December 7, 2022

Roche Diagnostics
Greg Mondics
Senior Regulatory Program Manager
9115 Hague Rd.
Indianapolis, Indiana 46250

Re: K221842
Trade/Device Name: Elecsys β-Amyloid (1-42) CSF II, Elecsys Phospho-Tau (181P) CSF
Regulation Number: 21 CFR 866.5840
Regulation Name: Alzheimer's Disease Pathology Assessment Test
Regulatory Class: Class II
Product Code: QSE
Dated: June 23, 2022
Received: June 24, 2022

Dear Greg Mondics:

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,

Ying Mao -S

Ying Mao, Ph.D.
Branch Chief
Division of Immunology and Hematology Devices
OHT7: Office of In Vitro Diagnostics
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: 06/30/2020
See PRA Statement below.

510(k) Number *(if known)*
K221842

Device Name
Elecsys β-Amyloid (1-42) CSF II
Elecsys Phospho-Tau (181P) CSF

Indications for Use *(Describe)*

Elecsys β-Amyloid (1-42) CSF II and Elecsys Phospho-Tau (181P) CSF are in vitro electrochemiluminescence immunoassays for the measurement of the β-Amyloid (1-42) (Abeta42) and Phospho-Tau (181P) (pTau181) protein concentrations in cerebrospinal fluid (CSF) from adult patients aged 55 years and older being evaluated for Alzheimer's disease (AD) and other causes of cognitive impairment to generate a pTau181/Abeta42 ratio value.
A negative result, defined as pTau181/Abeta42 ratio value below cut-off or an Abeta42 value above the measuring range, is consistent with a negative amyloid positron emission tomography (PET) scan result. A negative result reduces the likelihood that a patient's cognitive impairment is due to AD. A positive result, defined as pTau181/Abeta42 ratio value above cut-off, is consistent with a positive amyloid PET scan result. A positive result does not establish a diagnosis of AD or other cognitive disorder. The pTau181/Abeta42 ratio result is used as an adjunct to other clinical diagnostic evaluations.

Limitations of Use
The performance of the pTau181/Abeta42 ratio has not been established for:
- Predicting development of dementia or other neurologic conditions
- Monitoring responses to therapies

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.

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510(k) Summary

K221842

21 CFR 807.92(a)(1)	
Date this summary was prepared	12/7/2022
Submitter's Name	Roche Diagnostics
Submitter's Address	9115 Hague Rd., Indianapolis, IN 46250 United States
Submitter's phone and email	1-317-600-8647 greg.mondics@roche.com
Contact person	Greg Mondics

21 CFR 807.92(a)(2)	
Name of the Device (trade or proprietary as applicable) (and model numbers)	Elecsys β-Amyloid (1-42) CSF II (08821909160) Elecsys Phospho-Tau (181P) (08846693160)
Common name	Elecsys Phospho-Tau (181P) Elecsys β-Amyloid (1-42) CSF II ratio for amyloid PET concordance
Classification name	Alzheimer's disease pathology assessment test
Regulation Number	866.5840
Product Code	QSE

21 CFR 807.92(a)(3)	
Predicate Device	Lumipulse G β-Amyloid Ratio (1-42/1-40)
Submission where de novo was granted	DEN200072
Product Code	QSE

21 CFR 807.92(a)(4)
Device Description Summary
The Elecsys β-Amyloid (1-42) II immunoassay makes use of a two-step, double antigen sandwich principle using a biotinylated monoclonal β-Amyloid (1-42)-specific and monoclonal β-Amyloid (1-42) antibodies labeled with a ruthenium complex. The Elecsys β-Amyloid (1-42) II immunoassay is intended for the in vitro quantitative determination of β-Amyloid (1-42) (Abeta42) in human CSF. Similarly, the Elecsys Phospho-Tau (181P) immunoassay makes use of a two-step, double antigen sandwich principle using a biotinylated monoclonal Phospho-Tau (181P)-specific and monoclonal Phospho-Tau (181P) antibodies labeled with a ruthenium complex. The Elecsys Phospho-Tau (181P) immunoassay is intended for the in vitro quantitative determination of Phospho-Tau (181P) (pTau181) in human CSF.

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The assays are indicated for use with adult subjects being evaluated for Alzheimer's disease and other causes of cognitive impairment.
The results of the measurement of a CSF sample for each analyte in pg/mL is used in a ratio (pTau181/Abeta42). The assays manually calculated ratio result is compared to a cutoff to determine if the test result indicates an amyloid PET positive or negative result. The result does not establish a diagnosis of AD or other cognitive disorders. Additionally, the result does not predict development of dementia or other neurologic disorders, nor does the result monitor responses to therapies. Both assays are intended for use on the **cobas e** 601immunoassay analyzer.
Results for each assay are determined by an analyte specific calibration curve generated by 2-point calibration and a master curve provided via the reagent barcode or e-barcode.

21 CFR 807.92(a)(5)
Intended use/Indications for Use
Elecsys β-Amyloid (1-42) CSF II and Elecsys Phospho-Tau (181P) CSF are in vitro electrochemiluminescence immunoassays for the measurement of the β-Amyloid (1-42) (Abeta42) and Phospho-Tau (181P) (pTau181) protein concentrations in cerebrospinal fluid (CSF) from adult patients aged 55 years and older being evaluated for Alzheimer's disease (AD) and other causes of cognitive impairment to generate a pTau181/Abeta42 ratio value. A negative result, defined as pTau181/Abeta42 ratio value below cutoff or an Abeta42 value above the measuring range, is consistent with a negative amyloid positron emission tomography (PET) scan result. A negative result reduces the likelihood that a patient's cognitive impairment is due to AD. A positive result, defined as pTau181/Abeta42 ratio value above cut-off, is consistent with a positive amyloid PET scan result. A positive result does not establish a diagnosis of AD or other cognitive disorder. The pTau181/Abeta42 ratio result is used as an adjunct to other clinical diagnostic evaluations. **Limitations of Use** The performance of the pTau181/Abeta42 ratio has not been established for: ▪ Predicting development of dementia or other neurologic conditions ▪ Monitoring responses to therapies

21 CFR 807.92(a)(5)
Indications for Use Comparison
The Elecsys Phospho-Tau (181P) and Elecsys β-Amyloid (1-42) CSF II ratio for amyloid PET concordance is substantially equivalent to the Lumipulse G β-Amyloid Ratio (1-42/1-40). Both test systems measure a CSF sample and utilize two biomarkers in a ratio compared to cutoff(s) to determine a test result of amyloid PET positive or negative.

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21 CFR 807.92(a)(6)
Technological Comparison
The Roche Elecsys Phospho-Tau (181P) and Elecsys β-Amyloid (1-42) CSF II ratio for amyloid PET concordance is substantially equivalent to the predicate device Lumipulse G β-Amyloid Ratio (1-42/1-40). Both products measure the respective analytes in a very similar fashion utilizing monoclonal antibodies which bind with the analyte in the CSF sample and are both measured using luminescence technology.

21 CFR 807.92(b)
Non-Clinical Tests Summary
Elecsys β-Amyloid (1-42) CSF II
Precision: Precision were evaluated according to CLSI guidance EP05-A3. Testing consisted of 2 replicates of each control (PreciControl β-Amyloid (1-42) II), CSF single marker and ratio samples per run, 2 runs separated by 2 hours per day for 21 days on the **cobas e** 601. All samples met the predetermined acceptance criteria.
Lot-to-Lot Precision: Lot-to-lot precision was determined using Elecsys reagents, CSF samples (Abeta42 only and pTau181/Abeta42 ratio samples) and controls in a protocol with the following experimental design: 3 Lots of reagent at one site, 2 runs per day in triplicate each for 5 days (n = 90). All samples met the predetermined acceptance criteria.
Site-to-Site Reproducibility: Reproducibility was determined with a panel of human CSF samples (Abeta42 only and pTau181/Abeta42 ratio samples) and 2 controls. Samples were measured in triplicate using 1 reagent lot, in 2 runs for 5 days at 3 sites according to CLSI EP05-A3. All samples met the predetermined acceptance criteria.
Limit of Blank (LoB): The limit of Blank was determined according to CLSI EP17-A2. Experimental design included three reagent lots evaluated on one **cobas e** 601 analyzer, six runs over three days with one blank sample with ten replicates per run. CSF depleted of Abeta42 was used as the blank sample. The LoB claim in the labeling will be set to 50 pg/mL
Limit of Detection (LoD): The limit of Detection was determined according to CLSI EP17-A2. Five CSF samples with low-analyte concentrations (i.e., > LoB) were measured with three lots in duplicate determination in six runs, distributed over three days, on one **cobas e** 601 analyzer. The LoD claim in the labeling with be set to 100 pg/mL.
Limit of Quantitation (LoQ):

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The limit of Quantitation was determined according to CLSI EP17-A2. Six CSF samples with analyte concentrations close to the specified LoQ were measured as 5 replicates in one-fold determination in 5 runs over at least 5 days. All lots met the predetermined acceptance criterion and the LoQ claim in the labeling will be set to 150 pg/mL.

Linearity:

Linearity was performed according to CLSI EP06-ED2. Linearity was assessed on the **cobas e** 601 analyzer utilizing three dilution series that were prepared from three different human CSF samples. CSF depleted of Abeta42 was employed as dilution medium (sample low) to generate 13 sample levels for the linearity study. Each sample was measured 4-fold within one run and the measured concentrations were plotted against the expected sample concentrations. Linearity results confirm the measuring range claim of 150 pg/mL to 2500 pg/mL.

High Dose Hook Effect:

To determine the hook concentration, a dilution series of two high CSF sample (spiked with Abeta(1-12)-O2Oc4-Abeta(34-42)) dilute with analyte-depleted CSF were measured on one **cobas e** 601 analyzer. Each dilution series included 13 levels. Each sample was measured with 3-fold within one run and measured counts were plotted against the expected sample concentrations. The data supports the claim that there is no hook effect up to 6000 pg/mL.

Human Anti-Mouse Antibodies (HAMA):

The effect on quantitation of analyte in the presence of human anti-mouse antibodies was determined on the **cobas e** 601 analyzer. HAMA interference was assessed in three CSF sample pools: one with a low Abeta42 concentration, a second one with an elevated Abeta42 concentration and a third sample with a pTau181/Abeta42 ratio within 20% above or below the ratio cutoff. One aliquot of each CSF sample was spiked with the interfering substance (HAMA pool) at 120 µg/mL (1:10 dilution), and another aliquot was spiked with the same volume of the base pool. Both pools were tested in the same run in 2-fold determination. The mean value was used to compare the expected value with the measured value. The specification was fulfilled for the HAMA interference.

Endogenous Interferences:

The effect on the quantitation of β-Amyloid (1-42) and the ratio of Elecsys Phospho-Tau (181P) and Elecsys β-Amyloid (1-42) CSF II in the presence of nine interfering substances (Hemoglobin, Bilirubin, Intralipid, Biotin, Rheumatoid Factor (RF), Human Serum Albumin, IgG, IgM, IgA). A total of 4 CSF samples pools (low, medium, high and within 20% above or below the cutoff of pTau181/Abeta42 ratio) derived from residual CSF samples were prepared and used for each interference experiment. For each interfering substance, One aliquot of each CSF sample was spiked with interfering pool, another aliquot was spiked with the same volume of dilution pool. For each experiment, the interfering pool was diluted in n=11 dilution steps with the dilution pool. The recovery for each sample was calculated by comparison to the reference (unspiked) sample. All compounds met the acceptance criteria.

Exogenous (drugs) Interferences:

The effect on quantitation of the Abeta42 analyte in the presence of exogenous interfering substances using the Elecsys β-Amyloid (1-42) CSF II was determined on the **cobas e** 601 analyzer. Seventeen common and fourteen special pharmaceuticals were tested by spiking into two human CSF sample pools. These exogenous interferences were assessed in four CSF samples (low, medium, high and within 20% above or below the cutoff of the

pTau181/Abeta42 ratio). All CSF sample pools were divided into two aliquots. One was spiked with the potential interferent, the reference sample without interferent was spiked with the respective amount of solvent only. All compounds met the acceptance criteria.

Cross Reactivity / Analytical Specificity:

The Elecsys β-Amyloid (1-42) CSF II assay was evaluated for potential cross-reactivity to Abeta(1-40) and Abeta(1-38) peptides, representing Abeta isoforms of different lengths. The study was conducted on the **cobas e** 601 analyzer. Cross-interference was assessed in three CSF sample pools: one with a low Abeta42 concentration, a second one with an elevated Abeta42 concentration and a third sample with a pTau181/Abeta42 ratio within 20% above or below the ratio cutoff. Each pool was divided into two aliquots: One was spiked with 10,000 pg/mL of Abeta(1-40) or Abeta(1-38) as putative cross-reactants, respectively. The other aliquot served as a dilution pool. The interference and dilution pools were mixed in different ratios (0, 25, 50, 75, 100% interference) to assess cross-reactivity at different concentrations of the cross-reactant. Each level was tested in 5-fold measurements. The mean value was used to compare the expected value with the measured value. Ratio and single marker values are within specification.

Lot Calibration Stability:

A calibration should be stable for at least 28 days (4 weeks) when using a new reagent Rackpack of the same reagent lot. Please note "RackPack" refers to the reagent container. A set of 7 CSF samples covering the measuring range was generated for lot calibration testing. A fresh reagent Rackpack was placed on the analyzer and calibrated. Reference values for the samples tested were determined in two runs and in duplicates to obtain a robust reference at day 0. On day 36 (five weeks), a fresh kit (stored at 2-8°C) from the same lot was tested with the same samples, using the calibration established on day 0. Samples were tested in duplicates. The mean value was used to calculate the absolute deviation or percent recovery, respectively, compared to the value obtained at day 0. All samples are within specification.

On-Board Calibration Stability:

Onboard calibration stability should be stable for ≥ 7 days of a not consumed kit. A set of 6 CSF samples covering the measuring range was generated for onboard calibration stability testing. A fresh reagent Rackpack was placed on the analyzer and calibrated. Reference values for the samples tested were determined. The same samples were retested after 8 days with reagent bottles kept at 20°C +/- 3°C (on-board conditions) using the calibration established on day 0. Samples were tested in duplicates. The absolute or relative sample recovery was calculated by comparing the mean sample concentrations based on the two calibrations. All samples are within specification.

Reagent Stability after first opening:

Elecsys β-Amyloid (1-42) CSF II reagent kits can be used after first opening for up to 8 weeks when stored at 2-8°C. Reagent stability after first opening for the Elecsys β-Amyloid (1-42) CSF II assay was tested on one **cobas e** 601 analyzer. A set of 7 CSF samples covering the measuring range was generated for reagent stability after first opening testing. A fresh reagent Rackpack was placed on the analyzer and calibrated. Reference values for the samples tested were determined on day 0 in two runs to generate a more stable reference value at t=0. After the initial measurement the kit was removed from the analyzer and kept at 2-8 °C. On day 36 (5 weeks) and day 64 (9 weeks), the kit was placed on the analyzer again, calibrated and the test samples were determined in duplicates. The mean value was used to calculate the absolute

deviation or percent recovery, respectively, compared to the sample concentration obtained at day 0. All samples are within specification.

Reagent On-Board Stability:

Elecsys β-Amyloid (1-42) CSF II reagent kits can be stored on-board of the analyzers for up to 28 days. Reagent on-board stability for the Elecsys β-Amyloid (1-42) CSF II assay was tested on one **cobas e** 601 analyzer. A set of 6 CSF samples covering the measuring range was generated for reagent on-board stability testing. A fresh reagent Rackpack was placed on the analyzer and calibrated. Reference values for the samples tested were determined (day 0). Three more reagent Rackpacks were opened for approx. 1 hour, closed, and then stored at 20°C ± 3°C (on-board conditions). After 8, 22 and 29 days the kit was placed on the analyzer again, calibrated and the test samples were determined. Each following Rackpack was opened for the duration of the testing as well. Samples were tested in duplicates. The mean value was used to calculate the absolute deviation or percent recovery, respectively, compared to the sample concentration obtained at day 0. All samples are within specification.

Open Rackpack On-Board Stability:

Onboard stability of ≥ 25 hours for an open Rackpack was determined using a **cobas** e601 instrument. A set of 9 CSF samples covering the measuring range was generated for reagent stability testing. A fresh Rackpack was placed on a **cobas e** 601, calibrated and measured (t=0) and left open on the instrument for 26 hours and then measured again. Sample concentration was read off the same calibration curve. The absolute and relative recoveries of the determined sample concentrations (t=26 hours) to the reference (t=0) were calculated. All samples are within specification.

Reagent Shelf-life Stability:

Reagent shelf-life stability of the Elecsys β-Amyloid (1-42) CSF II Rackpack was determined on one **cobas e** 601 analyzer using three reagent lots. A set of 9 CSF samples covering the measuring range of the Elecsys β-Amyloid (1-42) CSF II assay was generated from native human CSF. To determine a robust reference value at time point t=0, the samples were measured in two independent runs and with double determination on a **cobas e** 601 analyzer. The median value from each sample at t=0 was calculated and set as a reference value. For the subsequent time points a new calibration is established and the samples are determined in one run in duplicates. The kits are continuously stored at 2-8 °C and aliquots of the above-mentioned samples are deep-frozen until the next measurement point. At each timepoint, absolute and relative recovery of the test samples with respect to the initial measurement at t=0 is evaluated. All samples are within specification.

Elecsys Phospho-Tau (181P)

Precision:

Precision were evaluated according to CLSI guidance EP05-A3. The protocol consisted of testing 2 replicates of each control (PC = PreciControl Phospho-Tau 181P), CSF single marker and ratio samples per run, 2 runs separated by 2 hours per day for 21 days. All samples met the predetermined acceptance criteria.

Lot-to-Lot precision:

Lot-to-lot precision was determined using Elecsys reagents, CSF samples (pTau181 only and pTau181/Abeta42 ratio samples) and controls in a protocol with the following experimental

design: 3 lots of reagent at one site, 2 runs per day in triplicate each for 5 days (n = 90). All samples met the predetermined acceptance criteria.

Site-to-Site Reproducibility:
Reproducibility was determined with a panel of human CSF samples (pTau181 only and pTau181/Abeta42 ratio samples) and 2 controls. Samples were measured in triplicate using 1 reagent lot in 2 runs for 5 days at 3 sites according to CLSI EP05-A3. All samples met the predetermined acceptance criteria.

Limit of Blank (LoB):
The limit of Blank was determined according to CLSI EP17-A2. Experimental design included three reagent lots evaluated on one **cobas e** 601 analyzer, six runs over three days with one blank sample with ten replicates per run. CSF depleted of Tau proteins was used as the blank sample. The LoB claim in the labeling will be set to 4 pg/mL

Limit of Detection (LoD):
The limit of Detection was determined according to CLSI EP17-A2. Five CSF samples with low-analyte concentrations (i.e., > LoB) were measured with three lots in duplicate determination in six runs, distributed over three days, on one **cobas e** 601 analyzer. The LoD claim in the labeling with be set to 8 pg/mL.

Limit of Quantitation (LoQ):
The limit of Quantitation was determined according to CLSI EP17-A2. Six CSF samples with analyte concentrations close to the specified LoQ were measured as 5 replicates in one-fold determination in 5 runs over at least 5 days. All lots met the predetermined acceptance criterion and the LoQ claim in the labeling will be set to 8 pg/mL.

Linearity:
Linearity was performed according to CLSI EP06-A. Linearity was assessed on the **cobas e** 601 analyzer utilizing three dilution series that were prepared from three different human CSF samples. CSF depleted of Tau proteins was employed as dilution medium (sample low) to generate 13 sample levels for the linearity study. Each sample was measured 3-fold within one run and the measured concentrations were plotted against the expected sample concentrations. All deviations from the linear regression model were within predetermined acceptance criteria for all three samples. The assay is linear over the measuring range of 8.0 pg/mL to 120 pg/mL.

High Dose Hook Effect:
To determine the hook concentration, a dilution series of two high CSF sample (spiked with Tau(172-205)[pThr181)amid) with analyte-depleted CSF were measured on one **cobas e** 601 analyzer. Each dilution series included 13 levels. Each sample was measured with 3-fold within one run and measured counts were plotted against the expected sample concentrations. The data supports the claim that there is no hook effect up to 300 pg/mL.

Human Anti-Mouse Antibodies (HAMA):
The effect on quantitation of analyte in the presence of human anti-mouse antibodies was determined on the **cobas e** 601 analyzer. HAMA interference was assessed in three CSF sample pools: one with a low pTau181 concentration, a second one with an elevated pTau181 concentration and a third sample with a pTau181/Abeta42 ratio within 20% above or below the ratio cutoff. One aliquot of each CSF sample was spiked with the interfering substance (HAMA pool) at 120 µg/mL (1:10 dilution), and another aliquot was spiked with the same volume of the base pool. Both pools were tested in the same run in 2-fold determination.

The mean value was used to compare the expected value with the measured value. The specification was fulfilled for the HAMA interference.

Endogenous Interferences:

The effect on the quantitation of pTau181 and the ratio of Elecsys Phospho-Tau (181P) and Elecsys β-Amyloid (1-42) CSF II in the presence of nine interfering substances (Hemoglobin, Bilirubin, Intralipid, Biotin, Rheumatoid Factor (RF), Human Serum Albumin, IgG, IgM, IgA). A total of 4 CSF samples pools (low, medium, high and within 20% above or below the cutoff of pTau181/Abeta42 ratio) derived from residual CSF samples were prepared and used for each interference experiment. For each interfering substance, One aliquot of each CSF sample was spiked with interfering pool, another aliquot was spiked with the same volume of dilution pool. For each experiment, the interfering pool was diluted in n=11 dilution steps with the dilution pool. The recovery for each sample was calculated by comparison to the reference (unspiked) sample. All compounds met the acceptance criteria.

Exogenous (drugs) Interferences:

The effect on quantitation of the pTau181 analyte in the presence of exogenous interfering substances using the Elecsys Phospho-Tau (181P) was determined on the **cobas e** 601 analyzer. Seventeen common and fourteen special pharmaceuticals were tested by spiking into two human CSF sample pools. These exogenous interferences were assessed in four CSF samples (low, medium, high and within 20% above or below the cutoff of the pTau181/Abeta42 ratio). All CSF sample pools were divided into two aliquots. One was spiked with the potential interferent, the reference sample without interferent was spiked with the respective amount of solvent only. All compounds met the acceptance criteria.

Cross Reactivity / Analytical Specificity:

Cross-interference was assessed in three CSF sample pools: one with a low pTau181 concentration, a second one with an elevated pTau181 concentration and a third sample with a pTau181/Abeta42 ratio within 20% above or below the ratio cutoff. Each pool was divided into two aliquots, one that was spiked with 1300 pg/mL non-phosphorylated Tau as putative cross reactant (Tau(172-205)amide; in-house synthesis) and one aliquot that served as dilution pool. The interference and dilution pools were mixed in different ratios (0, 25, 50, 75, 100% interference) to assess cross-reactivity at different concentrations of the cross-reactant. Each level was tested in 5-fold measurements. The mean value was used to compare the expected value with the measured value. Ratio and single marker values are within specification.

Lot Calibration Stability:

A calibration should be stable for at least 28 days (4 weeks) when using a new reagent Rackpack of the same reagent lot. Please note "RackPack" refers to the reagent container. A set of eight CSF samples covering the measuring range was generated for lot calibration testing. A fresh reagent Rackpack was placed on the analyzer and calibrated. Reference values for the samples tested were determined in two runs and in duplicates to obtain a robust reference at day 0. On day 36 (five weeks), a fresh kit (stored at 2-8°C) from the same lot was tested with the same samples, using the calibration established on day 0. Samples were tested in duplicates. The mean value was used to calculate the absolute deviation or percent recovery, respectively, compared to the value obtained at day 0. All samples are within specification.

On-Board Calibration Stability:

Onboard calibration stability should be stable for ≥ 7 days of a not consumed kit. A set of eight CSF samples covering the measuring range was generated for onboard calibration

stability testing. A fresh reagent Rackpack was placed on the analyzer and calibrated. Reference values for the samples tested were determined. The same samples were retested after 8 days with reagent bottles kept at 20°C +/- 3°C (on-board conditions) using the calibration established on day 0. Samples were tested in duplicates. The absolute or relative sample recovery was calculated by comparing the mean sample concentrations based on the two calibrations. All samples are within specification.

Reagent Stability after first opening:

Elecsys Phospho-Tau (181P) CSF reagent kits can be used after first opening for up to 8 weeks when stored at 2-8°C. Reagent stability after first opening for the Elecsys Phospho-Tau (181P) CSF assay was tested on one **cobas e** 601 analyzer. A set of eight CSF samples covering the measuring range was generated for reagent stability after first opening testing. A fresh reagent Rackpack was placed on the analyzer and calibrated. Reference values for the samples tested were determined on day 0 in two runs to generate a more stable reference value at t=0. After the initial measurement the kit was removed from the analyzer and kept at 2-8 °C. On day 36 (5 weeks) and day 64 (9 weeks), the kit was placed on the analyzer again, calibrated and the test samples were determined in duplicates. The mean value was used to calculate the absolute deviation or percent recovery, respectively, compared to the sample concentration obtained at day 0. All samples are within specification.

Reagent On-Board Stability:

Elecsys Phospho-Tau (181P) CSF reagent kits can be stored on-board of the analyzers for up to 28 days. Reagent on-board stability for the Elecsys Phospho-Tau (181P) CSF assay was tested on one **cobas e** 601 analyzer. A set of eight CSF samples covering the measuring range was generated for reagent on-board stability testing. A fresh reagent Rackpack was placed on the analyzer and calibrated. Reference values for the samples tested were determined (day 0). Three more reagent Rackpacks were opened for approx. 1 hour, closed, and then stored at 20°C ± 3°C (on-board conditions). After 8, 22 and 29 days the kit was placed on the analyzer again, calibrated and the test samples were determined. Each following Rackpack was opened for the duration of the testing as well. Samples were tested in duplicates. The mean value was used to calculate the absolute deviation or percent recovery, respectively, compared to the sample concentration obtained at day 0. All samples are within specification.

Open Rackpack On-Board Stability:

Onboard stability of $\geq$ 25 hours for an open Rackpack was determined using a **cobas** e601 instrument. A set of seven CSF samples covering the measuring range was generated for reagent stability testing. A fresh Rackpack was placed on a **cobas e** 601, calibrated and measured (t=0) and left open on the instrument for 26 hours and then measured again. Sample concentration was read off the same calibration curve. The absolute and relative recoveries of the determined sample concentrations (t=26 hours) to the reference (t=0) were calculated. All samples are within specification.

Reagent Shelf-life Stability:

Reagent shelf-life stability of the Elecsys Phospho-Tau (181P) CSF Rackpack was determined on one **cobas e** 601 analyzer using three reagent lots. A set of seven CSF samples covering the measuring range of the Elecsys Phospho-Tau (181P) CSF assay was generated from native human CSF. To determine a robust reference value at time point t=0, the samples were measured in two independent runs and with double determination on a **cobas e** 601 analyzer. The median value from each sample at t=0 was calculated and set as a reference value. For the subsequent time points a new calibration is established and the samples are determined in one

run in duplicates. The kits are continuously stored at 2-8 °C and aliquots of the above-mentioned samples are deep-frozen until the next measurement point. At each timepoint, absolute and relative recovery of the test samples with respect to the initial measurement at t=0 is evaluated. All samples are within specification.

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Clinical Tests Summary

BioFINDER cutoff setting cohort

The pTau181/Abeta42 ratio cut-off was defined based on the first-generation Elecsys Phospho-Tau (181P) CSF and Elecsys β-Amyloid (1-42) CSF assay results obtained in the retrospective samples from the Swedish BioFINDER1 study. The analysis population comprised a subset of 277 participants with mild cognitive symptoms for whom banked CSF samples and amyloid PET scan results obtained with the tracer [^{18}F]-Flutemetamol were available. Of the 277 subjects, 120 had subjective cognitive decline (SCD), 153 mild cognitive impairment (MCI) and for 4 patients no assignment was available. The ratio cut-off 0.022 was calculated based on the agreement with amyloid PET status by visual read. The resulting agreement rates percentages were:

- Positive Percent Agreement (PPA) 90.9 % (95 % CI: 83.9 % to 95.6 %)
- Negative Percent Agreement (NPA) 89.2 % (95 % CI: 83.5 % to 93.5 %)
- Overall Percent Agreement (OPA) 89.9 % (95 % CI: 85.7 % to 93.2 %)

Cutoff Adjustment for CSF preanalytical differences

Due to the susceptibility of Abeta42 to the use of different pre-analytical protocols for the handling of CSF, a pre-analytical bridging study was conducted to evaluate the differences between the cut-off determination (BioFINDER1) and the Alzheimer's Disease Neuroimaging Initiative (ADNI) cut-off validation studies. The purpose of the pre-analytical bridging study was to determine the conversion factor needed to adjust the optimal ratio cut-off defined in the BioFINDER1 samples prior to the cut-off validation study in order to account for pre-analytical differences between the BioFINDER1 and ADNI protocols. The pre-analytical bridging study (i.e. protocol comparison) was performed with the CSF samples from subjects undergoing diagnostic lumbar puncture due to suspicion of normal pressure hydrocephalus (N = 19 for pTau181 and N = 17 for Abeta42). The CSF samples were handled according to the BioFINDER and ADNI pre-analytical handling protocols.
No meaningful systematic differences were observed for CSF pTau181 measurements [0.7 % (95 % CI: -0.2 % to 1.6 %, p = 0.135)]. The mean percentage difference in CSF Abeta42 measurements was -24 % (95 % CI: -27 % to -20 %, p < 0.001). The upper 95 % confidence limit of the estimated percentage difference between ADNI and BioFINDER was used to

define the conversion factor for Abeta42 (Abeta42 [ADNI] = 0.8*Abeta42 [BioFINDER]) and to adjust the pTau181/Abeta42 cut-off from 0.022 to 0.028 ($0.022*0.8^{-1}=0.028$).

Clinical Validation in ADNI cohort

The pTau181/Abeta42 ratio cut-off was pre-specified and validated using retrospectively collected CSF samples in the Alzheimer's Disease Neuroimaging Initiative studies, ADNI-GO and ADNI2. All patients enrolled into ADNI2 and ADNIGO with baseline CSF sample and amyloid PET image available were considered eligible. Eligibility criteria were not reassessed, with the exception of the following:

- CSF sample volume approximately ≥ 0.4 mL
- CSF sample not visibly hemolyzed (confirmed by the site pre-analysis)

The analysis population included 646 participants with significant memory concerns (SMC, N = 94), early MCI (N = 272), late MCI (N = 152) and Alzheimer's Disease (AD, N = 128) with available banked CSF samples and amyloid PET scans ([^{18}F]florbetapir PET). The average age was 72 years (range 55-91), 46 % / 54 % of subjects were female/male and 50 % / 50 % of subjects were ApoE4 carriers/non-carriers.

The amyloid PET scans were read and interpreted by 3 trained readers and majority voting was used to classify each image as amyloid positive or negative, resulting in 347 (53.7 %) positive, and 299 (46.3 %) negative amyloid PET reads. The independent readers were blinded to any clinical information, including the patient's clinical status, diagnosis, and CSF biomarker measurements. Amyloid PET reads were conducted according to the approved instructions for use of the amyloid PET agent.

The agreements with visual read amyloid PET classification at the pre-specified ratio-cutoff of 0.028 are PPA = 88.2 (95% CI: 84.4 - 91.2); NPA = 92.6 (95% CI: 89.1 - 95.1)

The ratio of pTau181/Abeta42 had concordant predictions for amyloid status in 583 of 646 individuals (90.25 %). The number of cases with discordant CSF status compared to visual amyloid PET assessments was 63 (9.75 %), consisting mainly of pTau181/Abeta42 negative and visual amyloid PET-positive cases. The validation met the accceptance criteria.

Roche Generation 1 of the Elecsys β-Amyloid (1-42) CSF and version 1 of the Elecsys Phospho-Tau(181P) CSF assays were used for this study.

Cutoff Adjustment for assay updates and final CSF preanalytical handling

Compared with the corresponding first-generation assay, the Elecsys β-Amyloid (1-42) CSF II assay was re-standardized using certified reference materials (CRMs) ERM®-DA480/-481/-482/IFCC. Additionally, a new routine-use pre-analytical protocol for CSF handling was adopted for use with the Elecsys β-Amyloid (1-42) CSF II and Elecsys Phospho-Tau (181P)

CSF assays. Consequently, because of the changes in assay standardization and pre-analytical handling protocol, a second bridging study using CSF samples from subjects undergoing diagnostic lumbar puncture due to suspicion of normal pressure hydrocephalus (N = 25 for Abeta42 and N = 22 for pTau181) was performed to address systematic differences between results generated with the first and second assay version.

CSF samples were prepared according to the BioFINDER protocol and measured using the first generation assays. The BioFINDER cohort was utilized for cutoff setting. The values were compared with the values in CSF samples prepared according to the new routine use protocol and measured with the second version of the two assays. The CSF biomarker percentage measurements were highly correlated. No meaningful differences (< 3 %) were obtained for pTau181 in CSF. The mean percentage difference for Abeta42 was -6.32 % (95 % CI: -8.73 % to -3.90 %). The inverse value of the conversion factor (1/0.9368) was used for the adjustment of pTau181/Abeta42 ratio cut-off defined in the BioFINDER1 cohort. **The adjusted ratio cut-off is $0.022*0.9368^{-1} = 0.023$.**

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Substantial Equivalence Summary
The Elecsys Phospho-Tau (181P) CSF / Elecsys β-Amyloid (1-42) CSF II ratio for amyloid PET Concordance is substantially equivalent to the predicate device. Both devices have been adequately tested to demonstrate appropriate analytical performance for the assays utilized in the amyloid PET concordance ratios. Both devices provide a clinical result of amyloid PET positive or amyloid PET negative validated by clinical studies in the intended use population. The Roche device performs as well as or better than the legally marketed predicate device. Based on the comparison of the two devices, the Elecsys Phospho-Tau (181P) CSF / Elecsys β-Amyloid (1-42) CSF II ratio for amyloid PET Concordance is substantially equivalent to the predicate device.