March 31, 2022

Roche Diagnostics
Jane Phillips
Sr. Regulatory Program Manager
9115 Hague Road
Indianapolis, Indiana 46250

Re: K210546
Trade/Device Name: Elecsys proBNP II, Elecsys proBNP II STAT
Regulation Number: 21 CFR 862.1117
Regulation Name: B-Type Natriuretic Peptide Test System
Regulatory Class: Class II
Product Code: NBC
Dated: December 8, 2021
Received: December 8, 2021

Dear Jane Phillips:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database located at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803) for devices or postmarketing safety reporting (21 CFR 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Marianela Perez-Torres, Ph.D.
Deputy Director
Division of Chemistry
and Toxicology Devices
OHT7: Office of In Vitro Diagnostics
and Radiological Health
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

# Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 06/30/2023
*See PRA Statement below.*

510(k) Number *(if known)*
k210546

Device Name
Elecsys proBNP II

Indications for Use *(Describe)*
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.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

**CONTINUE ON A SEPARATE PAGE IF NEEDED.**

This section applies only to requirements of the Paperwork Reduction Act of 1995.

***DO NOT SEND YOUR COMPLETED FORM TO THE PRA STAFF EMAIL ADDRESS BELOW.***

The burden time for this collection of information is estimated to average 79 hours per response, including the time to review instructions, search existing data sources, gather and maintain the data needed and complete and review the collection of information. Send comments regarding this burden estimate or any other aspect of this information collection, including suggestions for reducing this burden, to:

Department of Health and Human Services
Food and Drug Administration
Office of Chief Information Officer
Paperwork Reduction Act (PRA) Staff
*PRAStaff@fda.hhs.gov*

*"An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB number."*

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

# Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 06/30/2023
*See PRA Statement below.*

510(k) Number *(if known)*
k210546

Device Name
Elecsys proBNP II STAT

Indications for Use *(Describe)*
Immunoassay for the in vitro quantitative determination of Nterminal proBrain 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.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

**CONTINUE ON A SEPARATE PAGE IF NEEDED.**

This section applies only to requirements of the Paperwork Reduction Act of 1995.

***DO NOT SEND YOUR COMPLETED FORM TO THE PRA STAFF EMAIL ADDRESS BELOW.***

The burden time for this collection of information is estimated to average 79 hours per response, including the time to review instructions, search existing data sources, gather and maintain the data needed and complete and review the collection of information. Send comments regarding this burden estimate or any other aspect of this information collection, including suggestions for reducing this burden, to:

Department of Health and Human Services
Food and Drug Administration
Office of Chief Information Officer
Paperwork Reduction Act (PRA) Staff
*PRAStaff@fda.hhs.gov*

*"An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB number."*

# 510(k) Summary

March 28th, 2022

U.S. Food and Drug Administration
Center for Devices and Radiological Heath
Document Mail Center – WO66 Room 0609
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002

---

**Purpose** In accordance with 21 CFR 807.87, Roche Diagnostics Corporation hereby submits official notification as required by Section 510(k) of the Federal Food, Drug and Cosmetics Act of our intention to market the device described in this Premarket Notification [510(k)].

## 510(k) Summary k210546

| | | |
|---|---|---|
| **Device Name** | Proprietary name: | Elecsys proBNP II and Elecsys proBNP II STAT |
| | Common name: | proBNP II and proBNP II STAT |

**Owner** Roche Diagnostics
9115 Hague Road
Indianapolis, IN 46250
Phone: 317-521-2000
Fax: 317-521-1413

**Contact** Jane Phillips, PhD

**Date**
March 28th, 2022

| Panel | Product Code | Classification Name | Regulation Citation |
|---|---|---|---|
| Clinical Chemistry | NBC | B-Type Natriuretic Peptide Test System | 862.1117 |

| | |
|---|---|
| **Substantial Equivalence** | The Elecsys proBNP II (updated assay) is substantially equivalent to the Elecsys proBNP II (old assay, k072437)<br><br>The Elecsys proBNP II (updated assay) STAT is substantially equivalent to the Elecsys proBNP II STAT (old assay, k092649) |
| **Intended Use** | **Elecsys proBNP II STAT**<br>Immunoassay for the in vitro quantitative determination of N-terminal proBrain 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.<br>The electrochemiluminescence immunoassay "ECLIA" is intended for use on cobas e immunoassay analyzers.<br><br>**Elecsys proBNP II**<br>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.<br>The electrochemiluminescence immunoassay "ECLIA" is intended for use on cobas e immunoassay analyzers. |

**Table 1: Similarities and Differences between the Elecsys proBNP II assays**

| Item | Elecsys proBNP II (old assay design) | Elecsys proBNP II (updated assay design) | Change description |
|---|---|---|---|
| Proprietary name | Elecsys proBNP II | Elecsys proBNP II | None |
| Technology | ECLIA | ECLIA | None |
| Test format | Sandwich | Sandwich | None |
| Test type | Quantitative | Quantitative | None |
| Assay protocol | R1 + R2 + sample, incubation, + beads, incubation | R1 + R2 + sample, incubation, + beads, incubation | None |
| Measuring Range | 5-35000 pg/ml | 36-35000 pg/ml | Changing the lower end of MR from LoD to LoQ |
| Biotin Tolerance | Up to 30 ng/mL | Up to 3500 ng/mL | Increase of biotin tolerance |

**Table 2: Similarities and Differences between the Elecsys proBNP STAT assays**

| Item | Elecsys proBNP II (old assay design) | Elecsys proBNP II (updated assay design) | Change description |
|---|---|---|---|
| Proprietary name | Elecsys proBNP II STAT | Elecsys proBNP II STAT | None |
| Technology | ECLIA | ECLIA | None |
| Test format | Sandwich | Sandwich | None |
| Test type | Quantitative | Quantitative | None |
| Assay protocol | R1 + R2 + sample+ beads, incubation | R1 + R2 + sample+ beads, incubation | None |
| Measuring Range | 5-35000 pg/ml | 36-35000 pg/ml | Changing the lower end of MR from LoD to LoQ |
| Biotin Tolerance | Up to 30 ng/mL | Up to 3500 ng/mL | Increase of biotin tolerance |

## Device Description

Elecsys proBNP II (updated assay) is a second-generation assay by Roche Diagnostics for the in vitro quantitative determination of N-terminal pro-Brain natriuretic peptide (NT-proBNP) in human serum and plasma with increased biotin tolerance. The electrochemiluminescence immunoassay “ECLIA” is intended for use on cobas e immunoassay analyzers.

The cobas e family of analyzers employs the electrochemiluminescence immunoassay "ECLIA" technology. The assays are an 18-minute (Elecsys proBNP II) and 9 minute (Elecsys proBNP II STAT) application following a sandwich principle using two monoclonal antibodies which are specifically directed against NT-proBNP.

**Changes to Reagent Composition**
For the neutralization of free biotin in serum and plasma, Roche developed an antibody, which binds to free biotin. The antibodies are specific for free biotin and do not bind to or interact with the biotin-linker conjugates.

## Analytical Performance

### Precision/Reproducibility

### Repeatability and Intermediate Precision

Precision measurements were conducted to evaluate repeatability (within-run precision) and the intermediate precision (within-laboratory precision) according to the CLSI guideline EP5-A3.

**Methods:**

The precision of the Elecsys proBNP II assay was evaluated on the **cobas e** 411 and proBNP II STAT assay was evaluated on one **cobas e** 601 analyzer at one internal site with three reagent lots over 21 days.

The protocol consisted of testing the eight serum samples and two controls in single determinations in four separate aliquots (divided into two runs per day) for 21 operating days (n=84). Repeatability and Intermediate Precision were calculated according to EP05-A3.

**Results:**

Lot 391671: Elecsys proBNP II

| **cobas e** 411 analyzer | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Repeatability | | | | Intermediate precision | | | |
| Sample (Serum) | Mean pg/mL | SD pg/mL | SD 95% UCL pg/mL | CV % | CV 95% UCL % | SD pg/mL | SD 95% UCL pg/mL | CV % | CV 95% UCL % |
| Human serum 1 | 55.9 | 2.62 | 3.20 | 4.7 | 5.7 | 4.35 | 5.35 | 7.8 | 9.6 |
| Human serum 2 | 129 | 3.07 | 3.76 | 2.4 | 2.9 | 7.40 | 9.61 | 5.7 | 7.5 |
| Human serum 3 | 423 | 8.91 | 10.9 | 2.1 | 2.6 | 18.0 | 22.3 | 4.3 | 5.3 |
| Human serum 4 | 925 | 23.0 | 28.1 | 2.5 | 3.0 | 44.3 | 55.6 | 4.8 | 6.0 |

| **cobas e** 411 analyzer | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Repeatability | | | | Intermediate precision | | | |
| Sample (Serum) | Mean pg/mL | SD pg/mL | SD 95% UCL pg/mL | CV % | CV 95% UCL % | SD pg/mL | SD 95% UCL pg/mL | CV % | CV 95% UCL % |
| Human serum 5 | 1924 | 43.8 | 53.5 | 2.3 | 2.8 | 88.8 | 110 | 4.6 | 5.7 |
| Human serum 6 | 15620 | 248 | 303 | 1.6 | 1.9 | 662 | 844 | 4.2 | 5.4 |
| Human serum 7 | 33526 | 778 | 950 | 2.3 | 2.8 | 1591 | 2010 | 4.7 | 6.0 |
| Human serum 8 | 337 | 5.11 | 6.24 | 1.5 | 1.8 | 11.4 | 14.4 | 3.4 | 4.3 |
| PreciControl Cardiac II 1 | 132 | 3.29 | 4.02 | 2.5 | 3.1 | 5.97 | 7.38 | 4.5 | 5.6 |
| PreciControl Cardiac II 2 | 4477 | 135 | 165 | 3.0 | 3.7 | 216 | 267 | 4.8 | 6.0 |

Lot 391674: Elecsys proBNP II STAT

| **cobas e** 601 analyzer | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Repeatability | | | | Intermediate precision | | | |
| Sample (Serum) | Mean pg/mL | SD pg/mL | SD 95% UCL | CV % | CV 95% UCL | SD pg/mL | SD 95% UCL | CV % | CV 95% UCL |
| Human serum 1 | 63.9 | 2.42 | 2.96 | 3.8 | 4.6 | 4.39 | 5.25 | 6.9 | 8.2 |
| Human serum 2 | 144 | 3.36 | 4.10 | 2.3 | 2.8 | 5.87 | 7.08 | 4.1 | 4.9 |
| Human serum 3 | 482 | 12.7 | 15.5 | 2.6 | 3.2 | 18.5 | 22.4 | 3.8 | 4.6 |
| Human serum 4 | 1060 | 24.6 | 30.1 | 2.3 | 2.8 | 34.4 | 40.9 | 3.2 | 3.9 |
| Human serum 5 | 2219 | 51.4 | 62.8 | 2.3 | 2.8 | 77.2 | 94.1 | 3.5 | 4.2 |
| Human serum 6 | 18371 | 405 | 494 | 2.2 | 2.7 | 654 | 801 | 3.6 | 4.4 |
| Human serum 7 | 33967 | 912 | 1114 | 2.7 | 3.3 | 1338 | 1608 | 3.9 | 4.7 |
| Human serum 8 | 331 | 5.01 | 6.12 | 1.5 | 1.8 | 12.7 | 16.1 | 3.8 | 4.9 |
| PreciControl Cardiac II 1 | 155 | 4.06 | 4.97 | 2.6 | 3.2 | 6.08 | 7.27 | 3.9 | 4.7 |
| PreciControl Cardiac II 2 | 5660 | 116 | 142 | 2.1 | 2.5 | 194 | 237 | 3.4 | 4.2 |

# Inter-Instrument Variability (CLSI EP5-A3)

**Methods (Inter-Instrument):**

This was done using the Elecsys proBNP II (updated assay) on three **cobas e** 411 analyzers and using the Elecsys proBNP II STAT (updated assay) on three **cobas e** 601 analyzers according to the precision model described in CLSI EP5-A3 guideline. One reagent lot of the updated assay was measured on 5 days in three laboratory sites.

A precision experiment according to the CLSI EP05-A3 setup (5-day model, 25 determinations total per sample pool, reagent lot, and site) was conducted at all 3 laboratories, for each of the assays. The sample panel was identical for both and consisted of 8 native human serum sample pools, and 2 concentration levels made of quality control material (PreciControl Cardiac II Level 1 and 2). The samples were not spiked.

**Results:**

Inter-Instrument Precision, proBNP II on the e411. Units = pg/mL

| Sample | N | Mean | SD | SD UCL | CV[%] | CV[%] UCL |
|---|---|---|---|---|---|---|
| **HS 1** | 75 | 55.2 | 1.02 | 2.06 | 1.8 | 3.7 |
| **HS 2** | 75 | 131 | 3.46 | 5.84 | 2.6 | 4.5 |
| **HS 3** | 75 | 332 | 5.50 | 10.3 | 1.7 | 3.1 |
| **HS 4** | 75 | 471 | 10.3 | 18.0 | 2.2 | 3.8 |
| **HS 5** | 75 | 963 | 13.2 | 27.1 | 1.4 | 2.8 |
| **HS 6** | 75 | 1773 | 20.0 | 41.1 | 1.1 | 2.3 |
| **HS 7** | 75 | 18,719 | 0.00 | N/A | 0.00 | N/A |
| **HS 8** | 75 | 31,425 | 0.00 | N/A | 0.00 | N/A |
| **PC 1** | 75 | 144 | 1.89 | 3.79 | 1.3 | 2.6 |
| **PC 2** | 75 | 4799 | 0.00 | N/A | 0.00 | N/A |

Inter-Instrument Precision, proBNP II STAT on the e601. Units = pg/mL

| Sample | N | Mean | SD | SD UCL | CV[%] | CV[%] UCL |
|---|---|---|---|---|---|---|
| **HS 1** | 75 | 52.1 | 3.91 | 6.45 | 7.5 | 12.4 |
| **HS 2** | 75 | 128 | 9.22 | 15.2 | 7.2 | 11.8 |
| **HS 3** | 75 | 317 | 23.8 | 38.9 | 7.5 | 12.3 |
| **HS 4** | 75 | 465 | 34.6 | 56.5 | 7.4 | 12.1 |
| **HS 5** | 75 | 961 | 74.3 | 122.7 | 7.7 | 12.7 |
| **HS 6** | 75 | 1845 | 134 | 220 | 7.3 | 11.9 |
| **HS 7** | 75 | 18,477 | 1466 | 2396 | 7.9 | 13.0 |
| **HS 8** | 75 | 30,089 | 2394 | 3914 | 8.0 | 13.0 |
| **PC 1** | 75 | 145 | 10.9 | 17.9 | 7.5 | 12.3 |
| **PC 2** | 75 | 5328 | 399 | 652 | 7.5 | 12.2 |

## Analytical Sensitivity

The analytical studies to establish the limit of blank (LoB), limit of detection (LoD) and limit of quantitation (LoQ) were conducted according to the experimental design described in CLSI EP-17-A2

## Limit of Blank (LoB) (CLSI EP17-A2)

LoB of the Elecsys proBNP II (updated assay) on the **cobas e** 411 analyzer and of Elecsys proBNP II STAT (updated assay) on the **cobas e** 601 was determined according to CLSI EP17-A2. Limit of Blank determines the highest observed measurement values for samples free of analyte. The Limit of Blank was determined as the 95th percentile of measurements of blank samples.

**Methods:**

In total 60 determinations of an analyte-free sample were obtained on one instrument over ≥ three days in 6 runs with 10-fold determination per run. Three lots of reagent were used in the experimental design.

As the analyzer does not report negative sample concentrations, the data set was truncated and the data were evaluated as the linear interpolation of the 57th and 58th ranked observation.

**Conclusion:**

All lots met the predetermined acceptance criterion of ≤ 8 pg/mL.

## Limit of Detection (LoD) (CLSI EP17-A2)

LoD of the Elecsys proBNP II (updated assay) on the **cobas e** 411 analyzer and of Elecsys proBNP II STAT (updated assay) on the **cobas e** 601 analyzer was determined according to CLSI EP17-A2. The LoD determines the lower limit for samples with analyte. The LoD was determined as the lowest amount of analyte in a sample that can be detected with a 95% probability.

**Methods:**

Five low-level human serum samples were measured on one instrument over ≥ three days in 6 runs with a two-fold determination per run. In total sixty determinations per sample per reagent lot. The experiment was conducted using three reagent lots.

A pooled estimate of the precision (SD total) for the 5 low level samples was calculated.

LoD was calculated according to EP17-A2, chapter 5.3.3.2 as:

LoD = LoB + 1.653 x SD total (of low analyte samples)

**Conclusion:**

All lots met the predetermined acceptance criterion of ≤ 10 pg/mL. The LoD claim in the labeling will be set to ≤ 10 pg/mL.

# Limit of Quantitation (CLSI EP17-A2)

The LoQ of the Elecsys proBNP II (updated assay) on the **cobas e** 411 analyzer and of the Elecsys proBNP II STAT (updated assay) on the **cobas e** 601 analyzer was determined according to CLSI Guideline EP17-A2.

The LoQ was determined as the lowest concentration of analyte which can be reproducibly measured with an intermediate precision of 20% CV.

**Methods:**

A low level sample set 9 native, unaltered serum samples using the Elecsys proBNP II on the **cobas e** 411 analyzer and 10 native, unaltered serum samples using the Elecsys proBNP II STAT on the **cobas e** 601 analyzer of known measurand concentration was tested in 5 replicates (aliquots) on one instrument in singleton per day, over 5 days with 1 run per day. Three lots of reagent were used in the LoQ experiment. A total of n=25 measured values were obtained for each sample. The mean value and the intermediate precision as coefficient of variation (CV) and standard deviation (SD) were calculated for each LoQ sample.

LoQ is defined as the mean value of that sample which is the first that fulfills the specification for the intermediate precision and for which no sample with higher concentration exists that exceeds this specification.

Data are summarized in the following tables, below.

**LoQ Results for Elecsys proBNP II**

| Reagent Lot | | LoQ [pg/mL] |
|---|---|---|
| 1 | 391671 | 33.7 |
| 2 | 391672 | 32.7 |
| 3 | 391673 | 35.7 |

**LoQ Results for proBNP II STAT**

| Reagent Lot | | LoQ [pg/mL] |
|---|---|---|
| 1 | 391674 | 8.98 |
| 2 | 391675 | 13.6 |
| 3 | 391676 | 7.28 |

# Linearity/Assay Reportable Range (CLSI EP06-Ed2)

The linearity study was conducted to demonstrate that measurements across the claimed measuring range for each parameter are linear. The study was performed according to CLSI guideline EP06-Ed2.

**Methods:**

One high analyte human, native serum sample above measuring rage was diluted with analyte free serum. 11 concentrations were prepared throughout the entire measuring range. Samples were assayed in 3-fold determination within a single run. The linearity data were analyzed as described below.

For each sample and its dilution levels a weighted least square regression by pooled variance is performed.

As one high analyte human, native serum sample above measuring range was diluted with analyte free serum (CLSI Design A1), a regression without intercept is chosen.

As variance increases with concentration, weighted linear regression is used.

As only 3 replicates are available at each dilution step, the weights are computed based on the pooled variance including also the replicates of the next higher as well as the next lower dilution step.

The result table is composed for each experiment, i.e. for the proBNP II (18min) on e411 and the proBNP II STAT on e601. The resulting linearity statistics and deviation results are given in the tables below (rounded data).

Linearity for Elecsys proBNP II

| Level | Rel Conc | Mean Conc | Expected Conc. | Predicted Conc. | Deviation | Deviation (%) |
|---|---|---|---|---|---|---|
| 1 | 0 | 8.417 | 9.911 | 8.424 | -0.007 | -0.084 |
| 2 | 0.001 | 22.792 | 25.371 | 21.567 | 1.225 | 5.681 |
| 3 | 0.002 | 55.455 | 63.428 | 53.916 | 1.538 | 2.853 |
| 4 | 0.004 | 132.273 | 158.569 | 134.791 | -2.518 | -1.868 |
| 5 | 0.01 | 310.973 | 396.423 | 336.977 | -26.004 | -7.717 |
| 6 | 0.026 | 780.863 | 1014.843 | 862.660 | -81.798 | -9.482 |
| 7 | 0.064 | 1939.433 | 2537.108 | 2156.651 | -217.217 | -10.072 |
| 8 | 0.16 | 4823.735 | 6342.77 | 5391.626 | -567.891 | -10.533 |
| 9 | 0.4 | 13053.629 | 15856.925 | 13479.066 | -425.437 | -3.156 |
| 10 | 0.7 | 24009.672 | 27749.618 | 23588.365 | 421.307 | 1.786 |
| 11 | 1 | 39642.311 | 39642.311 | 33697.665 | 5944.647 | 17.641 |

Linearity for Elecsys proBNP II STAT

| Level | Rel Conc | Mean Conc | Expected Conc. | Predicted Conc. | Deviation | Deviation (%) |
|---|---|---|---|---|---|---|
| 1 | 0 | 8.515 | 10.024 | 9.720 | -1.206 | -12.403 |
| 2 | 0.001 | 22.355 | 25.662 | 24.884 | -2.529 | -10.164 |
| 3 | 0.002 | 55.493 | 64.156 | 62.210 | -6.717 | -10.797 |
| 4 | 0.004 | 137.301 | 160.39 | 155.524 | -18.224 | -11.717 |
| 5 | 0.01 | 328.36 | 400.975 | 388.810 | -60.450 | -15.547 |
| 6 | 0.026 | 828.107 | 1026.495 | 995.354 | -167.248 | -16.803 |
| 7 | 0.064 | 2066.792 | 2566.238 | 2488.386 | -421.593 | -16.942 |
| 8 | 0.16 | 5351.93 | 6415.594 | 6220.964 | -869.034 | -13.969 |
| 9 | 0.4 | 14054.029 | 16038.985 | 15552.410 | -1498.381 | -9.634 |
| 10 | 0.7 | 25238.171 | 28068.223 | 27216.718 | -1978.547 | -7.270 |
| 11 | 1 | 40097.462 | 40097.462 | 38881.026 | 1216.436 | 3.129 |

# Endogenous Interference Studies

The purpose of these studies was to evaluate endogenous substances for potential interference with the parameters measured on the **cobas e** 411 for Elecsys proBNP II (updated assay) and on the **cobas e** 601 for Elecsys proBNP II STAT (updated assay).

**Method for Bilirubin, Hemoglobin and Lipemia:**

The effect on quantitation of analyte in the presence of endogenous interfering substances using the Elecsys proBNP II was determined on the **cobas e** 411 and the Elecsys proBNP II STAT was determined on the **cobas e** 601.

Endogenous interferences were determined by testing three different analyte concentration levels (low about 130 pg/mL, medium about 900 pg/mL, high about 20000 pg/mL) in human native serum samples. The high concentrations were spiked with recombinant human proBNP for the bilirubin and lipemia testing.

One aliquot of each serum sample was spiked with the interfering substance (= interference pool) and another aliquot was spiked (if applicable) with the same volume of the solvent of the interfering substance (= dilution pool). The interfering pool was then diluted into the dilution pool in 10 % increments. The recovery for each sample was calculated by comparison to the reference (unspiked sample).

**Results/Conclusion for both proBNP II and proBNP II STAT:**

| Interfering substance | No interference up to |
|---|---|
| Conjugated Bilirubin | 25.0 mg/dL |
| Unconjugated Bilirubin | 25.0 mg/dL |
| Hemoglobin | 1000 mg/dL |
| Lipemia | 1500 mg/dL |

**Method for Biotin:**

The effect on quantitation of analyte in the presence of Biotin using the Elecsys proBNP II was determined on the **cobas e** 411 and the Elecsys proBNP II STAT was determined on the **cobas e** 601.

Biotin interferences were determined by testing three different analyte concentration levels (low about 125 pg/mL, medium about 800 pg/mL, high about 18000 pg/mL) in human native serum samples.

One aliquot of each serum sample was spiked with 15000 ng/mL Biotin (= interference pool) and another aliquot was spiked (if applicable) with the same volume of the solvent of the interfering substance (= dilution pool). The interfering pool was then diluted into the dilution

pool in 11 steps. The recovery for each sample was calculated by comparison to the reference (unspiked sample).

**Results:**

Biotin: Elecsys proBNP II

| Volume (Relation) | | Measured concentration of analyte pg/mL | Expected concentration of analyte pg/mL | Concentration of interferent ng/mL | Recovery % |
|---|---|---|---|---|---|
| Sample 1a | Sample 1b | | | | |
| 100 | 0.0000 | 123 | 123 | 0.0000 | 100 |
| 96.7 | 3.33 | 124 | 123 | 500 | 101 |
| 93.3 | 6.67 | 127 | 123 | 1000 | 103 |
| 90.0 | 10.0 | 126 | 123 | 1500 | 102 |
| 86.7 | 13.3 | 126 | 123 | 2000 | 102 |
| 83.3 | 16.7 | 127 | 123 | 2500 | 103 |
| 80.0 | 20.0 | 127 | 123 | 3000 | 103 |
| 76.0 | 24.0 | 125 | 123 | 3600 | 102 |
| 66.7 | 33.3 | 116 | 123 | 5000 | 93.9 |
| 33.3 | 66.7 | 85.1 | 123 | 10000 | 69.1 |
| 0.0000 | 100 | 46.9 | 123 | 15000 | 38.1 |

**Conclusion:**

No interference was seen up to 5000 ng/mL biotin. All predetermined acceptance criteria were met. The claimed Biotin concentration at which no interference is observed is 3500 ng/mL.

**Method for Rheumatoid Factor:**

The effect on quantitation of analyte in the presence of Rheumatoid Factors using the Elecsys proBNP II was determined on the **cobas e** 411 and the Elecsys proBNP II STAT was determined on the **cobas e** 601.

Rheumatoid Factors interferences were determined by testing three different analyte concentration levels (low about 130 pg/mL, medium about 900 pg/mL, high about 20000 pg/mL) in human native serum samples. The high sample was spiked with recombinant human NT-proBNP.

One aliquot of each serum sample was spiked with 1500 IU/mL Rheumatoid Factors

(= interference pool) and another aliquot was spiked (if applicable) with the same volume of the solvent of the interfering substance (= dilution pool). The interfering pool was then diluted into the dilution pool in 10 % increments. The recovery for each sample was calculated by comparison to the reference (unspiked sample).

The claimed Rheumatoid factors concentration is 1500 IU/mL.

**Method for Albumin:**

The effect on quantitation of analyte in the presence of Albumin using the Elecsys proBNP II was determined on the **cobas e** 411 and the Elecsys proBNP II STAT was determined on the **cobas e** 601.

Albumin interferences were determined by testing three different analyte concentration levels (low about 140 pg/mL, medium about 1000 pg/mL, high about 23000 pg/mL) in human native serum samples. The high sample was spiked with human recombinant proBNP.

One aliquot of each serum sample was spiked with 7 g/dL Albumin (= interference pool) and another aliquot was spiked (if applicable) with the same volume of the solvent of the interfering substance (= dilution pool). The interfering pool was then diluted into the dilution pool in 10 % increments. The recovery for each sample was calculated by comparison to the reference (unspiked sample).

The claimed Albumin concentration is 7 g/dL.

# Cross-Reactivity

This study was conducted to evaluate the Elecsys proBNP II and proBNP II STAT assay on the **cobas e** 411 and 601, respectively, for potential cross-reactivity.

## Methods

To determine the analytical specificity of the Elecsys proBNP II (updated assay) and Elecsys proBNP II STAT (updated assay), two human native serum samples with low (about 150 pg/mL) and high (about 2500 pg/mL) analyte levels were aliquoted and spiked with potential cross-reactants. One aliquot was left unspiked to serve as a reference. Samples were measured on the **cobas e** 411 analyzer for Elecsys proBNP II and on the **cobas e** 601 analyzer for Elecsys proBNP II STAT and cross reactivity was calculated according to the formula:

$$\text{x percent cross reaction} = \frac{100 \times \text{simulated analyte conc.}}{\text{conc. of cross-reactant spiked}}$$

## Results:

| Cross-reactant | Concentration reactant | Low analyte level | | | High analyte level | | |
|---|---|---|---|---|---|---|---|
| | | Measured analyte concentration without Cross reactant [pg/mL] | Measured Analyte concentration with Cross reactant [pg/mL] | Recovery with x-reactant [%] | Measured analyte concentration without Cross reactant [pg/mL] | Measured Analyte concentration with Cross reactant [pg/mL] | Recovery with x-reactant [%] |
| Adrenomedullin | 1.0 ng/mL | 143 | 130 | 91 | 2655 | 2573 | 97 |
| Angiotensin I | 0.6 ng/mL | 145 | 144 | 99 | 2640 | 2615 | 99 |
| Angiotensin II | 0.6 ng/mL | 147 | 147 | 100 | 2657 | 2603 | 98 |
| Angiotensin III | 1.0 ng/mL | 147 | 147 | 100 | 2692 | 2617 | 97 |
| Arg-Vasopressin | 1.0 ng/mL | 139 | 139 | 100 | 2659 | 2647 | 100 |
| Endothelin | 20 pg/mL | 148 | 148 | 100 | 2680 | 2593 | 97 |
| Aldosterone | 0.6 ng/mL | 151 | 146 | 97 | 2655 | 2541 | 96 |
| Renin | 50 ng/mL | 144 | 136 | 94 | 2671 | 2498 | 94 |
| BNP32 | 3.5 µg/mL | 140 | 140 | 100 | 2655 | 2646 | 100 |
| CNP22 | 2.2 µg/mL | 143 | 130 | 91 | 2579 | 2467 | 96 |
| Urodilatin | 3.5 µg/mL | 141 | 142 | 100 | 2682 | 2668 | 99 |
| ANP 1-28 | 3.1 µg/mL | 141 | 142 | 101 | 2668 | 2642 | 99 |

| Cross-reactant | Concentration reactant | Low analyte level | | | High analyte level | | |
|---|---|---|---|---|---|---|---|
| | | Measured analyte concentration without Cross reactant [pg/mL] | Measured Analyte concentration with Cross reactant [pg/mL] | Recovery with x-reactant [%] | Measured analyte concentration without Cross reactant [pg/mL] | Measured Analyte concentration with Cross reactant [pg/mL] | Recovery with x-reactant [%] |
| NT-proANP (1-30) [preproANP (26-55)] | 3.5 µg/mL | 146 | 144 | 99 | 2677 | 2568 | 96 |
| NT-proANP (31-67) [preproANP 56-92] | 1.0 ng/mL | 146 | 141 | 97 | 2694 | 2622 | 97 |
| NT-proANP (79-98) [preproANP 104-123] | 1.0 ng/mL | 146 | 146 | 100 | 3077 | 3181 | 103 |

# Exogenous Interference – Drugs

The purpose of this study was to evaluate drugs for potential interference with the parameters measured on the **cobas e** 411 analyzer for Elecsys proBNP II (updated assay) and on the **cobas e** 601 analyzer for Elecsys proBNP II STAT (updated assay).

**Methods:**

Two human native serum samples with approximately 125 pg/mL and 2000 pg/mL were analyzed using the **cobas e** 411 and **cobas e** 601 analyzer. These samples were divided into an appropriate number of aliquots. One aliquot of each serum sample was spiked with the respective amount (volume) of the drug (= interference sample) and another aliquot was spiked (if applicable) with the same volume of the solvent of the respective drug (= reference sample). The recovery of the interference sample was calculated as percent recovery compared to the reference sample.

In total, 18 common and 33 special pharmaceutic compounds were analyzed and measured.

**Potentially Interfering Drugs and Test Concentrations**

| Common therapeutic drugs | Drug concentration [mg/L] |
|---|---|
| Acetylcystein | 150 |
| Ampicillin-Na | 1000 |
| Ascorbic acid | 300 |
| Cefoxitin | 2500 |

| Common therapeutic drugs | Drug concentration [mg/L] |
|---|---|
| Heparin | 5000 IU/L |
| Levodopa | 20 |
| Methyldopa | 20 |
| Metronidazole | 200 |
| Doxycycline | 50 |
| Acetylsalicylic Acid | 1000 |
| Rifampicin | 60 |
| Cyclosporine | 5 |
| Phenylbutazone | 400 |
| Acetaminophen | 200 |
| Ibuprofen | 500 |
| Theophylline | 100 |
| Intralipid | 10000 |
| Ca-Dobesilate | 200 |

| Special therapeutic drugs | Drug concentration [mg/L] |
|---|---|
| Carvedilol | 150 |
| Clopidogrel | 75 |
| Digoxin | 0.5 |
| Epinephrine | 0.37 |
| Insulin | 0.84 |
| Lidocaine | 100 |
| Lisinopril | 40 |
| Methylprednisolone | 80 |
| Metoprolol | 15 |
| Nifedipine | 60 |
| Marcumar | 6 |
| Propafenone | 900 |
| Reteplase | 1.12 |
| Simvastin | 40 |
| Spirolactone | 400 |
| Tolbutamide | 3000 |
| Torasemide | 200 |

| Special therapeutic drugs | Drug concentration [mg/L] |
|---|---|
| Verapamil | 120 |
| Propanolol | 0.32 |
| Enalapril | 40 |
| Captopril | 50 |
| Gentamycin | 500 |
| Lovostatin | 80 |
| Pravastatin | 40 |
| Bisoprolol | 10 |
| Glycerol nitrate | 192 |
| Molsidormin | 24 |
| Nicardipin | 90 |
| Streptokinase | 300 IE/mL |
| Urokinase | 600 IE/mL |
| Digitoxin | 0.3 |
| Sotalol | 320 |
| Low molecular weight heparin | 18 |

No interference was seen with the drugs tested.

# Matrix Comparisons

## Methods:

The effect on quantitation of analyte in the presence of anticoagulants with the Elecsys proBNP II (updated assay) was determined on the **cobas e** 411 and with the Elecsys proBNP II STAT (updated assay) was determined on the **cobas e** 601 by comparing values obtained from native samples (single donors) drawn into Serum, Li-Heparin and K2-EDTA plasma.

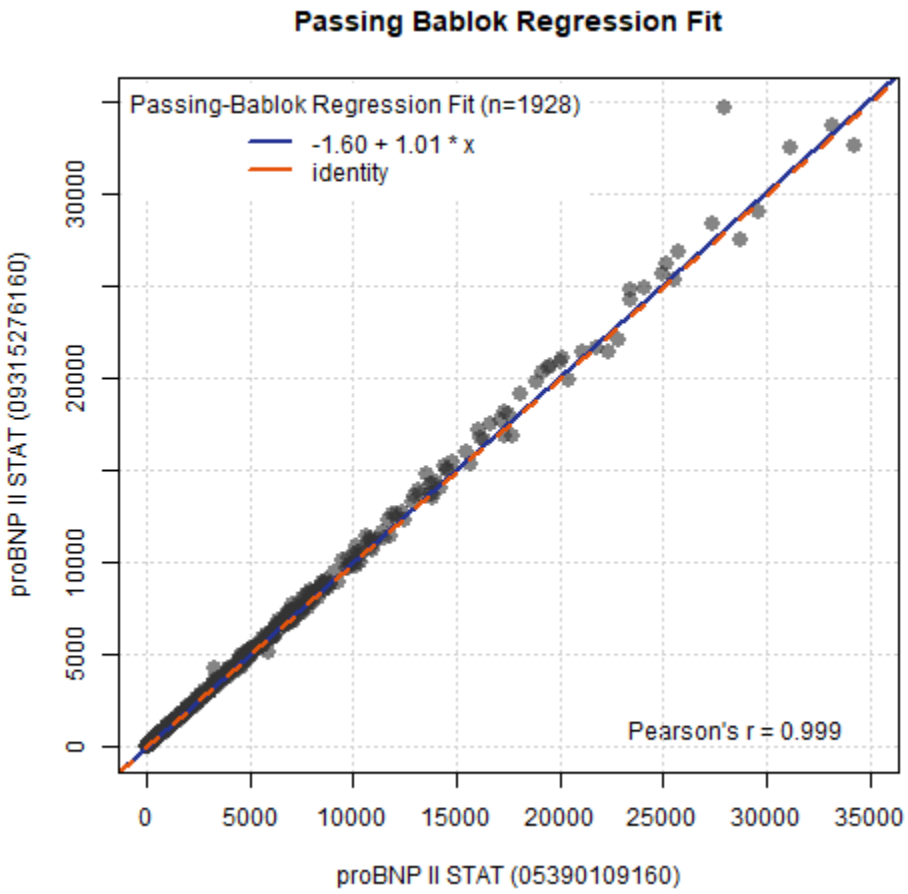
## Results:

### Sample Matrix Comparison for Elecsys proBNP II

| Elecsys proBNP II | Li Heparin | K2-EDTA |
|---|---|---|
| Slope<br>[95% LCL/UCL] | 0.990<br>[0.981/0.997] | 1.01<br>[0.994/1.01] |
| Intercept (pg/mL)<br>[95% LCL/UCL] | 0.898<br>[-0.981/3.45] | -0.985<br>[-4.23/2.21] |
| Correlation coefficient<br>Pearson's r | 0.998 | 0.999 |
| Absolute Bias at 125 pg/mL | -0.391 | -0.189 |
| % Bias at 125 pg/mL<br>[95% LCL/UCL] | -0.3<br>[-1.5/1.3] | -0.2<br>[-2.3/2.1] |
| Serum/plasma pairs | 98 | 111 |

## 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).

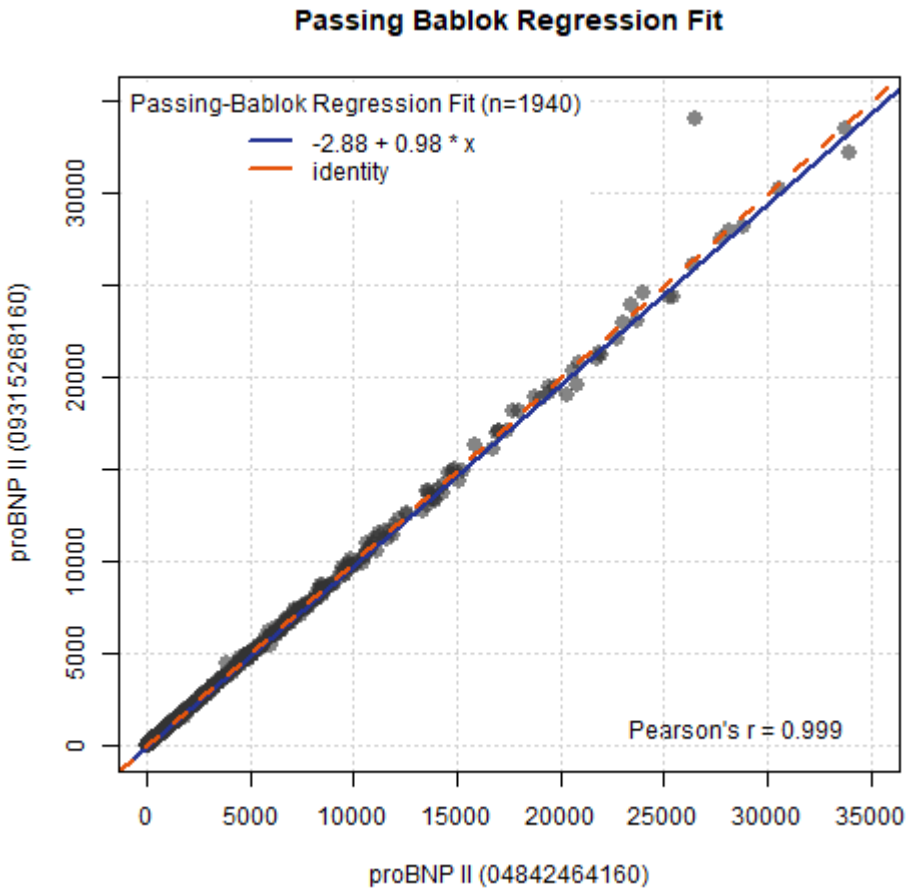



| Correlation | Estimate |
|---|---|
| Kendall's tau | 0.99 |
| Pearson's r | 1.00 |

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

| Passing-Bablok Regression | | | | | Predicted Relative Bias (%) | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Test Method** | **Comparison Method** | **N** | **Intercept (95% CI)** | **Slope (95% CI)** | **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)** |
| Biotin-remediated Elecsys proBNP II STAT (09315276160) | Elecsys proBNP II STAT (05390109160) | 1,928 | -1.60 (-2.09,-1.16) | 1.01 (1.00,1.01) | -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) |

We performed a method comparison study with 1940 subjects with the biotin remediated Elecsys proBNP II assay, 09315268160 (y) and the Elecsys proBNP II assay, 04842464160 (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, 18min)

| Passing-Bablok Regression | | | | | Predicted Relative Bias (%) | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Test Method | Comparison Method | N | Intercept (95% CI) | Slope (95% CI) | 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) |
| Biotin-remediated Elecsys proBNP II (09315268160) | Elecsys proBNP II (04842464160) | 1,940 | -2.88 (-3.20,-2.46) | 0.98 (0.98,0.98) | -4.2 (-4.4,-3.9) | -2.8 (-3.0,-2.7) | -2.5 (-2.6,-2.4) | -2.2 (-2.3,-2.1) | -2.0 (-2.2,-1.9) |

**Conclusion:** Testing demonstrated that the Elecsys proBNP II and Elecsys proBNP II STAT assays are safe and effective and are substantially equivalent to the assays cleared in k072437 and k092649. Biotin tolerance has been improved for patient safety.