510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K221842

B Applicant

Roche Diagnostics

C Proprietary and Established Names

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

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
QSE	Class II	21 CFR 866.5840 - Alzheimer's Disease Pathology Assessment Test	IM- Immunology

II Submission/Device Overview:

A Purpose for Submission:

New device

B Measurand:

β-amyloid (1-42)
Phospho-Tau (181P)

C Type of Test:

Fully automated, electrochemiluminescence immunoassay (ECLIA)

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) 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 (l-42) (Abeta42) and Phospho-Tau (181P) (pTau181) protein concentrations in cerebral spinal 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 cutoff, 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

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

cobas e 601 analyzer (cleared under K060373)

IV Device/System Characteristics:

A Device Description:

The test system consists of the Elecsys Phospho-Tau (181P) CSF and Elecsys β-Amyloid (1-42) CSF II immunoassays and the cobas e601 immunoassay analyzer. Each assay is packaged in a box individually and sold separately. The Elecsys Phospho-Tau (181P) CSF should be used only with the Elecsys β-Amyloid (1-42) CSF II to calculate the ratio of phospho-Tau (181P) (pTau181) to β-Amyloid (1-42) (Abeta42). The Elecsys Phospho-Tau (181P) CSF and Elecsys β-Amyloid (1-42) CSF II immunoassays are not intended to be used individually.

i) Elecsys Phospho-Tau (181P) CSF

The Elecsys Phospho-Tau (181P) CSF is used for the quantitative measurement of $pTau_{181}$ in human CSF with the intent that the result only be used to calculate the ratio of Elecsys Phospho-Tau (181P) CSF to Elecsys β-Amyloid (1-42) CSF II. The Elecsys Phospho-Tau (181P) CSF is not intended to be used individually. The reagent components of Elecsys Phospho-Tau(181P) CSF are as follows:

Elecsys Phospho-Tau (181P) CSF		
Component	**Volume**	**Contents**
M	Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL	Streptavidin-coated microparticles 0.72 mg/mL; preservative
R1	Anti-β-amyloid (1-42)-Ab~biotin (gray cap), 1 bottle, 6.5 mL	Biotinylated monoclonal anti-pTau antibody (rabbit/mouse) 2.5 mg/L; Tris buffer* > 14 mmol/L, pH 7.2; preservative
R2	Anti-Tau-Ab~Ru(bpy)^ (black cap), 1 bottle, 6.5 mL	Monoclonal anti-Tau antibody (mouse) labeled with ruthenium complex 2.0 mg/L; Tris buffer > 14 mmol/L, pH 7.2; preservative
*Tris(hydroxymethyl)aminomethane ^Tris(2,2'-bipyridyl)ruthenium(II)-complex (Ru(bpy)		

The following materials are required for the Elecsys Phospho-Tau (181P) CSF, but sold separately:

- CalSet Phospho-Tau (181P): 4 x 1.0 mL
  Cal1: approx. 10 pg/mL and Cal2: approx. 70 pg/mL
- PreciControl Phospho-Tau (181P): 6 x 1.0 mL
  Ctrl1: approx. 15 pg/mL and Ctrl2: approx. 50 pg/mL

The matrices for CalSet and PreciControl are identical and are based on Tris-buffer supplied with preservatives (0.11% w/v), detergent (0.1% w/v) and proteins (2% w/w).

ii) Elecsys β-Amyloid (1-42) CSF II

The Elecsys β-Amyloid (1-42) CSF II is used for the quantitative measurement of β-Amyloid (1-42) in human CSF with the intent that the result only be used to calculate the ratio of Elecsys Phospho-Tau (181P) CSF to Elecsys β-Amyloid (1-42) CSF II. The Elecsys β-Amyloid (1-42) CSF II is not intended to be used individually. The components of Elecsys β-Amyloid (1-42) CSF II are as follows:

Elecsys β-Amyloid (1-42) CSF II		
Component	**Volume**	**Contents**
M	Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL	Streptavidin-coated microparticles 0.72 mg/mL; preservative
R1	Anti-β-amyloid (1-42)-Ab~biotin (gray cap), 1 bottle, 6.5 mL	Biotinylated monoclonal anti-β-amyloid (1-42) antibody (mouse) 2.0 mg/L; phosphate buffer > 100 mmol/L, pH 7.2; preservative
R2	Anti-β-amyloid (1-42)-Ab~Ru(bpy)* (black cap), 1 bottle, 6.5 mL	Monoclonal anti-β-amyloid antibody (mouse) labeled with ruthenium complex 1.75 mg/L; phosphate buffer > 100 mmol/L, pH 7.2; preservative
*Tris(2,2'-bipyridyl)ruthenium(II)-complex (Ru(bpy)) ethane		

The following materials are required for the Elecsys β-Amyloid (1-42) CSF II, but sold separately:

- CalSet β-Amyloid (1-42) II; 4 x 1.0 mL
  Cal1: approx. 35 pg/mL and Cal2: approx. 500 pg/mL
- PreciControl β-Amyloid (1-42) II); 6 x 1.0 mL
  Ctrl1: approx. 500 pg/mL and Ctrl2: approx. 1600 pg/mL

The matrices for CalSet and PreciControl are identical and are based on phosphate-buffer supplied with preservatives (0.11% w/v), detergent (0.1% w/v) and proteins (2% w/w).

iii) Cobas e 601 immunoassay analyzer and software

The cobas e 601 is a module that is part of the cobas 6000 analyzer system (c6000). Cobas e 601 immunoassay analyzer is intended for in vitro diagnostic use and is designed to perform automated chemiluminescence immunoassays of specimens using Elecsys reagents, conducting various processes such as dispensing, agitation, and photometric measurement. The assays applied to the analyzer system have assay specific parameters which are contained in downloadable e-barcode files specific to the assay. The cobas e 601 uses software version 05.01 or above.

B Principle of Operation:

1. Specimen collection and handling

Refer to the package inserts of Elecsys Phospho-Tau (181P) and Elecsys β-Amyloid (1-42) CSF II.

2. Elecsys Phospho-Tau (181P) CSF

Elecsys Phospho-Tau (181P) CSF is an assay including a set of immunoassay reagents, for the quantitative measurement of pTau181 in human CSF specimens based on the sandwich principle. Total duration of assay run is 18 minutes and include the following steps:

a) First incubation: 50 µL of sample, a biotinylated monoclonal Tau antibody specific for phosphorylation at threonine 181 and a monoclonal Tau-specific antibody labeled with a ruthenium complex, Tris(2,2'-bipyridyl)ruthenium(II)-complex (Ru(bpy), react to form a sandwich complex.
b) Second incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
c) 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/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
d) The analyzer automatically calculates the pTau181 concentration of each sample in pg/mL 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.

3. Elecsys β-Amyloid (1-42) CSF II

Elecsys β-Amyloid (1-42) CSF II is an assay including a set of immunoassay reagents, for the quantitative measurement of Abeta42 in human CSF specimens based on the sandwich principle. Total duration of assay run is 18 minutes and include the following steps:

a) First incubation step: 50 µL of sample, a biotinylated monoclonal Abeta42-specific antibody and a monoclonal Abeta42-specific antibody labeled with a ruthenium complex, Tris(2,2'-bipyridyl)ruthenium(II)-complex (Ru(bpy), react to form a sandwich complex.
b) Second incubation step: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
c) 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/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
d) The analyzer automatically calculates the Abeta42 concentration of each sample in pg/mL 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.

4. Interpretation of Elecsys Phospho-Tau (181P) CSF/Elecsys β-Amyloid (1-42) CSF II ratio results

Results of the Elecsys Phospho-Tau (181P) CSF and Elecsys β-Amyloid (1-42) CSF II are reported separately by the analyzer. The results from the same patient CSF specimen are combined into a numerical ratio in a range of 0.003< Ratio <0.800 as shown below.

$$\text{Elecsys Phospho-Tau (181P) CSF/ Elecsys β-Amyloid (1-42) CSF II} = \frac{\text{pTau181 (results in pg/mL)}}{\text{Abeta42 (results in pg/mL)}} = \text{a numerical value ranging } 0.003 < \text{Ratio} < 0.800$$

The ratio of pTau181 to Abeta42 (pTau181/Abeta42) must be calculated by the operator because the instrument does not report the final result. The numerical ratio must be compared to the cut-off of 0.023. The final result (negative or positive) must be interpreted by the laboratory professional according to the table below:

Result	Interpretation
pTau181/Abeta42 ≤ 0.023*	A negative result consistent with a negative amyloid PET scan result
pTau181/Abeta42 > 0.023*	A positive result consistent with a positive amyloid PET scan result
Invalid result for either Elecsys Phospho-Tau(181P) CSF or Elecsys β-Amyloid(1-42) CSF II	Not reportable
Invalid results for both Elecsys Phospho-Tau(181P) CSF and Elecsys β-Amyloid(1-42) CSF II	Not reportable
*The ratio should be rounded to 4 decimal places before comparing against 0.023. If the concentrations of the analytes are outside the assay measuring range, the following rules apply: • If the Abeta42 concentration is below 150 pg/mL, or pTau181 concentration is below 8.0 pg/mL or above 120 pg/mL, the concentration should be set to the respective limit of the measuring range of each assay and the ratio should be calculated. • If the Abeta42 concentration is above 2500 pg/mL, the result is consistent with a negative amyloid PET scan result.	

Specimens that fail to meet the run validity criteria yield 'Invalid Result' outcomes. Specimens with 'Invalid Result' results for each assay may be retested. The retest result should then be used to calculate the ratio to obtain a negative or positive result. The qualitative results for the retested samples are re-interpreted according to the above table.

V Substantial Equivalence Information:

A Predicate Device Name(s):

Lumipulse ***G*** ß-Amyloid Ratio (1-42/1-40)

B Predicate 510(k) Number(s):

DEN200072

C Comparison with Predicate(s):

Device & Predicate Device(s):	**Device** **K221842**	**Predicate** **DEN200072**
Device Trade Name	Elecsys β-Amyloid (1-42) CSF II Elecsys Phospho-Tau (181P) CSF	Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40)
General Device Characteristic Similarities		
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 (l-42) (Abeta42) and Phospho-Tau (181P) (pTau181) protein concentrations in cerebral spinal 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 cutoff, 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	The Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) is an *in vitro* cerebral spinal fluid (CSF) test that combines the results of Lumipulse G P-Amyloid 1-42 and Lumipulse ***G*** β-Amyloid 1-40 assays into a ratio of β-amyloid 1−42 to β-amyloid 1−40 concentrations using the LUMIPULSE ***G*** 1200 System. The Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) is intended to be used in adult patients, aged 55 years and older, presenting with cognitive impairment who are being evaluated for Alzheimer's disease (AD) and other causes of cognitive decline. A test result ≥0.073 is a negative result which 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 test result ≤0.058 is a positive result which is consistent with a positive amyloid PET scan result. A positive result does not establish a diagnosis of AD or other cognitive disorder. A test result between 0.059 and 0.072 is considered as a likely positive result as it is more likely consistent with a positive amyloid PET scan result. A likely positive result does not establish a diagnosis of AD or other cognitive disorders and has increased uncertainty in regard to amyloid PET positivity. The Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) results must be interpreted in conjunction with other patient clinical information. This test is not intended as a screening or stand-alone diagnostic test.
Assay Format	Two-step sandwich	Same
Sample Type	Human CSF	Same

General Device Characteristic Differences		
Analyte	Phospho-Tau (181P) (pTau181) β-Amyloid (1-42) (Abeta42)	β-Amyloid (1-42) β-Amyloid (1-40)
Assay Type	Electrochemiluminescence immunoassay (ECLIA)	Chemiluminescent enzyme immunoassay (CLEIA)
Assay Antibodies	*Phospho-Tau (181P):* biotinylated monoclonal anti-pTau181 antibody (rabbit/mouse) and monoclonal anti-Tau antibody labeled with a ruthenium complex *β-Amyloid (1-42):* biotinylated monoclonal anti-Abeta42 antibody (mouse) and monoclonal anti-β-amyloid antibody (mouse) labeled with a ruthenium* complex	*β-Amyloid (1-42):* anti-β-amyloid(1−42) monoclonal antibody (mouse)-coated particles and biotinylated anti-β-amyloid antibody (mouse) *β-Amyloid (1-40):* anti-β-amyloid(1−40) monoclonal antibody (mouse)-coated particles and alkaline phosphatase (ALP)-labeled anti-β-amyloid antibody (mouse) conjugate
Substrate Solution	No substrate is added as there is no enzyme reaction.	AMPPD** is added as the substrate.
Assay Cut-off	One ratio cut-off	Two ratio cut-offs
Assay Output	Negative and Positive	Negative, Likely Positive and Positive
Ratio Calculation and Test Result Interpretation	Operator calculates the ratio of pTau181 to Abeta42. The final test result (negative or positive relative to the cut-off) must be interpreted by the laboratory professional according to the instructions provided in the package insert	A web-based Calculator Tool calculates the ratio of Aβ1-42 to Aβ1-40 and reports the final test result (negative, positive or likely positive relative to the two cut-offs). If the ratio is not calculated by the Calculator Tool, the result is manually reported as '*ratio undetermined*'
Instrument	cobas e 601 analyzer	LUMIPULSE ***G*** l200 System
Calibrators	*Phospho-Tau (181P):* CalSet Phospho-Tau(181P): ~10 pg/mL (Cal 1) ~70 pg/mL (Cal 2) *β-Amyloid (1-42):* CalSet β-Amyloid(1-42) II: ~35 pg/mL (Cal 1); ~500 pg/mL (Cal 2)	*β-Amyloid (1-42):* Lumipulse ***G*** β-Amyloid 1-42 Calibrators Set: 30, 129, and 2,335 pg/mL *β-Amyloid (1-40):* Lumipulse ***G*** β-Amyloid 1-40 Calibrators Set: 0, 500, and 30,000 pg/mL
Controls	*Phospho-Tau (181P):* PreciControl Phospho-Tau(181P) (sold separately): approx.15 pg/mL (Ctrl 1) and approx. 50 pg/mL (Ctrl 2) *β-Amyloid (1-42):* PreciControl β-Amyloid(1-42) II (sold	*β-Amyloid (1-42):* Lumipulse ***G*** β-Amyloid Controls (sold separately): 4,000, 10,000, and 20,000 pg/mL *β-Amyloid (1-40):* Lumipulse ***G*** β-Amyloid Controls (sold

	separately): approx. 500 pg/mL (Ctrl 1) and approx. 1600 pg/mL (Ctrl 2)	separately): 274, 548, and 1,027 pg/mL
Traceability/ Standardization	*Phospho-Tau (181P):* Standardized against a purified reference material Tau(172−205) [pThr181]amide, absolutely quantified via amino acid analysis. *β-Amyloid (1-42):* Standardized against three certified reference materials: ERM-DA480/IFCC, ERM-DA481/IFCC and ERM-DA482/IFCC	*β-Amyloid (1-42):* Standardized against three certified reference materials: ERM-DA480/IFCC, ERM-DA481/IFCC and ERM-DA482/IFCC *β-Amyloid (1-40):* Traceable to INNOTEST β-Amyloid 1-40
Measuring Range	*Phospho-Tau (181P):* 8.0−120 pg/mL *β-Amyloid (1-42):* 150−2500 pg/mL	*β-Amyloid (1-42):* 38−2203.5 pg/mL *β-Amyloid (1-40):* 156.3−28450.3 pg/mL
No high-dose hook effect	*Phospho-Tau (181P):* Up to 300 pg/mL *β-Amyloid (1-42):* Up to 6000 pg/mL	*β-Amyloid (1-42):* Up to 159,000 pg/mL *β-Amyloid (1-40):* Up to 150,000 pg/mL
Detection Limit	*Phospho-Tau (181P):* Limit of Blank = 4.0 pg/mL Limit of Detection = 8.0 pg/mL Limit of Quantitation = 8.0 pg/mL *β-Amyloid (1-42):* Limit of Blank = 50.0 pg/mL Limit of Detection = 100.0 pg/mL Limit of Quantitation = 150.0 pg/mL	*β-Amyloid (1-42):* Limit of Blank = 2.2 pg/mL Limit of Detection = 11.6 pg/mL Limit of Quantitation = 38.0 pg/mL *β-Amyloid(1-40):* Limit of Blank = 0.97 pg/mL Limit of Detection = 33.0 pg/mL Limit of Quantitation = 158.0 pg/mL
Reagent Stability	Unopened: 12 months at 2−8 °C After opening: 8 weeks at 2−8 °C On-board: 28 days	Unopened: 19 months at 2−10 °C On-board: 15 days
Sample Stability	14 days at 2−8 °C 5 days at 15−25 °C	8 days at 2−8 °C 48 hours at 23−27 °C 2 weeks at -30− -10 °C 1 month at -80 °C Up to three freeze/thaw cycles
Sample Volume	50 µL	40 µL
* Tris(2,2'-bipyridyl)ruthenium(II)-complex (Ru(bpy)) **AMPPD= 3-(2' -spiroadamantane)-4-methoxy-4-(3"-phosphoryloxy) phenyl-I, 2-dioxetane disodium salt		

VI Standards/Guidance Documents Referenced:

- CLSI EP05-A3: Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition
- CLSI EP06, 2nd ed.: Evaluation of the Linearity of Quantitative Measurement Procedures − Second Edition
- CLSI EP07, 3rd ed.: Interference Testing in Clinical Chemistry; Approved Guideline – Third Edition
- CLSI EP12-A2: User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline – Second Edition
- CLSI EP17-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline − Second Edition
- EP28-A3c: Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline – Third Edition
- CLSI EP37: Supplemental Tables for Interference Testing in Clinical Chemistry: Approved Guideline; First Edition

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

All results met the manufacturer's pre-determined acceptance criteria.

1. Precision/Reproducibility

A study was conducted per CLSI guideline EP05-A3 to evaluate precision including the reproducibility of the Elecsys Phospho-Tau (181P) CSF and the Elecsys β-Amyloid (1-42) CSF II and the ratio of pTau181/Abeta42 derived from the measurements with these two immunoassays. A panel of seven human CSF pools was prepared to achieve target concentrations of pTau181 and Abeta42 that cover the respective measuring ranges of the individual immunoassays. In addition, a second panel of five human CSF pools with different pTau181/Abeta42 ratio levels (Ratio CSF) that reflect the natural combinations of CSF pTau181 and CSF Abeta42 in the intended use population was prepared and tested with both immunoassays: one CSF pool with a pTau181/Abeta42 value no more than 20% below the ratio cut-off, one CSF pool with a pTau181/Abeta42 value no more than 20% above the ratio cut-off and three CSF pools with pTau181/Abeta42 ratio values outside the ratio cut-off range. These sample panels were used to evaluate *i)* within-laboratory precision, *ii)* lot-to-lot precision, and *iii)* site-to-site reproducibility, as described below:

(i) Within-laboratory precision

To evaluate within-laboratory precision, each panel member was tested in two replicates per run, two runs separated by two hours per day for 21 days at a single

site, using one cobas e 601 immunoassay analyzer and one reagent lot (N=84 per sample). The samples were run in randomized order on the analyzer. In addition, two control levels were run for run validity. The results are summarized in the tables below for each immunoassay (*a* and *b*) separately and the ratio of pTau181 to Abeta42 (*c*):

a. Elecsys Phospho-Tau (181P) CSF

		Within-Run		Between-Run		Between-Day		Within-Laboratory	
Panel member	**Mean (pg/mL)**	SD	%CV	SD	%CV	SD	%CV	SD	%CV
CSF 1	15.5	0.26	1.7	0.12	0.8	0.26	1.7	0.39	2.5
CSF 2	22.3	0.27	1.2	0.31	1.4	0.14	0.6	0.43	2.0
CSF 3	26.3	0.40	1.5	0.25	0.9	0.25	0.9	0.53	2.0
CSF 4	33.3	0.58	1.7	0.00	0.0	0.21	0.6	0.62	1.9
CSF 5	58.4	0.76	1.3	0.61	1.0	0.00	0.0	0.98	1.7
CSF 6	108	1.54	1.4	0.88	0.8	0.20	0.2	1.79	1.7
CSF 7	115	1.53	1.3	1.10	1.0	0.48	0.4	1.94	1.7
PC Level 1	14.2	0.28	1.9	0.19	1.3	0.41	2.9	0.53	3.7
PC Level 2	47.7	0.79	1.7	0.34	0.7	0.46	1.0	0.97	2.0
CSF 1 sample was a diluted native CSF pool. CSF 2−4 samples were native CSF pools. CSF 5, 6 and 7 samples were spiked native CSF pools. PC=PreciControl									

b. Elecsys β-Amyloid (1-42) CSF II

		Within-Run		Between-Run		Between-Day		Within-Laboratory	
Panel member	**Mean (pg/mL)**	SD	%CV	SD	%CV	SD	%CV	SD	%CV
CSF 1	302	3.3	1.1	3.0	1.0	3.5	1.2	5.7	1.9
CSF 2	793	7.9	1.0	9.6	1.2	12.9	1.6	17.9	2.3
CSF 3	1027	11.5	1.1	13.6	1.3	14.7	1.4	23.1	2.2
CSF 4	1305	14.7	1.1	18.5	1.4	26.8	2.1	35.7	2.7
CSF 5	1243	15.3	1.2	17.7	1.4	16.3	1.3	28.5	2.3
CSF 6	2374	40.3	1.7	23.2	1.0	53.3	2.2	70.7	3.0
CSF 7	2317	52.4	2.3	30.7	1.3	32.9	1.4	69.0	3.0
PC Level 1	515	3.9	0.8	7.1	1.4	9.2	1.8	12.2	2.4
PC Level 2	1767	29.9	1.7	29.5	1.7	40.4	2.3	58.3	3.3
CSF 1, 2, 4 and 5 samples were native CSF pools. CSF 3 sample was a diluted native CSF pool. CSF 6 and 7 samples were spiked native CSF pools. PC=PreciControl									

c. pTau181/Abeta42 Ratio

		Within-Run		Between-Run		Between-Day		Within-Laboratory	
Panel member	**Mean Ratio**	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Ratio CSF 1	0.021	0.0005	2.3	0.0003	1.5	0.0005	2.6	0.0008	3.8
Ratio CSF 2	0.028	0.0006	2.0	0.0006	2.1	0.0003	1.1	0.0009	3.1
Ratio CSF 3	0.038	0.0006	1.6	0.0004	1.0	0.0008	2.1	0.0010	2.8
Ratio CSF 4	0.041	0.0008	2.0	0.0004	0.9	0.0008	1.8	0.0010	2.8
Ratio CSF 5	0.054	0.0009	1.6	0.0008	1.4	0.0007	1.3	0.0010	2.5
Ratio CSF 1 and 5 samples were diluted native CSF pools. Ratio CSF 2 and 3 samples were spiked native CSF pools. Ratio CSF sample 4 was a native CSF pool. *The ratio values were rounded to 4 decimal places before comparing against 0.023									

The ratio of pTau181 to Abeta42 for each panel member was further analyzed to evaluate qualitative agreement. The % correct call was calculated for each panel member based on the number of pTau181/Abeta42 result above the ratio cut-off and summarized in the table below.

Panel member	**Mean Ratio***	**Total Replicates (N)**	**Qualitative agreement**	
			Number of ratio results > 0.023	**%Ratio Positive Result**
Ratio CSF 1	0.021	84	0	0.0 (0/84)
Ratio CSF 2	0.028	84	84	100.0 (84/84)
Ratio CSF 3	0.038	84	84	100.0 (84/84)
Ratio CSF 4	0.041	84	84	100.0 (84/84)
Ratio CSF 5	0.054	84	84	100.0 (84/84)
Ratio CSF 1 and 5 samples were diluted native CSF pools. Ratio CSF 2 and 3 samples were spiked native CSF pools. Ratio CSF 4 sample was a native CSF pool. *The ratio values were rounded to 4 decimal places before comparing against 0.023.				

(ii) Lot-to-lot precision

To evaluate between-lot precision, each panel member was tested in three replicates per run, two runs separated by two hours per day for five, not necessarily consecutive, days at a single site, using one cobas e 601 analyzer and three reagent lots (N=90 per sample). The three reagent lots were evaluated in a subsequent fashion with separate days for each lot. The samples were run in randomized order on the analyzer. In addition, two control levels were included for run validity. The results are summarized in the tables below for each immunoassay separately (*a* and *b*) and the ratio of pTau181 to Abeta42 (*c*):

a. Elecsys Phospho-Tau (181P) CSF

		Within-Run		Between-Run		Between-Day		Between-Lot		Total	
Panel member	**Mean (pg/mL)**	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
CSF 1	15.5	0.22	1.4	0.22	1.4	0.00	0.0	0.46	2.9	0.55	3.6
CSF 2	20.9	0.28	1.3	0.31	1.5	0.00	0.0	0.45	2.1	0.61	2.9
CSF 3	26.2	0.42	1.6	0.39	1.5	0.00	0.0	0.36	1.4	0.68	2.6
CSF 4	30.2	0.50	1.6	0.44	1.5	0.00	0.0	0.32	1.1	0.74	2.4
CSF 5	59.3	0.83	1.4	0.75	1.3	0.00	0.0	0.62	1.0	1.28	2.2
CSF 6	115	1.49	1.3	1.13	1.0	0.87	0.8	0.83	0.7	2.22	1.9
PC Level 1	14.1	0.23	1.7	0.14	1.0	0.00	0.0	0.28	2.0	0.39	2.8
PC Level 2	48.1	0.59	1.2	0.65	1.4	0.00	0.0	1.11	2.3	1.42	3.0

CSF 1, 2, 4 and 5 samples were native CSF pools. CSF 6 and 7 samples were spiked native CSF pools. CSF 3 was a diluted native CSF pool. PC=PreciControl

b. Elecsys β-Amyloid (1-42) CSF II

		Within-Run		Between-Run		Between-Day		Between-Lot		Total	
Panel member	**Mean (pg/mL)**	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
CSF 1	322	9.8	3.0	14.3	4.4	15.3	4.8	25.1	7.8	34.1	10.6
CSF 2	886	20.4	2.3	20.8	2.4	0.0	0.0	35.6	4.0	46.0	5.2
CSF 3	1042	19.0	1.8	15.5	1.5	18.8	1.8	33.8	3.2	45.8	4.4
CSF 4	1235	28.3	2.3	16.9	1.4	20.8	1.7	39.3	3.2	55.4	4.5
CSF 5	1447	27.3	1.9	26.5	1.8	21.7	1.5	36.8	2.5	57.2	4.0
CSF 6	2391	56.7	2.4	60.9	2.6	52.5	2.2	45.4	1.9	108.0	4.5
PC Level 1	546	4.7	0.9	6.7	1.2	6.0	1.1	27.5	5.0	29.3	5.4
PC Level 2	1892	19.8	1.1	31.6	1.7	0.0	0.0	55.1	3.0	66.5	3.5

CSF 1−4 samples were native CSF pools. CSF 5 and 6 were spiked native CSF pool. CSF 7 sample was created but was deemed not appropriate for testing. PC=PreciControl

c. pTau181/Abeta42 Ratio

		Within-Run		Between-Run		Between-Day		Between-Lot		Total	
Panel member	**Mean Ratio***	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Ratio CSF 1	0.021	0.0003	1.7	0.0003	1.5	0.0003	1.6	0.0010	4.7	0.0011	5.4
Ratio CSF 2	0.024	0.0006	2.5	0.0001	0.6	0.0000	0.0	0.0010	4.2	0.0012	5.0
Ratio CSF 3	0.036	0.0007	1.8	0.0006	1.6	0.0002	0.5	0.0020	5.4	0.0021	5.9
Ratio CSF 4	0.039	0.0007	1.8	0.0000	0.0	0.0004	1.0	0.0016	4.2	0.0018	4.7

		Within-Run		Between-Run		Between-Day		Between-Lot		Total	
Panel member	**Mean Ratio***	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Ratio CSF 5	0.037	0.0007	1.8	0.0002	0.7	0.0002	0.5	0.0013	3.4	0.0015	3.9

Ratio CSF 1, 2, 4 and 5 samples were spiked native CSF pools. Ratio CSF 3 sample was a native CSF pool.
* The ratio values were rounded to 4 decimal places before comparing against 0.023.

The ratio of pTau181 to Abeta42 for each panel member was further analyzed to evaluate qualitative agreement. The % correct call was calculated for each panel member based on the number of pTau181/Abeta42 result above the ratio cut-off and summarized in the table below.

Panel member	**Mean Ratio***	**Total Replicates (N)**	**Qualitative agreement**	
			Number of ratio results > 0.023	**%Ratio Positive Result**
Ratio CSF 1	0.021	90	0	0.0 (0/90)
Ratio CSF 2	0.024	90	85	94.4 (85/90)
Ratio CSF 3	0.036	90	90	100.0 (90/90)
Ratio CSF 4	0.039	90	90	100.0 (90/90)
Ratio CSF 5	0.037	90	90	100.0 (90/90)

Ratio CSF 1, 2, 4 and 5 samples were spiked native CSF pools. Ratio CSF 3 sample was a native CSF pool.
* The ratio values were rounded to 4 decimal places before comparing against 0.023.

(iii) Site-to-site reproducibility:

To evaluate site-to-site reproducibility, each panel member was tested in three replicates per run, two runs per day with a minimum of two hours idle time, for five days at three sites with one cobas e 601 immunoassay analyzer at each site, using one reagent lot (N=90 per sample). All participating laboratories represent the intended use sites (hospitals, clinics and/or commercial labs). The results are summarized in the tables below for each immunoassay separately (*a* and *b*) and the ratio of pTau181 to Abeta42 (*c*) :

a. Elecsys Phospho-Tau (181P) CSF

		Within-Run		Between-Run		Between-Day		Between-Site		Total	
Panel member	**Mean (pg/mL)**	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
CSF 1	16.5	0.27	1.7	0.33	2.0	0.17	1.1	0.32	2.0	0.57	3.4
CSF 2	22.3	0.35	1.6	0.36	1.6	0.22	1.0	0.40	1.8	0.68	3.1
CSF 3	27.8	0.57	2.0	0.46	1.7	0.00	0.0	0.62	2.2	0.96	3.4

		Within-Run		Between-Run		Between-Day		Between-Site		Total	
Panel member	**Mean (pg/mL)**	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
CSF 4	33.6	0.63	1.9	0.61	1.8	0.24	0.7	0.31	0.9	0.96	2.8
CSF 5	61.2	0.97	1.6	0.90	1.5	0.81	1.3	0.71	1.2	1.71	2.8
CSF 6	118.0	1.76	1.5	2.06	1.7	0.55	0.5	1.45	1.2	3.12	2.6
PC Level 1	15.4	0.35	2.3	0.29	1.9	0.18	1.2	0.22	1.4	0.54	3.5
PC Level 2	54.2	0.89	1.6	0.98	1.8	0.56	1.0	0.64	1.2	1.58	2.9
CSF 1– 3 samples were diluted native CSF pools. CSF 4 sample was a native CSF pool. CSF 5 and 6 samples were spiked native CSF pools. PC=PreciControl											

b. Elecsys β-Amyloid (1-42) CSF II

		Within-Run		Between-Run		Between-Day		Between-Site		Total	
Panel member	**Mean (pg/mL)**	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
CSF 1	397	3.9	1.0	5.8	1.5	5.2	1.3	0.0	0.0	8.7	2.2
CSF 2	856	7.9	1.0	15.7	1.8	7.7	0.9	0.0	0.0	19.2	2.2
CSF 3	1120	12.7	1.1	16.3	1.5	4.8	0.4	0.0	0.0	21.2	1.9
CSF 4	1291	14.5	1.1	27.4	2.1	0.0	0.0	5.2	0.4	31.4	2.4
CSF 5	1358	17.6	1.3	22.3	1.6	0.0	0.0	0.0	0.0	28.4	2.1
CSF 6	2231	39.2	1.8	70.2	3.2	76.4	3.4	0.0	0.0	111	5.0
CSF 7	2448	32.4	1.3	45.5	1.9	20.3	0.8	15.4	0.6	61.4	2.5
PC Level 1	590	4.8	0.8	10.2	1.7	10.6	1.8	10.2	1.7	18.6	3.2
PC Level 2	1751	18.3	1.0	34.0	1.9	18.4	1.1	42.4	2.4	60.2	3.4
CSF 1–4 samples were native CSF pools. CSF 5–7 samples were spiked native CSF pools PC=PreciControl											

c. pTau181/Abeta42 Ratio

		Within-Run		Between-Run		Between-Day		Between-Site		Total	
Panel member	**Mean Ratio***	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Ratio CSF 1	0.018	0.0005	2.9	0.0004	1.9	0.0002	1.4	0.0003	1.9	0.0008	4.2
Ratio CSF 2	0.031	0.0009	2.9	0.0010	3.2	0.0007	2.3	0.0012	3.8	0.0019	6.1
Ratio CSF 3	0.049	0.0009	1.8	0.0009	1.9	0.0008	1.7	0.0010	1.9	0.0018	3.6
Ratio CSF 4	0.047	0.0010	2.1	0.0005	1.0	0.0006	1.4	0.0007	1.5	0.0014	3.0
Ratio CSF 5	0.047	0.0010	2.1	0.0003	0.6	0.0007	1.5	0.0007	1.6	0.0014	3.1
Ratio CSF 6	0.020	0.0004	2.0	0.0009	4.6	0.0000	0.0	0.0008	3.9	0.0013	6.4

		Within-Run		Between-Run		Between-Day		Between-Site		Total	
Panel member	**Mean Ratio***	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Ratio CSF 7	0.024	0.0006	2.6	0.0010	4.0	0.0000	0.0	0.0007	3.0	0.0014	5.6
Ratio CSF 1 sample was a native CSF pool. Ratio CSF 2−7 samples were spiked native CSF pools *The ratio values were rounded to 4 decimal places before comparing against 0.023											

The ratio of pTau181 to Abeta42 for each panel member was further analyzed to evaluate qualitative agreement. The % correct call was calculated for each panel member based on the number of pTau181/Abeta42 result above the ratio cut-off and summarized in the table below.

Panel member	**Mean Ratio***	**Total Replicates (N)**	**Qualitative agreement**	
			Number of ratio results > 0.023	**%Ratio Positive Result**
Ratio CSF 1	0.018	90	0	0.0 (0/90)
Ratio CSF 2	0.031	90	90	100.0 (90/90)
Ratio CSF 3	0.049	90	90	100.0 (90/90)
Ratio CSF 4	0.047	90	90	100.0 (90/90)
Ratio CSF 5	0.047	90	90	100.0 (90/90)
Ratio CSF 6	0.020	90	4	4.4 (4/90)
Ratio CSF 7	0.024	90	83/90	92.2 (83/90)
Ratio CSF 1 was a native CSF pool, Ratio CSF 2−7 samples were spiked native CSF pools *The ratio values were rounded to 4 decimal places before comparing against 0.023				

(iv) pTau181/Abeta42 Precision Simulation:

The within-laboratory (intermediate) precision was evaluated with only two pTau181 to Abeta42 combinations within the ratio cut-off range. Because different combinations of pTau181 and Abeta42 can lead to a ratio value in the cut-off range and because different combinations correspond to different measurement random variabilities, a simulation study was conducted to *(i)* evaluate the expected intermediate- and within-run precision for pTau181/Abeta42 ratio values corresponding to a broad range of hypothetically possible pTau181 and Abeta42 combinations and *(ii)* compare the simulated results with the acceptance criteria for the intermediate precision and repeatability defined for the pTau181/Abeta42 ratio. The variance profile of Panel members 1−7 tested for within-laboratory and within-run precision was considered for simulation.

- The simulated values of the intermediate imprecision results in terms of %CV ranged between 2.23 and 3.85% for all simulated ratio values. For ratio values within the cut-off range (0.023 ± 20%), the intermediate imprecision (in %CV) ranged between 3.01 and 3.53%. The intermediate imprecision met the acceptance criteria.

- The simulated values of the with-run imprecision results in terms of %CV ranged between 1.52 and 2.41% for all simulated ratio values. For ratio values within the cut-off range (0.023 ± 20%), the intermediate imprecision (in %CV) ranged between 1.87 and 2.27%. The within-run precision also met the acceptance criteria.

2. Linearity

The linearity of the Elecsys Phospho-Tau(181P) CSF and Elecsys β-Amyloid(1-42) CSF II on the cobas e 601 analyzer was evaluated in accordance with the CLSI guideline EP06-Ed2. Three high native CSF pools (for pTau181) and three high native CSF samples (for Abeta42) were each diluted with an analyte-depleted CSF sample (for pTau181) or a low CSF sample pool (for Abeta42) to create a series of 13 dilutions of pTau181 or Abeta42 concentrations that span across the measuring range of the respective assays. Each sample dilution was measured in one run with four (for Abeta42) or three (for pTau181) replicates using one reagent lot of each assay. For each sample, the mean value of the measured values, predicted value and the deviation from linearity were calculated. The linear range is defined by the mean of the measurements for the lowest and for the highest sample and is confirmed if all sample levels are within specification for allowable deviation and precision. Percent deviations from linearity were calculated as differences between the observed values and the predicted values divided by the predicted values. The table below summarizes the regression statistics based on the pooled results of the three dilution experiments per analyte. For the pooled analysis based on the entire data set, percent deviations from linearity were within ±15%.

Assay	Range (pg/mL)	Slope (95%CI)	Intercept (95% CI)	R^2
Elecsys Phospho-Tau (181P) CSF	7.8 – 134.7	0.98 (0.97 – 0.99)	0	0.999
Elecsys β-Amyloid (1-42) CSF II	24.8 – 2572.0	0.92 (0.90 – 0.93)	2.08 (-1.16 – 5.31)	0.999

Linearity results support the measuring range claims of 8.0 pg/mL–120.0 pg/mL for Elecsys Phospho-Tau(181P) CSF and 150.0 pg/mL−2500.0 pg/mL for Elecsys β-Amyloid(1-42) CSF II.

High-Dose Hook Effect

The high-dose hook effect was evaluated for the Elecsys Phospho-Tau (181P) CSF and Elecsys β-Amyloid (1-42) CSF II using three reagent lots. To determine the hook concentration, a dilution series of two CSF sample pools spiked with a high concentration stock of Tau(172-205)[pThr181)amide or Abeta(1-12)-O2Oc4-Abeta(34-42) to reach concentrations above the specified hook concentration was measured on a cobas e 601 analyzer. The spiked samples were diluted with a CSF sample depleted of pTau181 and Abeta42 to create 13 levels and each sample level was measured in triplicates within one run. The measured counts were plotted against the expected sample concentrations. The data

supports the claim that there is no high-dose hook effect up to 300.0 pg/mL for pTau181 and up to 6000.0 pg/mL for Abeta42.

3. Analytical Specificity/Interference

(i) Interference

Interference studies were performed in accordance with the CLSI guideline EP07, 3rd ed. To assess test performance in the presence of potentially interfering substances, a panel of three CSF sample pools with pTau181 and Abeta42 concentrations covering the respective measuring ranges of the individual assays (i.e., lower range, mid-range and upper range). A CSF pool with $pTau_{181}/A\beta_{1-42}$ value no more than 20% below or no more than 20% above the ratio cut-off was also included. Analyte spiking (Tau(172-205) [pThr181) amide and Abeta(1-12)-O2Oc4-β-Amyloid (34-42)) was utilized for the two highest levels of pTau181 and Abeta42 and for the ratio sample.

All CSF sample pools were divided into two aliquots: one was spiked with the potential interferent (i.e., the test sample) and the other (i.e., the control sample) without interferent was spiked with the respective amount of solvent used to create the interfering substances panel. The pTau181 and Abeta42 levels in the test and control samples were measured in five replicates in the same run on a cobas e 601 analyzer. The %Interference of each analyte was calculated by determining the percent differences between the test and control samples; $\%Interference = [(Mean_{test} - Mean_{control})/Mean_{control}] \times 100\%$.

A total of 10 endogenous interfering substances (hemoglobin, bilirubin, intralipid, biotin, rheumatoid factor (RF), human serum albumin, IgG, IgM, IgA and human anti-mouse antibodies (HAMA)) and 31 exogenous interfering substances (17 common and 14 special pharmaceuticals) was evaluated. No significant interference was observed (≤10% difference from the control sample) for Elecsys Phospho-Tau (181P) CSF and Elecsys β-Amyloid (1-42) CSF II and for the pTau181/Abeta42 ratio up to the concentrations of the potential interfering substances tested as shown in the tables below:

Endogenous Interferent	**Interferent Concentration***
Bilirubin[&]	0.9 mg/L
Biotin[^]	1200.0 ng/mL
HAMA (IgG and IgM)[#]	120.0 µg/mL
Hemoglobin	150.0 mg/L
Human serum albumin	1.5 g/L
Immunoglobulin G (IgG)	0.6 g/L
Immunoglobulin M (IgM)	0.015 g/L
Immunoglobulin A (IgA)	0.06 g/L
Intralipid[@]	300.0 mg/L
Rheumatoid factor[%]	4.0 IU/mL

* Recommended test concentrations were based on the scientific literature
& Bilirubin consists of a mixture of unconjugated and conjugated forms
^ Biotin was tested at 3600 ng/mL. The data supports no interference up to 1200 ng/mL
# HAMA (human anti-mouse antibodies) was tested at the indicated test concentration in duplicates in the same run using three CSF sample pools: one with low analyte concentration, a second one with elevated analyte concentration and a third sample with pTau181/Aβ1-42 value within 20% above or below the ratio cut-off
% Rheumatoid factor: The data supports no interference up to 12 IU/mL. The specification in the labeling sets a conservative value of 4.0 IU/mL

Exogenous Interferent	Interferent Concentration*
Acetaminophen	156.0 mg/dL
Acetylcysteine	150.0 mg/dL
Acetylsalicylic Acid	30.0 mg/dL
Ampicillin-Na	75.0 mg/dL
Ascorbic Acid	52.5 mg/dL
Atorvastatin	0.75 mg/dL
Cefoxitin	750.0 mg/dL
Cyclosporine	1.8 mg/dL
Digoxin	0.04 mg/dL
Donepezil	30.0 mg/dL
Doxycycline	18.0 mg/dL
Escitalopram	0.19 mg/dL
Esomeprazole	6.9 mg/dL
Furosemide	15.9 mg/dL
Galantamine	250 mg/dL
Heparin	1100.0 IU/dL
Hydrochlorothiazide	1.1 mg/dL
Ibuprofen	219.0 mg/dL
Itraconazol	0.06 mg/dL
Levodopa	7.5 mg/dL
Lisinopril	0.25 mg/dL
Memantine	0.12 mg/dL
Metformin	12.0 mg/dL
Methyldopa	22.5 mg/dL
Metronidazole	123.0 mg/dL
Metoprolol	1.5 mg/dL
Phenylbutazone	107.0 mg/dL
Rifampicin	48.0 mg/dL
Rivastigmine	45.0 mg/dL
Simvastatin	1.7 mg/dL
Theophylline	60.0 mg/dL
*Recommended test concentrations were based on CLSI EP37 and the scientific literature	

An analysis was conducted to further evaluate the expected influence of each potential interfering substance listed in the table above on the pTau181/Abeta42 ratio resulting from different combinations of the single biomarker level (i.e., lower range, mid-range and upper range of the respective assay), based on the results observed in the interference testing for single biomarkers. For each interfering substance concentration and each of the nine $pTau_{181}$ and $A\beta_{1-42}$ biomarker level combinations, a control ratio ($Ratio_{control}$) and spiked ratio ($Ratio_{test}$) were calculated. The %Interference was then calculated as follows: $\%Interference = [(Ratio_{test} - Ratio_{control})/ Ratio_{control}] \times 100\%$. The results showed that for all endogenous interfering substances listed in the table above and for all nine combinations of biomarker levels, the %Interference values were between -6.8% and 8.0%. For all exogenous interfering substances, the %Interference values were between -4.4% and 8.0%. Therefore, for all nine pTau181 and Abeta42 combinations the %Interferences were within ±10%.

(ii) Cross-reactivity

To assess test performance in the presence of putative cross-reactants, three pooled human CSF samples: one with a low analyte concentration (low level CSF sample pool), a second with an elevated analyte concentration (high level CSF sample pool) and a third with pTau181/Abeta42 value (Ratio CSF) within 20% above or below the ratio cut-off. Each pool was divided into two aliquots: one was spiked with a cross-reactant (test pool) and the other aliquot without the cross-reactant served as a dilution pool. The concentration of each cross-reactant was chosen to reflect maximum physiological concentration. To assess cross-reactivity at different concentrations of the cross-reactant, the test and dilution pools were mixed in different ratios (0, 25, 50, 75, 100%). Each level of cross-reactant was tested in five replicates on a cobas e 601 analyzer and the mean value of the test pool was used to compare to that of the control (unspiked) pool. For the Ratio CSF pool, the mean values were used to calculate the pTau181/Abeta42 ratio in the respective test and control samples. The percentage of cross reactivity was calculated using the following formula:

$$\%\text{cross-reactivity} = [(\text{mean of test sample} - \text{mean of control sample}) / (\text{cross-reactant concentration})] \times 100$$

The results are summarized below for each assay (*a* and *b*) and the ratio of the analytes (*c*):

a. Elecsys Phospho-Tau (181P) CSF

The Elecsys Phospho-Tau(181P) CSF was evaluated for potential cross-reactivity to Tau (172-205)amide, a non-phosphorylated peptide corresponding to amino acids 174-188 of the human Tau441 (2N4R) protein, at the highest test concentration of 1300.0 pg/mL which is the reported concentration in the intended use population[1]. The mean % cross-reactivity values for Tau(172-205)amide tested at five different

[1] Skillbäck T, Rosén C, Asztely F, Mattsson N, Blennow K, Zetterberg H. Diagnostic Performance of Cerebrospinal Fluid Total Tau and Phosphorylated Tau in Creutzfeldt-Jakob Disease: Results From the Swedish Mortality Registry. JAMA Neurol. 2014;71(4):476–483. doi:10.1001/jamaneurol.2013.6455

concentrations in two levels (Low and High) of pooled human CSF samples are summarized in the table below:

		Low level CSF sample pool		**High level CSF sample pool**	
Cross-reactant	Cross-reactant concentration (pg/mL)	Measured Concentration (pg/mL)	Mean Cross-reactivity (%)	Measured Concentration (pg/mL)	Mean Cross-reactivity (%)
Tau (172-205) amide	0.0	13.6	–	40.1	–
	325.0	13.9	0.10	39.9	-0.05
	650.0	14.1	0.07	40.3	0.03
	975.0	14.2	0.06	40.7	0.07
	1300.0	14.3	0.05	41.5	0.11

The Elecsys Phospho-Tau (181P) CSF showed no significant cross-reactivity (within ±1% difference of test from control sample) up to 1300 pg/mL for the tested cross-reactant.

To assess the specificity of the Phospho-Tau (181P) CSF capture antibody towards all other possible epitopes and threonine phosphorylation sites across the entire human protein, a panel of 36 synthetic phospho-peptides (each 15 amino acids in length) derived from regions of human Tau441 around all threonine phosphorylation sites was evaluated for interaction with the capture antibody by surface plasmon resonance. A peptide corresponding to amino acids 174-188 of the human Tau protein covering the non-phosphorylated threonine at amino acid 181(Thr181) was included as a negative control. Results showed that of the 36 phospho-peptides, only the peptide covering the phosphorylated threonine at amino acid 181 (pThr181) interacts with the capture antibody. Additionally, a synthetic peptide covering the phosphorylated serine at amino acid 185 (pSer185) failed to bind to the capture antibody demonstrating the high specificity of this antibody for pThr181 on the human Tau protein.

To further assess the specificity of the Elecsys Phospho-Tau (181P) CSF, the purified full-length recombinant human Tau441 protein and a variant form of Tau411 carrying the mutation T181A [Tau441(T181A) with a threonine to alanine substitution at amino acid 181] that is not phosphorylatable at amino acid 181 were spiked at equal concentrations in analyte-depleted CSF and diluted across the measuring range of Elecsys Phospho-Tau (181P) CSF. The resulting dilution series obtained for each protein was measured in two replicates using one cobas e 601 analyzer and the results are presented in the table below:

	Tau441			**Tau441(T181A)**		
Sample	Concentration (pg/mL)		Mean (pg/mL)	Concentration (pg/mL)		Mean (pg/mL)
	Replicate 1	Replicate 2		Replicate 1	Replicate 2	
1	1.6	1.8	1.7	0	0	0
2	8.1	8.3	8.2	0	0	0
3	27.3	27.8	27.6	0	0	0
4	93.4	94.8	94.1	0	0	0

b. Elecsys β-Amyloid(1-42) CSF II

The Elecsys β-Amyloid (1-42) CSF II was evaluated for potential cross-reactivity to Abeta1-38 and Abeta1-40 species representing Abeta isoforms of different lengths. Two pooled human CSF samples with low and high concentrations of Abeta42 were spiked with the potential cross-reactive Abeta species at the highest test concentration of 10,000 pg/mL, which is two-fold higher than the reported concentration of Abeta1-40[2], the major species of Abeta, in the brain. The mean % cross-reactivity values for the cross-reactive Abeta species tested at five different concentrations in two levels of pooled human CSF samples are summarized below:

		Low level CSF sample pool		**High level CSF sample pool**	
Cross-reactants	Cross-reactant concentration (pg/mL)	Measured Concentration (pg/mL)	Mean % Cross-reactivity	Measured Concentration (pg/mL)	Mean % Cross-reactivity
Abeta1-38	0.0	434	–	1323	–
	2500.0	434	0.00	1317	-0.24
	5000.0	434	0.00	1311	-0.24
	7500.0	429	-0.07	1312	-0.14
	10000.0	425	-0.09	1281	-0.42
Abeta1-40	0.0	420	–	1271	–
	2500.0	419	-0.03	1265	-0.25
	5000.0	412	-0.16	1258	-0.26
	7500.0	408	-0.16	1246	-0.33
	10000.0	401	-0.19	1225	-0.46

The Elecsys β-Amyloid (1-42) CSF II showed no significant cross-reactivity (within ±1% difference of test from control sample) up to 10,000 pg/mL for the tested cross-reactants.

c. pTau181/Abeta42 Ratio

A Ratio CSF sample pool within 20% of the ratio cut-off (0.023) was spiked with Tau(172-205)amide and the two cross-reactive Abeta species, Abeta1-38 and Abeta1-40. A control sample was spiked only with the appropriate solvent used to prepare the cross-reactants. The %cross-reactivity for each cross-reactant tested at five different concentrations in the Ratio CSF sample pool is summarized below:

[2] Korecka M, Figurski MJ, Landau SM, Brylska M, Alexander J, Blennow K, Zetterberg H, Jagust WJ, Trojanowski JQ, Shaw LM; Alzheimer's Disease Neuroimaging Initiative. Analytical and Clinical Performance of Amyloid-Beta Peptides Measurements in CSF of ADNIGO/2 Participants by an LC-MS/MS Reference Method. Clin Chem. 2020 Apr 1;66(4):587-597. doi: 10.1093/clinchem/hvaa012. PMID: 32087019; PMCID: PMC7108496.

		Ratio CSF sample pool within 20% of the ratio cut-off	
Cross-reactants	Cross-reactant concentration	Measured Ratio*	% Cross-Reactivity
Tau (172-205) amide	0.0	0.022	–
	325.0	0.024	6.2×10^{-4}
	650.0	0.025	4.6×10^{-4}
	975.0	0.025	3.1×10^{-4}
	1300.0	0.024	1.5×10^{-4}
Abeta1-38	0.0	0.023	–
	2500.0	0.023	0.0×10^{-4}
	5000.0	0.024	0.1×10^{-4}
	7500.0	0.024	0.2×10^{-4}
	10000.0	0.025	0.1×10^{-4}
Abeta1-40	0.0	0.023	–
	2500.0	0.023	0.0×10^{-4}
	5000.0	0.024	0.4×10^{-4}
	7500.0	0.024	0.1×10^{-4}
	10000.0	0.025	0.2×10^{-4}
*The ratio values should be rounded to 4 decimal places before comparing against 0.023			

The ratio of pTau181 to Abeta42 was not significantly impacted (within ± 1% difference of test from control sample) by each cross-reactant up to the indicated test concentrations.

4. Assay Reportable Range

Elecsys Phospho-Tau (181P) CSF: 8.0−120.0 pg/mL
Elecsys β-Amyloid (1-42) CSF II: 150.0−2500.0 pg/mL

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods)

1) Traceability

The Elecsys β-Amyloid (1-42) CSF II was reference standardized against three certified European Reference Materials (ERM): ERM®-DA480/IFCC, ERM®-DA481 /IFCC and ERM®- DA482/IFC[3,4]. Reference Standardization for the Elecsys Phospho-Tau (181P) CSF was performed against a purified reference material Tau(172−205)[pThr181]amide. The purified reference material was absolutely quantified via amino acid analysis. Value assignment was based on weighted pTau reference material traceable to National Institute of Standards and Technology (NIST) amino acid reference calibrators.

2) Stability

[3] Boulo S, Kuhlmann J, Andreasson U, *et al*., (2020). First amyloid β1-42 certified reference material for re-calibrating commercial immunoassays. Alzheimers Dement.:16(11):1493-1503. 10.1002/alz.12145.
[4] Kuhlmann J, Andreasson U, Pannee L, *et al.,* (2017) Csf aβ(1-42) - an excellent but complicated alzheimer's biomarker - a route to standardisation. Clin. Chim. Acta 467, 27–33

a) Calibration

(i) Lot Calibration Stability

A panel of eight CSF samples with pTau181 levels (16.2−115.3 pg/mL) and another panel of seven CSF samples with Abeta42 levels (210.2−2368.6 pg/mL) covering the respective measuring ranges of the individual CSF assays were generated for lot calibration testing. A fresh reagent Rackpack was placed on the analyzer and calibrated. Baseline 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. The results support the calibration is stable up to 28 days (4 weeks) when using a new reagent Rackpack of the same reagent lot.

(ii) On-Board Calibration Stability

A panel of eight CSF samples with pTau181 levels (16.2−115.3 pg/mL) and another panel of six CSF samples with Abeta42 levels (210.2−2368.6 pg/mL) covering the respective measuring ranges of the individual CSF assays were generated for onboard calibration stability testing. A fresh reagent Rackpack was placed on the analyzer and calibrated. Baseline 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 specifications. The results support that onboard calibration stability is stable up to 7 days when using the same reagent kit on the analyzer.

b) Reagent

(i) Shelf-life Stability

Reagent shelf-life stability of the Elecsys Phospho-Tau (181P) CSF and Elecsys β-Amyloid (1-42) CSF II Rackpack was determined on one cobas e601 analyzer using three reagent lots. A panel of seven CSF samples with pTau181 levels (16.5−124.1 pg/mL) and another panel of nine CSF samples with Abeta42 levels (210.2−2565.2 pg/mL) covering the respective measuring ranges of the individual CSF assays were generated from native human CSF. To determine a robust baseline value at timepoint t=0 (T_0), the samples were measured in two independent runs and with double determination on a cobas e 601 analyzer. The mean value from each sample at T_0 was calculated and set as a baseline value. For the subsequent time points of 6.5, 9.5, 12.5 and 15.5 months, a new calibration was established, and the sample values were determined in one run in duplicates. At each timepoint, absolute and relative recovery of the test samples with respect to the initial measurement at T_0 was evaluated. All samples tested with the three reagent lots were within ±10%. The results support that the Elecsys Phospho-Tau (181P) CSF and Elecsys β-Amyloid (1-42) CSF II Rackpack reagent kits can be stored unopened for up to 15 months when stored at 2−8°C.

(ii) Stability after first opening:

Reagent stability after first opening for the Elecsys Phospho-Tau (181P) CSF assay and Elecsys β-Amyloid (1-42) CSF II Rackpack was tested using one reagent lot on one cobas e 601 analyzer. A panel of eight CSF samples with pTau181 levels (16.2−115.3 pg/mL) and another panel of seven CSF samples with Abeta42 levels (210.2−2368.6 pg/mL) covering the respective measuring ranges of the individual CSF assays were generated for reagent stability after first opening testing. A fresh reagent kit was placed on the analyzer and calibrated. Baseline 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 reagent kit was removed from the analyzer and kept at 2−8 °C. On day 36 (5 weeks) and day 64 (9 weeks), the reagent 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 T_0. All samples in both panels were within ±10%. The results support that the Elecsys Phospho-Tau (181P) CSF and Elecsys β-Amyloid (1-42) CSF II Rackpack reagent kits can be used after first opening for up to 8 weeks when stored at 2−8°C.

(iii) On-Board Stability

Reagent on-board stability for the Elecsys Phospho-Tau (181P) CSF assay and Elecsys β-Amyloid (1-42) CSF II Rackpack was tested on one cobas e 601 analyzer using one reagent lot. A panel of eight CSF samples with pTau181 levels (16.2−115.3 pg/mL) and another panel of six CSF samples with Abeta42 levels (210.2−2368.6 pg/mL) covering the respective measuring ranges of the individual CSF assays were generated for reagent on-board stability testing. A fresh reagent kit was placed on the analyzer and calibrated. Baseline values for the samples tested were determined (T_0). Three more reagent kits were opened for approximately 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 T_0. All samples in both panels were within ±10%. The results support that the Elecsys Phospho-Tau (181P) CSF and Elecsys β-Amyloid (1-42) CSF II Rackpack reagent kits can be stored on-board of the analyzer for up to 28 days.

(iv) On-Board In Use Stability

On-board stability for an open Rackpack was determined using one reagent lot on a cobas e601 instrument. A panel of seven CSF samples with pTau181 levels (16.2−115.3 pg/mL) and another panel of nine CSF samples with $A\beta_{1-42}$ levels (160.7−2565.2 pg/mL) covering the respective measuring ranges of the individual CSF assays were 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 at the test point to the baseline (T_0) were calculated. All samples in both panels were within ±10%. The results support that the Elecsys Phospho-Tau(181P) CSF and Elecsys β-Amyloid(1-42) CSF II Rackpack reagent kits can be left opened and stored on-board of the analyzer for 25 hours.

(v) Transport Stability

Reagent transport stability for the Elecsys Phospho-Tau (181P) CSF and Elecsys β-Amyloid (1-42) CSF II Rackpack was tested in one run using one cobas e 601 analyzer using one reagent lot. A panel of eight CSF samples with pTau181 levels (16.2−115.3 pg/mL) and another panel of seven CSF samples with Aβ1-42 levels (160.7−2368.6 pg/mL) covering the respective measuring ranges of the individual CSF assays were generated for transport stress testing. The reagent was stressed in the original container at 25°C for 192 hours (eight days for Elecsys Phospho-Tau (181P) CSF assay and seven days for Elecsys β-Amyloid (1-42) CSF II Rackpack) in a drying cabinet. The unstressed baseline kit was kept at 2−8 °C. Samples were determined in duplicates and the mean values were determined and the absolute or relative deviation from the values obtained with the unstressed baseline were calculated. The results support that the Elecsys Phospho-Tau (181P) CSF and Elecsys β-Amyloid (1-42) CSF II Rackpack reagent kits can be stored at 25°C for up to 168 hours (7 days).

c) Specimen

(i) Specimen storage stability at 15−25ºC

A panel of 13 CSF samples with varying pTau181 levels and Abeta42 levels were freshly collected in accordance with the routine-use pre-analytical procedure for fresh CSF samples described in the package inserts of Elecsys Phospho-Tau (181P) CSF and Elecsys β-Amyloid (1-42) CSF II. Most of the samples were from patients representing the intended use population. The panel included at least one sample in each of the low, medium, and high concentration ranges of pTau181 and Abeta42 and at least two samples with a ratio value of pTau181/Abeta42 near (± 20%) the cut-off (0.023 pg/mL). After the T_0 baseline measurements of five aliquots per sample (T_0=within 6 hours. after the lumbar puncture), samples were placed into storage at 25ºC. At the defined T_1 (1−3 days of storage after the baseline measurements) and T_2 (6−8 days after the baseline measurements), at least two aliquots of each sample were measured. All samples tested at T_1 were within specifications (≤10% difference from baseline) for pTau181 and Abeta42. One sample tested at T_2 had an average recovery of 87.9% for Abeta42 after 8 days of storage. The recommended sample storage duration at 15−25°C is 5 days.

(ii) Specimen storage stability at 2−8ºC

A panel of 15 CSF samples with varying pTau181 levels and Abeta42 levels as described above for the sample panel tested for storage stability at 15−25ºC were freshly collected in accordance with the routine-use pre-analytical procedure. Most of the samples were from patients representing the intended use population. One sample was excluded from the analysis because the donor failed to meet the

inclusion criteria for age. After the T_0 baseline measurements of five aliquots per sample (T_0=within 6 hours after the lumbar puncture), samples were placed into storage at 6ºC. Two aliquots per sample were measured at the defined T1 (1−5 days after the baseline measurements), another aliquot at T2 (6−12 days after the baseline measurements) and the remaining two at T_3 (13−15 days after the baseline measurements). All samples tested at T_1, T_2 and T_3 were within specifications (≤ 10% difference from baseline) for pTau181 and Abeta42. The recommended sample storage duration at 2−8ºC is 14 days in the package inserts of Elecsys Phospho-Tau (181P) CSF and Elecsys β-Amyloid (1-42) CSF II.

6. Detection Limit

The Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ) studies were conducted in accordance with the CLSI guideline EP17-A2. The studies evaluated three lots of Elecsys Phospho-Tau (181P) CSF and three lots of Elecsys β-Amyloid (1-42) CSF II on one cobas e 601 analyzer. A description of each study and the results obtained are summarized below for each immunoassay:

(i) Elecsys Phospho-Tau (181P) CSF

For LoB, a CSF sample depleted of pTau181 and Abeta42 was tested in six runs, distributed over three days, with 10 replicates per run to reach a total of 60 measurements for each reagent lot. The LoB was determined as the 95th percentile of the measurements obtained. The LoB values from the three lots were 0.000, 0.000 and 0.009 pg/mL. The claimed LoB is 4.0 pg/mL.

For LoD, five low-level CSF samples with target concentrations of pTau181 above LoB (from 4.1 to 8.0 pg/mL) were tested in six runs, distributed over three days, with two replicates per run to reach a total of 12 replicates per sample or 60 replicates for all samples for each reagent lot. The LoD values from the three lots were 0.28, 0.40 and 0.27 pg/mL. The claimed LoD is 8.0 pg/mL.

For LoQ, six low-level CSF samples with target concentrations of pTau181 close to the specified LoQ (from 6.0 to 16.2 pg/mL) were tested in five runs distributed over at least five days with five replicates per run to reach a total of 25 measurements per sample (150 measurements for all samples) for each reagent lot. The LoQ was estimated based on the lowest concentration of p-Tau181 which can be quantified with an intermediate precision of no more than 20% CV. The LoQ values from the three lots were 6.55, 6.35 and 5.73 pg/mL. The claimed LoQ is 8.0 pg/mL.

(ii) Elecsys β-Amyloid (1-42) CSF II

For LoB, a CSF sample depleted of pTau181 and Abeta42 was tested in six runs, distributed over three days, with 10 replicates per run to reach a total of 60 measurements for each reagent lot. The LoB was determined as the 95th percentile of the measurements obtained. The LoB values from the three lots were 14.6, 22.0 and 23.5 pg/mL. The claimed LoB is 50.0 pg/mL.

For LoD, five low-level CSF samples with target concentrations of Abeta42 above LoB (from 70.4 to 179.2 pg/mL) were tested in six runs, distributed over three days, with two measurements per run to reach a total of 12 measurements per sample or 60 measurements for all samples for each reagent lot. The LoD values from the three lots were 16.5, 24.2 and 25.9 pg/mL. The claimed LoD is 100.0 pg/mL.

For LoQ, six low-level CSF samples with target concentrations of Abeta42 close to the specified LoQ (from 70.4 to 300.9) were tested in five runs distributed over at least five days with five replicates per run to reach a total of 25 measurements per sample or 150 measurements for all samples for each reagent lot. The LoQ was estimated based on the lowest concentration of Abeta42 which can be quantified with an intermediate precision of no more than 20% CV. The LoQ values from the three lots were 73.0, 74.9 and 60.1 pg/mL. The claimed LoQ is 150.0 pg/mL

7. Assay Cut-Off

1) Determination of the pTau181/Abeta42 ratio cut-off

The pTau181/Abeta42 ratio cut-off was defined based on the first-generation Elecsys Phospho-Tau (181P) CSF (Version 1 or V1) and Elecsys β-Amyloid (1-42) CSF (Generation 1 or Gen1) assay results obtained in the retrospective CSF samples from the Swedish BioFINDER1 study which are distinct from those evaluated in the pivotal clinical validation study (see Section C '*Clinical Studies*' below). The procedures of CSF collection, processing and handling were conducted according to a standardized BioFINDER1 protocol. 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 with mild cognitive impairment (MCI) and 4 with missing SCD/MCI classification. Images were analyzed by visual read and scored as either amyloid PET positive or amyloid PET negative. Amyloid PET positivity was found in 110 of 277 patients (39.7%) and amyloid PET negativity was found in 167 of 277 patients (60.3%). The ratio cut-off of 0.022 pg/mL was calculated based on the agreement with amyloid PET status by visual read. The resulting agreement rates with amyloid PET were:

- Positive Percent Agreement (PPA) 90.9% (95% CI: 83.9%−95.6%)
- Negative Percent Agreement (NPA) 89.2% (95% CI: 83.5%−93.5%)
- Total Percent Agreement (TPA) 89.9% (95% CI: 85.7%−93.2%)

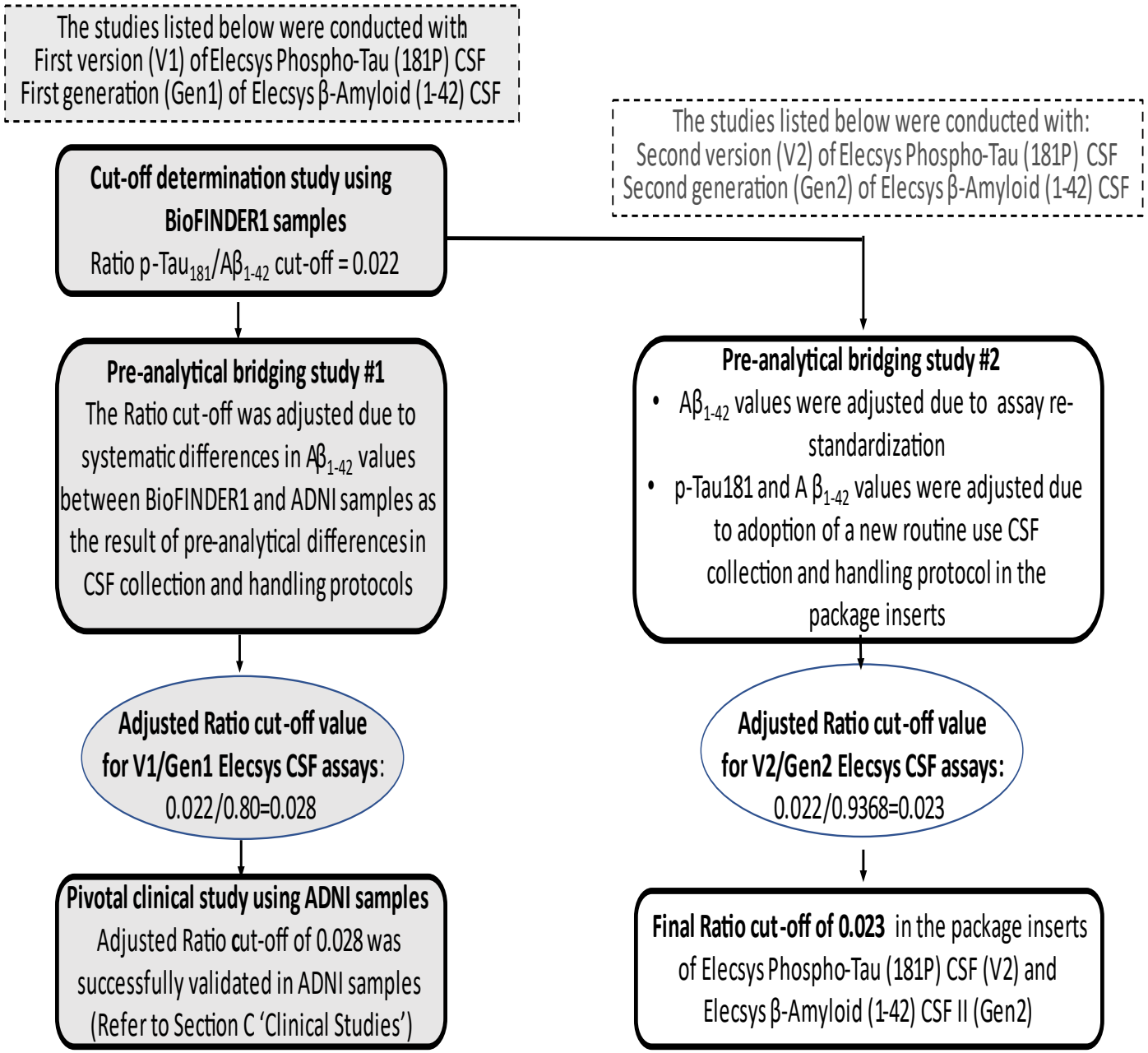
2) Adjustment of pTau181/Abeta42 ratio cut-off for CSF pre-analytical differences in pre-analytical bridging study #1

Due to the susceptibility of Abeta42 to the use of different pre-analytical protocols for the handling of CSF[5], 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 study (see Section C '*Clinical Studies*' below). The purpose of the pre-analytical bridging study was to determine the

[5] Vanderstichele H *et al*., (2012) Standardization of preanalytical aspects of cerebrospinal fluid biomarker testing for Alzheimer's disease diagnosis: a consensus paper from the Alzheimer's Biomarkers Standardization Initiative. Alzheimers Dement. 8(1):65-73.

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 BioFINDER1 and ADNI pre-analytical handling protocols. Results showed that small (not statically significant) systematic differences were observed for CSF pTau181 measurements [0.7% (95% CI: (-0.2%; 1.6%), p=0.135)]. The mean percentage difference in CSF Abeta42 measurements was -24% (95% CI: (-27%; -20%), p<0.001). The upper limit of the 95% CI for the estimated percentage difference between ADNI and BioFINDER1 was used to define the conversion factor for Abeta42 (Abeta42 [ADNI] = 0.8 x Abeta42 [BioFINDER1]) and to adjust the pTau181/Abeta42 cut-off from 0.022 to 0.028 (0.022 x (1/0.8) = 0.028). Results from this study showed that the systematic difference in pre-analytical factors between different handling procedures can have an impact of as much as 24% on the measured Abeta42 concentrations. These results demonstrated the need to develop a simple and standardized pre-analytical handling procedure for routine use as described below under item 3. The pre-specified pTau181/Abeta42 ratio cut-off of 0.028 was validated using retrospectively collected CSF samples in the Alzheimer's Disease Neuroimaging Initiative studies, ADNI-GO and ADNI2 (refer to clinical performance data presented in Section C 'Clinical Studies' below).

3) *Adjustment of pTau181/Abeta42 ratio cut-off for assay upgrades and adoption of final CSF pre-analytical handling in pre-analytical bridging study #2*
Compared with the corresponding first-generation assay (Gen1), the Elecsys β-Amyloid(1-42) CSF II (Gen2) was re-standardized using certified reference materials (CRMs) ERM®-DA480/481/482/IFCC. Additionally, a new routine-use pre-analytical protocol for fresh CSF sample handling (described in the '*Specimen collection and preparation'* section of the package inserts) was adopted for use with Elecsys β-Amyloid 1-42) CSF II and Elecsys Phospho-Tau (181P) CSF (V2). Consequently, because of the changes in assay standardization and pre-analytical protocol, a second bridging study using CSF samples from subjects undergoing diagnostic lumbar puncture due to suspicion of normal pressure hydrocephalus (N=22 for pTau181 and N=25 for Abeta42) was performed to address systematic differences between results generated with the first and second assay version. CSF samples were prepared according to the BioFINDER1 protocol and measured using the first-generation assays. The BioFINDER1 cohort was utilized for setting the pTau181/Abeta42 cut-off as described above under item 1. 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. Small differences (< 3%) were obtained for pTau181 in CSF. The mean percentage difference for Abeta42 was -6.32% (95% CI: (-8.73%; -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 x (1/0.9368) = 0.023 which is the final ratio cut-off that is provided in the package insert. An overview of the ratio cut-off values - original and after adjustment for different pre-analytical procedures and assay versions - is summarized in the diagram below:



B Comparison Studies:

1. Method Comparison with Predicate Device

   Refer to Section C on 'Clinical Studies' below.

2. Matrix Comparison

   Not applicable.

C Clinical Studies:

1. Clinical Sensitivity and Clinical Specificity

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 ADNI-GO with baseline CSF sample and PET image available were considered eligible. Eligibility criteria were not reassessed, except for the following: *(i)* CSF sample volume approximately ≥ 0.4 mL and *(ii)* 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 Mild Cognitive Impairment (N=272, EMCI), late MCI (N=152, LMCI) and AD (N=128) with available banked CSF samples and 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. Roche Generation 1 of the Elecsys β-Amyloid (1-42) CSF and version 1 of the Elecsys Phospho-Tau (181P) CSF were used in the pivotal clinical validation study.

The amyloid PET scans were randomly assigned, read and interpreted by three trained readers, each reading independent of each other's, and majority voting was used to classify each image as amyloid positive or negative, resulting in 347 (54%) positive, and 299 (46%) negative amyloid PET reads. The independent readers were blinded to any clinical information, including the patient's clinical status, diagnosis, and CSF biomarker measurements. PET reads were conducted according to the approved instructions for use of the PET agent. The inter-reader visual read agreement and amyloid PET scan positivity in the diagnostic groups are summarized in the tables below.

Inter-reader visual read agreement		
Agreement Rate	**Mean (%)**	**Min-Max (%)**
Positive Percent Agreement (PPA)	94.0	92.1−96.9
Negative Percent Agreement (NPA)	94.9	86.8−100.0
Total Percent Agreement (TPA)	93.7	83.7−100.0
Reader 1 vs Reader 2 TPA	96.9	95.3−98.1
Reader 1 vs Reader 3 TPA	93.0	90.8−94.9
Reader 2 vs Reader 3 TPA	92.1	89.8−94.1

Percentage of positive amyloid PET scans in the diagnostic groups	
Diagnostic groups	**PET scans rated positive [%]**
Significant memory concern (SMC)	25.5
Early mild cognitive impairment (EMCI)	39.3
Late mild cognitive impairment (LCMI)	67.1
Alzheimer's disease (AD)	89.1

The demographic and clinical characteristics of the study subjects according to diagnostic groups and PET scan status are presented in the table below.

		Diagnostic Groups*				Visual Amyloid PET Read		Total = 646 Total % (N)
		SMC = 94 % (N)	EMCI = 272 % (N)	LMCI = 152 % (N)	AD = 128 % (N)	Positive =347 % (N)	Negative =299 % (N)	
Cohort	ADNIGO	0.0% (0)	42.3% (115)	0.0% (0)	0.0% (0)	24.4% (73)	12.1% (42)	17.8% (115)
	ADNI2	100.0% (94)	57.7% (157)	100.0% (152)	100.0% (128)	75.6% (226)	87.9% (305)	82.2% (531)
Sex	Male	40.4% (38)	55.9% (152)	53.9% (82)	59.4% (76)	55.6% (193)	51.8% (155)	53.9% (348)
	Female	59.6% (56)	44.1% (120)	46.1% (70)	40.6% (52)	44.4% (154)	48.2% (144)	46.1% (298)
Age	55–59 years	1.1% (1)	5.9% (16)	5.3% (8)	5.5% (7)	4.0% (14)	6.0% (18)	5.0% (32)
	60–69 years	43.6% (41)	40.1% (109)	29.6% (45)	20.3% (26)	24.8% (86)	45.2% (135)	34.2% (221)
	70–79 years	46.8% (44)	40.1% (109)	52.6% (80)	49.2% (63)	53.3% (185)	37.1% (111)	45.8% (296)
	≥80 years	8.5% (8)	14.0% (38)	12.5% (19)	25.0% (32)	17.9% (62)	11.7% (35)	15.0% (97)
	Mean	72.1	71.1	72.1	74.3	-	-	72.1
	Median	71.4	70.9	72.8	75.1	-	-	72.4
	Min.	59.7	55.0	55.0	55.6	-	-	55
	Max.	85.3	88.6	91.4	90.3	-	-	90
Race	White	93.6% (88)	93.4% (254)	94.7% (144)	93.0% (119)	92.0% (275)	95.1% (330)	93.7% (605)
	Asian	0.0% (0)	1.5% (4)	0.7% (1)	3.1% (4)	1.7% (5)	1.2% (4)	1.4% (9)
	African American	3.2% (3)	1.8% (5)	3.3% (5)	3.1% (4)	3.0% (9)	2.3% (8)	2.6% (17)
	Others^	3.2% (3)	2.6% (7)	1.3% (2)	0.8% (1)	2.7% (8)	1.4% (5)	2.0% (13)
MMSE@	<18	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)
	18-23	0.0% (0)	0.4% (1)	0.0% (0)	53.9% (69)	2.0% (6)	18.4% (64)	10.8% (70)
	24-30	100% (94)	99.6% (271)	100% (152)	46.1% (59)	98.0% (293)	81.6% (283)	89.2% (576)
	Mean	29.0	28.3	27.6	23.2	-	-	27.2
	Median	29	29	28	23	-	-	28
	Min.	24	23	24	19	-	-	19
	Max.	30	30	30	26	-	-	30

Apolipo-protein E (ApoE) Risk Alleles	0	66.0 (62)	57.7 (157)	42.1 (64)	32.8 (42)	3.0 (6)	18.3 (17)	50.3 (325)
	1	33.0 (31)	35.0 (95)	40.8 (62)	46.9 (60)	1.5 (3)	1.1 (1)	38.4 (248)
	2	1.0 (1)	7.3 (20)	17.1 (26)	20.3 (26)	31.7 (63)	60.2 (56)	11.3 (73)
Years of Education	Mean	16.7	15.9	16.7	15.7	-	-	16.2
	Median	17	16	17	16	-	-	16
	Min	8	10	9	9	-	-	8
	Max	20	20	20	20	-	-	20
Visual Amyloid PET Read	Positive	25.5% (24)	39.3% (107)	67.1% (102)	89.1% (114)	0% (0)	100% (347)	53.7% (347)
	Negative	74.5% (70)	60.7% (165)	32.9% (50)	9.6% (10.9)	100% (299)	0% (0)	46.3% (299)

* Significant memory concern (SMC); Early mild cognitive impairment (EMCI); Late mild cognitive impairment (LCMI); Alzheimer's disease (AD)
@ Mini-Mental State Examination
^ Others include Hawaiian / Pacific Islander, American Indian / Alaskan, and 'More than one race'

Results:

1) Clinical Performance

The agreements with visual read amyloid PET classification at the pre-specified ratio cut-off of 0.028 are summarized in the table below.

		Visual Amyloid PET Read		
		Positive	**Negative**	**Total**
Elecsys Phospho-Tau (181P) CSF and Elecsys β-Amyloid (1-42) CSF II	**Positive (Ratio result>0.028)***	306	22	328
	Negative (Ratio result ≤0.028)*	41	277	318
	Total	347	299	646
Performance Measures		**Point Estimates % (95% CI)**		
Positive Percent Agreement (PPA)		88.2 (306/347) (84.4−91.2)*		
Negative Percent Agreement (NPA)		92.6 (277/299) (89.1−95.1)*		
Total Percent Agreement (TPA)		90.2 (583/646) (87.7−92.3)*		
Prevalence		53.7 (347/646) (59.9-57.5)*		
Positive Predictive Value (PPV)		93.3 (306/328) (90.3– 95.4)**		

Performance Measures	Point Estimates % (95% CI)
Negative Predictive Value (NPV)	87.1 (277/318) (83.5–90.0)**
Positive Likelihood Ratio (LR+)	12.0 (8.0–18.0)***
Negative Likelihood Ratio (LR-)	0.13 (0.10–0.17)***

* 95%CI are calculated using a Wilson score method for binomial proportions
** 95%CI are calculated using 95%CI for the corresponding likelihood ratio and prevalence
*** 95%CI are calculated using an asymptotic method for ratios of two independent binomial proportions

Of the 347 subjects with an amyloid PET scan positive result, 306 also had a positive pTau181/Abeta42 ratio result. The positive percent agreement (PPA) was 88.2% (306/347) with 95% CI: (84.4 – 91.2). The remaining 41 PET scan positive subjects had a negative pTau181/Abeta42 ratio result. The rate of false negative (FN) results was 11.8% (41/347). Of the 299 subjects with a PET scan negative result, 277 also had a negative pTau181/Abeta42 ratio result. The negative percent agreement (NPA) was 92.6% (277/299) with a 95% CI: (89.1–95.1). The remaining 22 amyloid PET scan negative subjects had a positive pTau181/Abeta42 ratio result. The rate of false positive (FP) results was 7.4% (22/299). The total percent agreement (TPA) was 90.3% (583/646) with a 95% CI: 87.7–92.3. The number of cases with discordant CSF status compared to visual amyloid PET assessments was 63 (9.7%). The Positive Predictive Value (PPV) was 93.3% with a 95% CI: 90.3–95.4 and the Negative Predictive Value (NPV) was 87.1% with a 95% CI: 83.5–89.6 based on the amyloid PET positivity rate of 53.7% (347/646).

2) Sub-group Analysis

a. Agreement with visual amyloid PET by diagnostic groups

The clinical performance measures of the ratio results generated from the measurements with the Elecsys Phospho-Tau (181P) CSF and Elecsys β-Amyloid (1-42) CSF II immunoassays are stratified by diagnostic groups and summarized in the table below.

Total= 646	**Diagnosis***			
	SMC	**EMCI**	**LMCI**	**AD**
N (% Total)	94 (14.6)	272 (42.1)	152 (23.5)	128 (19.8)
Visual Amyloid PET Read Positive (% N) (95% CI)	24 (25.5) (17.8−35.2)	107 (39.3) (33.7−45.3)	102 (67.1) (59.3−74.1)	114 (89.1) (82.5−93.4)
PPA (n/N) (95% CI)	66.7 (16/24) (46.7−82.0)	79.4 (85/107) (70.8−86.0)	90.2 (92/102) (82.9−94.6)	99.1 (113/114) (95.2−99.8)
NPA (n/N) (95% CI)	92.9 (65/70) (84.3−96.9)	94.6 (156/165) (90.0−97.1)	88.0 (44/50) (76.2−94.4)	85.7 (12/14) (60.1−96.0)

	SMC	EMCI	LMCI	AD
TPA (n/N) (95% CI)	86.2 (81/94) (77.8−91.7)	88.6 (241/272) (84.3−91.9)	89.5 (136/152) (83.6−93.4)	97.7 (125/128) (93.3−99.2)
PPV (n/N) (95% CI)	76.2 (16/21) (56.6−88.7)	90.4 (85/94) (83.3−94.7)	93.9 (92/98) (87.7−97.0)	98.3 (113/115) (95.3−99.5)
NPV (n/N) (95% CI)	89.0 (65/73) (82.2−93.6)	87.6 (156/178) (82.8−91.1)	81.5 (44/54) (71.0−89.1)	92.3 (12/13) (68.2−98.6)
LR+ (95% CI)	9.3 (3.8−22.8)	14.6 (7.7−27.7)	7.5 (3.5−16.0)	6.9 (2.5−24.8)
LR- (95% CI)	0.36 (0.20−0.63)	0.22 (0.15−0.32)	0.11 (0.06−0.20)	0.009 0.002−0.057)

*Diagnostic groups: SMC=significant memory concern, EMCI=early mild cognitive impairment, LMCI=late mild cognitive impairment, AD=Alzheimer's disease. Levels of MCI (early or late) were determined using the Wechsler Memory Scale Logical Memory II.

Across diagnostic groups, the estimates of TPA and PPA increase as the disease progresses from SMC (66.7%) to AD (99.1%). The estimate of NPV was slightly higher in EMCI (94.6%) and SMC (92.9%) compared to LMCI (88.0%) and AD (85.7%). The estimate of PPV also increases from SMC (76.2%) to AD (98.3%) whereas NPV was higher in AD (92.3%) compared to SMC (89.0%), EMCI (87.6%) and LMCI (81.5%). By contrast, the estimate of LR- decreases from SMC (0.36) to AD (0.01) and the estimate of LR+ was higher in EMCI (14.6) compared to AD (6.9), LMCI (7.5) and SMC (9.3).

b. Agreement with visual amyloid PET by sex

The clinical performance measures of the ratio results generated from the measurements with Elecsys Phospho-Tau (181P) CSF and Elecsys β-Amyloid (1-42) CSF II immunoassays are stratified by sex and summarized in the table below.

Total= 646	**Sex**	
	Male	**Female**
N (% Total)	348 (53.9)	298 (46.1)
Visual Amyloid PET Read Positive (% N) (95% CI)	193 (55.5) (50.2–60.6)	154 (51.7) (46.0−57.3)
PPA (n/N) (95% CI)	87.0 (168/193) (81.6–91.1)	89.6 (138/154) (83.8−93.5)
NPA (n/N) (95% CI)	92.3 (143/155) (87.0−95.5)	93.1 (134/144) (87.7−96.2)
TPA (n/N) (95% CI)	89.4 (311/348) (85.7−92.2)	91.3 (272/298) (87.5−94.0)

	Male	Female
PPV (n/N) (95% CI)	93.3 (168/180) (89.2−96.0)	93.2 (138/148) (88.6−96.2)
NPV (n/N) (95% CI	85.1 (143/168) (80.0−89.3)	89.3 (134/150) (84.3−93.1)
LR+ (95% CI)	11.2 (6.6−19.4)	12.9 (7.3−23.6)
LR- (95% CI)	0.14 (0.10−0.20)	0.11 (0.07−0.17)

The estimate of TPA was similar for males (89.4%) and females (91.3%). The estimates of PPA and NPA were 87.0% and 92.3%, respectively, for males and 89.6% and 93.1%, respectively, in females. The estimates of PPV and NPV were also similar in males (93.3% and 85.1%) and females (93.2% and 89.3%).

c. Agreement with visual amyloid PET by age

The clinical performance measures of the ratio results generated from the measurements with the Elecsys Phospho-Tau (181P) CSF and Elecsys β-Amyloid (1-42) CSF II immunoassays are stratified by age groups and summarized in the table below.

Total= 646	**Age**			
	55−59 years	**60−69 years**	**70−79 years**	**≥80 years**
N (% Total)	32 (5.0)	221 (34.2)	296 (45.8)	97 (15.0)
Visual Amyloid PET Read Positive (% N) (95% CI)	14 (43.8) (28.2–60.7)	86 (38.9) (32.7–45.5)	185 (62.5) (56.9–67.8)	62 (63.9) (54.0–72.8)
PPA (n/N) (95% CI)	92.9 (13/14) (68.5−98.7)	89.5 (77/86) (81.3−94.4)	87.0 (161/185) (81.4−91.1)	88.7 (55/62) (78.5−94.4)
NPA (n/N) (95% CI)	94.4 (17/18) (74.2−99.0	94.1 (127/135) (88.7−97.0)	91.0 (101/111) (84.2−95.0)	91.4 (32/35) (77.6−97.0)
TPA (n/N) (95% CI)	93.8 (30/32) (79.9−98.3)	92.3 (204/221) (88.0−95.1)	88.5 (262/296) (84.4−91.7)	89.7 (87/97) (82.1−94.3)
PPV (n/N) (95% CI)	92.9 (13/14) (73.1−98.7)	90.6 (77/85) (83.4−95.0)	94.2 (161/171) (90.1−96.7)	94.8 (55/58) (87.4−98.2)
NPV (n/N) (95% CI	94.4 (17/18) (79.0−99.0)	93.4 (127/136) (88.7−96.4)	80.8 (101/125) (74.5−86.1)	82.1 (32/39) (70.3−90.3)
LR+ (95% CI)	16.7 (3.5−96.5)	15.1 (7.9−29.7)	9.7 (5.5−17.6)	10.4 (3.9−30.2)
LR- (95% CI)	0.08 (0.01−0.34)	0.11 (0.06−0.20	0.14 (0.10−0.21)	0.12 (0.06−0.24)

The estimate of TPA decreases slightly with age, from 93.8% in the '55−59 years' age group to 88.5% in the '70−79 years' age group. The estimates of PPA and NPA followed a similar trend as TPA across the age groups.

d. Agreement with visual amyloid PET by race

The clinical performance measures of the ratio results generated from the measurements with the Elecsys Phospho-Tau (181P) CSF and Elecsys β-Amyloid (1-42) CSF II immunoassays are stratified by race and summarized in the table below.

Total= 646*	**Race**			
	White	**African American**	**Asian**	**Others[#]**
N (% Total)	605 (93.7)	17 (2.6)	9 (1.4)	13 (2.0)
Visual Amyloid PET Read Positive (% N) (95% CI)	330 (54.5) (50.6−58.5)	8 (47.1) (26.2−69.0)	4 (44.4) (18.9−73.3)	5 (38.5) (17.7−64.5)
PPA (n/N) (95% CI)	88.2 (291/330) (84.3−91.2)	100.0 (8/8) (67.6−100)	100.0 (4/4) (51.0−100)	60.0 (3/5) (23.1−88.2)
NPA (n/N) (95% CI)	92.0 (253/275) (88.2−94.7)	100 (9/9) (67.6−100)	100 (5/5) (56.6−100)	100 (8/8) (67.6−100)
TPA (n/N) (95% CI)	89.9 (544/605) (87.3−92.1)	100 (17/17) (81.6−100)	100 (9/9) (70.1−100)	84.6 (11/13) (57.8−95.7)
PPV (n/N) (95% CI)	93.0 (291/313) (89.9−95.2)	100 (8/8) (74.0−100)	100 (4/4) (62.5−100)	100 (3/3) (47.7−100)
NPV (n/N) (95% CI	86.6 (253/292) (82.9− 89.8)	100 (9/9) (76.9−100)	100 (5/5) (70.6−100)	80.0 (8/10) (66.7−93.5)
LR+ (95% CI)	11.0 (7.4−16.5)	+Inf (3.21 - +Inf)	+Inf (2.08 - +Inf)	+Inf (1.46 - +Inf)
LR- (95% CI)	0.13 (0.10− 0.17)	0.00 (0.00-0.34)	0.00 (0.00-0.52)	0.40 (0.11−0.80)

*2 of 646 subjects had missing information about race
[#]Including Hawaiian / Pacific Islander
+Inf means Infinity, large positive number

The validation study was conducted in cohorts that are primarily Caucasian. Sample sizes from other races are not sufficient to provide reliable estimates of the clinical performance by race. For white patients, the estimates of PPA, NPV and TPA were 88.2%, 92.0%, and 89.9%, respectively. The same estimates for African Americans, Asians and Others had high uncertainty due to limited numbers of patients in the study.

In conclusion, the data of the clinical performance study conducted with the first version (V1) of Elecsys Phospho-Tau (181P) CSF and first generation (Gen1) of Elecsys β-Amyloid (1-42) CSF support that a positive result, defined as pTau181/Abeta42 ratio value above the pre-specified ratio cut-off of 0.028 is consistent with an amyloid PET scan positive result and negative result, defined as pTau181/Abeta42 ratio value below the pre-specified ratio cut-off of 0.028 is consistent with a amyloid PET scan negative result. Compared with the corresponding first-generation Gen1 assay, the Elecsys β-Amyloid(1-42) CSF II (second generation; Gen2) was re-standardized using certified reference materials (CRMs), ERM®-DA480/-481/-482/IFCC. Additionally, a new routine-use pre-analytical protocol for CSF handling (as described in the '*Specimen collection and preparation'* section of the package inserts) was adopted for use with Elecsys Phospho-Tau(181P) CSF (second version;V2) and Elecsys β-Amyloid(1-42) CSF II. Consequently, because of the changes in assay standardization and pre-analytical handling protocol, a pre-analytical bridging study was performed to adjust the ratio cut-off to 0.023 as described in Section A.7 Assay Cut-off. This final ratio cut-off was included in the package inserts of the Elecsys Phospho-Tau (181P) CSF (V2) and Elecsys β-Amyloid (1-42) CSF II.

2. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

   Not applicable

D Clinical Cut-Off

Refer to Assay Cut-Off in Section VII.A, item 7.

E Expected Values/Reference Range

A reference interval study was performed in accordance with the CLSI guideline EP28-A3c. This study had two parts:

1) *Study Part 1 (Retrospective data analysis):* The data analyzed were generated from CSF samples collected from 115 (69 female and 46 male) cognitive healthy normal subjects aged 66 to 75 years, with a Mini-Mental State Exam (MMSE) score ≥ 29, in the Swedish BioFINDER1 study according to the BioFINDER CSF pre-analytical handling protocol and measured with the first-generation Elecsys Phospho-Tau (181P) CSF and Elecsys β-Amyloid (1-42) CSF assays (for description of different assay versions, refer to Assay Cut-Off in Section A, item 7). A bridging factor established in pre-analytical bridging study#2 (also described in Assay Cut-off in Section A.7) was applied to Abeta42 results (prior to determining reference ranges) to adjust for differences in assay versions and sample pre-analytical handling between Study Part 1 and Study Part 2 (see below). In this cognitively normal reference population, 19.1% (22/115) of the results were above (test-positive) and 81% were below (test-negative) the pTau181/Abeta42 ratio of 0.023. The median, 2.5th, 5th, 95th, and 97.5th percentile of pTau181, Abeta42 and pTau181/Abeta42 for the reference population and the subgroups stratified by sex and age are calculated and shown in the tables below.

Age 66-75 years (N=115)					
	2.5th Percentile	**5th Percentile**	**Median**	**95th Percentile**	**97.5th Percentile**
pTau181 (pg/mL)	8.5	9.6	17.5	31.3	40.9
Abeta42 (pg/mL)	514.1	564.2	1296.0	>2500.0	>2500.0
pTau181/Abeta42^	0.007	0.008	0.012	0.039*	0.047*
^ The ratio values were rounded to 4 decimal places before comparing against 0.023 * Based on values back-calculated using the instrument calibration curve.					

Age 66-70 years (N=43)					
	2.5th Percentile	**5th Percentile**	**Median**	**95th Percentile**	**97.5th Percentile**
pTau181 (pg/mL)	8.0	8.4	15.9	28.5	33.1
Abeta42 (pg/mL)	536.3	583.2	1446.0	>2500.0	>2500.0
pTau181/Abeta42^	0.006	0.007	0.010	0.026	0.035*
Age 71-75 years (N=72)					
	2.5th Percentile	**5th Percentile**	**Median**	**95th Percentile**	**97.5th Percentile**
pTau181 (pg/mL)	10.2	11.6	18.4	35.8	42.8
Abeta42 (pg/mL)	480.1	530.0	1203.5	2292.6	>2500.0
pTau181/Abeta42^	0.008	0.009	0.014	0.043	0.052*
^ The ratio values were rounded to 4 decimal places before comparing against 0.023 * Based on values back-calculated using the instrument calibration curve.					

The 95% reference intervals established in Study Part 1 are as follows: 8.5−40.9 pg/mL for pTau181, 514.1− >2500 pg/mL for Abeta42 and 0.0074−0.0473 for pTau181/Abeta42. Considering the number of females (N=69) and males (N=46) evaluated, the 2.5th and 97.5th percentiles for pTau181/Abeta42 obtained in females (0.007 and 0.050) are relatively close to the corresponding values (0.007 and 0.048) in males. Regarding age, a slight trend of decreasing Abeta42 but increasing pTau181 and pTau181/Abeta42 was observed over the age of 70 years. The 2.5th percentiles for pTau181 and pTau181/Abeta42 in cognitive healthy normal subjects aged 66 to 70 (N=43) were 8.0 g/mL and 0.006, respectively, compared to 10.2 pg/mL and 0.008, respectively, in those aged 71−75 (N=72). The 97.5th percentiles for pTau181 and pTau181/Abeta42 were also higher in the 71−75 years age group (42.8 pg/mL and 0.052, respectively) compared to the 66−70 years age group (33.1 pg/mL and 0.035, respectively). In contrast, the 2.5th and 97.5th percentiles for Abeta42 were lower in the 71−75 years age group (480.1 pg/mL and >2500 pg/mL, respectively) compared to the 66−70 years age group (536.3 pg/mL and >2500 pg/mL, respectively).

2) *Study Part 2 (Prospective study in a U.S. cohort):* Verification of the reference ranges established in Study Part 1 was performed according to CLSI Guideline EP28-A3c. In total,

20 CSF samples were prospectively collected from eligible subjects in the Emory Healthy Brain Study (EHBS) according to the pre-analytical protocol for routine use and measured with the second-generation Elecsys Phospho-Tau (181P) CSF and Elecsys β-Amyloid (1-42) CSF II assays. The subjects (65% females/35% males) aged 60−75 (mean 66.1 ± 4.36) years had a Montreal Cognitive Assessment score 25 or higher[6,7] (mean 28.0 ± 1.59). The table below shows the ranges of pTau181, Abeta42 and pTau181/Abeta42 in the CSF samples. Two of the 20 test results (20%) were above the pTau181/Abeta42 ratio of 0.023.

	Minimum Value	Maximum Value
pTau181 (pg/mL)	8.8	33.7
Abeta42 (pg/mL)	565.8	>2500
pTau181/Abeta42^	0.0057	0.0390*
^ The ratio values were rounded to 4 decimal places before comparing against 0.023 * Based on values back-calculated using the instrument calibration curve.		

For pTau181/Abeta42, 19 out of 20 measurements were within the established reference range from 0.0074−0.0473. One measurement for pTau181/Abeta42 (0.0057) is below the lower 95% reference limit of 0.0074. For $pTau_{181}$ and $A\beta_{1-42}$, all 20 measurements were within the respective reference ranges. Because the verification data met the pre-specified study acceptance criteria (i.e., $n \leq 2$ results fall outside of the reference ranges for pTau181, Abeta42 and pTau181/Abeta42 established in the BioFINDER1 cohort), the reference ranges are verified in the EHBS cohort.

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary, determine its own reference ranges.

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.

[6] Nasreddine et al., (2005) The Montreal Cognitive Assessment, MoCA: A Brief Screening Tool For Mild Cognitive Impairment. J Am Geriatr Soc. 53(4):695-9. doi: 10.1111/j.1532-5415.2005.53221.x.

[7] Trzepacz at al., (2015) Relationship between the Montreal Cognitive Assessment and Mini-mental State Examination for assessment of mild cognitive impairment in older adults. BMC Geriatr. 15:107. doi: 10.1186/s12877-015-0103-3.