



**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY**

I Background Information:

A 510(k) Number

K231348

B Applicant

Roche Diagnostics

C Proprietary and Established Names

Elecsys β -Amyloid (1-42) CSF II

Elecsys Total-Tau 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)

Total Tau

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 Total-Tau CSF are in vitro electrochemiluminescence immunoassays for the measurement of the β -Amyloid (1-42) (Abeta42) and Total-Tau (tTau) 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 tTau/Abeta42 ratio value.

A negative result, defined as tTau/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 tTau/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 tTau/Abeta42 ratio result is used as an adjunct to other clinical diagnostic evaluations.

Limitations of Use

The performance of the tTau/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 Elecsys Total-Tau CSF and Elecsys β -Amyloid (1-42) CSF II and the cobas e 601 immunoassay analyzer. Each assay is packaged in a box individually and sold separately. The Elecsys Total-Tau CSF should be used only with the Elecsys β -Amyloid (1-42) CSF II to calculate the ratio tTau to Abeta42 (tTau/Abeta42). The Elecsys Total-Tau CSF and Elecsys β -Amyloid (1-42) CSF II immunoassays are not intended to be used individually. Every Elecsys reagent set has a barcoded label containing specific information for calibration of the

particular reagent lot. The predefined master curve is adapted to the analyzer using the relevant CalSet.

i) Elecsys Total-Tau CSF

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

Elecsys Total-Tau 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-Tau-Ab~biotin (gray cap), 1 bottle, 6.5 mL	Two biotinylated monoclonal anti-Tau antibodies (mouse), each 1.25 mg/L; Tris* buffer > 14 mmol/L, pH 7.2; preservative
R2	Anti-Tau-Ab~Ru(bpy) ⁺ (black cap), 1 bottle, 6.5 mL	A 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 Total-Tau CSF but sold separately.

- CalSet Total-Tau (4 x 1.0 mL):
Cal1 (2 x 1.0 mL): approx. 30 pg/mL and Cal2 (2 x 1.0 mL): approx. 400 pg/mL
- PreciControl Total-Tau (6 x 1.0 mL):
Ctrl1 (3 x 1.0 mL) : approx. 200 pg/mL and Ctrl2 (3 x 1.0 mL): approx. 500 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 Total-Tau CSF to Elecsys β -Amyloid (1-42) CSF II. The Elecsys β -Amyloid (1-42) CSF II is not intended to be used individually.

Refer to K221842 for description of components in Elecsys β -Amyloid (1-42) CSF II.

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). The 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 Total-Tau and Elecsys β -Amyloid (1-42) CSF II.

2. Elecsys Total-Tau CSF

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

- First incubation: 50 μ L of sample, a biotinylated monoclonal antibody specific for Tau 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.
- Second incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- 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.
- The analyzer automatically calculates the tTau 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

Refer to K221842 for a description of the principle of operation for Elecsys β -Amyloid (1-42) CSF II

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

Results of Elecsys Total-Tau 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 $0.032 < \text{Ratio} < 8.667$ as shown below.

$$\frac{\text{Elecsys Total-Tau CSF/}}{\text{Elecsys } \beta\text{-Amyloid(1-42) CSF II}} = \frac{\text{tTau (results in pg/mL)}/}{\text{Abeta42 (results in pg/mL)}} = \text{a numerical value ranging } 0.032 < \text{Ratio} < 8.667$$

The ratio of tTau to Abeta42 (tTau/Abeta42) must be calculated by the operator because the instrument does not report the final result. The numerical ratio must be compared to the

cutoff of 0.28 The final result (negative or positive) must be interpreted by the laboratory professional according to the table below:

Result	Interpretation
$t\text{Tau}/\text{Abeta}42 \leq 0.28^*$	A negative result consistent with a negative β -Amyloid PET scan result
$t\text{Tau}/\text{Abeta}42 > 0.28^*$	A positive result consistent with a positive β -Amyloid PET scan result
Invalid result for either Elecsys Total-Tau CSF or Elecsys β -Amyloid (1-42) CSF II	Not reportable
Invalid results for both Elecsys Total-Tau CSF and Elecsys β -Amyloid (1-42) CSF II	Not reportable
* The ratio should be rounded to 3 decimal places before comparing against 0.28. 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 tTau concentration is below 80 pg/mL or above 1300 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 β-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):

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

B Predicate 510(k) Number(s):

K221842

C Comparison with Predicate(s):

Device & Predicate Device(s):	Device K231348	Predicate K221842
Device Trade Name	Elecsys β -Amyloid (1-42) CSF II Elecsys Total-Tau CSF	Elecsys β -Amyloid (1-42) CSF II Elecsys Phospho-Tau (181P) CSF
General Device Characteristic Similarities		
Intended Use/Indications For Use	Elecsys β-Amyloid (1-42) CSF II and Elecsys Total-Tau CSF are in vitro electrochemiluminescence immunoassays for the measurement of the β-Amyloid (1-42) (Abeta42) and Total-Tau (tTau) 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 tTau/Abeta42 ratio value. A negative result, defined as tTau/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 tTau/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 tTau/Abeta42 ratio result is used as an adjunct to other clinical diagnostic evaluations. Limitations of Use The performance of the tTau/Abeta42 ratio has not been established for: • Predicting development of dementia or other neurologic conditions • Monitoring responses to therapies	Elecsys β-Amyloid (1-42) CSF II and Elecsys Phospho-Tau (181P) CSF are in vitro electrochemiluminescence immunoassays for the measurement of the β-Amyloid (1-42) (Abeta42) and Phospho-Tau (181P) (pTau181) protein concentrations in 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
Assay Format	Two-step sandwich	Same
Assay Type	Electrochemiluminescence immunoassay (ECLIA)	Same
Sample Type	Human CSF	Same
Sample Volume	50 μ L	Same
Instrument	cobas e 601 analyzer	Same
Reagent Stability	Unopened: 12 months at 2–8 °C After opening: 8 weeks at 2–8 °C On-board: 28 days	Same
Sample Stability	14 days at 2–8 °C 5 days at 15–25 °C 8 weeks at -15 – -25 °C 1 freeze/thaw cycle	Same

Total duration of Assay	18 minutes	Same
General Device Characteristic Differences		
Analyte	Total-Tau and β -Amyloid (1-42)	Phospho-Tau (181P) and β -Amyloid (1-42)
Assay Antibodies	<i>Total-Tau:</i> Two biotinylated monoclonal anti-Tau antibodies (mouse) and monoclonal anti-Tau antibody labeled with ruthenium complex <i>β-Amyloid (1-42):</i> A biotinylated monoclonal anti-β-amyloid (1-42) antibody (mouse) and monoclonal anti-β-amyloid antibody (mouse) labeled with a ruthenium* complex	<i>Phospho-Tau (181P):</i> A biotinylated monoclonal anti-pTau (181P) antibody (rabbit/mouse) and monoclonal anti-Tau antibody labeled with ruthenium complex <i>β-Amyloid (1-42):</i> Same
Calibrators	<i>Total-Tau:</i> CalSet Total-Tau (sold separately): approx. 30 pg/mL (Cal 1) and approx. 400 pg/mL (Cal 2) <i>β-Amyloid (1-42):</i> CalSet β-Amyloid (1-42) II (sold separately): approx. 35 pg/mL (Cal 1) and approx. 500 pg/mL (Cal 2)	<i>Phospho-Tau (181P):</i> CalSet Phospho-Tau (181P) (sold separately): approx. 10 pg/mL (Cal 1) and approx. 70 pg/mL (Cal 2) <i>β-Amyloid (1-42):</i> Same
Controls	<i>Total-Tau:</i> PeciControl Total-Tau (sold separately): approx. 200 pg/mL (Ctrl 1) and approx. 500 pg/mL (Ctrl 2) <i>β-Amyloid (1-42):</i> PeciControl β-Amyloid (1-42) II (sold separately): approx. 500 pg/mL (Ctrl 1) and approx. 1600 pg/mL (Ctrl 2)	<i>Phospho-Tau (181P):</i> PeciControl Phospho-Tau (181P) (sold separately): approx. 15 pg/mL (Ctrl 1) and approx. 50 pg/mL (Ctrl 2) <i>β-Amyloid (1-42):</i> Same
Traceability/ Standardization	<i>Total-Tau:</i> Standardized against a reference method. Calibrator values are based on weighted purified reference tTau material, traceable to National Institute of Standards and Technology (NIST) amino acid reference calibrators <i>β-Amyloid (1-42):</i> Standardized against three certified reference materials: ERM-DA480/IFCC, ERM-DA481/IFCC and ERM-DA482/IFCC	<i>Phospho-Tau (181P):</i> Standardized against a purified reference material Tau(172–205) [pThr181]amide, absolutely quantified via amino acid analysis <i>β-Amyloid (1-42):</i> Same
Measuring Range	<i>Total-Tau:</i> 80–1300 pg/mL <i>β-Amyloid (1-42):</i> 150–2500 pg/mL	<i>Phospho-Tau (181P):</i> 8.0–120 pg/mL <i>β-Amyloid (1-42):</i> Same
No high-dose hook effect	<i>Total-Tau:</i> Up to 3000 pg/mL <i>β-Amyloid (1-42):</i> Up to 6000 pg/mL	<i>Phospho-Tau (181P):</i> Up to 300 pg/mL <i>β-Amyloid (1-42):</i> Same

Detection Limit	<i>Total-Tau:</i> Limit of Blank = 30.0 pg/mL Limit of Detection = 60.0 pg/mL Limit of Quantitation = 80.0 pg/mL <i>β-Amyloid (1-42):</i> Limit of Blank = 50.0 pg/mL Limit of Detection = 100.0 pg/mL Limit of Quantitation = 150.0 pg/mL	<i>Phospho-Tau (181P):</i> Limit of Blank = 4.0 pg/mL Limit of Detection = 8.0 pg/mL Limit of Quantitation = 8.0 pg/mL <i>β-Amyloid (1-42):</i> Same
* Tris(2,2'-bipyridyl)ruthenium(II)-complex (Ru(bpy))		

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 reproducibility of the Elecsys Total-Tau CSF and Elecsys β-Amyloid (1-42) CSF II immunoassays and the ratio of tTau/Abeta42 derived from measurements with these two immunoassays. A panel of human CSF pools was prepared to achieve target concentrations of tTau and Abeta42 that cover the respective measuring ranges of the individual immunoassays. In addition, a second panel of human CSF pools with different tTau/Abeta42 ratio levels (Ratio CSF) that reflect the natural combinations of CSF tTau and Abeta42 in the intended use population was prepared and tested with both immunoassays: one CSF pool with a tTau/ Abeta42 value no more than 20% below the

ratio cut-off, one CSF pool with a tTau/Abeta42 value no more than 20% above the ratio cut-off and three CSF pools with tTau/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 tTau to Abeta42 (*c*):

a. Elecsys Total-Tau CSF

Panel member	Mean (pg/mL)	Within-Run		Between-Run		Between-Day		Within-Laboratory	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
CSF 1	96.8	0.99	1.0	1.4	1.4	0.71	0.7	1.8	1.9
CSF 2	280	4.3	1.5	2.5	0.9	2.0	0.7	5.4	1.9
CSF 3	325	4.0	1.2	3.9	1.2	2.8	0.9	6.3	1.9
CSF 4	345	3.4	1.0	5.0	1.4	12.0	3.5	13.4	3.9
CSF 5	584	9.9	1.7	4.1	0.7	6.9	1.2	12.7	2.2
CSF 6	1210	20.2	1.7	19.3	1.6	50.2	4.1	57.4	4.7
CSF 7	1224	19.3	1.6	17.4	1.4	51.4	4.2	57.6	4.7
PC Level 1	183	2.4	1.3	2.3	1.3	4.22	2.3	5.40	3.0
PC Level 2	454	6.2	1.4	6.7	1.5	4.10	0.9	10.0	2.2
CSF 2, 3 and 4 were native CSF pools. CSF 1 was a diluted CSF pool. CSF 5, 6 and 7 were CSF pools spiked with tTau calibrator peptide. PC=PreciControl									

b. Elecsys β -Amyloid(1-42) CSF II

Refer to K221842 for within-laboratory precision of Elecsys β -Amyloid (1-42) CSF II.

c. tTau/Abeta42 Ratio

Panel member	Mean Ratio*	Within-Run		Between-Run		Between-Day		Within-Laboratory	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
Ratio CSF 1	0.23	0.004	1.8	0.003	1.3	0.001	0.4	0.005	2.2
Ratio CSF 2	0.31	0.005	1.7	0.005	1.6	0.004	1.2	0.008	2.6
Ratio CSF 3	0.43	0.009	2.0	0.008	1.9	0.003	0.7	0.012	2.8
Ratio CSF 4	0.47	0.008	1.6	0.007	1.6	0.005	1.1	0.012	2.5
Ratio CSF 5	0.52	0.010	1.9	0.006	1.1	0.001	0.2	0.011	2.2
Ratio CSF 3 was a native CSF pool. Ratio CSF 1, 2 and 5 were CSF pools spiked with a synthetic A β peptide. Ratio CSF 4 was a CSF pool spiked with tTau calibrator peptide. *The ratio values were rounded to 3 decimal places before comparing against 0.28.									

The ratio of tTau 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 tTau/ Abeta42 results above the ratio cut-off and summarized in the table below.

Panel member	Mean Ratio*	Total Replicates (N)	Qualitative agreement	
			Number of Ratio results >0.28	%Ratio Positive Result
Ratio CSF 1	0.23	84	0	0.0 (0/84)
Ratio CSF 2	0.31	84	84	100 (84/84)
Ratio CSF 3	0.43	84	84	100 (84/84)
Ratio CSF 4	0.47	84	84	100 (84/84)
Ratio CSF 5	0.52	84	84	100 (84/84)
Ratio CSF 3 was a native CSF pool. Ratio CSF 1, 2 and 5 were CSF pools spiked with a synthetic A β peptide. Ratio CSF 4 was a CSF pool spiked with the tTau calibrator peptide. *The ratio values were rounded to 3 decimal places before comparing against 0.28.				

(ii) Lot-to-lot precision

To evaluate between-lot reproducibility, 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 tTau to Abeta42 (*c*):

a. Elecsys Total-Tau CSF

Panel member	Mean (pg/mL)	Within-Run		Between-Run		Between-Day		Between-Lot		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
CSF 1	102	1.0	1.0	1.8	1.8	0.0	0.0	2.3	2.2	3.1	3.1
CSF 2	236	2.4	1.0	4.9	2.1	0.0	0.0	6.2	2.6	8.3	3.5
CSF 3	292	2.7	0.9	5.6	1.9	0.0	0.0	8.8	3.0	10.7	3.7
CSF 4	349	3.5	1.0	7.3	2.1	0.0	0.0	10.9	3.1	13.5	3.9
CSF 5	627	6.6	1.1	13.0	2.1	0.0	0.0	17.3	2.8	22.6	3.6
CSF 6	1180	13.4	1.1	26.2	2.2	0.0	0.0	46.7	4.0	55.2	4.7
PC Level 1	195	2.1	1.1	3.8	1.9	0.0	0.0	5.2	2.7	6.8	3.5
PC Level 2	491	5.1	1.0	9.4	1.9	0.0	0.0	16.5	3.4	19.7	4.0
CSF 2, 3 and 4 were native CSF pools. CSF1 was a diluted CSF pool. CSF 5, 6 and 7 were CSF pools spiked with the tTau calibrator peptide. PC=PreciControl											

b. Elecsys β -Amyloid(1-42) CSF II

Refer to K221842 for lot-to-lot precision of Elecsys β -Amyloid(1-42) CSF II.

c. tTau/Abeta42 Ratio

Panel member	Mean Ratio*	Within-Run		Between-Run		Between-Day		Between-Lot		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Ratio CSF 1	0.24	0.003	1.3	0.005	2.0	0.002	1.0	0.014	6.0	0.016	6.5
Ratio CSF 2	0.31	0.005	1.6	0.005	1.6	0.0	0.0	0.020	6.5	0.021	6.9
Ratio CSF 3	0.46	0.006	1.2	0.005	1.2	0.004	1.0	0.037	7.9	0.038	8.1
Ratio CSF 4	0.43	0.006	1.3	0.007	1.7	0.0	0.0	0.031	7.4	0.033	7.7
Ratio CSF 5	0.42	0.005	1.2	0.008	1.8	0.003	0.7	0.026	6.2	0.028	6.6
Ratio CSF 3 was a native CSF pool. Ratio CSF 1, 2 and 4 were CSF pools spiked with a synthetic Tau peptide. Ratio CSF 5 sample was a CSF pool spiked with the tTau calibrator peptide and Abeta42peptide. *The ratio values were rounded to 3 decimal places before comparing against 0.28.											

The ratio of tTau 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 tTau/Abeta42 results above the ratio cut-off and summarized in the table below.

Panel member	Mean Ratio*	Total Replicates (N)	Qualitative agreement	
			Number of ratio results >0.28	%Ratio Positive Result
Ratio CSF 1	0.24	90	90	0.0 (0/90)
Ratio CSF 2	0.31	90	84	93.3 (84/90)
Ratio CSF 3	0.46	90	90	100 (90/90)
Ratio CSF 4	0.43	90	90	100 (90/90)
Ratio CSF 5	0.42	90	90	100 (90/90)
Ratio CSF 3 was a native CSF pool. Ratio CSF 1, 2 and 4 were CSF pools spiked with a synthetic Tau peptide. Ratio CSF 5 sample was a CSF pool spiked with the tTau calibrator peptide and Abeta42 peptide.				
*The ratio values were rounded to 3 decimal places before comparing against 0.28.				

(iii) Site-to-site reproducibility:

To evaluate between-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 5 days at three sites with one cobas e 601 analyzer at each site, using one reagent lot (N=90 per sample or 150 replicates per site per day). 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 tTau to Abeta42 (*c*) :

a. Elecsys Total-Tau CSF

Panel member	Mean (pg/mL)	Within-Run		Between-Run		Between-Day		Between-Site		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
CSF 1	102	1.4	1.4	0.5	0.5	1.8	1.8	0.0	0.0	2.4	2.3
CSF 2	259	2.0	0.8	1.8	0.7	3.6	1.4	1.8	0.7	4.8	1.9
CSF 3	316	3.3	1.1	0.6	0.2	4.6	1.5	3.3	1.1	6.6	2.1
CSF 4	385	4.8	1.3	3.5	0.9	6.1	1.6	4.0	1.0	9.4	2.4

Panel member	Mean (pg/mL)	Within-Run		Between-Run		Between-Day		Between-Site		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
CSF 5	662	7.5	1.1	4.5	0.7	10.1	1.5	9.7	1.5	16.5	2.5
CSF 6	1248	15.5	1.2	11.1	0.9	15.6	1.2	18.5	1.5	30.8	2.5
CSF 7	1270	16.9	1.3	13.0	1.0	20.2	1.6	26.0	2.0	39.2	3.1
PC Level 1	197	3.2	1.6	1.8	0.9	3.4	1.7	1.7	0.9	5.3	2.7
PC Level 2	492	7.5	1.5	3.2	0.7	8.0	1.6	6.5	1.3	13.2	2.7
CSF 2, 3 and 4 were native CSF pools. CSF1 was a diluted CSF pool. CSF 5, 6 and 7 were CSF pools spiked with the tTau calibrator peptide. PC=PreciControl											

b. Elecsys β -Amyloid(1-42) CSF II

Refer to K221842 for site-to-site reproducibility of Elecsys β -Amyloid(1-42) CSF II.

c. tTau/Abeta42 Ratio

Panel member	Mean Ratio*	Within-Run		Between-Run		Between-Day		Between-Site		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Ratio CSF 1	0.24	0.004	1.7	0.005	2.1	0.003	1.3	0.005	2.0	0.009	3.6
Ratio CSF 2	0.38	0.010	2.7	0.010	2.6	0.006	1.7	0.007	1.9	0.017	4.5
Ratio CSF 3	0.47	0.006	1.3	0.007	1.4	0.002	0.4	0.007	1.5	0.012	2.5
Ratio CSF 4	0.49	0.010	1.9	0.005	0.9	0.002	0.3	0.004	0.8	0.011	2.3
Ratio CSF 5	0.51	0.010	1.9	0.007	1.4	0.0	0.0	0.005	1.0	0.013	2.5
Ratio CSF 6	0.30	0.004	1.2	0.005	1.7	0.002	0.8	0.007	2.3	0.010	3.2
Ratio CSF 3 was a native CSF pool. Ratio CSF 1, 2 and 4 were CSF pools spiked with a synthetic Tau peptide. Ratio CSF 5 sample was a CSF pool spiked with the tTau calibrator peptide and Abeta42 peptide. *The ratio values were rounded to 3 decimal places before comparing against 0.28.											

The ratio of tTau 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 tTau/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 >0.28	%Ratio Positive Result
Ratio CSF 1	0.24	90	0	0.0 (0/90)
Ratio CSF 2	0.38	90	90	100 (90/90)
Ratio CSF 3	0.47	90	90	100 (90/90)
Ratio CSF 4	0.49	90	90	100 (90/90)
Ratio CSF 5	0.51	90	90	100 (90/90)
Ratio CSF 6	0.30	90	90	100 (90/90)
Ratio CSF 3 was a native CSF pool. Ratio CSF 1, 2 and 4 were CSF pools spiked with a synthetic Tau peptide. Ratio CSF 5 sample was a CSF pool spiked with the tTau calibrator peptide and Abeta42 peptide *The ratio values were rounded to 3 decimal places before comparing against 0.28.				

(iv) tTau/Abeta42 Precision Simulation:

The within-laboratory (intermediate) precision was evaluated with only two tTau to Abeta42 combinations within the ratio cut-off range. Because different combinations of tTau 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 tTau/Abeta42 ratio values corresponding to a broad range of hypothetically possible tTau and Abeta42 combinations and (ii) compare the simulated results with the acceptance criteria for the intermediate precision and repeatability defined for the tTau/Abeta42 ratio. The variance profile of panel members tested for within-laboratory and within-run precision were considered for simulation.

- The simulated values of the intermediate imprecision results in terms of %CV ranged between 2.18 and 5.00% for all simulated ratio values. For ratio values within the cut-off range ($0.28 \pm 20\%$), the intermediate imprecision (in %CV) ranged between 2.30 and 4.56%. The intermediate imprecision met the acceptance criteria.
- The simulated values of the with-run imprecision results in terms of %CV ranged between 1.25 and 2.43% for all simulated ratio values. For ratio values within the cut-off range ($0.28 \pm 20\%$), the intermediate imprecision (in %CV) ranged between 1.33 and 2.33%. The within-run precision also met the acceptance criteria.

2. Linearity

The linearity of Elecsys Total-Tau CSF on the cobas e 601 analyzer was evaluated in accordance with the CLSI guideline EP06-Ed2. Three high native CSF pools with tTau concentrations of 1571 pg/mL, 1514 pg/mL and 1550 pg/mL were each diluted with an analyte-depleted CSF sample to create a series of 13 dilutions of tTau concentrations that

span across the measuring range. Each sample dilution was measured in one run with three replicates using one reagent lot. 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. For the pooled analysis based on the entire data set, percent deviations from linearity were within $\pm 15\%$.

Assay	Range (pg/mL)	Slope (95%CI)	Intercept (95% CI)	R ²
Elecsys Total-Tau CSF	3.4 – 1610	1.01 (0.99 – 1.02)	0.95 (-0.53 – 2.44)	0.998

Linearity results support the measuring range claim of 80.0 pg/mL – 1300 pg/mL for Elecsys Total-Tau CSF.

Refer to K221842 for linearity results and measuring range claim for Elecsys β -Amyloid(1-42) CSF II.

High-Dose Hook Effect

The high-dose hook effect was evaluated for Elecsys Total-Tau CSF using three reagent lots. To determine the hook concentration, a dilution series of two native CSF samples spiked with a high concentration stock of tTau calibrator peptide to reach concentrations above the specified hook concentration was measured on one cobas e 601 analyzer. The spiked samples were diluted with a CSF sample depleted of tTau 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 3000 pg/mL for tTau.

Refer to K221842 for high dose hook effect for Elecsys β -Amyloid(1-42) CSF II.

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 tTau concentrations covering the measuring range (i.e., lower range, mid-range and upper range). A CSF pool with tTau/Abeta42 value no more than 20% below or no more than 20% above the ratio cut-off was also included. Analyte spiking (tTau calibrator peptide) was utilized for the two highest levels of tTau 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 reference sample) without

interferent was spiked with the respective amount of solvent used to create the interfering substances panel. The tTau levels in the test and reference samples were measured in five replicates in the same run on one cobas e 601 analyzer. The recovery of each analyte was calculated by determining the percent differences between the test and reference samples.

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 ($\leq 10\%$ difference from the reference sample) for Elecsys Total-Tau CSF and the ratio of tTau/Abeta42 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 3600.0 ng/mL¹ and % interference was from -2.5% to +16%. The specification in the labeling sets a value of 1200.0 mg/L. [#] 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 tTau/Abeta42 value within 20% above or below the ratio cut-off. [%] Rheumatoid factor was tested at 12.0 IU/mL. The data supports no interference up to 12.0 IU/mL. However, the specification in the labeling sets a conservative value of 4.0 IU/mL.	

Exogenous Interferent	Interferent Concentration*
Acetaminophen	156.0 mg/L
Acetylcysteine	150.0 mg/L
Acetylsalicylic Acid	30.0 mg/L
Ampicillin-Na	75.0 mg/L
Ascorbic Acid	52.5 mg/L

¹ FDA Guidance: Testing for Biotin Interference in In Vitro Diagnostic Devices (available at <https://www.fda.gov/media/127915/download>)

Exogenous Interferent	Interferent Concentration*
Atorvastatin	0.75 mg/L
Cefoxitin	750.0 mg/L
Cyclosporine	1.8 mg/L
Digoxin	0.04 mg/L
Donepezil	30.0 mg/L
Doxycycline	18.0 mg/L
Escitalopram	0.19 mg/L
Esomeprazole	6.9 mg/L
Furosemide	15.9 mg/L
Galantamine	250 mg/L
Heparin	495 IU/L
Hydrochlorothiazide	1.1 mg/L
Ibuprofen	219.0 mg/L
Itraconazol	0.06 mg/L
Levodopa	7.5 mg/L
Lisinopril	0.25 mg/L
Memantine	0.12 mg/L
Metformin	12.0 mg/L
Methyldopa	22.5 mg/L
Metronidazole	123.0 mg/L
Metoprolol	1.5 mg/L
Phenylbutazone	107.0 mg/L
Rifampicin	48.0 mg/L
Rivastigmine	45.0 mg/L
Simvastatin	1.7 mg/L
Theophylline	60.0 mg/L
*Recommended test concentrations were based on CLSI EP37 and the scientific literature	

Refer to K221842 for interference testing with Elecsys β -Amyloid(1-42) CSF II.

An analysis was conducted to further evaluate the expected influence of each potential interfering substance on the tTau/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 empirical results observed in the interference testing for single biomarkers. For each interfering substance concentration and each of the nine tTau and Abeta42 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}] x 100%. The results showed that for all tTau181 and Abeta42 combinations, the %Interferences were within $\pm 10\%$ for all endogenous and exogenous interfering substances listed in the tables above, except for biotin (at 3600 ng/mL) which exhibited %Interference value up to +16%.

(ii) Cross-reactivity

A BLAST search revealed that the highest sequence identity between Tau-441 (longest human tau isoform) and human non-Tau proteins was observed for microtubule

associated protein 2 (MAP2; 53.9% sequence identity) and microtubule associated protein 4 (MAP4; 63.1% sequence identity). Both MAP sequences were aligned with Tau-441 and the epitopes of the Tau specific antibodies that are used in the Elecsys Total-Tau CSF Assay. The alignment showed that all three epitopes are not present in the MAP2 and MAP4 proteins. No potential cross reactants were identified for the Elecsys Total-Tau CSF Assay.

Refer to K221842 for cross-reactivity testing of A β peptides representing A β isoforms of different lengths with Elecsys β -Amyloid(1-42) CSF II.

To assess potential cross-reactivity of tTau/Abeta42 ratio, a Ratio CSF sample pool within 20% above or below the ratio cut-off (0.28) was spiked with two Abeta42 species, Abeta38 and Abeta40, representing Abeta isoforms of different lengths. The 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 the potential cross-reactive Abeta species tested was 10,000 pg/mL) which is two-fold higher than the reported concentration of A β ₁₋₄₀, the major species of Abeta found in the brain². To assess cross-reactivity at varying 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 reference (unspiked) pool. The percentage of cross-reactivity was calculated using the following formula:

$$\% \text{ Cross-reactivity} = \left[\frac{(\text{mean of cross-reactant sample} - \text{mean of reference sample})}{(\text{cross-reactant concentration})} \right] \times 100$$

The %cross-reactivity for each cross-reactant tested at five different concentrations in the Ratio CSF sample pool is summarized below:

		Ratio CSF sample pool within 20% of the ratio cut-off	
Cross-reactants	Cross-reactant concentration	Measured Ratio*	% Cross-reactivity
Abeta38	0.0	0.275	—
	2500.0	0.277	0.0 x 10 ⁻⁴
	5000.0	0.281	0.1 x 10 ⁻⁴
	7500.0	0.284	0.1 x 10 ⁻⁴
	10000.0	0.285	0.1 x 10 ⁻⁴
Abeta40	0.0	0.281	—
	2500.0	0.280	0.0 x 10 ⁻⁴
	5000.0	0.282	0.0 x 10 ⁻⁴
	7500.0	0.286	0.1 x 10 ⁻⁴
	10000.0	0.291	0.1 x 10 ⁻⁴

*The ratio values should be rounded to 3 decimal places before comparing against 0.28

The ratio of tTau to Abeta42 was not significantly impacted (within $\pm 1\%$ difference of test from control sample) by the cross-reactive Abeta species up to 10000 pg/mL.

² Korecka M, *et al.*, 2020. Analytical and Clinical Performance of Amyloid-Beta Peptides Measurements in CSF of ADNIGO/2 Participants by an LC-MS/MS Reference Method. Clin Chem. ;66(4):587-597.

iii) Inclusivity

Antibody epitope–target sequence alignments showed that the antibodies used in Elecsys Total-Tau CSF bind to epitopes which are present in all six human tau isoforms. Surface plasmon resonance experiments demonstrated that all antibodies showed significant binding to all six human tau isoforms. All antibodies also showed affinity to the Tau 156-227 peptide that represents a truncated Tau found in CSF³ and detected high molecular weight oligomerized/aggregated Tau forms⁴.

4. Assay Reportable Range

Elecsys Total-Tau CSF: 80 – 1300 pg/mL

Elecsys β -Amyloid (1-42): 150 – 2500 pg/mL (Refer to K221842)

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

1) Traceability

The Elecsys Total-Tau CSF assay has been standardized against a reference method. Calibrator values are based on weighted purified reference tTau material, traceable to NIST amino acid reference calibrators. The purified reference material was absolutely quantified via amino acid analysis.

Refer to K221842 for traceability of Elecsys β -Amyloid(1-42) CSF II.

2) Stability

a) Calibration

(i) Lot Calibration Stability

A panel of seven CSF samples with tTau levels (102–1072 pg/mL) covering the measuring range was generated for lot calibration testing. A fresh reagent Rackpack was placed on the analyzer and calibrated. Reference values for the samples tested were determined in two runs and in duplicates to obtain a robust reference at day 0. On day 36 (5 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 Elecsys Total-Tau CSF Rackpack of the same reagent lot.

Refer to K221842 for lot calibration stability of Elecsys β -Amyloid (1-42) CSF II.

³ Kanmert *et al.* 2015 C-Terminally Truncated Forms of Tau, But Not Full-Length Tau or Its C-Terminal Fragments, Are Released from Neurons Independently of Cell Death. *The Journal of Neuroscience*, 35:10851–10856

⁴ Lasagna-Reeves *et al.*, 2012 Identification of oligomers at early stages of tau aggregation in Alzheimer's disease. *FASEB J* 26; 1946–1959

(ii) On-Board Calibration Stability

A panel of seven CSF samples with tTau levels (102 –1072 pg/mL) covering the measuring range was generated for onboard calibration stability testing. A fresh reagent Rackpack was placed on the analyzer and calibrated. Reference values for the samples tested were determined. The same samples were retested after 8 days with reagent bottles kept at $20^{\circ}\text{C} \pm 3^{\circ}\text{C}$ (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 Elecsys Total-Tau CSF Rackpack reagent kit on the analyzer.

Refer to K221842 for on-board calibration stability of the Elecsys β -Amyloid (1-42) CSF II.

b) Reagent

(i) Shelf-life Stability

Reagent shelf-life stability of the Elecsys Total-Tau CSF Rackpack was determined on one cobas e 601 analyzer using three reagent lots. A panel of six CSF samples with tTau levels (161–1293 pg/mL) covering the measuring ranges was generated from native human CSF. To determine a robust reference value at time point $t=0$, the samples were measured in two independent runs and with double determination on a cobas e 601 analyzer. The median value from each sample at $t=0$ was calculated and set as a reference value. For the subsequent time points 6, 9, 12 and 24 months, a new calibration was established, and the samples were measured in one run in duplicates. For the 6-, 9-, 12- and 15-months' time points, a safety margin of +14 days was added to the measurement timing. For the 24-month time point, a safety margin of +1 month was added to the measurement timing. At each time point, 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 $\pm 10\%$. The results support that the Elecsys Total-Tau CSF Rackpack reagent kit can be stored unopened for up to 24 months when stored at $2-8^{\circ}\text{C}$.

Refer to K221842 for shelf-life stability of Elecsys β -Amyloid (1-42) CSF II.

(ii) Stability after first opening:

Reagent stability after first opening for the Elecsys Total-Tau CSF Rackpack was tested using one reagent lot on one cobas e 601 analyzer. A panel of seven CSF samples with tTau levels (102–1072 pg/mL) covering the measuring ranges was generated for reagent stability after first opening testing. A fresh reagent kit was placed on the analyzer and calibrated. Reference values for the samples tested were determined on day 0 in two runs to generate a more stable reference value at $t=0$. After the initial measurement, the reagent kit was removed from the analyzer and kept at $2-8^{\circ}\text{C}$. 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 tested were within $\pm 10\%$. The results support that the Elecsys Total-Tau CSF Rackpack reagent kit can be used after first opening for up to 8 weeks when stored at $2-8^{\circ}\text{C}$.

Refer to K221842 for stability after first opening of Elecsys β -Amyloid (1-42) CSF II.

(iii) On-Board Stability

Reagent on-board stability for the Elecsys Total-Tau CSF Rackpack was tested on one cobas e 601 analyzer using one reagent lot. A panel of six CSF samples with tTau levels (102–1072 pg/mL) covering the measuring range was generated for reagent on-board stability testing. A fresh reagent kit was placed on the analyzer and calibrated. Reference values for the samples tested were determined (day 0). Three more reagent kits were opened for approx. 1 hour, closed, and then stored at $20^{\circ}\text{C} \pm 3^{\circ}\text{C}$ (on-board conditions). After 8, 22 and 29 days the kit was placed on the analyzer again, calibrated and the test samples were determined. Each following Rackpack was opened for the duration of the testing as well. Samples were tested in duplicates. The mean value was used to calculate the absolute deviation or percent recovery, respectively, compared to the sample concentration obtained at day 0. All samples tested were within $\pm 10\%$. The results support that the Elecsys Total-Tau CSF Rackpack reagent kit can be stored on-board of the analyzer for up to 28 days.

Refer to K221842 for on-board stability of Elecsys β -Amyloid (1-42) CSF II.

(iv) On-Board In Use Stability

On-board stability for an open Rackpack was determined using one reagent lot on a cobas e 601 analyzer. A panel of six CSF samples with tTau levels (102–1072 pg/mL) covering the measuring range was generated for reagent stability testing. A fresh Rackpack was placed on a cobas e 601, calibrated and measured ($t=0$) and left open on the instrument for 26 hours and then measured again. Sample concentration was read off the same calibration curve. The absolute and relative recoveries of the determined sample concentrations ($t=26$ hours) to the reference ($t=0$) were calculated. All samples tested were within specifications. The Elecsys Total-Tau CSF Rackpack reagent kits can be left opened and stored on-board of the analyzer for 25 hours.

Refer to K221842 for on-board in use stability of Elecsys β -Amyloid (1-42) CSF II.

(v) Transport Stability

Reagent transport stability for the Elecsys Total-Tau CSF Rackpack was tested in one run using one reagent lot on a cobas e 601 analyzer. A panel of seven CSF samples with tTau levels (102–1072 pg/mL) covering the measuring ranges was generated for transport stress testing. The reagent was stressed in the original container at $35^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 192 hours (8 days) in a drying cabinet. The unstressed reference kit was kept at $2-8^{\circ}\text{C}$. Samples were determined in

duplicates and the median values were determined and the absolute or relative deviation from the values obtained with the unstressed reference were calculated. The Elecsys Total-Tau CSF assay Rackpack reagent kit can be stored at 35°C for up to 168 hours (7 days).

Refer to K221842 for transport stability of Elecsys β -Amyloid (1-42) CSF II.

c) Specimen

(i) Specimen storage stability at 15–25°C

A panel of 13 CSF samples with varying tTau levels were freshly collected in accordance with the routine-use pre-analytical procedure for fresh CSF samples described in the package insert of Elecsys Total-Tau CSF. Most of the samples were from patients representing the intended use population. The panel included at least one sample in each of the low (<150 pg/mL), medium (150–250 pg/mL), and high (>250 pg/mL) concentration range of tTau and at least two samples with a ratio value of tTau/A β ₁₋₄₂ near ($\pm$ 20%) the cut-off (0.28). After the T0 baseline measurements (T0=within 6 hours after the lumbar puncture) of five aliquots per sample, all samples were placed into storage at 25°C within 2 hours. At the defined T1 (1–3 days of storage after the baseline measurements) and T2 (6–8 days after the baseline measurements), at least two aliquots of each sample were re-measured. All samples tested at T1 were within specifications for tTau. The recommended sample storage duration at 15–25°C is 5 days.

Refer to K221842 for stability of Abeta42 in specimens stored at 15–25°C.

(ii) Specimen storage stability at 2–8°C

A panel of 15 CSF samples with varying tTau levels as described above for the sample panel tested for storage stability at 15–25°C were freshly collected in accordance with the routine-use pre-analytical procedure. Most of the samples were from patients representing the intended use population. After the T0 baseline measurements (T0=within 6 hours after the lumbar puncture) of five aliquots per sample, all samples were placed into storage at 2–8°C within 2 hours. Two aliquots per sample were re-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 T3 (13–15 days after the baseline measurements). For samples where only four aliquots were employed, T2 was not measured. All samples tested at T1, T2 and T3 were within specifications for tTau. The recommended sample storage duration at 2–8°C is 14 days in the package inserts of Elecsys Total-Tau CSF.

Refer to K221842 for stability of Abeta42 in specimens stored at 2–8°C.

(iii) Specimen storage stability at -20°C $\pm$ 5°C and stability after freeze and thaw:

A panel of 42 CSF samples with varying tTau and Abeta42 levels were freshly collected in accordance with the routine-use pre-analytical procedure for fresh CSF samples described in the package insert of Elecsys Total-Tau CSF and Elecsys β -Amyloid (1-42) CSF II. Most of the samples were from patients

representing the intended use population. After the T0 baseline measurements (T0=within 6 hours after the lumbar puncture) of five aliquots per sample, samples were frozen at $-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$ within 2 hours. At the defined T1 (at least 5 weeks and maximally 8 weeks after freezing) and T2 (frozen for at least 13 weeks), two and three aliquots of each sample were measured. For measurements at T1 and T2, the respective aliquots were thawed at room temperature for 30 minutes, followed by roller-mixing for 20 minutes, before being placed on the analyzer. The measurements obtained at T1 was compared with those at T0 to show freeze-thaw stability. Comparison of measurements obtained at T2 with that at T0 showed storage stability at $-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$. All samples tested were within specifications and support stability of tTau and Abeta42 after one freeze/thaw cycle and storage for up to 12 weeks at $-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$. The recommended sample storage duration is 8 weeks at $-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$ with one freeze/thaw cycle in the package inserts of Elecsys Total-Tau 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 Total-Tau 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 Total-Tau CSF

For LoB, a CSF sample depleted of tTau 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 4.0, 3.9 and 4.0 pg/mL. The claimed LoB is 30.0 pg/mL.

For LoD, five low-level CSF samples with target concentrations of tTau 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 5.5, 5.5 and 6.1 pg/mL. The claimed LoD is 60.0 pg/mL.

For LoQ, six low-level CSF samples with target concentrations of tTau close to the specified LoQ (from 34.6 to 154 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 tTau which can be quantified with an intermediate precision of no more than 20 %CV. The LoQ values from the three lots were 34.6, 34.0 and 35.6 pg/mL. The claimed LoQ is 80.0 pg/mL.

(ii) Elecsys β -Amyloid (1-42) CSF II

Refer to K221842 for LoB, LoD and LoQ of Elecsys β -Amyloid (1-42) CSF II.

7. Assay Cut-Off

1) *Determination of the tTau/Aβ₁₋₄₂ ratio cut-off for distinguishing amyloid PET positive and amyloid PET negative patients by visual read*

The tTau/Abeta42 ratio cut-off was defined based on the first-generation Elecsys Total-Tau CSF and Elecsys β-Amyloid (1-42) CSF 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 BioFINDER 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 [¹⁸F]-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 0.26 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% (100/110, 95% CI: 83.9% – 95.6%)
- Negative Percent Agreement (NPA) 89.2% (149/167, 95% CI: 83.5% – 93.5%)
- Overall Percent Agreement (OPA) 89.9% (249/277, 95% CI: 85.7% – 93.2%)

2) *Adjustment of the tTau/Aβ₁₋₄₂ ratio cut-off for amyloid positivity due to CSF pre-analytical differences (Pre-analytical bridging study #1)*

Due to the susceptibility of Abeta42 to the use of different pre-analytical protocols for the handling of CSF⁵, 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 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=20 for tTau and N=17 for Abeta42). The CSF samples were handled according to the BioFINDER and ADNI pre-analytical handling protocols. Results showed that no meaningful systematic differences were observed for CSF tTau measurements [1.60 % (95 % CI: -0.54% – 1.75%, p = 0.285)]. 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

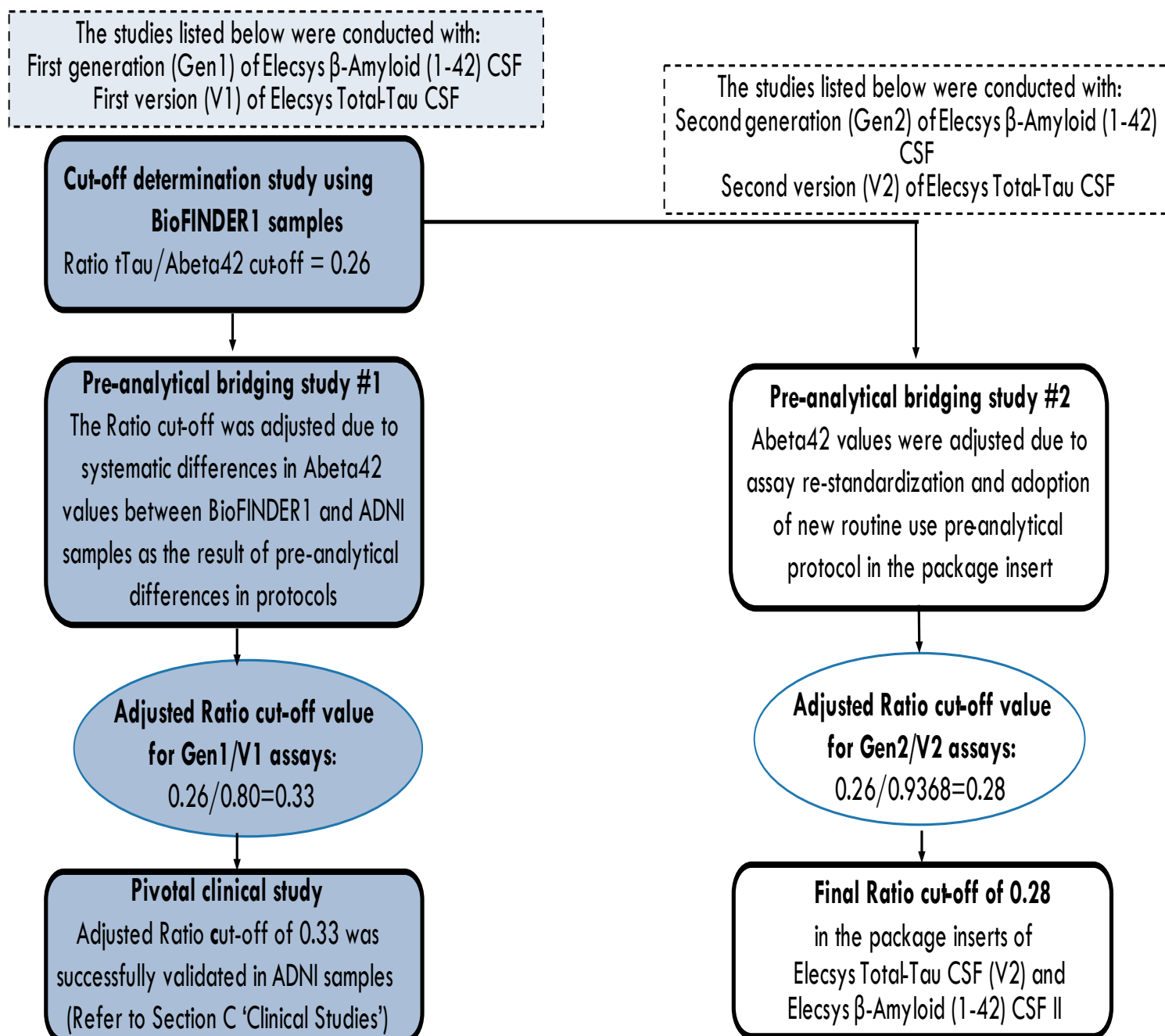
⁵ Vanderstichele H *et al.*, (2012) Standardization of preanalytical aspects of cerebrospinal fluid biomarker testing for Alzheimer's disease diagnosis: a consensus paper from the Alzheimer's Biomarkers Standardization Initiative. *Alzheimers Dement.* 8(1):65-73.

BioFINDER was used to define the conversion factor for Abeta42 ($\text{Abeta42 [ADNI]} = 0.8 \times \text{Abeta42 [BioFINDER]}$) and to adjust the tTau/Abeta42 cut-off from 0.26 to 0.33 ($0.26 \times (1/0.8) = 0.33$). Results from this study showed that the systematic difference in preanalytical 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 (see below).

3) *Adjustment of the tTau/ $\text{A}\beta_{1-42}$ ratio cut-off due to assay upgrades and adoption of final CSF pre-analytical handling (Pre-analytical bridging study #2).*

Compared with the corresponding first-generation assay, Elecsys β -Amyloid (1-42) CSF II 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 Total-Tau CSF. 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=24 for tTau 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 BioFINDER protocol and measured using the first-generation assays. The BioFINDER cohort was utilized for setting the tTau/Abeta42 cut-off as described above under 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. No meaningful differences (< 3%) were obtained for tTau 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 tTau/Abeta42 ratio cut-off defined in the BioFINDER1 cohort. The adjusted ratio cut-off is $0.26 \times (1/0.9368) = 0.28$ 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 tTau/Abeta42 ratio cut-off was pre-specified and validated using retrospectively collected CSF samples in the Alzheimer's Disease Neuroimaging Initiative studies, ADNI-GO and ADNI2. All patients enrolled into ADNI2 and ADNIGO with baseline CSF sample and PET image available were considered eligible. Eligibility criteria were not reassessed, with the exception of 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 Total-Tau CSF were used for the cut-off 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 [%]
SMC	25.5
EMCI	39.3
LCMI	67.1
AD	89.1
SMC=significant memory concern, EMCI=early mild cognitive impairment, LCMI=late mild cognitive impairment, AD=Alzheimer's disease	

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)	LCMI = 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

Ethnicity	Hispanic/ Latino	1.1% (1)	4.4% (12)	1.3% (2)	4.7% (6)	3.2% (11)	3.3% (10)	3.3% (21)
	Not Hispanic/ Latino	96.8% (91)	95.2% (259)	98.7% (150)	94.5% (121)	96.3% (334)	96.0% (287)	96.1% (621)
	Unknown	2.1% (2)	0.4% (1)	0% (0)	0.8% (1)	0.6% (2)	0.7% (2)	0.6% (4)
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)	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)
	18-23	0% (0)	0.4% (1)	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 0.33 are summarized in the table below.

		Visual Amyloid PET Read		
		Positive	Negative	Total
Elecsys Total Tau CSF and Elecsys β-Amyloid (1-42) CSF II	Positive (Ratio result>0.33)	295	18	313
	Negative (Ratio result ≤0.33)	52	281	333
	Total	347	299	646
Performance Measures		Point Estimates % (95% CI)		
Positive Percent Agreement (PPA)		85.0 (295/347) (80.9 – 88.4)*		
Negative Percent Agreement (NPA)		94.0 (281/299) (90.7 – 96.2)*		
Total Percent Agreement (TPA)		89.2 (576/646) (86.5 – 91.3)*		
Prevalence		53.7 (347/646) (59.9 – 57.5)*		
Positive Predictive Value (PPV)		94.2 (295/313) (91.3 – 96.3)**		
Negative Predictive Value (NPV)		84.4 (281/333) (80.8 – 87.4)**		
Positive Likelihood Ratio (LR+)		14.1 (9.0 – 22.1)***		
Negative Likelihood Ratio (LR-)		0.16 (0.12 – 0.21)***		
* 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 a PET scan positive result, 295 also had a positive tTau/Abeta42 ratio result. The positive percent agreement (PPA) was 85.0% (295/347) with 95% CI: 80.9 – 88.4. The remaining 52 PET scan positive subjects had a negative tTau/Abeta42 ratio result. The rate of false negative (FN) results was 15.0% (52/347). Of the 299 subjects with a PET scan negative result, 277 also had a negative tTau/Abeta42 ratio result. The negative percent

agreement (NPA) was 94.0% (281/299) with a 95% CI: 90.7 – 96.2. The remaining 18 PET scan negative subjects had a positive tTau/Abeta42 ratio result. The rate of false positive (FP) results was 6.0% (18/299). The total percent agreement (TPA) was 89.2% (576/646). The number of cases with discordant CSF status compared to visual PET assessments was 70 (10.8%). The Positive Predictive Value (PPV) was 94.2% with a 95% CI: 91.3 – 96.3 and the Negative Predictive Value (NPV) was 84.4% with a 95% CI: 80.8 – 87.4 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 Total-Tau 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)	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)	62.5 (15/24) (42.7 – 78.8)	72.9 (78/107) (63.8 – 80.4)	89.2 (91/102) (81.7 – 93.9)	97.4 (111/114) (92.5 – 99.1)
NPA (n/N) (95% CI)	92.9 (65/70) (84.3 – 96.9)	96.4 (159/165) (92.3 – 98.3)	90.0 (45/50) (78.6 – 95.7)	85.7 (12/14) (60.1 – 96.0)
TPA (n/N) (95% CI)	85.1 (80/94) (76.5 – 90.9)	87.1 (237/272) (82.6 – 90.6)	89.5 (136/152) (83.6 – 93.4)	96.1 (123/128) (91.2 – 98.3)
PPV (n/N) (95% CI)	75.0 (15/20) (55.2 – 88.1)	92.9 (78/84) (85.5 – 96.6)	94.8 (91/96) (88.8 – 97.7)	98.2 (111/113) (95.1 – 99.5)
NPV (n/N) (95% CI)	87.8 (65/74) (81.1 – 92.4)	84.6 (159/188) (80.2 – 88.0)	80.4 (45/56) (70.0 – 87.5)	80.0 (12/15) (57.7 – 92.5)
LR+ (95% CI)	8.8 (3.6 – 21.5)	20.1 (9.1 – 44.4)	8.9 (3.9 – 20.6)	6.8 (2.4 – 24.6)
LR- (95% CI)	0.40 (0.24 – 0.68)	0.28 (0.21 – 0.38)	0.12 (0.07 – 0.21)	0.03 (0.01 – 0.09)

*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) are determined using the Wechsler Memory Scale Logical Memory II.

Across diagnostic groups, the estimates of PPA and TPA increase as the disease progresses from SMC (62.5% and 85.1%) to AD (97.4% and 96.1%). The estimate of NPA was slightly higher in EMCI (96.46%) and SMC (92.9%) compared to LMCI (90.0%) and AD (85.7%). The estimate of PPV also increases from SMC (75.02%) to AD (98.2%) whereas NPV was higher in SMC (87.8%) compared to EMCI (84.6%), LMCI (80.4%), and AD (80.0%). By contrast, the estimate of LR- decreases from SMC (0.40) to AD (0.03) and the estimate of LR+ was higher in EMCI (20.1) compared to AD (6.8), SMC (8.8) and LMCI (8.9).

b. Agreement with visual Amyloid PET by sex

The clinical performance measures of the ratio results generated from the measurements with Elecsys Total-Tau 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)	193 (55.5) (50.2 – 60.6)	154 (51.7) (46.0 – 57.3)
PPA (n/N) (95% CI)	82.9 (160/193) (77.0 – 87.6)	87.7 (135/154) (81.5 – 92.0)
NPA (n/N) (95% CI)	94.2 (146/155) (89.3 – 96.9)	93.8 (135/144) (88.5 – 96.7)
TPA (n/N) (95% CI)	87.9 (306/348) (84.1 – 90.9)	90.6 (270/298) (86.8 – 93.4)
PPV (n/N) (95% CI)	94.7 (160/169) (90.4 – 97.1)	93.8 (135/144) (88.8 – 96.6)
NPV (n/N) (95% CI)	81.6 (146/179) (76.3 – 86.1)	87.7 (135/154) (82.4 – 91.2)
LR+ (95% CI)	14.3 (7.6 – 27.0)	14.0 (7.4 – 26.5)
LR- (95% CI)	0.18 (0.13 – 0.25)	0.13 (0.09 – 0.20)

The estimate of TPA was 87.9% in males and 90.6% in females. The estimates of PPA and NPA were 82.9% and 94.2%, respectively, for males and 87.7% and 93.8%,

respectively, in females. The estimates of PPV and NPV were 94.7% and 81.6%, respectively, in males and 93.8% and 87.7%, respectively in females.

c. Agreement with visual Amyloid PET by age

The clinical performance measures of the ratio results generated from the measurements with the Elecsys Total-Tau 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 yrs	60–69 yrs	70–79 yrs	≥80 yrs
N (% Total)	32 (5.0)	221 (34.2)	296 (45.8)	97 (15.0)
Visual Amyloid PET Read Positive (% N)	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)	87.2 (75/86) (78.5 – 92.7)	83.8 (155/185) (77.8 – 88.4)	83.9 (52/62) (72.8 – 91.0)
NPA (n/N) (95% CI)	94.4 (17/18) (74.2 – 99.0)	96.3 (130/135) (91.6 – 98.4)	91.9 (102/111) (85.3 – 95.7)	91.4 (32/35) (77.6 – 97.0)
TPA (n/N) (95% CI)	93.8 (30/32) (79.2 – 99.2)	92.8 (205/221) (88.6 – 95.5)	86.8 (257/296) (82.5 – 90.2)	86.6 (84/97) (78.4 – 92.0)
PPV (n/N) (95% CI)	92.9 (13/14) (73.1 – 98.7)	93.8 (75/80) (86.3 – 97.3)	94.5 (155/164) (90.2 – 97.0)	94.5 (52/55) (85.4 – 98.1)
NPV (n/N) (95% CI)	94.4 (17/18) (79.1 – 99.2)	92.2 (130/141) (87.2 – 95.2)	77.3 (102/132) (70.6 – 82.2)	76.2 (32/42) (64.6 – 85.0)
LR+ (95% CI)	16.7 (3.5 – 96.5)	23.5 (9.9 – 55.9)	10.3 (5.5 – 19.4)	9.8 (3.3 – 29.0)
LR- (95% CI)	0.08 (0.01 – 0.34)	0.13 (0.08 – 0.23)	0.18 (0.13 – 0.25)	0.18 (0.10 – 0.31)

The estimate of TPA decreases slightly with age, from 93.8% in the ‘55–59 years’ age group to 86.6% in the ‘≥80 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 Total-Tau 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)	15 (2.0)
Visual Amyloid PET Read Positive (% N)	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)	85.2 (281/330) (80.9 – 88.6)	100.0 (8/8) (67.6 – 100)	100.0 (4/4) (51.0 – 100)	40.0 (2/5) (11.8 – 76.9)
NPA (n/N) (95% CI)	93.5 (257/275) (89.9 – 95.8)	100 (9/9) (70.1 – 100)	100 (5/5) (56.6 – 100)	100 (10/10) (72.3 – 100)
TPA (n/N) (95% CI)	88.9 (538/605) (86.2 – 91.2)	100 (17/17) (81.6 – 100)	100 (9/9) (70.1 – 100)	80.0 (12/15) (54.8 – 93.0)
PPV (n/N) (95% CI)	94.0 (281/299) (90.9 – 96.1)	100 (8/8) (74.0 – 100)	100 (4/4) (62.5 – 100)	100 (2/2) (42.2 – 100)
NPV (n/N) (95% CI)	84.0 (257/306) (79.9 – 87.4)	100 (9/9) (76.8 – 100)	100 (5/5) (70.6 – 100)	76.9 (10/13) (61.9 – 87.3)
LR+ (95% CI)	13.0 (8.3 – 20.4)	+Inf [^] (3.21 – +Inf [^])	+Inf [^] (2.08 – +Inf [^])	+Inf [^] (1.46 – +Inf [^])
LR- (95% CI)	0.16 (0.12 – 0.21)	0.00 (0.00-0.34)	0.00 (0.00 – 0.52)	0.60 (0.29 – 1.23)
*2 of 646 subjects had missing information about race (unknown)				
[#] Including Hawaiian / Pacific Islander and 2 unknowns				
[^] +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 85.2%, 93.5%, and 88.9%, 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 Total-Tau CSF and first generation (Gen1) of Elecsys β -Amyloid (1-42) CSF support that a positive result, defined as tTau/Abeta42 ratio value above the pre-specified ratio cut-off of 0.33 is consistent with a PET scan positive result and negative result, defined

as tTau/Abeta42 ratio value below the pre-specified ratio cut-off of 0.33 is consistent with a 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 Total-Tau 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 0.28 as described in Section A.7 Assay Cut-off. This final ratio cut-off (0.28) was included in the package inserts of the Elecsys Total-Tau 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 (mean=71.5 years), with a Mini-Mental State Exam (MMSE) score ≥ 29 previously measured in the Swedish BioFINDER1 study according to the BioFINDER CSF pre-analytical handling protocol using the first-generation Elecsys Total-Tau CSF and Elecsys β -Amyloid (1-42) CSF (for description of different assay versions, refer to Assay Cut-Off in Section A, item 7). A conversion factor (0.9368) 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 intervals 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, 17% of the results were above (test-positive) and 83% were below (test-negative) the tTau/Abeta42 ratio of 0.28. The median, 2.5th, 5th, 95th, and 97.5th percentile of tTau and tTau/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)					
	2.5 th Percentile	5 th Percentile	Median	95 th Percentile	97.5 th Percentile
tTau (pg/mL)	102.6	118.2	203.0	375.4	430.7
tTau /Abeta42 ^	0.09	0.09	0.14	0.41	0.50
^ The ratio values were rounded to 3 decimal places before comparing against 0.28					

Age 66 – 70 years (N=43)					
	2.5 th Percentile	5 th Percentile	Median	95 th Percentile	97.5 th Percentile
tTau (pg/mL)	81.9	99.7	191.0	342.0	373.9
tTau /Abeta42 [^]	0.08	0.09	0.12	0.30	0.40
Age 71 – 75 years (N=72)					
	2.5 th Percentile	5 th Percentile	Median	95 th Percentile	97.5 th Percentile
tTau (pg/mL)	118.4	138.3	218.0	411.2	441.6
tTau /Abeta42 [^]	0.09	0.11	0.18	0.45	0.54
[^] The ratio values were rounded to 3 decimal places before comparing against 0.28					

The 95% reference intervals established in Study Part 1 are as follows: 102.6–430.7 pg/mL for tTau and 0.09–0.50 for tTau/Abeta42. Considering the number of females (N=69) and males (N=46) evaluated, the 2.5th and 97.5th percentiles for tTau/Abeta42 obtained in females (0.09 and 0.05) are not different compared to the corresponding values (0.09 and 0.05) in males. Regarding age, a slight trend of increasing tTau and tTau/Abeta42 was observed over the age of 70 years. The 2.5th percentiles for tTau and tTau/Abeta42 in cognitive healthy normal subjects aged 66 to 70 (N=43) were 81.9 pg/mL and 0.08, respectively, compared to 118.4 pg/mL and 0.09, respectively, in those aged 71–75 (N=72). The 97.5th percentiles for tTau and tTau/Abeta42 were also higher in the 71–75 years age group (441.6 pg/mL and 0.54, respectively) compared to the 66–70 years age group (373.9 pg/mL and 0.40, respectively).

Refer to K221842 for the 95% reference intervals established in Study Part 1 for Abeta42.

- 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 Total-Tau CSF and Elecsys β -Amyloid (1-42) CSF II. The subjects (65% females/35% males) aged 60–75 (mean 66.1 $\pm$ 4.36) years had a Montreal Cognitive Assessment score 25 or higher^{6,7} (mean 28.0 $\pm$ 1.59). The table below shows the ranges of tTau and tTau/Abeta42 ratio in the CSF samples.

	Minimum Value	Maximum Value
tTau (pg/mL)	117.0	371.0
tTau/Abeta42 [^]	0.09	0.41
[^] The ratio values were rounded to 3 decimal places before comparing against 0.28		

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.

For tTau and tTau/Abeta42, all 20 measurements were within the respective reference ranges: 102.6–430.7 pg/mL for tTau and 0.09 – 0.50 for tTau/Abeta42. Because the verification data met the pre-specified study acceptance criteria (i.e., $n \leq 2$ results fall outside of the reference ranges for tTau and tTau/Abeta42 established in the BioFINDER1 cohort), the reference ranges are verified in the EHBS cohort.

Refer to K221842 for the 95% reference intervals verification in Study Part 2 for Abeta42.

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.