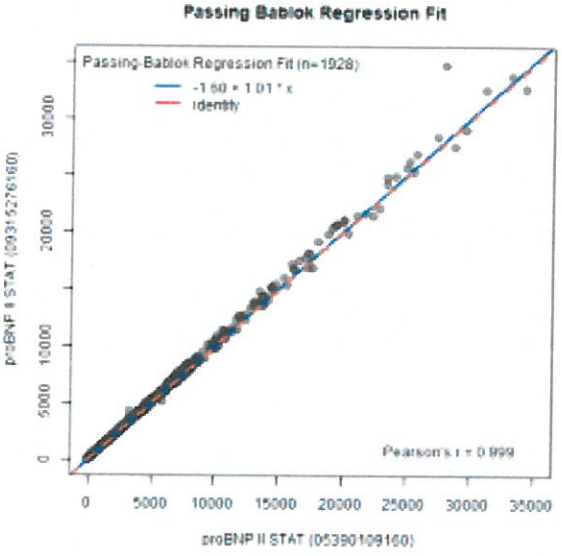
cobas e 601 and **cobas e** 602 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean	SD	CV	SD	CV
	pg/mL	pg/mL	%	pg/mL	%
HS[b] 1	63.9	2.42	3.8	4.39	6.9
HS 2	144	3.36	2.3	5.87	4.1
HS 3	331	5.01	1.5	12.7	3.8
HS 4	482	12.7	2.6	18.5	3.8
HS 5	1060	24.6	2.3	34.4	3.2
HS 6	2219	51.4	2.3	77.2	3.5
HS 7	18371	405	2.2	654	3.6
HS 8	33967	912	2.7	1338	3.9
PC CARD[c] II1	155	4.06	2.6	6.08	3.9
PC CARD II2	5660	116	2.1	194	3.4

b) HS = human serum

c) PC CARD = PreciControl Cardiac

Method comparison

We performed a method comparison study with 1928 subjects with the biotin-remediated Elecsys proBNP II STAT assay, 09315276160 (y) and the Elecsys proBNP II STAT assay, 05390109160 (x).



Correlation	Estimate
Kendall's tau	0.99
Pearson's r	1.00

Regression results and relative bias at selected NT-proBNP concentration levels (all, STAT)

Passing-Bablok Regression[20]				
Test Method	**Comparison Method**	**N**	**Intercept (95 % CI)**	**Slope (95 % CI)**
Biotin-remediated Elecsys proBNP II STAT (09315276160)	Elecsys proBNP II STAT (05390109160)	1928	-1.60 (-2.09, -1.16)	1.01 (1.00, 1.01)

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)**
-0.8 (-1.0, -0.5)	-0.0 (-0.2, 0.2)	0.2 (-0.1, 0.4)	0.3 (0.1, 0.6)	0.4 (0.2, 0.7)

A comparison of the biotin-remediated Elecsys proBNP II STAT assay, REF 09315276160 (y) with the biotin-remediated 18 minute assay, REF 09315268160 (x) was done using clinical samples:

Number of samples measured: 155

Passing/Bablok[20]

y = 1.03x + 10.7

Slope 95 % confidence between 1.01 and 1.05, intercept 95 % confidence between -1.43 and 44.1.

r = 0.99

The sample concentrations were between 36 and 35000 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)**
11.5 (2.9, 37.4)	6.5 (3.0, 16.9)	5.3 (3.0, 11.9)	4.1 (2.6, 7.0)	3.6 (2.2, 5.0)

Elecsys proBNP II STAT

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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

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For further information, please refer to the appropriate 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

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

FOR US CUSTOMERS ONLY: LIMITED WARRANTY

Roche Diagnostics warrants that this product will meet the specifications stated in the labeling when used in accordance with such labeling and will be free from defects in material and workmanship until the expiration date printed on the label. THIS LIMITED WARRANTY IS IN LIEU OF ANY OTHER WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR PARTICULAR PURPOSE. IN NO EVENT SHALL ROCHE DIAGNOSTICS BE LIABLE FOR INCIDENTAL, INDIRECT, SPECIAL OR CONSEQUENTIAL DAMAGES.

Elecsys proBNP II STAT

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New Vision Medical Laboratories St. Rita's Medical Center Lima, Ohio Technical Procedure		Procedure Number: nvml.chem.t.359.0 Policy Initiated: 02.25.14

SUBJECT: **proBNP**

PROCEDURE: (Addendum to the above pages)

1. **Rejection Criteria:**
   - Specimens that are improperly labeled or of the incorrect specimen type must be rejected.
2. **Quality Control:**
   - Controls must be run on each day that this test is requested and are good for 24 hours unless trouble is encountered that requires another evaluation. See posted Quality Control information sheet for levels of control to be run. Follow guidelines for Quality Control using the 2 SD ranges, which are posted and available in the LIS.
3. **Quality Control Failure:**

   1. Unacceptable QC results should be entered into the computer along with appropriate actions taken.

   2. Various corrective actions include:

      a. Check reagent expiration dates and check for proper storage conditions.

      b. Rerun QC (use same reagent).

      c. Calibrate and run freshest control material

      d. Open new reagent and run qc

      e. Call hotline for assistance and document actions taken

      f. Inform a Technical Specialist

4. **Calculations:**
   - N/A
5. **Interpretation:**
   - No interpretation is required.
6. **Normal Range:**

Age	Normal
<75 years	<125 pg/mL
>75 Years	<450 pg/mL

7. **Analytical Measureable Range:**
   - 36 - 35,000 pg/ml
8. **Clinical Reportable Range**
   - 36 - 70,000 pg/ml
9. **Critical Values:**
   - None Stated
10. **Reporting Results:**
    - Results may be entered directly into the LIS.
11. **Backup for Inoperable System:**
    - Use backup analyzer.

12. **Referral of Specimens:**
    - Use backup analyzer.
13. **Submission and Handling of Referral Specimens:**
    - See requirements of reference lab.
14. **Method Validation:**
    - This test was validated by means of startup studies (on file). Subsequent validation is by virtue of QC and CAP surveys.

11-25-22

12/20/22