510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K252163

B Applicant

Roche Diagnostics

C Proprietary and Established Names

Elecsys Phospho-Tau (181P) Plasma

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
SET	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:

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 Phospho-Tau (181P) Plasma is an vitro electrochemiluminescence immunoassay (ECLIA) intended for the measurement of the phosphorylated Tau 181 protein in human plasma on **cobas e** immunoassay analyzers.

The Elecsys Phospho-Tau (181P) Plasma assay result is intended to aid in the initial assessment for Alzheimer's disease and other causes of cognitive decline in adult patients aged 55 years and older, presenting with signs, symptoms, or complaints of cognitive decline. The result should be interpreted in conjunction with other clinical information.

A negative test result is consistent with a negative amyloid positron emission tomography (PET) scan result and reduced likelihood that a patient's cognitive impairment is due to amyloid pathology. These patients should be investigated for other causes of cognitive decline.

A positive test result may not be consistent with a positive amyloid PET scan result. Patients with an initial positive result should be further investigated to determine whether the amyloid pathology can be a cause of cognitive impairment.

Limitations of Use
The Elecsys Phospho-Tau (181P) Plasma is not recommended for patients with signs, symptoms, or complaints of cognitive decline, who are already referred to the specialist.
The performance of Elecsys Phospho-Tau (181P) Plasma 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 801 analyzer (cleared under K162606)

IV Device/System Characteristics:

A Device Description:

Elecsys Phospho-Tau (181P) Plasma is an assay including a set of immunoassay reagents for the measurement of the phosphorylated Tau 181 protein in human plasma (K2-EDTA). The reagent

components packaged in the **cobas e** pack are ready-for-use and are supplied in snap-cap plastic bottles compatible with the system.

Component	Volume	Contents
M	Streptavidin-coated microparticles, 1 bottle, 6.4 mL	Streptavidin-coated microparticles 0.72 mg/mL; preservative
R1	Anti-pTau-Ab~biotin 1 bottle, 7.0 mL	Biotinylated monoclonal anti-pTau antibody (mouse/human) 2.9 mg/L; HEPES buffer* 50 mmol/L, pH 7.4; preservative
R2	Anti-Tau-Ab~Ru(bpy)^ 1 bottle, 7.0 mL	Monoclonal anti-tau antibody (mouse) labeled with ruthenium complex 4.5 mg/L; HEPES buffer* 50 mmol/L, pH 7.4; preservative
* [4-(2-hydroxyethyl)-piperazine]-ethane sulfonic acid ^Tris(2,2'-bipyridyl)ruthenium(II)-complex (Ru(bpy)		

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

- CalSet Phospho-Tau (181P) Plasma (4 x 1.0 mL):
  Cal1: approx. 1.00 pg/mL (2 bottles, each for 1.0 mL)
  Cal2: approx. 8.00 pg/mL (2 bottles, each for 1.0 mL)

- PreciControl Phospho-Tau (181P) Plasma (6 x 1.0 mL):
  Ctrl1: approx. 0.700 pg/mL (3 bottles, each for 1.0 mL)
  Ctrl2: approx. 2.00 pg/mL (3 bottles, each for 1.0 mL)

B Principle of Operation:

1. Specimen collection and preparation

   Refer to the package insert of Elecsys Phospho-Tau (181P) Plasma.

2. Elecsys Phospho-Tau (181P) Plasma

   Elecsys Phospho-Tau (181P) Plasma is a serological, sandwich principal immunoassay to be used on the cobas e immunoassay family of analyzers with an 18-minute test time and include the following steps:

   a) First incubation: 30 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 II M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

d) Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the cobas link. The analyzer automatically calculates the analyte concentration of each sample in pg/mL.

3. Interpretation of Elecsys Phospho-Tau (181P) Plasma results

Results of the Elecsys Phospho-Tau (181P) Plasma are reported by the instrument. The result must be interpreted by the laboratory professional according to the table below:

Elecsys Phospho-Tau (181P) Plasma result	**Interpretation**
Negative (≤ 0.722 pg/mL)	A negative result is consistent with a negative amyloid positron emission tomography (PET) scan result. These patients should be investigated for other causes of cognitive decline.
Positive (> 0.722 pg/mL)	A positive result may not be consistent with a positive PET scan result. Patients with an initial positive result should be further investigated to determine whether the amyloid pathology can be a cause of cognitive impairment.

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 K252163	Predicate K221842
Device Trade Name	Elecsys Phospho-Tau (181P) Plasma	Elecsys β-Amyloid (1-42) CSF II, Elecsys Phospho-Tau (181P) CSF
General Device Characteristic Similarities		
Intended Use/ Indications For Use	Elecsys Phospho-Tau (181P) Plasma is an vitro electrochemiluminescence immunoassay (ECLIA) intended for the measurement of the phosphorylated Tau 181 protein in human plasma on **cobas e** immunoassay analyzers. The Elecsys Phospho-Tau (181P) Plasma assay result is intended to aid in the initial assessment for Alzheimer's disease and other causes of cognitive decline in adult patients aged 55 years and older, presenting with signs, symptoms, or complaints of cognitive decline. The result should be interpreted in conjunction with other clinical information. A negative test result is consistent with a negative amyloid positron emission tomography (PET) scan result and reduced likelihood that a patient's cognitive impairment is due to amyloid pathology. These patients should be investigated for other causes of cognitive decline. A positive test result may not be consistent with a positive amyloid PET scan result. Patients with an initial positive result should be further investigated to determine whether the amyloid pathology can be a cause of cognitive impairment Limitations of Use The Elecsys Phospho-Tau (181P) Plasma is not recommended for patients with signs, symptoms, or complaints of cognitive decline, who are already referred to the specialist.	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

Device & Predicate Device(s):	Device K252163	Predicate K221842
Device Trade Name	Elecsys Phospho-Tau (181P) Plasma	Elecsys β-Amyloid (1-42) CSF II, Elecsys Phospho-Tau (181P) CSF
	The performance of Elecsys Phospho-Tau (181P) Plasma 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
Assay Output	Negative and Positive (relative to cut-off)	Same
Total Duration of Assay	18 minutes	Same
Traceability/ Standardization	Standardized against a purified reference material Tau(172-205)[pThr181]amide, absolutely quantified via amino acid analysis. Calibrator values are based on weighted pTau reference material, traceable to National Institute of Standards and Technology (NIST) amino acid reference calibrators	Same
Reagent Stability	On-board: 16 weeks	Same
General Device Characteristic Differences		
Analyte	pTau181	pTau181 and β-Amyloid (1-42)
Sample Matrix	Human K2-EDTA plasma, K3-EDTA plasma, and K2-EDTA plasma drawn in tubes containing separating gel	Human CSF
Result Calculation and Interpretation	The analyzer automatically calculates pTau181 concentration in pg/mL for each sample	Operator calculates the ratio of pTau181 to β-Amyloid (1-42) for each sample
Reagent Composition	**M** - Streptavidin-coated microparticles, 1 bottle, 6.4 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative. **R1** - Anti-pTau Ab~biotin, 1 bottle, 7.0 mL: Biotinylated monoclonal anti-pTau antibody (mouse/human) 2.9 mg/L; HEPES* buffer 50 mmol/L, pH 7.4; preservative.	*Elecsys Phospho-Tau (181P) CSF:* **M** - Streptavidin-coated microparticles, 1 bottle, 6.1 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative. **R1** - Anti-pTau Ab~biotin, 1 bottle, 6.8 mL: Biotinylated monoclonal anti-pTau antibody (rabbit/mouse) 2.5

Device & Predicate Device(s):	Device K252163	Predicate K221842
Device Trade Name	Elecsys Phospho-Tau (181P) Plasma	Elecsys β-Amyloid (1-42) CSF II, Elecsys Phospho-Tau (181P) CSF
	R2 - Anti-Tau-Ab~Ru(bpy), 1 bottle, 7.0 mL: Monoclonal anti-Tau antibody (mouse) labeled with ruthenium complex 4.5 mg/L; HEPES* buffer 50 mmol/L, pH 7.4; preservative. *[4-(2-hydroxyethyl)-piperazine]-ethanesulfonic acid	mg/L;Trisb) buffer > 14 mmol/L, pH 7.2; preservative. **R2** - Anti-Tau-Ab~Ru(bpy), 1 bottle, 6.8 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
Sample Volume	30 µL	50 µL
Instrument	**cobas e** 801	**cobas e** 601
Calibrators	CalSet Phospho-Tau (181P) Plasma: Cal1: approx. 1.0 pg/mL and Cal2: approx. 8.0 pg/mL	*Elecsys Phospho-Tau (181P) CSF:* CalSet Phospho-Tau (181P): Cal1: approx. 10 pg/mL and Cal2: approx. 70 pg/mL
Controls	PreciControl Phospho-Tau (181P) Plasma: Ctrl1: approx. 0.700 pg/mL and Ctrl2: approx. 2.00 pg/mL	*Elecsys Phospho-Tau (181P) CSF:* PreciControl Phospho-Tau (181P): Ctrl1: approx. 15 pg/mL and Ctrl2: approx. 50 pg/mL
Measuring Range	0.300−10.0 pg/mL	*Elecsys Phospho-Tau (181P) CSF:* 8.0−120.0 pg/mL
No high-dose hook effect	Up to 150 pg/mL	*Elecsys Phospho-Tau (181P) CSF:* Up to 300.0 pg/mL
Detection Limit	Limit of Blank = 0.250 pg/mL Limit of Detection = 0.300 pg/mL Limit of Quantitation = 0.300 pg/mL	*Elecsys Phospho-Tau (181P) CSF:* Limit of Blank = 4.0 pg/mL Limit of Detection = 8.0 pg/mL Limit of Quantitation = 8.0 pg/mL
Reagent Stability	Unopened: 15 months at 2−8 °C	*Elecsys Phospho-Tau (181P) CSF:* Unopened: 12 months at 2−8 °C
Sample Stability	2 days at 20°C−25 °C 7 days at 2°C−8 °C 3 months at -15°C−-25 °C Freeze only once	*Elecsys Phospho-Tau (181P) CSF:* 5 days at 15°C−25 °C 14 days at 2°C−8°C

VI Standards/Guidance Documents Referenced:

The following Clinical and Laboratory Standards Institute (CLSI) guidelines were used:

- 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, 3rd ed.: Evaluation of Qualitative, Binary Output Examination Performance – Third Edition
- CLSI EP17-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline − Second Edition
- CLSI EP25-Ed2A: Evaluation of Stability of In Vitro Medical Laboratory Reagents-Second Edition
- CLSI EP28-A3c: Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline – Third Edition
- CLSI EP35, 1st ed.: Assessment of Equivalence or Suitability of Specimen Types for Medical Laboratory Measurement Procedures - First Edition
- CLSI EP37, 1st ed.: 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) Plasma using a panel of eight or nine human K2-EDTA plasma samples with pTau181 concentrations that span the analytical measuring range of the assay. The same sample panel 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** 801 analyzer and one reagent lot to yield a total of 84 replicates per sample. In addition, two control levels were run for run validity. The results are summarized in the table below.

			Within-Run		Between-Run		Between-Day		Within-Laboratory	
Panel Member	**N**	**Mean (pg/mL)**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
Plasma 1	84	0.545	0.026	4.7	0.005	0.9	0.000	0.0	0.026	4.8
Plasma 2	84	0.622	0.032	5.2	0.000	0.0	0.000	0.0	0.032	5.2
Plasma 3	84	0.624	0.025	4.0	0.007	1.1	0.009	1.4	0.027	4.3
Plasma 4	84	0.828	0.026	3.2	0.011	1.4	0.009	1.1	0.030	3.6
Plasma 5	84	0.972	0.029	2.9	0.013	1.3	0.011	1.2	0.033	3.4
Plasma 6	84	1.02	0.032	3.1	0.015	1.5	0.021	2.1	0.041	4.0
Plasma 7	84	1.09	0.035	3.2	0.000	0.0	0.017	1.6	0.039	3.6
Plasma 8	84	5.19	0.085	1.6	0.000	0.0	0.062	1.2	0.105	2.0
Plasma 9	84	9.22	0.146	1.6	0.064	0.7	0.123	1.3	0.202	2.2
PC Level 1	84	0.543	0.028	5.2	0.000	0.0	0.009	1.7	0.029	5.5
PC Level 2	84	1.95	0.037	1.9	0.022	1.1	0.020	1.0	0.048	2.4

Panel members 1−5 were native samples, panel members 6 and 7 were native pooled samples, panel member 8 was a native sample spiked with Tau(172-205)[pThr181], and panel member 9 was a pooled sample spiked with Tau(172-205)[pThr181].
PC=PreciControl

Qualitative agreement: A total of 84 replicates of each panel member were performed to evaluate qualitative agreement. The percentage of negative test results was calculated for each panel member based on the number of Elecsys Phospho-Tau (181P) Plasma results below the cut-off. Results are summarized in the table below.

Panel member	**Mean (pg/mL)**	**Total replicates**	**Qualitative agreement**	
			Number of Results ≤ 0.722 pg/mL	**% Negative Results**
Plasma 1	0.545[A]	84	84	100 (84/84)
Plasma 2	0.622[B]	84	84	100 (84/84)
Plasma 3	0.624[B]	84	84	100 (84/84)
Plasma 4	0.828[B]	84	0	0 (0/84)
Plasma 5	0.972[C]	84	0	0 (0/84)
Plasma 6	1.02[C]	84	0	0 (0/84)
Plasma 7	1.09[C]	84	0	0 (0/84)
Plasma 8	5.19[C]	84	0	0 (0/84)
Plasma 9	9.22[C]	84	0	0 (0/84)

[A]Below cutoff; [B]Cut-off ± 20%; [C]Above cutoff

(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** 801 analyzer and three reagent lots (N=90 per sample for all lots combined). 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 table below.

		Within-Run		Between-Run		Between-Day		Between-Lot		Total	
Panel Member	**Mean (pg/mL)**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
Plasma 1	0.518	0.022	4.3	0.006	1.1	0.027	5.3	0.032	6.1	0.048	9.2
Plasma 2	0.601	0.066	11.0	0.022	3.6	0.033	5.5	0.026	4.3	0.081	13.6
Plasma 3	0.658	0.020	3.1	0.006	1.0	0.014	2.2	0.043	6.5	0.050	7.5
Plasma 4	0.840	0.020	2.4	0.010	1.2	0.022	2.6	0.057	6.8	0.066	7.8
Plasma 5	1.07	0.027	2.5	0.000	0.0	0.011	1.0	0.064	6.0	0.070	6.6
Plasma 6	5.11	0.060	1.2	0.041	0.8	0.050	1.0	0.175	3.4	0.196	3.8
Plasma 7	9.16	0.080	0.9	0.032	0.3	0.077	0.8	0.261	2.8	0.285	3.1
Plasma 8	1.23	0.025	2.1	0.000	0.0	0.017	1.4	0.041	3.3	0.051	4.2
PC Level 1	0.626	0.020	3.2	0.011	1.8	0.014	2.3	0.053	8.5	0.060	9.5
PC Level 2	2.06	0.029	1.4	0.008	0.4	0.021	1.0	0.113	5.5	0.119	5.8
Panel members 1−5 were native samples, panel members 6 and 7 were native pooled samples, and panel member 8 was a native sample spiked with Tau(172-205)[pThr181]. PC=PreciControl											

Qualitative agreement: A total of 90 replicates of each panel member were performed to evaluate qualitative agreement. The percentage of negative test results was calculated for each panel member based on the number of Elecsys Phospho-Tau (181P) Plasma results below the cut-off. Results are summarized in the table below.

Panel member	**Mean (pg/mL)**	**Total replicates**	**Qualitative agreement**	
			Number of Results ≤ 0.722 pg/mL	**% Negative Results**
Plasma 1	0.518[A]	90	90	100 (90/90)
Plasma 2	0.601[B]	90	89	98.9 (89/90)
Plasma 3	0.658[B]	90	82	91.1(82/90)
Plasma 4	0.840[B]	90	0	0 (0/90)
Plasma 5	1.07[C]	90	0	0(0/90)
Plasma 6	5.11[C]	90	0	0 (0/90)
Plasma 7	9.16[C]	90	0	0 (0/90)
Plasma 8	1.23[C]	90	0	0 (0/90)
[A]Below cutoff; [B]Cut-off ± 20%; [C]Above cutoff				

(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** 801 analyzer at each site, using one reagent lot (N=90 per sample). The results are summarized in the table below.

		Within-Run		Between-Run		Between-Day		Between-Site		Total	
Panel Member	**Mean (pg/mL)**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
Plasma 1	0.462	0.015	3.2	0.007	1.6	0.000	0.0	0.043	9.3	0.046	9.9
Plasma 2	0.540	0.020	3.6	0.007	1.2	0.006	1.1	0.040	7.4	0.045	8.4
Plasma 3	0.623	0.016	2.6	0.006	1.0	0.006	1.0	0.043	6.9	0.047	7.6
Plasma 4	0.790	0.021	2.7	0.000	0.0	0.008	1.0	0.037	4.7	0.044	5.5
Plasma 5	1.02	0.024	2.4	0.000	0.0	0.003	0.2	0.045	4.4	0.051	5.0
Plasma 6	4.94	0.048	1.0	0.047	0.9	0.000	0.0	0.118	2.4	0.136	2.7
Plasma 7	8.94	0.086	1.0	0.032	0.4	0.051	0.6	0.180	2.0	0.208	2.3
Plasma 8	1.15	0.026	2.3	0.017	1.5	0.000	0.0	0.059	5.1	0.067	5.8
PC Level 1	0.62	0.021	3.5	0.005	0.8	0.010	1.7	0.010	1.7	0.026	4.3
PC Level 2	2.03	0.031	1.5	0.000	0.0	0.014	0.7	0.009	0.5	0.035	1.7
Panel members 1−5 were native samples, panel members 6 and 7 were native pooled samples, and panel member 8 was a native sample spiked with Tau(172-205)[pThr181]. PC=PreciControl											

Qualitative agreement: A total of 90 replicates of each panel member were performed to evaluate qualitative agreement. The percentage of negative test results was calculated for each panel member based on the number of Elecsys Phospho-Tau (181P) Plasma results below the cut-off. Results are summarized in the table below.

Panel member	**Mean (pg/mL)**	**Total replicates**	**Qualitative agreement**	
			Number of Results ≤ 0.722 pg/mL	**% Negative Results**
Human plasma 1	0.462[A]	90	90	100 (90/90)
Human plasma 2	0.540[A]	90	90	100 (90/90)
Human plasma 3	0.623[B]	90	90	100 (90/90)
Human plasma 4	0.790[B]	90	4	4.44 (4/90)
Human plasma 5	1.02[C]	90	0	0 (0/90)
Human plasma 6	4.94[C]	90	0	0 (0/90)
Human plasma 7	8.94[C]	90	0	0 (0/90)
Human plasma 8	1.15[C]	90	0	0 (0/90)
[A]Below cutoff; [B]Cut-off ±20%; [C]Above cutoff				

2. Linearity:

The linearity of the Elecsys Phospho-Tau(181P) Plasma on the **cobas e** 801 analyzer was evaluated in accordance with the CLSI guideline EP06-Ed2. Three high native K2-EDTA plasma samples spiked with Tau(172-205)[pThr181] and a native sample that covers at least 50% of the measuring range were each mixed in varying proportion with a low analyte plasma sample to create a series of 10 dilutions of pTau181 concentrations that span across the measuring range of the assay. Each sample dilution was measured in one run with four 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 highest sample levels and is confirmed if all sample levels are within the specifications for allowable deviation and precision. Percent deviations from linearity (%DL) were calculated as differences between the observed values and the predicted values divided by the predicted values. A weighted regression analysis was performed to determine the predicted values. The table below summarizes the regression statistics for each sample separately and for the four samples combined. The %DLs were within ± 10% for each dilution level in all four sample panels.

Panel Member	Range (pg/mL)	Predicted Line
Panel 1	0.321–10.9	Y=1.012*X
Panel 2	0.261–10.8	Y=0.949*X
Panel 3	0.266–10.9	Y=0.994*X
Panel 4 (patient sample)	0.248–5.92	Y=0.993*X
Combined Panels 1–4	0.248–10.9	Y=0.998*X

Linearity results support the measuring range claim of 0.300 pg/mL–10.0 pg/mL for the Elecsys Phospho-Tau(181P) Plasma.

High-Dose Hook Effect

The high-dose hook effect was evaluated for the Elecsys Phospho-Tau (181P) Plasma using one reagent lot on a **cobas e** 801 analyzer. To determine the hook concentration, two K2-EDTA plasma sample pools spiked with a high concentration stock of Tau(172-205)[pThr181] were used to prepare a dilution series. For each sample, a dilution series was performed with depleted human K2-EDTA plasma, and each sample level was measured in triplicates within one run. The hook concentration corresponds to the pTau181 concentration that generates a signal ≥ 10% above the upper limit of the measuring range. The measured counts were plotted against the expected sample concentrations. No hook effect was seen up to 158 pg/mL for Sample 1 and up to 153 pg/mL for Sample 2. The data supports the claim that there is no high-dose hook effect up to 150.0 pg/mL.

3. Analytical Specificity/Interference:

(i) Interference

The effect of potential endogenous and exogenous interfering substances to the Elecsys Phospho-Tau (181P) Plasma assay was evaluated in accordance with the CLSI guidelines EP07, 3rd ed. and EP37, 1st ed. Four human K2-EDTA plasma sample pools with pTau181 concentrations spanning the measuring range of the assay (i.e., one low, one high, one no more than 20% above and one no more than 20% below the cut-off) were evaluated for interference with a panel of 10 endogenous substances: bilirubin, biotin, human anti-mouse antibodies (HAMAs), hemoglobin, human serum albumin, IgG, IgM, IgA intralipid, and rheumatoid factor (RFs). Three human plasma sample pools (i.e., one low, one no more than 20% above and one no more than 20% below the cut-off) were tested with a panel of 43 exogenous substances: 17 common and 26 special pharmaceuticals listed in the table below. Native human plasma pools were used for the low and near cut-off concentration samples. The high human plasma pools were spiked with the Tau(172-205)[pThr181]. All plasma

sample pools were divided into two parts: 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 levels in the test and control samples were measured in five replicates with one lot of reagents in the same run on a **cobas e** 801 analyzer. The %interference was calculated by comparing measurements of the test and control samples. No significant interference was determined for %interference within ±10%. Non-significant interferences were observed for the Elecsys Phospho-Tau (181P) Plasma up to the concentrations of the potential interfering substances tested as shown in the tables below.

Endogenous Interferents	Interferent Concentration
Bilirubin[&]	66.0 mg/dL
Biotin[^]	1200 ng/mL
HAMA (mixture of IgG and IgM)[#]	1200.0 µg/mL
Hemoglobin*	500.0 mg/dL
Human serum albumin	7.0 g/dL
Immunoglobulin G (IgG)	2.0 g/dL
Immunoglobulin M (IgM)	0.35 g/dL
Immunoglobulin A (IgA)	0.6 g/dL
Intralipid	2000.0 mg/dL
Rheumatoid factors	1200 IU/mL
[&] 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 * Hemoglobin was tested at 1000 mg/dL. The data supports no interference up to 1000 mg/dL. The claim was set to 500 mg/dL # Human anti-mouse antibodies (HAMAs) were tested at the indicated test concentration in five replicates in the same run using two plasma sample pools: one with low pTau181 concentration and the other with pTau concentration no more than 20% above the cut-off	

Exogenous Interferents	Interferent Concentration
Common drugs	
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
Cefoxitin	750.0 mg/L
Cyclosporine	1.8 mg/L
Doxycycline	18.0 mg/L
Heparin	3300 IU/L
Ibuprofen	219.0 mg/L
Itraconazole	30.0 mg/L
Levodopa	7.5 mg/L
Methyldopa	22.5 mg/L
Metronidazole	123.0 mg/L
Phenylbutazone	321.0 mg/L

Exogenous Interferents	Interferent Concentration
Rifampicin	48.0 mg/L
Theophylline	60.0 mg/L
Special drugs	
Adalimumab	240.0 mg/L
Albuterol	0.045 mg/L
Amlodipine	0.08 mg/L
Atorvastatin	0.75 mg/L
Clopidogrel	45.0 mg/L
Digoxin	0.039 mg/L
Donepezil	30.0 mg/L
Escitalopram	0.192 mgL
Esomeprazole	6.9 mg/L
Fluticasone	0.00126 mg/L
Formoterol	0.000273 mg/L
Furosemide	15.9 mg/L
Galantamine	250.0 mg/L
Hydrochlorothiazide	1.13 mg/L
Insulin	111.0 mU/L
Lisinopril	0.246 mg/L
Losartan	3.24 mg/L
Memantine	0.117 mg/L
Metformin	12.0 mg/L
Metoprolol	1.5 mg/L
Montelukast	4.45 mg/L
Prednisone	0.1 mg/L
Rivastigmine	45.0 mg/L
Rivaroxaban	2.7 mg/L
Simvastatin	1.68 mg/L
Sitagliptin	1.15 mg/L

(ii) Cross-reactivity

The performance of the Elecsys Phospho-Tau (181P) Plasma assay in the presence of putative cross-reactants was assessed in two human K2-EDTA plasma sample pools, one with pTau181 concentration within 20% of the cut-off (sample pool 1) and another with pTau181 concentration more than 20% above the cut-off (sample pool 2). Each pool was divided into two aliquots: one was spiked with 60 pg/mL synthetic non-phosphorylated tau, Tau(172-205), antigen peptide, or 60 pg/mL pTau175 peptide (test samples) and the other aliquot without the cross-reactant served as a reference sample. The concentration of each cross-reactant was chosen to reflect maximum physiological concentration. Each sample was tested in five replicates on a **cobas e** 801 analyzer and the mean value of each test sample was compared to that of the reference (unspiked) sample. The percentage of cross reactivity was calculated using the following formula:

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

The mean %cross-reactivity values for Tau(172-205) and pTau175 peptides are summarized in the table below.

		Plasma sample pool 1		**Plasma sample pool 2**	
Cross-reactant	Cross-reactant concentration (pg/mL)	Measured Concentration (pg/mL)	Cross-reactivity (%)	Measured Concentration (pg/mL)	Cross-reactivity (%)
Non-phosphorylated Tau (172-205) peptide)	0.0	0.854	n/a	1.01	n/a
	60.0	0.880	0.0433	0.978	-0.0556
Phosphorylated Tau175 peptide	0.0	0.870	n/a	0.991	n/a
	60.0	0.870	0.000629	0.986	-0.00750

The Elecsys Phospho-Tau (181P) Plasma showed no significant cross-reactivity (within ±1% difference of test from reference sample). The results support that Elecsys Phospho-Tau (181P) Plasma is highly specific for human pTau181.

4. Assay Reportable Range:

The Elecsys Phospho-Tau (181P) Plasma assay has a measuring interval of 0.300-10.0 pg/mL. Values below the Limit of Quantitation (LoQ) are reported as <0.300 pg/mL. Values above the measuring range are reported as >10.0 pg/mL.

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

1) Traceability

Standardization for Elecsys Phospho-Tau (181P) Plasma was performed against a purified reference material human Tau(172−205)[pThr181]. The purified reference material was absolutely quantified via amino acid analysis. The amino acid analysis was calibrated with the Certified Reference Material traceable to National Institute of Standards and Technology (NIST) SRM2389a.

2) Stability

a) Calibration

(i) Lot Calibration Frequency

A panel of 13 pooled human K2-EDTA plasma samples with pTau181 levels covering the analytical measuring range was generated for lot calibration testing on a **cobas e** 801 analyzer. The low concentration samples were native, and the middle and high concentration samples were spiked with Tau(172−205)[pThr181].

A fresh Elecsys Phospho-Tau (181P) Plasma reagent lot was placed on the analyzer and calibrated on day 0. Baseline values for the samples tested were determined in two runs and in duplicates to obtain robust values on day 0 (time point 0). After 5, 12, and 13 weeks, the same panel of samples was tested in duplicates with a new reagent kit (stored at 2-8°C) of the same lot. Results of the samples at 13 weeks, T13, were compared against the results of the samples at day 0, T0, using a Passing-Bablok regression analysis: slope was 0.971 (with 95%CI: 0.956–1.001), intercept was 0.0022 (with 95%CI: -0.0403–0.0292), and %bias at the cut-off of 0.722 pg/mL was -2.6%. The results of the study support the lot calibration stability claim of 12 weeks when using a new Elecsys Phospho-Tau (181P) Plasma reagent kit of the same lot.

(ii) On-Board Calibration Frequency

A panel of 13 pooled human K2-EDTA plasma samples with pTau181 levels covering the analytical measuring range was generated for on-board calibration stability testing on a **cobas e** 801 analyzer. The low concentration samples were native, and the middle and high concentration samples were spiked with Tau(172−205)[pThr181]. A fresh Elecsys Phospho-Tau (181P) Plasma reagent kit was placed on the analyzer and calibrated on day 0. Baseline values for the samples tested were determined in two runs and in duplicates to obtain a robust reference at day 0 (time point 0). The same samples were tested in duplicates after 8, 15, 22, 28 and 29 days with the same reagent kept in on-board condition using the calibration established on day 0. The mean recovery compared to the mean reference value (time point 0) was determined for all plasma samples using the calibration curve established on day 0. The specification (within ±10.0% deviation from time point 0) for on-board calibration stability was met for 29 day. The results support the on-board calibration stability claim of 28 days when using the same reagent kit kept in on-board condition.

b) Reagent

(i) Shelf-life Stability

Reagent shelf-life stability of the Elecsys Phospho-Tau (181P) Plasma was determined on a **cobas e** 801 analyzer using three reagent lots and a panel of eight pooled human K2-EDTA plasma samples with pTau181 concentrations covering the analytical measuring range of the assay. The low concentration samples were native, and the middle and high concentration samples were spiked with Tau(172−205)[pThr181]. To determine a robust baseline value at timepoint 0, the samples were measured in two independent runs and with double determination at the same day. The mean value from each sample at T_0 was calculated and set as a baseline value. For the subsequent time points of 6, 9, 15 and 27 months, a new calibration was established, and the sample values were determined in one run in duplicates. At each timepoint, mean recovery of the test samples with respect to the initial measurement at timepoint 0 was evaluated. All samples tested with the three reagent lots were within specification (within ±10.0% deviation from timepoint 0). The results support that the Elecsys Phospho-Tau (181P) Plasma reagent kit can be stored unopened for up to 15 months when stored at 2−8°C.

(ii) On-Board Stability

Reagent on-board stability for the Elecsys Phospho-Tau (181P) Plasma was tested on a **cobas e** 801 analyzer using one reagent lot and a panel of 13 pooled human K2-EDTA plasma samples with pTau181 concentrations covering the analytical measuring range. The low concentration samples were native, and the middle and high concentration samples were spiked with Tau(172−205)[pThr181]. A freshly opened reagent kit was placed on the analyzer and calibrated. Baseline values for the sample panel tested were determined in duplicate at timepoint 0. After 4, 8, 12, 16 and 17 weeks on the **cobas e** 801 analyzer (reagent kit kept at 10°C ± 2°C), frozen aliquots of the same samples were measured again in duplicate with the stressed kit. The mean recovery compared to the mean reference value (time point 0) was determined for all samples. The specification (within ±10.0% deviation from time point 0) for reagent on-board stability was met for 17 weeks. The results support that the Elecsys Phospho-Tau (181P) Plasma reagent kits can be stored on-board of the analyzer for up to 16 weeks.

(iii) Transport (Shipping) Stability

Reagent transport stability for the Elecsys Phospho-Tau (181P) Plasma was tested on a **cobas e** 801 analyzer using one reagent lot and a panel of 13 pooled human K2-EDTA plasma samples with pTau181 concentrations covering the analytical measuring range. The low concentration samples were native, and the middle and high concentration samples were spiked with Tau(172−205)[pThr181]. The **cobas e** pack was stressed in the original container for 7 days (168 hours) at 25°C. The unstressed **cobas e** pack was kept at 2−8°C. Stability was assessed by testing the sample panel in duplicates and comparing mean recovery in the stressed and non-stressed reagent kits. The specification (within ±10.0% deviation from non-stressed reagent kit) for reagent transport stability was met for 1 week. The results support the reagent transport stability claim of 7 days at 25°C.

c) Specimen

(i) Specimen storage stability

The stability of pTau181 in K2-EDTA and K3-EDTA plasma was evaluated under various storage conditions on a **cobas e** 801 analyzer with a panel of 10 human plasma samples derived from whole blood that was freshly collected (never frozen) in accordance with the specimen collection and preparation procedure described in the package insert of the Elecsys Phospho-Tau (181P) Plasma. The following storage conditions were evaluated: room temperature (15°C−25°C), refrigerated (2°C−4°C) and frozen (-15°C−-20°C). The plasma samples in the panel were from patients representing the intended use population. The panel included at least one plasma sample in each of the low (0.300−0.800 pg/mL), medium (0.800−2.00 pg/mL), and high (2.00−10.0 pg/mL) concentration ranges and two samples around the cut-off. All plasma samples were aliquoted. The baseline measurement (time point 0) was carried out using two aliquots per

sample matrix type for each plasma sample in the panel. Five replicates of each aliquot were tested, and the two aliquots were measured in separate runs. The baseline measurements were completed within six hours after the initial blood draw. Of the remaining aliquots, five aliquots per sample matrix type were placed into each of the three storage conditions for the duration indicated in the table below.

Storage condition	Measurement Time Points (T)*				
	T1	T2	T3	T4	T5
Room temperature (15°C−25°C)	9 hours	24 hours	40 hours	2 days	3 days
Refrigerated (2°C−4°C)	2 days	4 days	6 days	7 days	8 days
Frozen (-15°C− -20°C)	3 weeks	6 weeks	9 weeks	12 weeks	13 weeks
*Time since aliquot was placed in storage					

At the defined time points, one aliquot per sample matrix type for each plasma sample in the panel was measured in five replicates. Linear regression analysis of the mean recoveries of aliquots at all time points under different temperatures was performed to determine the appropriate storage duration. All samples tested at each time point were within specification (within ±10% difference from baseline). The plasma sample stability claim for each storage condition is summarized in the table below.

K2-EDTA and K3-EDTA Plasma Sample Stability		
Room temperature (15°C−25°C)	**Refrigerated (2°C−4°C)**	**Frozen (-15 °C− -20°C)**
2 days	7 days	12 weeks

(ii) Specimen stability upon freezing and thawing

One aliquot per sample matrix type for each plasma sample in the same panel used for specimen storage stability testing was used to evaluate the effect of one freeze-thaw (F/T) cycle on pTau181 measurement by the Elecsys Phospho-Tau (181P) Plasma. The aliquots were frozen at -20°C after sample aliquoting for at least overnight and up to three days and thawed at room temperature (15°C−25°C) on a roller mixer for 15−30 minutes. Five replicates of each sample aliquot were tested on a **cobas e** analyzer. The mean recoveries of all aliquots were within specification (within ±10% difference from baseline). The data show that pTau181 in K2-EDTA and K3-EDTA plasma samples is stable after one F/T cycle at -20°C.

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) Plasma on one **cobas e** 801 analyzer. A description of each study and the results obtained are summarized below:

For the evaluation of LoB, five zero-level (blank) human K2-EDTA plasma sample pools, four from analyte-depleted and one native, were tested in six runs, distributed over six 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 LoB was determined as the 95th percentile of the measurements obtained for each reagent lot. The LoB values from the three lots were 0.129, 0.154 and 0.187 pg/mL. The claimed LoB is 0.250 pg/mL.

For the evaluation of LoD, five low-level native, K2-EDTA human plasma samples with target concentrations of pTau181 between 0.0877 to 0.737 pg/mL were tested in six runs, distributed over six 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 0.173, 0.192 and 0.225 pg/mL. The claimed LoD is 0.300 pg/mL.

For the evaluation of LoQ, seven low-level native, K2-EDTA human plasma samples with target mean concentrations of pTau181 between 0.109 to 0.564 pg/mL) were tested in five runs distributed over five days with five replicates per run to reach a total of 25 measurements per sample (175 measurements for all samples) for each reagent lot. The LoQ was estimated based on the lowest concentration of pTau181 which can be quantified with an intermediate precision of no more than 20% CV. The LoQ values from the three lots were 0.179, 0.210 and 0.259 pg/mL. The claimed LoQ is 0.300 pg/mL.

7. Assay Cut-Off:

The cut-off of 0.722 pg/mL was selected for determination of negative (≤0.722 pg/mL) and positive (>0.722 pg/mL) Elecsys Phospho-Tau (181P) Plasma assay results.

B Comparison Studies:

1. Method Comparison with Predicate Device:

Refer to Section C on 'Clinical Studies' below.

2. Matrix Comparison:

Sample matrix equivalence was evaluated in accordance with the CLSI guideline EP35, 1st ed. for samples collected with five different blood collection tube types: K2-EDTA, K3-EDTA, and K2-EDTA plasma gel separating tube (PST). Fifty-six sample pairs per tube type with pTau181 concentrations that span across the measuring range were tested in singleton with one reagent lot of Elecsys Phospho-Tau (181P) Plasma on a **cobas e** 801 analyzer. Samples with high pTau181 concentration were obtained by spiking of native plasma samples with Tau(172-205)[pThr181]. Among the 56 sample pairs measured, four were spiked, while 52 remained native. Passing-Bablok regression analysis was performed using pTau181 concentrations measured in samples collected with the primary tube (K2-EDTA

without separating gel) compared to those in samples collected with each of the other tube types tested as summarized in the table below.

Candidate Tube	N of Total Samples	Range (pg/mL)	Slope	Intercept	Bias at cut-off = 0.722 pg/mL	%Bias at cut-off = 0.722 pg/mL
K3-EDTA vs K2-EDTA*	56	0.386−8.75 0.406−8.95*	0.954	-0.0161	-0.0493	-6.8%
K2-EDTA plasma gel separating tube (PST) vs K2 EDTA*	56	0.381−8.78 0.406−8.95*	1.005	-0.0218	-0.0182	-2.5%
*The reference for all sample types was K2-EDTA plasma drawn into K2-EDTA plasma primary tubes						

C Clinical Studies:

1. Clinical Sensitivity and Clinical Specificity

The study enrolled 312 subjects reflective of primary care with valid the Elecsys Phospho-Tau (181P) Plasma and amyloid PET results, with an average age of 69.1 years (range 55–80 years) who presented with cognitive complaints or impairment, subjective or objective, of unknown cause, at eight geographically diverse intended use enrollment sites across U.S. (n=6) and Europe (n=2). A total of 299 (95.8%) subjects were from U.S. sites and 13 (4.2%) from Europe sites. The study subjects were categorized into three diagnostic groups based on cognitive test results and clinical assessments: 41.0% (128/312) subjective cognitive decline (SCD), 56.1% (175/312) mild cognitive impairment (MCI), and 0.962% (3/312) mild dementia. The diagnostic category was unknown in six (6) subjects (1.92%). The pre-dementia AD diagnostic groups (SCD and MCI) represent 97.1% (303/312) of the study population.

The study population consisted of 40.7% (127/312) males with a mean age of 69.1 years (range 57−80 years with a median age of 69 years), and 59.3% females (185/312) with a mean age of 69.1 years (range 55-80 years with a median 69 years). Regarding race, 59.0% were White, 34.0% were Black or African American, 1.6% were Asian, and 5.4% identified as Other. Regarding ethnicity, 66.3% of participants were Not Hispanic or Latino, 29.5% were Hispanic or Latino, and 4.17% of the participants' ethnicity was missing.

Cognitive Assessments (Quick Dementia Rating System, Mini-Mental State Examination, Clinical Dementia Rating (CDR) Global, CDR–Sum of Boxes), Imaging (amyloid PET, Magnetic Resonance Imaging) and Questionnaires (Medical History, Medication, Quality of Life, Physical Activity, Socio-demographics) were collected from the enrolled subjects. The study population included participants with comorbidities frequently encountered in clinical practice such as cardiovascular disease (56.1%), diabetes (25.6%), depression (19.9%),

kidney disease (2.2%), or history of cerebrovascular accident (3.5%) or cancer (12.5%), among others. The demographic and clinical characteristics including comorbidities based on the patient's medical history are summarized in the table below for the entire study population.

	Diagnostic Groups#				Visual Amyloid PET Read		Total
	SCD*	MCI *	Mild Dementia	Missing	Positive	Negative	
N (%Total)	128 (41.0%)	175 (56.1%)	3 (0.962%)	6 (1.92%)	41 (13.1%)	271 (86.9%)	312 (100%)
Sex							
Male	53 (41.4%)	73 (41.7%)	1 (33.3%)	0 (0%)	20 (48.8%)	107 (39.5%)	127 (40.7%)
Female	75 (58.6%)	102 (58.3%)	2 (66.7%)	6 (100%)	21 (51.2%)	164 (60.5%)	185 (59.3%)
Age (years)							
55–70 years	89 (69.5%)	81 (46.3%)	1 (33.3%)	4 (66.7%)	12 (29.3%)	163 (60.1%)	175 (56.1%)
71–80 years	39 (30.5%)	94 (53.7%)	2 (66.7%)	2 (33.3%)	29 (70.7%)	108 (39.9%)	137 (43.9%)
Mean (SD)	66.6 (5.87)	70.9 (6.27)	68.7 (9.29)	69.3 (6.98)	73.5 (5.1)	68.4 (6.4)	69.1 (6.5)
Median	66.0	72.0	73.0	68.0	75.0	68.0	69.0
Q1−Q3	62.3−71.0	67.0−76.0	58.0−75.0	62.8−76.8	69.5−78.0	63.0−74.0	64.0−75.0
Min−Max	55.0−80.0	56.0−80.0	58.0−75.0	62.0−79.0	59.0−80.0	55.0−80.0	55.0−80.0
Race							
White	74 (57.8%)	106 (60.6%)	1 (33.3%)	3 (50.0%)	37 (90.2%)	147 (54.2%)	184 (59.0%)
Black or African American	51 (39.9%)	51 (29.1%)	2 (66.7%)	2 (33.3%)	4 (9.8%)	102 (37.6%)	106 (34.0%)
Asian	0 (0%)	5 (2.9%)	0 (0%)	0 (0%)	0 (0%)	5 (1.9%)	5 (1.6%)
Other@	3 (2.3%)	13 (7.4%)	0 (0%)	1 (16.7%)	0 (0%)	17 (6.3%)	17 (5.4%)
Quick Dementia Rating System (QDRS)							
Mean (SD)	3.03 (1.59)	4.40 (2.64)	5.17 (1.04)	4.58 (1.28)	3.9 (2.4)	3.8 (2.3)	3.9 (2.3)
Median	2.50	4.00	5.50	4.50	3.00	3.00	3.00
Q1−Q3	2.00−3.50	2.50−6.00	4.00−6.00	3.38−5.75	2.5−4.8	2.0−5.0	2.0−5.0
Min−Max	0.500−10.5	0.500−11.5	4.00−6.00	3.00−6.50	1.0−11.5	0.50−11.0	0.50−11.5
Mini-Mental State Examination (MMSE)							
< 25	27 (21.1%)	48 (27.4%)	1 (33.3%)	2 (33.3%)	13 (31.7%)	65 (24.0%)	78 (25.0%)
25–27	77 (60.2%)	69 (39.4%)	2 (66.7%)	2 (33.3%)	15 (36.6%)	135 (49.8%)	150 (48.1%)
28–30	24 (18.8%)	58 (33.1%)	0 (0%)	2 (33.3%)	13 (31.7%)	71 (26.2%)	84 (26.9%)
Mean (SD)	25.9 (1.89)	26.2 (2.42)	24.3 (2.89)	26.5 (3.02)	26.1 (2.5)	26.0 (2.2)	26.1 (2.23)
Median	26.0	26.0	26.0	27.0	26.0	26.0	26.0
Q1−Q3	25.0−27.0	24.0−28.0	21.0−26.0	23.5−29.3	24.0−29.0	25.0−28.0	24.3−28.0
Min−Max	21.0−30.0	21.0−30.0	21.0−26.0	22.0−30.0	21.0−30.0	21.0−30.0	21.0−30.0

	Diagnostic Groups				Visual Amyloid PET Read		Total
	SCD*	MCI *	Mild Dementia	Missing	Positive	Negative	
Clinical Dementia Rating (CDR) Global							
0	15 (11.7%)	3 (1.71%)	0 (0%)	0 (0%)	3 (7.3%)	15 (5.5%)	18 (5.8%)
0.5	111 (86.7%)	165 (94.3%)	0 (0%)	0 (0%)	34 (82.9%)	242 (89.3%)	276 (88.5%)
1	2 (1.56%)	7 (4.00%)	3 (100%)	0 (0%)	2 (4.9%)	10 (3.7%)	12 (3.9%)
1.5	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
2	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
2.5	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
3	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Missing	0 (0%)	0 (0%)	0 (0%)	6 (100%)	2 (4.88%)	4 (1.48%)	6 (1.92%)
Clinical Dementia Rating–Sum of Boxes (CDR–SB)							
Mean (SD)	1.86 (1.18)	2.18 (1.31)	5.00 (0.500)	NA	1.90 (1.6)	2.11 (1.3)	2.08 (1.3)
Median	2.00	2.00	5.00	NA	1.5	2.0	2.0
Q1−Q3	1.00−2.50	1.00−3.00	4.50−5.50	NA	1.0−2.5	1.0−3.0	1.0−3.0
Min−Max	0−7.50	0−7.00	4.50−5.50	NA	0−7.0	0−7.5	0−7.5
Missing	13 (10.2%)	4 (2.29%)	0 (0%)	6 (100%)	2 (4.9%)	21 (7.8%)	23 (7.4%)
Years of Education							
Mean (SD)	13.4 (3.4	15.2 (3.6)	15.0 (2.7)	17.0 (2.10)	16.2 (3.3)	14.2 (3.6)	14.5 (3.6)
Median	12.0	15.0	16.0	17.0	16.0	14.0	14.0
Q1–Q3	12.0−16.0	12.0−18.0	12.0−17.0	15.5−18.5	14.0−18.0	12.0−16.0	12.0−16.0
Min–Max	5.00−24.0	7.00−37.0	12.0−17.0	14.0−20.0	10.0−25.0	5.00−37.0	5.00−37.0
Apolipoprotein E4 (ApoE4) Status[#]							
Reactive	43 (33.6%)	56 (32.0%)	1 (33.3%)	1 (16.7%)	23 (56.1%)	78 (28.8%)	101 (32.4%)
Nonreactive	85 (66.4%)	119 (68.0%)	2 (66.7%)	5 (83.3%)	18 (43.9%)	193 (71.2%)	211 (67.6%)
Body Mass Index [kg/m2]							
Mean (SD)	29.4 (5.6)	28.4 (5.2)	25.9 (4.8)	26.2 (3.7)	26.8 (4.1)	29.0 (5.5)	28.7 (5.4)
Median	28.5	27.5	24.8	27.1	25.8	28.3	27.8
Q1−Q3	25.7−32.2	24.9−31.2	21.8−31.2	22.4−29.2	23.9−29.1	25.4−31.8	25.2−31.4
Min−Max	16.5−46.5	18.5−48.8	21.8−31.2	21.0−30.6	20.7−37.6	16.5−48.8	16.5−48.8
Creatinine [mg/dL]							
Mean (SD)	0.92 (0.36)	0.91 (0.25)	0.89 (0.085)	0.75 (0.078)	0.89 (0.20)	0.91 (0.31)	0.91 (0.30)
Median	0.83	0.86	0.91	0.74	0.83	0.85	0.84
Q1−Q3	0.74−1.0	0.72−1.1	0.80−0.97	0.71−0.83	0.74−1.0	0.735−1.0	0.735−1.0
Min−Max	0.31−3.3	0.45−1.9	0.80−0.97	0.63−0.86	0.633−1.45	0.305−3.26	0.305−3.26
Estimated Glomerular Filtration Rate (eGFR)							
Mean (SD)	81.7 (17.5)	78.8 (16.8)	77.9 (12.0)	85.1 (10.8)	80.6 (14.0)	80.0 (17.4)	80.1 (17.0)
Median	84.9	81.9	77.4	85.6	86.1	82.0	83.4
Q1−Q3	75.4−93.2	67.3−91.6	66.2−90.2	75.7−93.7	71.4−90.4	70.7−92.8	70.7−91.9
Min−Max	14.3−109	38.4−112	66.2−90.2	70.0−100	42.0−95.3	14.3−112	14.3−112

	Diagnostic Groups				Visual Amyloid PET Read		Total
	SCD*	MCI *	Mild Dementia	Missing	Positive	Negative	
Comorbidities							
Cerebrovascular Accident	2 (1.56%)	8 (4.57%)	0 (0%)	1 (16.7%)	1 (2.44%)	10 (3.69%)	11 (3.53%)
Cardiovascular Disease[$]	75 (58.6%)	96 (54.9%)	1 (33.3%)	3 (50.0%)	15 (36.6%)	160(59.0%)	175 (56.1%)
Diabetes	35 (27.3%)	42 (24.0%)	1 (33.3%)	2 (33.3%)	7 (17.1%)	73 (26.9%)	80 (25.6%)
Metabolic Conditions^	15 (11.7%)	55 (31.4%)	1 (33.3%)	1 (16.7%)	11 (26.8%)	61 (22.5%)	72 (23.1%)
Respiratory Conditions^^	6 (4.69%)	32 (18.3%)	0 (0%)	2 (33.3%)	5 (12.2%)	35 (12.9%)	40 (12.8%)
Kidney Disease	0 (0%)	7 (4.00%)	0 (0%)	0 (0%)	1 (2.4%)	6 (2.2%)	7 (2.2%)
Liver Disorder	3 (2.34%)	1 (0.571%)	0 (0%)	0 (0%)	0 (0%)	4 (1.48%)	4 (1.3%)
Cancers[%]	4 (3.13%)	35 (20.0%)	0 (0%)	0 (0%)	8 (19.5%)	31 (11.4%)	39 (12.5%)
Inflammatory and Immune Disorders[&]	4 (3.13%)	18 (10.3%)	0 (0%)	0 (0%)	1 (2.4%)	21 (7.8%)	22 (7.1%)
Depression	11 (8.59%)	48 (27.4%)	1 (33.3%)	2 (33.3%)	8 (19.5%)	54 (19.9%)	62 (19.9%)
Hearing Loss	7 (5.47%)	59 (33.7%)	0 (0%)	0 (0%)	16 (39.0%)	50 (18.5%)	66 (21.2%)
Vision Loss	20 (15.6%)	116 (66.3%)	3 (100%)	2 (33.3%)	18 (43.9%)	123 (45.4%)	141 (45.2%)
Post-acute COVID-19 Syndrome	2 (1.56%)	2 (1.14%)	0 (0%)	1 (16.7%)	40 (97.6%)	267 (98.5%)	307 (98.4%)
Clinical Diagnosis							
SCD*					6 (14.6%)	122 (45.0%)	128 (41.0%)
MCI*					33 (80.5%)	142 (52.4%)	175 (56.1%)
Mild Dementia					0 (0%)	3 (1.1%)	3 (0.96%)
Missing					2 (4.9%)	4 (1.5%)	6 (1.9%)
Visual Amyloid PET Read							
Positive	6 (4.7%)	33 (18.9%)	0 (0%)	2 (33.3%)			
Negative	122 (95.3%)	142 (81.1%	3 (100%)	4 (66.7%)			

[#] The diagnostic category was unknown in six subjects

***** SCD: subjective cognitive decline; MCI: mild cognitive impairment

[@] Including Middle Eastern, American Indian /Alaska Native and Native Hawaiian

[#] Based on plasma ApoE4 levels (protein)

[$] Cardiovascular diseases other than cerebrovascular accident include atrial fibrillation, cardiac failure, coronary artery disease, and hypertension

^ Metabolic conditions other than diabetes include thyroid disorder, parathyroid disorder, and hypovitaminosis. Including seven subjects (2.24%) with missing results

^^Including seven subjects (2.24%) with missing results

[%] Including current, in remission, and resolved

[&] Inflammatory bowel disease and other autoimmune or inflammatory disorders

Detection of amyloid pathology

A total of 313 subjects underwent amyloid PET scans using FDA approved amyloid tracers (18F-florbetapir, 18F-Florbetaben or 18F-Flutemetamol). The amyloid PET scans were randomly assigned, read and interpreted by three trained readers out of a pool of five, each reading independent of each other's, and majority result was used to classify each image as amyloid positive or negative, resulting in 41 (13.1%) positive, and 271 (86.9%) negative amyloid PET reads. The independent readers were blinded to any clinical information, including the patient's clinical status, diagnosis, and plasma and/or CSF biomarker measurements. PET reads were conducted according to the approved instructions for use of the amyloid tracers. Positive concordance occurred in 28 cases (8.95%), while discordant positive readings occurred in 13 cases (4.15%), and discordant negative readings in 11 cases (3.51%). Negative concordance occurred in 260 cases (83.1%). There were no cases with one or two missing ratings, and only 1 case (0.319%) had three missing ratings. Because one scan was not readable, the total available amyloid PET scans were 312. The number and proportion of concordant and discordant visual PET ratings are summarized in the table below.

Reader concordance	**All (N=313, 100%)**
Concordant positive (+/+/+)	n=28 (8.95%)
Discordant positive (+/+/-)	n=13 (4.15%)
Discordant negative (+/-/-)	n=11 (3.51%)
Concordant negative (-/-/-)	n=260 (83.1%)
Missing (one missing rating)	n=0 (0%)
Missing (two missing ratings)	n=0 (0%)
Missing (three missing ratings)	n=1 (0.319%)

The inter-reader visual read agreement between the five readers with each image read by a selection of three readers is summarized in the table below. The Positive Percent Agreement (PPA) between readers was 81.6% on average (range: 53.8%−100%), the Negative Percent Agreement (NPA) was 97.1% on average (range: 91.5%−100%), and the Total Percent Agreement (TPA) was 94.9% on average (range: 92.3%− 96.6%). The total available amyloid PET scans were 312.

Inter-reader visual read agreement		
Agreement Rate	**Mean (%)**	**Min−Max (%)**
Positive Percent Agreement (PPA)	81.6	53.8−100
Negative Percent Agreement (NPA)	97.1	91.5−100
Total Percent Agreement (TPA)	94.9	92.3−96.6
TPA with 95% CIs between readers		

Reader 1 vs Reader 2	92.7% (86.3%–96.3%)
Reader 1 vs Reader 3	93.6% (87.4%–96.9%)
Reader 1 vs Reader 4	96.5% (90.2%–98.8%)
Reader 1 vs Reader 5	96.3% (89.5%–98.7%)
Reader 2 vs Reader 3	95.6% (89.2%–98.3%)
Reader 2 vs Reader 4	94.1% (87.6%–97.2%)
Reader 2 vs Reader 5	92.3% (84.2%–96.4%)
Reader 3 vs Reader 4	96.6% (90.5%–98.8%)
Reader 3 vs Reader 5	96.5% (91.3%–98.6%)
Reader 4 vs Reader 5	94.9% (87.7%–98.0%)

The time difference between blood collection and PET imaging exhibited a mean of 52.2 days (SD = 34.8), a median of 39.0 days, and ranged from -15 to 179 days. Timing was consistent between PET positive (mean = 45.8 days; SD = 29.9) and PET negative (mean = 53.1 days; SD = 35.5) participants.

		PET Positive (N=41, 13.1%)	**PET Negative (N=271, 86.9%)**	**All (N=312, 100%)**
Days between blood collection and PET	**Mean (SD)**	45.8 (29.9)	53.1 (35.5)	52.2 (34.8)
	Median (Min.–Max.)	35 (8–112)	39 (-15–179)	39 (-15–179)

Plasma Sampling and Analysis

The analysis of plasma biomarkers among 312 participants with valid PET results revealed that PET Positive individuals had higher mean pTau181p levels (N=41; 1.29 pg/mL) compared to PET Negative individuals (N=271; 0.828 pg/mL) as shown in the table below.

		PET Positive (N=41, 13.1%)	**PET Negative (N=271, 86.9%)**	**All (N=312, 100%)**
pTau181p (pg/mL)	**Mean (SD)**	1.29 (0.446)	0.828 (0.417)	0.888 (0.448)
	Median (Min.–Max.)	1.23 (0.649–2.59)	0.716 (0.300–3.22)	0.763 (0.300–3.22)

Results:

1) Clinical Performance

To estimate clinical performance measures, the Elecsys Phospho-Tau (181P) Plasma result was compared to the majority amyloid PET scan result for each patient. The agreement with visual read amyloid PET classification at the pre-specified cut-off of 0.722 pg/mL is

summarized in the table below. The prevalence of amyloid positivity based on amyloid PET was 13.1% in the study population. The agreement rates percentages and likelihood ratios are also provided in the table below.

		Visual Amyloid PET Read		
		Positive	**Negative**	**Total**
Elecsys Phospho-Tau (181P) Plasma	**Above Cut-off (> 0.722 pg/mL)**	38	132	170
	Below Cut-off (≤ 0.722 pg/mL)	3	139	142
	Total	41	271	312

Performance Measures	**Point Estimates % (95% CI)**
Positive Percent Agreement (PPA)	92.7 (38/41) (80.6−97.5)*
Negative Percent Agreement (NPA)	51.3 (139/271) (45.4−57.2)*
Total Percent Agreement (TPA)	56.7% (177/312) (51.2−62.1)*
Prevalence of Visual Amyloid PET Positive	13.1 (41/312) (9.8−17.3)*
Positive Predictive Value (PPV)	22.4 (38/170) (19.5−25.0)**
Negative Predictive Value (NPV)	97.9 (139/142) (94.5−99.3)**
Positive Likelihood Ratio (LR+)	1.9 (1.601–2.200)***
Negative Likelihood Ratio (LR-)	0.14 (0.049–0.382)***

* 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 312 evaluable primary care subjects, 41 had amyloid PET scan positive results. Of the 41 primary care subjects with an amyloid PET scan positive result, 38 also had a positive Elecsys Phospho-Tau (181P) Plasma result. The positive percent agreement (PPA) was 92.7 (38/41) with 95% CI: 80.6%−97.5%. The remaining 3 PET scan positive subjects had a negative Elecsys Phospho-Tau (181P) Plasma result. The rate of false negative results was 7.3% (3/41). Of the 271 primary care subjects with PET scan negative results, 139 also had a negative Elecsys Phospho-Tau (181P) Plasma result. The negative percent agreement (NPA) was 51.3% (139/271) with a 95% CI: 45.4%−57.2%. The remaining 132 amyloid PET scan negative subjects had a positive Elecsys Phospho-Tau (181P) Plasma result. The rate of false positive results was 48.7% (132/271). The total percent agreement (TPA) was 56.7% (177/312) with a 95% CI: 51.2%−62.1%. Overall, there were 142 primary care subjects with a negative Elecsys Phospho-Tau (181P) Plasma result. Of these, 139 had amyloid PET scan negative results. The Negative Predictive Value (NPV) of the assay was 97.9% (139/142)

with a 95% CI: 94.0%–99.3% based on the amyloid PET positivity rate of 13.1% (41/312). The Likelihood Ratio Negative (LR-) of Elecsys Phospho-Tau (181P) Plasma was 0.143 with a 95% CI: 0.049–0.382. The potential benefit of the Elecsys Phospho-Tau (181P) Plasma would be a reduction in unnecessary amyloid PET scan by 45.5% (142/312). The Positive Predictive Value (PPV) was 22.4% (38/170) with a 95% CI: 19.5%–25.0%. The Likelihood Ratio positive (LR+) of Elecsys Phospho-Tau (181P) Plasma was 1.903 with a 95% CI: 1.601%–2.200%). The results showed that the Elecsys Phospho-Tau (181P) Plasma assay is characterized by high PPA and high NPV which support clinical usefulness as an aid in ruling out amyloid pathology in patients presenting with complaints or impairment and a negative Elecsys Phospho-Tau (181P) Plasma result.

In conclusion, the data of the pivotal clinical validation study support the intended use of Elecsys Phospho-Tau (181P) Plasma as an aid in the initial assessment for Alzheimer's disease and other causes of cognitive in adult subjects, aged 55 years and older, presenting with signs, symptoms, or complaints of cognitive decline. The cut-off of the Elecsys Phospho-Tau (181P) Plasma is clinically validated for aiding to rule out amyloid pathology, thereby providing a reliable tool that, although not diagnostic on its own, facilitates further evaluation and management of patients with cognitive complaints or impairment, subjective or objective, of unknown cause.

2) Sub-group Analysis

a. Agreement with amyloid PET status stratified by diagnostic groups

The clinical performance measures of the Elecsys Phospho-Tau (181P) Plasma assay are stratified by clinical diagnosis subgroups and summarized in the table below.

Agreement with PET status by diagnostic groups (N=306)[#]			
	SCD^	**MCI^**	**Mild Dementia**
N (% Total)	128 (41.8%)	175 (57.2%)	3 (0.980%)
Visual Amyloid PET Read Negative (n/N) (95% CI)*	95.3% (122/128) (90.2%–97.8%)	81.1% (142/175) (74.7%–86.2%)	100% (3/3) (43.9%–100%)
PPA (n/N) (95% CI)*	83.3% (5/6) (43.7%–97.0%)	97.0% (32/33) (84.7%–99.5%)	N/A^ (0/0)
NPA (n/N) (95% CI)*	54.9% (67/122) (46.1%–63.5%)	47.9% (68/142) (39.8%–56.1%)	66.7% (2/3) (20.8%–93.9%)
TPA (n/N) (95% CI)*	56.3% (72/128) (47.6%–64.5%)	57.1% (100/175) (49.7%–64.2%)	66.7% (2/3) (20.8%–93.9%)
PPV	8.3%	30.2%	0%

Agreement with PET status by diagnostic groups (N=306)[#]			
	SCD^	**MCI^**	**Mild Dementia**
(n/N) (95% CI)**	(5/60) (4.4%−10.8%)	(32/106) (26.5%−34.0%)	(0/1) (0%−79.3%)
NPV (n/N) (95% CI)**	98.5% (67/68) (95.1%−99.7%)	98.6% (68/69) (93.0%−99.70%)	100% (2/2) (34.2%−100%)
LR+ (95% CI)***	1.85 (0.94−2.47)	1.86 (1.55-2.20)	N/A^
LR- (95% CI)***	0.304 (0.054−1.050)	0.063 (0.011−0.324)	N/A^
Rule-out Rate (%N) (95% CI)*	53.1% (68/128) (44.5%− 61.6%)	39.4% (69/175) (32.5%−46.8%)	66.7% (2/3) (20.8%− 93.9%)

[#] The diagnostic category was unknown in 6 subjects
^ SCD: subjective cognitive decline; MCI: mild cognitive impairment; N/A; not applicable
* 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

The estimates of PPA were 83.3% for SCD and 97.0% for MCI. The estimates of NPV were 98.5% for SCD and 98.6% for MCI. The rule-out rate decreases from 53.1% for SCD to 39.4% for MCI. The same estimates for mild dementia had high uncertainty due to limited numbers of patients in the study.

b. Agreement with amyloid PET status stratified by sex

The clinical performance measures of the Elecsys Phospho-Tau (181P) Plasma assay are stratified by sex and summarized in the table below.

Agreement with PET status by sex (N=312)		
	Male	**Female**
N **(% Total)**	127 (40.7%)	185 (59.3%)
Visual Amyloid PET Read Negative (n/N) (95% CI)*	84.3% (107/127) (76.9%−89.6%)	88.6% (164/185) (83.3%−92.5%)
PPA (n/N) (95% CI)*	90.0% (18/20) (69.9%−97.2%)	95.2% (20/21) (77.3%− 99.2%)
NPA (n/N) (95% CI)*	53.3% (57/107) (43.9%−62.4%)	50.0% (82/164) (42.4%−57.6%)

Agreement with PET status by sex (N=312)		
	Male	**Female**
TPA (n/N) (95% CI)*	59.1% (75/127) (50.4%−67.2%)	55.1% (102/185) (47.9%−62.1%)
PPV (n/N) (95% CI)**	26.5% (18/68) (21.0%−31.5%)	19.6% (20/102) (16.1%−22.6%)
NPV (n/N) (95% CI)**	96.6% (57/59) (90.3%−99.0%)	98.8% (82/83) (94.4%−99.8%)
LR+ (95% CI)***	1.93 (1.42−2.46)	1.91 (1.50−2.28)
LR- (95% CI)***	0.188 (0.052−0.578)	0.095 (0.017−0.659)
Rule-out Rate (%N) (95% CI)*	46.5% (59/127) (38.0%−55.1%)	44.9% (83/185) (37.9%−52.1%)
* 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		

The estimates of PPA and NPV were comparable for males (90% and 96.6%) and females (95.2% and 98.8%). The rule-out rate was also similar between males (46.5%) and females (44.9%).

c. Agreement with amyloid PET status stratified by age

The clinical performance measures of the Elecsys Phospho-Tau (181P) Plasma assay are stratified by age groups and summarized in the table below.

Agreement with PET status by age groups (N=312)		
	55 to 70 years	**71 to 80 years**
N **(% Total)**	175 (56.1%)	137 (43.9%)
Visual Amyloid PET Read Negative (n/ N) (95% CI)*	93.1% (163/175) (88.4%−96.0%)	78.8% (108/137) (71.3%−84.8%)
PPA (n/N) (95% CI)*	100% (12/12) (75.8%−100%)	89.7% (26/29) (73.6%−96.4%)
NPA (n/N) (95% CI)*	63.8% (104/163) (56.2%−70.8%)	32.4% (35/108) (24.3%−41.7%)

Agreement with PET status by age groups (N=312)		
	55 to 70 years	**71 to 80 years**
TPA (n/N) (95% CI)*	66.3% (116/175) (59.0%−72.9%)	44.5% (61/137) (36.5%−52.9%)
PPV (n/N) (95% CI)**	16.9% (12/71) (13.0%−20.1%)	26.3% (26/99) (22.2%−29.8%)
NPV (n/N) (95% CI)**	100% (104/104) (96.7%−100%)	92.1% (35/38) (81.3%−97.2%)
LR+ (95% CI)***	2.76 (2.03−3.43)	1.33 (1.06−1.58)
LR- (95% CI)***	0.000 (0.000−0.383)	0.319 (0.107−0.855)
Rule-out Rate (%N) (95% CI)*	59.4% (104/175) (52.0% − 66.4%)	27.7% (38/137) (20.9%−35.8%)
* 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		

The estimate of PPA decreases slightly with age from 100% in the '55−70 years' age group to 89.7% in the '71−80 years' age group. The estimates of NPA followed a similar trend as PPA across the age groups, decreasing from 100% to 92.1%. The rule-out rate decreases from 59.4% for the '55−70 years' age group to 27.7% for the '71−80 years' age group.

d. Agreement with amyloid PET status stratified by race

The clinical performance measures of the Elecsys Phospho-Tau (181P) Plasma assay are stratified by race and summarized in the table below.

Agreement with PET status by race (N=312)				
	White	**Black**	**Asian**	**Other@**
N **(% Total)**	184 (59.0%)	106 (34.0%)	5 (1.6%)	17 (5.4%)
Visual Amyloid PET Read Negative (n/ N) (95% CI)*	79.9% (147/184) (73.5%−85.0%)	96.2% (102/106) (90.7%−98.5%)	100% 5/5 (56.6%−100%)	100% 17/17 (81.6%−100%)
PPA (n/N) (95% CI)*	94.6% (35/37) (82.3%−98.5%)	75.0% (3/4) (30.1%−95.4%)	N/A^ (0/0)	N/A^ (0/0)

Agreement with PET status by race (N=312)				
	White	**Black**	**Asian**	**Other[@]**
NPA (n/N) (95% CI)*	44.9% (66/147) (37.1%−53.0%)	56.9% (58/102) (47.2%−66.1%)	40.0% (2/5) (11.8%−76.9%)	76.5% (13/17) (52.7%−90.4%)
TPA (n/N) (95% CI)*	54.9% (101/184) (47.7%−61.9%)	57.5% (61/106) (48.0%−66.5%)	40.0% (2/5) (11.8%−76.9%)	76.5% (13/17) (52.7%−90.4%)
PPV (n/N) (95% CI)**	30.2% (35/116) (26.5%−33.9%)	6.4% (3/47) (2.6%−9.1%)	0.0% (0/3) (0.0%−56.1%)	0% (0/4) (0%−49.0%)
NPV (n/N) (95% CI)**	97.1% (66/68) (90.8%−99.2%)	98.3% (58/59) (95.3%−99.7%)	100% (2/2) (34.2%−100%)	100% (13/13) (77.2%−100%)
LR+ (95% CI)***	1.72 (1.43−2.03)	1.74 (0.70−2.54)	N/A^	N/A^
LR- (95% CI)***	0.1204 (0.0329−0.4012)	0.440 (0.079−1.270)	N/A^	N/A^
Rule-out Rate (%N) (95% CI)*	37.0% (68/184) (30.3%−44.1%)	55.7% (59/106) (46.2%−64.8%)	40.0% (2/5) (11.8%−76.9%)	76.5% (13/17) (52.7%−90.4%)

[@] Others include Middle Eastern, Hawaiian / Pacific Islander, and American Indian/Alaskan
^ N/A; not applicable
* 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

The validation study was conducted in a cohort that is primarily White (n=184; 59.0%) and Black or African American (n=106; 34.0%). For White, the estimates of PPA and NPV were 94.6% and 97.1%, respectively. For Black or African American, the estimates of PPA and NPV were 75.0% and 98.3%, respectively. The rule-out rates for White and Black or African American were 37.0% and 55.7%, respectively. Sample sizes from other races are not sufficient to provide reliable estimates of the clinical performance by race.

In conclusion, the data of the clinical performance study support that a Elecsys Phospho-Tau (181P) Plasma result below the cut-off of 0.722 pg/mL is consistent with an amyloid PET scan negative result.

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

Not applicable

D Clinical Cut-Off:

Refer to Assay Cut-Off.

E Expected Values/Reference Range:

A reference interval of the Elecsys Phospho-Tau (181P) Plasma was determined in cognitively normal subjects in accordance with the CLSI guideline EP28-A3c. The subjects were apparently cognitively normal individuals that have had no record of cognitive complaints or impairment and completed the QDRS cognitive assessment with a score of zero. The study was conducted in the U.S and included a total of 174 subjects (45.5% females and 54.6% males) who were between 50–80 years old (inclusive), with an average age of 65.1 years. Regarding race, 139 are Whites (79.9%), 17 Blacks (9.8%), 7 Asians (4.0%), 10 Others (6.31%) and 1 not reported. Due to the high prevalence of comorbidities and medication intake in the average 55–80 years-old population, subjects with symptoms and morbidities other than SCD, MCI, mild dementia or unstable illness or receiving non-investigational medications were not excluded from this study. By contrast, subjects with chronic kidney disease, history of myocardial infraction or history of stroke were excluded due to their reported associations with plasma pTau181 levels[1]. The K2-EDTA plasma specimens collected from the prospectively enrolled subjects were measured with Elecsys Phospho-Tau (181P) Plasma on the **cobas e** 801. The results of the cognitively normal subjects tested with Elecsys Phospho-Tau (181P) Plasma are summarized in the table below.

		Age groups	
	All	**55 to 70 years**	**71 to 80 years**
N (%)	174 (100%)	129 (74.1%)	45 (25.9%)
Mean (SD) (pg/mL)	0.774 (0.460)	0.703 (0.307)	0.976 (0.707)
Median (pg/mL)	0.658	0.631	0.718
Range (pg/mL)	0.300–3.72	0.300–1.94	0.300–3.72
2.5th, 97.5th Percentile	0.323−1.91	0.323−1.52	0.358−3.43
N (%) > 0.722 pg/mL	68 (39.1%)	46 (35.7%)	22 (48.9%)

The established 95% reference interval is 0.323–1.91 pg/mL for plasma pTau181. Across the age groups, 39.1% of cognitively normal subjects have Elecsys Phospho-Tau (181P) Plasma test results above the cut-off. The percentage of cognitively normal subjects with Elecsys Phospho-Tau (181P) Plasma test results above the cut-off increased with age from 35.7% at ages between 55 to 70 years to 48.9% at ages between 71 to 80 years. The medians for Elecsys Phospho-Tau (181P) Plasma test result did not differ significantly across race, and the 95% reference interval of Elecsys Phospho-Tau (181P) Plasma test result is comparable in males and females as shown in the table below.

	Cognitively Normal					
	Race^				**Sex**	
	White	**Black**	**Asian**	**Other**	**Male**	**Female**
N (%)	139 (79.9%)	17 (9.8%)	7 (4.0%)	10 (5.7%)	95 (54.6%)	79 (45.4%)
Mean (SD)	0.805 (0.491)	0.697 (0.308)	0.650 (0.229)	0.581 (0.285)	0.836 (0.533)	0.699 (0.341)
Median	0.667	0.594	0.668	0.484	0.701	0.594

[1] Mielke MM, Dage JL, Frank RD, et al. Performance of plasma phosphorylated tau 181 and 217in the community. Nat Med. 2022;28:1398-405. PMID: 35618838

	Cognitively Normal					
	Race^				Sex	
	White	**Black**	**Asian**	**Other**	**Male**	**Female**
Range	0.300−3.72	0.300−1.59	0.300−0.98	0.320−1.18	0.300–3.72	0.300–1.94
2.5th, 97.5th Percentile	0.358−1.94	N/A*, N/A*	N/A*, N/A*	N/A*, N/A*	0.342−1.96	0.300−1.91
N (%) >0.722 pg/mL	58 (41.7%)	6 (35.3%)	2 (28.6%)	2 (20.0%)	45 (47.4%)	23 (29.1%)

^No race reported for 1 subject with mean pTau181 of 0.559 pg/mL
*Results listed as not applicable (N/A) are due to an insufficient number of samples to properly calculate the value

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.