08846693501V2.0

Elecsys Phospho-Tau (181P) CSF

cobas®

REF		Σ	SYSTEM
			cobas e 411
08846693160	08846693501	60	**cobas e** 601
			cobas e 602

English

For use in the USA only

System information

For **cobas e** 411 analyzer: test number 1670

For **cobas e** 601 and **cobas e** 602 analyzers: Application Code Number 179

Caution

The Elecsys Phospho-Tau (181P) CSF assay is not intended to be used as a stand-alone test and should only be used with the Elecsys β-Amyloid (1-42) CSF II assay to calculate the ratio of phosphorylated Tau (181P) (pTau181) to β-Amyloid (1-42) (Abeta42) in CSF (pTau181/Abeta42 ratio). Use of another manufacturer's pTau181 CSF assay may result in significantly different pTau181/Abeta42 ratios because the measured pTau181 value in a given sample, determined with assays from different manufacturers, can vary due to differences in assay methods and reagent. Values determined from samples by different assay methods and on different **cobas e** platforms cannot be used interchangeably.

The performance of the test for African American, Asian, and other races had high uncertainty due to the limited number of patients studied.

The ratio can only be used with the Elecsys Phospho-Tau (181P) CSF and Elecsys β-Amyloid (1-42) CSF II assay values generated from the **cobas e** immunoassay analyzers.

Due to the sticky properties of the Abeta42 protein, the cut-off for the ratio pTau181/Abeta42 (calculated based on results of the Elecsys Phospho-Tau (181P) CSF and the Elecsys β-Amyloid (1-42) CSF II assays) provided in this document is only valid if the required pre-analytical handling procedure (described in the "Specimen collection and preparation" section) is strictly followed.

A positive pTau181/Abeta42 ratio result in CSF does not establish a diagnosis of Alzheimer's disease (AD) and should always be interpreted in conjunction with clinical information.

Intended use

Elecsys β-Amyloid (1-42) CSF II and Elecsys Phospho-Tau (181P) CSF are in vitro electrochemiluminescence immunoassays for the measurement of the β-Amyloid (1-42) (Abeta42) and Phospho-Tau (181P) (pTau181) protein concentrations in cerebrospinal fluid (CSF) from adult patients aged 55 years and older being evaluated for Alzheimer's disease (AD) and other causes of cognitive impairment to generate a pTau181/Abeta42 ratio value.

A negative result, defined as pTau181/Abeta42 ratio value below cutoff or an Abeta42 value above the measuring range, is consistent with a negative amyloid positron emission tomography (PET) scan result. A negative result reduces the likelihood that a patient's cognitive impairment is due to AD. A positive result, defined as pTau181/Abeta42 ratio value above cut-off, is consistent with a positive amyloid PET scan result. A positive result does not establish a diagnosis of AD or other cognitive disorder. The pTau181/Abeta42 ratio result is used as an adjunct to other clinical diagnostic evaluations.

Limitations of use

The performance of the pTau181/Abeta42 ratio has not been established for:

- Predicting development of dementia or other neurologic conditions
- Monitoring responses to therapies

Summary

Tau (tubulin-associated unit) protein is one of the two hallmarks of AD, besides Abeta42. Tau is found as 6 molecular isoforms in the human brain. These isoforms are coded by a single gene on chromosome 17 and generated by alternative splicing of its pre-mRNA. The Tau from all these isoforms is called total Tau (tTau). The most common post-translational modification of Tau proteins is phosphorylation. Phosphorylation changes the shape of the Tau molecule and regulates its biological activity. During neurodegeneration abnormal phosphorylation leads to formation of intracellular neurofibrillary tangles (NFTs) composed of the Tau protein that has undergone hyper-phosphorylation, and developed aggregates of hyper-phosphorylated Tau proteins called Phospho-Tau (pTau).[1,2]

The Elecsys Phospho-Tau (181P) CSF assay is designed to quantify the concentration of the protein or fragments of Tau protein phosphorylated at threonine 181 in human CSF.

Clinical relevance of pTau181

In AD, numerous studies show that while Abeta42 levels in CSF decrease to around half the level in controls, CSF pTau181 levels increase around 2-3 fold in mild-moderate AD patients compared to age-matched controls.[3,4] High CSF pTau181 levels are also associated with a faster progression from mild cognitive impairment (MCI) to AD with more rapid cognitive decline in AD patients[5] and in mild AD dementia cases.[6]

pTau181 as a biomarker in CSF might be useful in detecting the likely progression of MCI to AD[7] and has most power when used in combination with Abeta42 in CSF.

The use of AD biomarkers has been included in the new consensus research diagnostic criteria for AD, MCI, and preclinical AD, proposed by the National Institute on Aging (NIA) and the Alzheimer's Association. These new criteria take into account that AD dementia is part of a continuum of clinical and biological phenomena.[8,9] The new International Working Group 2 (IWG 2) criteria recommend the use of either CSF biomarker or amyloid PET imaging for evaluation of AD patients.[10] In Europe, the Committee for Medicinal Products for Human Use (CHMP) published a number of positive opinions on the use of biomarkers in the context of AD for enrichment of clinical trials in pre-clinical dementia and mild to moderate AD.[11,12]

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 50 µL of sample, a biotinylated monoclonal antibody specific for phosphorylation at threonine 181 and a monoclonal Tau-specific antibody labeled with a ruthenium complex[a)] react to form a sandwich complex.
- 2nd 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.
- Results are determined 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.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Reagents - working solutions

The reagent rackpack is labeled as pTau.

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-pTau Ab~biotin (gray cap), 1 bottle, 6.5 mL: Biotinylated monoclonal anti-pTau antibody (rabbit/mouse) 2.5 mg/L; Tris[b)] buffer > 14 mmol/L, pH 7.2; preservative.

Elecsys Phospho-Tau (181P) CSF

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R2 Anti-Tau-Ab~Ru(bpy)$_3^{2+}$ (black cap), 1 bottle, 6.5 mL:
Monoclonal anti-Tau antibody (mouse) labeled with ruthenium complex 2.0 mg/L; Tris buffer > 14 mmol/L, pH 7.2; preservative.

b) Tris(hydroxymethyl)aminomethane

Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material.
Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.
Safety data sheet available for professional user on request.

For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H317 May cause an allergic skin reaction.

Prevention:

P261 Avoid breathing mist or vapours.

P272 Contaminated work clothing should not be allowed out of the workplace.

P280 Wear protective gloves.

Response:

P333 + P313 If skin irritation or rash occurs: Get medical advice/attention.

P362 + P364 Take off contaminated clothing and wash it before reuse.

Disposal:

P501 Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

Contact phone: 1-800-428-2336

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent handling

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

All information required for correct operation is read in from the respective reagent barcodes.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the Elecsys reagent kit **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	8 weeks
on the analyzers	28 days
open rackpack on the analyzers	25 hours

Specimen collection and preparation

Only the specimen listed below is acceptable for use with this assay.

Please follow the steps listed below for CSF sample collection and measurement.

The technical notes are an essential part of the instructions and must be read thoroughly before completing each step.

Steps	Technical notes
1. **Perform lumbar puncture (LP)** using gravity drip collection method.	Avoid the use of syringes or tubings. Perform LP before noon.
2. **Do not use the first 2 mL of CSF for Elecsys AD Biomarker measurement.**	None
3. **Subsequently collect at least 2.5 mL of CSF directly into the** CSF tube REF 63.614.625 (Sarstedt) for AD biomarker measurements (Note: 2.5 mL filling volume corresponds to filling up to the mark on the tube).	Each sample should be visually inspected for hemolysis. Do not use CSF samples which appear reddish for measurement of Elecsys AD biomarkers. Instead, collect additional clear (non-hemolytic) CSF in a new CSF tube. Collection of CSF for other purposes can follow thereafter, if required.
4. Do not process the CSF sample before transport to the measuring site (i.e. no inverting, no tube transfers, no aliquoting, and normally no centrifugation) until measurement.	It is strongly recommended that the sample be kept at 2-8 °C or -15 to -25 °C during transport and storage up to the time of measurement. Samples can be stored at -15 to -25 °C for up to 8 weeks or 2-8 °C for up to 14 days. If transport and storage at -15 to -25 °C or 2-8 °C is not feasible, the sample can be transported/stored at room temperature (15-25 °C). If this is the case, measurement is to be performed within 5 days after sample draw. Note that samples cannot be frozen at -80 °C.
5. For samples stored at -15 to -25 °C, thaw samples for 30 minutes at room temperature on a roller mixer.	Only one freeze/thaw is acceptable.
6. Measurement on the **cobas e** 601 analyzer: Directly place the CSF sample tube on the analyzer for measurement. To prevent evaporation, only open the sample tube immediately before measurement.	

Stability of CSF samples: Stable for 8 weeks at -15 to -25 °C (one freeze/thaw cycle), 14 days at 2-8 °C and 5 days at 15-25 °C.

Do not use CSF samples that show impurities with hemolyzed blood and are visibly colored red. Centrifuge samples containing precipitates before performing the assay.

Ensure the calibrators and controls are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples, calibrators and controls on the analyzers should be analyzed/measured within 2 hours.

Please always keep calibrators, controls and samples capped if not in use.

Elecsys Phospho-Tau (181P) CSF cobas®

Sample stability claims were established by experimental data by the manufacturer or based on reference literature and only for the temperatures/time frames as stated in the method sheet. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- REF 07357044190, CalSet Phospho-Tau (181P), for 4 x 1.0 mL
- REF 07357052190, PreciControl Phospho-Tau (181P), for 6 x 1.0 mL
- REF 63.614.625, 2.5 mL Low bind False bottom tube, Sarstedt (for CSF collection)
- General laboratory equipment
- **cobas e** analyzer

Additional materials for **cobas e** 411 analyzer:

- REF 11662988122, ProCell, 6 x 380 mL system buffer
- REF 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- REF 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- REF 11933159001, Adapter for SysClean
- REF 11706802001, AssayCup, 60 x 60 reaction cups
- REF 11706799001, AssayTip, 30 x 120 pipette tips
- REF 11800507001, Clean-Liner

Additional materials for **cobas e** 601 and **cobas e** 602 analyzers:

- REF 04880340190, ProCell M, 2 x 2 L system buffer
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- REF 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- REF 03004899190, PreClean M, 5 x 600 mL detection cleaning solution
- REF 12102137001, AssayTip/AssayCup, 48 magazines x 84 reaction cups or pipette tips, waste bags
- REF 03023150001, WasteLiner, waste bags
- REF 03027651001, SysClean Adapter M

Additional materials for all analyzers:

- REF 11298500160, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers.

cobas e 601 and **cobas e** 602 analyzers: PreClean M solution is necessary.

Bring the cooled reagents to approximately 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the bottles.

Calibration

Traceability: This method has been 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.

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.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer).

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Renewed calibration is recommended as follows:

- after 4 weeks when using the same reagent lot
- after 7 days (when using the same reagent kit on the analyzer)
- as required: e.g. quality control findings outside the defined limits

Quality control

For quality control, use PreciControl Phospho-Tau (181P).

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.

Special care needs to be taken to ensure that the accuracy and precision of the testing stays within acceptable limits. Besides meeting the PreciControl Phospho-Tau (181P) target ranges provided, the user needs to ensure that the systematic bias with respect to the assigned target value is within ± 10 %, the intermediate precision CV is ≤ 10 % and the maximal total error is within ± 26.5 % (TE = |bias| + 1.65*CV). It is recommended to use quality control rule software.

For those users who are not familiar with the special QC setup and application, detailed information is available in the brochure "**Implementation Guidance of Statistical Quality Control Rules for Quantitative Assays**," which is available via dialog.roche.com. This brochure explains e.g. how to check if the maximal total error is within the allowed range based on the local QC results, besides other useful information.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

If necessary, repeat the measurement of the samples concerned.

Calculation

The analyzer automatically calculates the analyte concentration of each sample in pg/mL.

Expected Values

Reference ranges were defined based on the first-generation Elecsys β-Amyloid (1-42) CSF and Elecsys Phospho-Tau (181P) CSF assay results from 115 (46 male and 69 female) cognitively normal healthy controls 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.[13] For each CSF sample, the levels of Abeta42 and pTau181 were determined in parallel on the **cobas e** 601. As detailed in the **Clinical performance (item iv)** section, a conversion factor (0.9368) was determined and applied to the Abeta42 values to account for bias in measurement results observed for the second-generation Elecsys β-Amyloid (1-42) CSF II assay, which is due to re-standardization to a certified reference material and adoption of a routine-use protocol for CSF collection and storage as described in the **Specimen collection and preparation** section. The median, 2.5th, 5th, 95th, and 97.5th percentile of pTau181 and the ratio of pTau181/Abeta42 for the reference population are calculated and shown in the table below.

	2.5th Pctl[c)]	**5th Pctl**	**Median**	**95th Pctl**	**97.5th Pctl**
pTau181 [pg/mL]	8.5	9.6	17.5	31.3	40.9
pTau181/Abeta42	0.007	0.008	0.012	0.039	0.047
Age 66-70 years (N = 43)					
pTau181 [pg/mL]	8.0	8.4	15.9	28.5	33.1
pTau181/Abeta42	0.006	0.007	0.010	0.026	0.035

Elecsys Phospho-Tau (181P) CSF cobas®

	2.5th Pctl[c)]	5th Pctl	Median	95th Pctl	97.5th Pctl
Age 71-75 years (N = 72)					
pTau181 [pg/mL]	10.2	11.6	18.4	35.8	42.8
pTau181/Abeta42	0.008	0.009	0.014	0.043	0.052

c) Pctl = percentile

In this cognitively normal reference population, 81 % were below and 19 % were above the pTau181/Abeta42 cutoff indicative of a negative or positive amyloid PET scan in combination with cognitive impairment (see section below). The medians for pTau181 and the ratio pTau181/Abeta42 did not differ significantly by gender. A slight trend of increasing pTau181 and pTau181/Abeta42 ratio levels was observed with age, especially over the age of 70 years.

A prospective study was conducted to verify the reference ranges (intervals between the calculated 2.5 % and 97.5 % distributions quantiles) using the final Elecsys CSF assays. Measurements were performed in prospectively collected fresh CSF samples (prepared according to the pre-analytical protocol for routine-use) obtained from 20 cognitively normal healthy donors aged 60 to 73 years with a Montreal Cognitive Assessment (MoCA) (this is a cognitive screening test)[14] score ≥ 25 enrolled in the Emory Healthy Brain Study (EHBS).

For pTau181, all 20 samples fell within the range and for the ratio pTau181/Abeta42, 19 of the 20 samples fell within the range established with BioFINDER1. Since less than two results fell outside the reference range established in the BioFINDER1 cohort, the expected values are successfully verified in the EHBS cohort.

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

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

Results of the Elecsys β-Amyloid (1-42) CSF II and Elecsys Phospho-Tau (181P) CSF assays are reported separately by the instrument. The ratio of pTau181/Abeta42 must be calculated by the operator because the instrument does not report the final result. The final result (negative or positive) must be interpreted by the laboratory professional according to the table below:

pTau181/Abeta42[d)]	Interpretation
≤ 0.023	A negative result consistent with a negative amyloid PET scan result.
> 0.023	A positive result consistent with a positive amyloid PET scan result.
Invalid result for either Elecsys Phospho-Tau (181P) CSF or Elecsys β-Amyloid (1-42) CSF II	Not reportable
Invalid results for both Elecsys Phospho-Tau (181P) CSF and Elecsys β-Amyloid (1-42) CSF II	Not reportable

d) The ratio should be rounded to 4 decimal places before comparing against 0.023. If the concentrations of the analytes are outside the measuring range, the following rules apply: In cases Abeta42 < 150 pg/mL, pTau181 < 8.0 pg/mL, pTau181 > 120 pg/mL, the value should be set to the respective limit of the measuring range and the ratio should be calculated. If the Abeta42 value is > 2500 pg/mL, the result is consistent with a negative amyloid PET scan result.

Specimens that fail to meet the run validity criteria yield 'Invalid Result' outcomes. Specimens with 'Invalid Result' results for either 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 table above.

Performance Characteristics

Measuring range

8.0-120 pg/mL (defined by the Limit of Quantitation and the maximum of the master curve). Values below the Limit of Quantitation are reported as < 8.0 pg/mL. Values above the measuring range are reported as > 120 pg/mL.

The numerical value of ratio pTau181/Abeta42 ranges from 0.003 (8/2500) to 0.800 (120/150).

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 4 pg/mL

Limit of Detection = 8 pg/mL

Limit of Quantitation = 8.0 pg/mL

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 20.0 %.

Linearity

The linearity study for Elecsys Phospho-Tau (181P) CSF was assessed on the **cobas e** 601 analyzer; 3 independent dilution series covering the measuring range were prepared from native human CSF samples. Linear regression was performed in accordance with CLSI EP06-Ed2. Regression statistics are based on the pooled results of 3 dilution experiments. All deviations from linearity were within ± 15 %.

Assay	Range [pg/mL]	Slope (95 % CI)	Intercept	R^2
Elecsys Phospho-Tau (181P) CSF	7.84 - 134.7	0.981 (0.972, 0.991)	0	0.9994

High-dose hook effect

High-dose hook effect was evaluated using two spiked CSF samples with analyte concentrations ≥ 300 pg/mL. Dilution series were measured with 3 lots on one **cobas e** 601 analyzer.

There is no high-dose hook effect at pTau181 concentrations up to 300 pg/mL.

Interference

The effect on quantitation of analyte in the presence of endogenous interfering substances was determined on the **cobas e** 601 analyzer using human CSF. For each interfering substance a total of four CSF sample pools (low, medium, high and within 20 % above or below the cutoff of pTau181/Abeta42 ratio) were prepared and tested in N = 5 determinations. The mean value was used to calculate the relative (%) or absolute (pg/mL) deviation from the reference sample.

The effect of the following endogenous substances and pharmaceutical compounds on assay performance was tested. Interferences were tested up to the listed concentrations and no impact on results was observed.

Endogenous substances

Compound	Concentration tested
Bilirubin	0.9 mg/L
Hemoglobin	150 mg/L
Intralipid	300 mg/L
Biotin	≤ 4912 nmol/L or ≤ 1200 ng/mL
Rheumatoid factors	4 IU/mL[e)]
IgG	0.6 g/L
IgA	0.06 g/L
IgM	0.015 g/L
Human Serum Albumin	1.5 g/L

e) The data supports no interference up to 12 IU/mL. However, a conservative value is specified.

Elecsys Phospho-Tau (181P) CSF cobas®

Criterion: Deviation within ± 3 pg/mL of initial value ≤ 25 pg/mL and within ± 10 % of initial value > 25 pg/mL. pTau181/Abeta42 ratio specification: Recovery within 82-122 %.

Biotin interference

This assay has no biotin interference in CSF concentrations up to 1200 ng/mL. Pharmacokinetic studies have shown that serum concentrations of biotin can reach up to 355 ng/mL within the first hour after biotin ingestion for subjects consuming supplements of 20 mg biotin per day[15] and up to 1160 ng/mL for subjects after a single dose of 300 mg biotin.[16]

Pharmaceutical substances

The effect on quantitation of the pTau181 analyte in the presence of exogenous interfering substances using the Elecsys Phospho-Tau (181P) CSF assay was determined on the **cobas e** 601 analyzer. Pharmaceuticals were tested by spiking into four CSF sample pools (low, medium, high and within 20 % above or below the cutoff of pTau181/Abeta42 ratio). Each sample was tested in N = 5 determinations and the mean value was used to calculate the relative (%) or absolute (pg/mL) deviation from the reference sample.

In vitro tests were performed on 17 commonly used pharmaceuticals. No interference with the assay was found.

Commonly used pharmaceuticals

Pharmaceutical	Concentration tested [mg/L]
Acetaminophen	156
Acetylcysteine	150
Acetylsalicylic acid	30
Ampicillin-Na	75
Ascorbic acid	52.5
Cefoxitin	750
Cyclosporine	1.8
Doxycycline	18
Heparin	1100 IU/L
Ibuprofen	219
Itraconazole	0.06
Levodopa	7.5
Methyldopa	22.5
Metronidazole	123
Phenylbutazone	107
Rifampicin	48
Theophylline	60

Criterion: Deviation within ± 3 pg/mL of initial value ≤ 25 pg/mL and recovery within 100 ± 10 % of initial value > 25 pg/mL. Specification for pTau181/Abeta42 ratio: Recovery within 82-122 %.

In addition, the following 14 special drugs were tested. No interference with the assay was found.

Special drugs

Drug	Concentration tested [mg/L]
Atorvastatin	0.75
Digoxin	0.04
Donepezil	30
Escitalopram	0.19
Esomeprazole	6.9
Furosemide	15.9
Galantamine	250
Hydrochlorothiazide	1.13
Lisinopril	0.246
Memantine	0.117
Metformin	12
Metoprolol	1.5
Rivastigmine	45
Simvastatin	1.68

Criterion: Deviation within ± 3 pg/mL of initial value ≤ 25 pg/mL and recovery within 100 ± 10 % of initial value > 25 pg/mL. Specification for pTau181/Abeta42 ratio: Recovery within 82-122 %.

Drug interferences are measured based on recommendations given in CLSI guidelines EP07 and EP37 and other published literature. Effects of concentrations exceeding these recommendations have not been characterized.

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Precision

Precision was determined using Elecsys reagents, CSF samples (pTau181 only and pTau181/Abeta42 ratio samples) and controls in a protocol (EP05-A3) of the CLSI: 2 runs per day in duplicate each for 21 days (N = 84). The following results were obtained:

Elecsys Phospho-Tau (181P) CSF precision data

cobas e 601 analyzer					
		Within-run		Between-run	
Sample	Mean [pg/mL]	SD [pg/mL]	CV [%]	SD [pg/mL]	CV [%]
CSF 1	15.5	0.26	1.7	0.12	0.8
CSF 2	22.3	0.27	1.2	0.31	1.4
CSF 3	26.3	0.40	1.5	0.25	0.9
CSF 4	33.3	0.58	1.7	0.00	0.0
CSF 5	58.4	0.76	1.3	0.61	1.0
CSF 6	108	1.54	1.4	0.88	0.8
CSF 7	115	1.53	1.3	1.10	1.0
PC[f] Phospho-Tau (181P) Level 1	14.2	0.28	1.9	0.19	1.3
PC Phospho-Tau (181P) Level 2	47.7	0.79	1.7	0.34	0.7

f) PC = PreciControl

Elecsys Phospho-Tau (181P) CSF precision data, continued

cobas e 601 analyzer					
		Between-day		Within laboratory	
Sample	Mean [pg/mL]	SD [pg/mL]	CV [%]	SD [pg/mL]	CV [%]
CSF 1	15.5	0.26	1.7	0.39	2.5
CSF 2	22.3	0.14	0.6	0.43	2.0
CSF 3	26.3	0.25	0.9	0.53	2.0
CSF 4	33.3	0.21	0.6	0.62	1.9
CSF 5	58.4	0.00	0.0	0.98	1.7

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cobas e 601 analyzer					
		Between-day		**Within laboratory**	
Sample	**Mean [pg/mL]**	**SD [pg/mL]**	**CV [%]**	**SD [pg/mL]**	**CV [%]**
CSF 6	108	0.20	0.2	1.79	1.7
CSF 7	115	0.48	0.4	1.94	1.7
PC Phospho-Tau (181P) Level 1	14.2	0.41	2.9	0.53	3.7
PC Phospho-Tau (181P) Level 2	47.7	0.46	1.0	0.97	2.0

pTau181/Abeta42 ratio precision data

cobas e 601 analyzer					
		Within-run		**Between-run**	
Sample	**Mean**	**SD**	**CV [%]**	**SD**	**CV [%]**
Ratio Sample 1	0.021	0.0005	2.3	0.0003	1.5
Ratio Sample 2	0.028	0.0006	2.0	0.0006	2.1
Ratio Sample 3	0.038	0.0006	1.6	0.0004	1.0
Ratio Sample 4	0.041	0.0008	2.0	0.0004	0.9
Ratio Sample 5	0.054	0.0009	1.6	0.0008	1.4

pTau181/Abeta42 ratio precision data, continued

cobas e 601 analyzer					
		Between-day		**Within laboratory**	
Sample	**Mean**	**SD**	**CV [%]**	**SD**	**CV [%]**
Ratio Sample 1	0.021	0.0005	2.6	0.0008	3.8
Ratio Sample 2	0.028	0.0003	1.1	0.0009	3.1
Ratio Sample 3	0.038	0.0008	2.1	0.001	2.8
Ratio Sample 4	0.041	0.0008	1.8	0.001	2.8
Ratio Sample 5	0.054	0.0007	1.3	0.001	2.5

Precision was determined using Elecsys reagents, CSF samples (pTau181 only and pTau181/Abeta42 ratio samples) and controls in a protocol (EP05-A3) of the CLSI: each sample was measured 5 times on each of 5 days (N = 25). The following results were obtained on a **cobas e** 411 analyzer:

Elecsys Phospho-Tau (181P) CSF precision data

cobas e 411 analyzer					
		Within-run		**Between-day**	
Sample	**Mean [pg/mL]**	**SD [pg/mL]**	**CV [%]**	**SD [pg/mL]**	**CV [%]**
CSF 1	16.8	0.36	2.1	0.07	0.4
CSF 2	22.2	0.40	1.8	0.25	1.1
CSF 3	27.3	0.38	1.4	0.43	1.6
CSF 4	30.8	0.97	3.2	0.79	2.6
CSF 5	64.1	0.95	1.5	0.41	0.6
CSF 6	113	2.10	1.8	0.98	0.9
PC Phospho-Tau (181P) Level 1	15.0	0.36	2.4	0.00	0.0
PC Phospho-Tau (181P) Level 2	53.4	1.75	3.3	0.00	0.0

Elecsys Phospho-Tau (181P) CSF precision data, continued

cobas e 411 analyzer			
		Within laboratory	
Sample	**Mean [pg/mL]**	**SD [pg/mL]**	**CV [%]**
CSF 1	16.8	0.37	2.2
CSF 2	22.2	0.47	2.1
CSF 3	27.3	0.57	2.1
CSF 4	30.8	1.25	4.1
CSF 5	64.1	1.03	1.6
CSF 6	113	2.32	2.0
PC Phospho-Tau (181P) Level 1	15.0	0.36	2.4
PC Phospho-Tau (181P) Level 2	53.4	1.75	3.3

pTau181/Abeta42 ratio precision data

cobas e 411 analyzer					
		Within-run		**Between-day**	
Sample	**Mean**	**SD**	**CV [%]**	**SD**	**CV [%]**
Ratio Sample 1	0.021	0.0005	2.3	0.0008	3.6
Ratio Sample 2	0.024	0.001	4.7	0.001	6.0
Ratio Sample 3	0.036	0.0009	2.4	0.001	2.7
Ratio Sample 4	0.038	0.0006	1.6	0.0009	2.4
Ratio Sample 5	0.037	0.0008	2.1	0.0007	2.0

pTau181/Abeta42 ratio precision data, continued

cobas e 411 analyzer			
		Within laboratory	
Sample	**Mean**	**SD**	**CV [%]**
Ratio Sample 1	0.021	0.0009	4.3
Ratio Sample 2	0.024	0.002	7.6
Ratio Sample 3	0.036	0.001	3.6
Ratio Sample 4	0.038	0.001	2.9
Ratio Sample 5	0.037	0.001	2.8

Lot-to-lot precision

Lot-to-lot precision was determined using **cobas e** 601, Elecsys reagents, CSF samples (pTau181 only and pTau181/Abeta42 ratio samples) and controls in a protocol with the following experimental design: 3 lots of reagent at one site, 2 runs per day in triplicate each for 5 days (N = 90) according to CLSI EP05-A3. The following results were obtained:

Elecsys Phospho-Tau (181P) CSF lot-to-lot precision data

cobas e 601 analyzer					
		Within-run		**Between-run**	
Sample	**Mean [pg/mL]**	**SD [pg/mL]**	**CV [%]**	**SD [pg/mL]**	**CV [%]**
CSF 1	15.5	0.22	1.4	0.22	1.4
CSF 2	20.9	0.28	1.3	0.31	1.5
CSF 3	26.2	0.42	1.6	0.39	1.5
CSF 4	30.2	0.50	1.6	0.44	1.5

Elecsys Phospho-Tau (181P) CSF cobas®

cobas e 601 analyzer					
		Within-run		**Between-run**	
Sample	**Mean [pg/mL]**	**SD [pg/mL]**	**CV [%]**	**SD [pg/mL]**	**CV [%]**
CSF 5	59.3	0.83	1.4	0.75	1.3
CSF 6	115	1.49	1.3	1.13	1.0
PC Phospho-Tau (181P) Level 1	14.1	0.23	1.7	0.14	1.0
PC Phospho-Tau (181P) Level 2	48.1	0.59	1.2	0.65	1.4

Elecsys Phospho-Tau (181P) CSF lot-to-lot precision data, continued

cobas e 601 analyzer					
		Between-day		**Between-lot**	
Sample	**Mean [pg/mL]**	**SD [pg/mL]**	**CV [%]**	**SD [pg/mL]**	**CV [%]**
CSF 1	15.5	0.0	0.0	0.46	2.9
CSF 2	20.9	0.0	0.0	0.45	2.1
CSF 3	26.2	0.0	0.0	0.36	1.4
CSF 4	30.2	0.0	0.0	0.32	1.1
CSF 5	59.3	0.0	0.0	0.62	1.0
CSF 6	115	0.87	0.8	0.83	0.7
PC Phospho-Tau (181P) Level 1	14.1	0.0	0.0	0.28	2.0
PC Phospho-Tau (181P) Level 2	48.1	0.0	0.0	1.11	2.3

Elecsys Phospho-Tau (181P) CSF lot-to-lot precision data, continued

cobas e 601 analyzer			
		Total	
Sample	**Mean [pg/mL]**	**SD [pg/mL]**	**CV [%]**
CSF 1	15.5	0.55	3.6
CSF 2	20.9	0.61	2.9
CSF 3	26.2	0.68	2.6
CSF 4	30.2	0.74	2.4
CSF 5	59.3	1.28	2.2
CSF 6	115	2.22	1.9
PC Phospho-Tau (181P) Level 1	14.1	0.39	2.8
PC Phospho-Tau (181P) Level 2	48.1	1.42	3.0

pTau181/Abeta42 ratio lot-to-lot precision data

cobas e 601 analyzer					
		Within-run		**Between-run**	
Sample	**Mean**	**SD**	**CV [%]**	**SD**	**CV [%]**
Ratio Sample 1	0.021	0.0003	1.7	0.0003	1.5
Ratio Sample 2	0.024	0.0006	2.5	0.0001	0.6
Ratio Sample 3	0.036	0.0007	1.8	0.0006	1.6
Ratio Sample 4	0.039	0.0007	1.8	0.0	0.0
Ratio Sample 5	0.037	0.0007	1.8	0.0002	0.7

pTau181/Abeta42 ratio lot-to-lot precision data, continued

cobas e 601 analyzer					
		Between-day		**Between-lot**	
Sample	**Mean**	**SD**	**CV [%]**	**SD**	**CV [%]**
Ratio Sample 1	0.021	0.0003	1.6	0.0010	4.7
Ratio Sample 2	0.024	0.0	0.0	0.0010	4.2
Ratio Sample 3	0.036	0.0002	0.5	0.0019	5.4
Ratio Sample 4	0.039	0.0004	1.0	0.0016	4.2
Ratio Sample 5	0.037	0.0002	0.5	0.0013	3.4

pTau181/Abeta42 ratio lot-to-lot precision data, continued

cobas e 601 analyzer			
		Total	
Sample	**Mean**	**SD**	**CV [%]**
Ratio Sample 1	0.021	0.0011	5.4
Ratio Sample 2	0.024	0.0012	5.0
Ratio Sample 3	0.036	0.0021	5.9
Ratio Sample 4	0.039	0.0018	4.7
Ratio Sample 5	0.037	0.0015	3.9

Site-to-site reproducibility

Reproducibility was determined with a panel of human CSF samples (pTau181 only and pTau181/Abeta42 ratio samples) and 2 controls. Samples were measured on a **cobas e** 601 analyzer in triplicate using 1 reagent lot in 2 runs for 5 days at 3 sites according to CLSI EP05-A3. The following results were obtained:

Elecsys Phospho-Tau (181P) CSF site-to-site reproducibility data

cobas e 601 analyzer					
		Within-run		**Between-run**	
Sample	**Mean [pg/mL]**	**SD [pg/mL]**	**CV [%]**	**SD [pg/mL]**	**CV [%]**
CSF 1	16.5	0.27	1.7	0.33	2.0
CSF 2	22.3	0.35	1.6	0.36	1.6
CSF 3	27.8	0.57	2.0	0.46	1.7
CSF 4	33.6	0.63	1.9	0.61	1.8
CSF 5	61.2	0.97	1.6	0.90	1.5
CSF 6	118	1.76	1.5	2.06	1.7
PC Phospho-Tau (181P) Level 1	15.4	0.35	2.3	0.29	1.9
PC Phospho-Tau (181P) Level 2	54.2	0.89	1.6	0.98	1.8

Elecsys Phospho-Tau (181P) CSF

Elecsys Phospho-Tau (181P) CSF site-to-site reproducibility data, continued

cobas e 601 analyzer					
		Between-day		**Between-site**	
Sample	**Mean [pg/mL]**	**SD [pg/mL]**	**CV [%]**	**SD [pg/mL]**	**CV [%]**
CSF 1	16.5	0.17	1.1	0.32	2.0
CSF 2	22.3	0.22	1.0	0.40	1.8
CSF 3	27.8	0.0	0.0	0.62	2.2
CSF 4	33.6	0.24	0.7	0.31	0.9
CSF 5	61.2	0.81	1.3	0.71	1.2
CSF 6	118	0.55	0.5	1.45	1.2
PC Phospho-Tau (181P) Level 1	15.4	0.18	1.2	0.22	1.4
PC Phospho-Tau (181P) Level 2	54.2	0.56	1.0	0.64	1.2

Elecsys Phospho-Tau (181P) CSF site-to-site reproducibility data, continued

cobas e 601 analyzer			
		Total	
Sample	**Mean [pg/mL]**	**SD [pg/mL]**	**CV [%]**
CSF 1	16.5	0.57	3.4
CSF 2	22.3	0.68	3.1
CSF 3	27.8	0.96	3.4
CSF 4	33.6	0.96	2.8
CSF 5	61.2	1.71	2.8
CSF 6	118	3.12	2.6
PC Phospho-Tau (181P) Level 1	15.4	0.54	3.5
PC Phospho-Tau (181P) Level 2	54.2	1.58	2.9

pTau181/Abeta42 ratio site-to-site reproducibility data

cobas e 601 analyzer					
		Within-run		**Between-run**	
Sample	**Mean**	**SD**	**CV [%]**	**SD**	**CV [%]**
Ratio Sample 1	0.018	0.0005	2.9	0.0004	1.9
Ratio Sample 2	0.031	0.0009	2.9	0.0010	3.2
Ratio Sample 3	0.049	0.0009	1.8	0.0009	1.9
Ratio Sample 4	0.047	0.0010	2.1	0.0005	1.0
Ratio Sample 5	0.047	0.0010	2.1	0.0003	0.6
Ratio Sample 6	0.020	0.0004	2.0	0.0009	4.6
Ratio Sample 7	0.024	0.0006	2.6	0.0010	4.0

pTau181/Abeta42 ratio site-to-site reproducibility data, continued

cobas e 601 analyzer					
		Between-day		**Between-site**	
Sample	**Mean**	**SD**	**CV [%]**	**SD**	**CV [%]**
Ratio Sample 1	0.018	0.0002	1.4	0.0003	1.9
Ratio Sample 2	0.031	0.0007	2.3	0.0012	3.8
Ratio Sample 3	0.049	0.0008	1.7	0.0010	1.9
Ratio Sample 4	0.047	0.0006	1.4	0.0007	1.5
Ratio Sample 5	0.047	0.0007	1.5	0.0007	1.6
Ratio Sample 6	0.020	0.0	0.0	0.0008	3.9
Ratio Sample 7	0.024	0.0	0.0	0.0007	3.0

pTau181/Abeta42 site-to-site reproducibility data, continued

cobas e 601 analyzer			
		Total	
Sample	**Mean**	**SD**	**CV [%]**
Ratio Sample 1	0.018	0.0008	4.2
Ratio Sample 2	0.031	0.0019	6.1
Ratio Sample 3	0.049	0.0018	3.6
Ratio Sample 4	0.047	0.0014	3.0
Ratio Sample 5	0.047	0.0014	3.1
Ratio Sample 6	0.020	0.0013	6.4
Ratio Sample 7	0.024	0.0014	5.6

Analytical specificity

Potential cross-reactivity to pTau species, represented by the pTau isoform Tau (172-205), was evaluated at the highest test concentration of 1300 pg/mL. % Cross-reactivity was calculated using the formula:

% cross-reactivity = [(mean of cross-reactant sample – mean of reference sample) / cross reactant concentration] x 100

The % cross-reactivity values for the cross-reactive pTau species is summarized below. The results showed no significant cross-reactivity up to 1300 pg/mL of the tested cross-reactant.

A ratio CSF sample pool within 20 % of the ratio cutoff (0.023) was also spiked with the cross-reactive pTau species at concentrations up to 1300 pg/mL. The ratio value was not significantly impacted by the cross-reactive pTau species.

Cross-reactant	**Concentration tested [pg/mL]**	**Cross-reactivity [%]**
Tau(172-205)amide	1300	≤ 0.11

Clinical performance

i) Definition of the ratio cutoff for distinguishing amyloid PET positive and amyloid PET negative patients by visual read

The pTau181/Abeta42 ratio cutoff was defined based on the first-generation Elecsys Phospho-Tau (181P) CSF and Elecsys β-Amyloid (1-42) CSF assay results obtained in the retrospective samples from the Swedish BioFINDER1 study.[13] The analysis population comprised a subset of 277 participants with mild cognitive symptoms for whom banked CSF samples and amyloid PET scan results obtained with the tracer [^{18}F]-Flutemetamol were available. Of the 277 subjects, 120 had subjective cognitive decline (SCD), 153 mild cognitive impairment (MCI) and for 4 patients no SCD/MCI assignment was available. The ratio cutoff 0.022 was calculated based on the agreement with amyloid PET status by visual read. The resulting agreement rates percentages were:

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

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ii) Adjustment of the defined ratio cutoff for amyloid positivity due to differences in pre-analytical handling protocols

Due to the susceptibility of Abeta42 to the use of different pre-analytical protocols for the handling of CSF, a pre-analytical bridging study was conducted to evaluate the differences between the cutoff determination (BioFINDER1) and the Alzheimer's Disease Neuroimaging Initiative (ADNI)[17] cutoff validation studies.[18] The purpose of the pre-analytical bridging study was to determine the conversion factor needed to adjust the optimal ratio cutoff defined in the BioFINDER1 samples prior to the cutoff validation study in order to account for pre-analytical differences between the BioFINDER1 and ADNI protocols. The pre-analytical bridging study (i.e. protocol comparison) was performed with the CSF samples from subjects undergoing diagnostic lumbar puncture due to suspicion of normal pressure hydrocephalus (N = 19 for pTau181 and N = 17 for Abeta42). The CSF samples were handled according to the BioFINDER and ADNI pre-analytical handling protocols.

No meaningful systematic differences were observed for CSF pTau181 measurements [0.7 % (95 % CI: -0.2 % to 1.6 %, p = 0.135)]. The mean percentage difference in CSF Abeta42 measurements was -24 % (95 % CI: -27 % to -20 %, p < 0.001). The upper 95 % confidence limit of the estimated percentage difference between ADNI and BioFINDER was used to define the conversion factor for Abeta42 (Abeta42 [ADNI] = 0.8*Abeta42 [BioFINDER]) and to adjust the pTau181/Abeta42 cutoff from 0.022 to 0.028 ($0.022*0.8^{-1} = 0.028$).

iii) Validation of the adjusted ratio cutoff for amyloid positivity

The pTau181/Abeta42 ratio cutoff was pre-specified and validated using retrospectively collected CSF samples in the Alzheimer's Disease Neuroimaging Initiative studies, ADNI-GO and ADNI2. The ADNI study[17] eligibility criteria are summarized in the table below.

Table 1: ADNI study eligibility criteria

ADNI Cohort			
	SMC[g)]	MCI	AD dementia
Inclusion criteria			
MMSE Score	24-30	24-30	20-26
Age (years)	65-90	55-90	55-90
Other	Score within normal range for cognition (or CDR[h)] = 0) but indicate that they have a concern, and exhibit slight forgetfulness	Report a subjective memory concern either autonomously or via an informant or clinician. No significant levels of impairment in other cognitive domains, essentially preserved activities of daily living and no signs of dementia	Meets the NINCDS/ADRDA[i)] criteria for probable AD
Exclusion criteria			
Significant neurological disease, major depression or history of schizophrenia, history of alcohol or substance abuse or dependence within the past 3 years. Participation in clinical studies involving neuropsychological measures being collected more than 1 time per year.			

g) SMC = Significant Memory Concern

h) CDR = Clinical Dementia Rating

i) National Institute of Neurological and Communicative Disorders and Stroke / Alzheimer's Disease and Related Disorders Association

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:

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

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

Table 2: Characteristics and demographics of analysis population with valid visual amyloid PET read-out and pTau181/Abeta42 ratio

	SMC [N = 94]	EMCI [N = 272]	LMCI [N = 152]	AD [N = 128]	All [N = 646]
Cohort					
ADNIGO	N = 0	N = 115	N = 0	N = 0	N = 115
ADNI2	N = 94	N = 157	N = 152	N = 128	N = 531
Age [years]					
Mean	72.1	71.1	72.2	74.3	72.1
SD	5.43	7.37	7.43	8.35	7.42
Min-Max	59.7 - 85.3	55.0 - 88.6	55.0 - 91.4	55.6 - 90.3	55.0 - 91.4
Age [categorized]					
55-59 years	N = 1 (1.1 %)	N = 16 (5.9 %)	N = 8 (5.3 %)	N = 7 (5.5 %)	N = 32 (5.0 %)
60-69 years	N = 41 (43.6 %)	N = 109 (40.1 %)	N = 45 (29.6 %)	N = 26 (20.3 %)	N = 221 (34.2 %)
70-79 years	N = 44 (46.8 %)	N = 109 (40.1 %)	N = 80 (52.6 %)	N = 63 (49.2 %)	N = 296 (45.8 %)
≥ 80 years	N = 8 (8.5 %)	N = 38 (14.0 %)	N = 19 (12.5 %)	N = 32 (25.0 %)	N = 97 (15.0 %)
Gender					
Male	N = 38 (40.4 %)	N = 152 (55.9 %)	N = 82 (53.9 %)	N = 76 (59.4 %)	N = 348 (53.9 %)
Female	N = 56 (59.6 %)	N = 120 (44.1 %)	N = 70 (46.1 %)	N = 52 (40.6 %)	N = 298 (46.1 %)
Education [years]					
Mean	16.7	15.9	16.7	15.7	16.2
SD	2.47	2.64	2.53	2.65	2.62
Min-Max	8.00 - 20.0	10.0 - 20.0	9.00 - 20.0	9.00 - 20.0	8.00 - 20.0
ApoE4 risk alleles					
0	N = 62 (66.0 %)	N = 157 (57.7 %)	N = 64 (42.1 %)	N = 42 (32.8 %)	N = 325 (50.3 %)
1	N = 31 (33.0 %)	N = 95 (34.9 %)	N = 62 (40.8 %)	N = 60 (46.9 %)	N = 248 (38.4 %)
2	N = 1 (1.1 %)	N = 20 (7.4 %)	N = 26 (17.1 %)	N = 26 (20.3 %)	N = 73 (11.3 %)
MMSE					
Mean	29.0	28.3	27.6	23.2	27.2
SD	1.24	1.58	1.83	2.05	2.68
Min-Max	24.0 - 30.0	23.0 - 30.0	24.0 - 30.0	19.0 - 26.0	19.0 - 30.0
< 18	N = 0 (0 %)	N = 0 (0 %)	N = 0 (0 %)	N = 0 (0 %)	N = 0 (0 %)
18-23	N = 0 (0 %)	N = 1 (0.4 %)	N = 0 (0 %)	N = 69 (53.9 %)	N = 70 (10.8 %)
24-30	N = 94 (100 %)	N =271 (99.6 %)	N = 152 (100 %)	N = 59 (46.1 %)	N = 576 (89.2 %)

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	SMC [N = 94]	EMCI [N = 272]	LMCI [N = 152]	AD [N = 128]	All [N = 646]
Race					
White	N = 88 (93.6 %)	N = 254 (93.4 %)	N = 144 (94.7 %)	N = 119 (93.0 %)	N = 605 (93.7 %)
Asian	N = 0 (0 %)	N = 4 (1.5 %)	N = 1 (0.7 %)	N = 4 (3.1 %)	N = 9 (1.4 %)
African American	N = 3 (3.2 %)	N = 5 (1.8 %)	N = 5 (3.3 %)	N = 4 (3.1 %)	N = 17 (2.6 %)
Others[j)]	N = 3 (3.2 %)	N = 7 (2.6 %)	N = 2 (1.3 %)	N = 1 (0.8 %)	N = 13 (2.0 %)
Unknown	N = 0 (0 %)	N = 2 (0.7 %)	N = 0 (0 %)	N = 0 (0 %)	N = 2 (0.3 %)

j) including Hawaiian / Pacific Islander, Am Indian / Alaskan, and more than one race

The time interval between the CSF sampling and conducting the amyloid PET scan did not exceed ± 154 days. For 50 % of the subjects the time difference was within ± 6 days.

The amyloid PET scans were read and interpreted by 3 trained readers.

The independent readers were blinded to any clinical information, including the patient's clinical status, diagnosis, and CSF biomarker measurements. In total, 653 amyloid PET images with matching CSF samples were available for subjects from the primary study population. Six images failed the initial quality control procedure and for one image the evaluation result of one reader was missing. For the remaining 646 scans all visual amyloid PET results were available.

The overall percent agreements between the visual amyloid PET results provided by single readers (inter-reader agreements) were in the range of 92.1 % to 96.9 %. The positive percent inter-reader agreements were in the range 86.8 % to 100 % and the negative percent agreements in the range of 82.8 % to 100 %.

The majority voting of three readers was used to classify each image as amyloid positive or negative, resulting in 347 (53.7 %) positive, and 299 (46.3 %) negative amyloid PET reads. The observed prevalences of amyloid positive results within AD was 89.1 % (114/128), 67.1 % (102/152) and 39.3 % (107/272) within these with LMCI and EMCI, and 25.5 % (24/94) within SMC cohort.

The measurements of the 2 AD biomarkers in CSF samples were performed using first-generation Elecsys Phospho-Tau (181P) CSF and Elecsys β-Amyloid (1-42) CSF assays.

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

Table 3: Agreement with visual read amyloid PET classification at pre-specified ratio cutoff

pTau181/Abeta42 ratio result	amyloid PET [Visual read]		Total
	Positive	Negative	
Positive	306	22	328
Negative	41	277	318
Total	347	299	646

Table 4: Agreement rates percentages and likelihood ratios for entire study population

Agreement rates [%] (n/N) (95 % CI)	
PPA[k)]	88.2 (306/347) (84.4 - 91.2)[l)]
NPA[m)]	92.6 (277/299) (89.1 - 95.1)[l)]
OPA[n)]	90.2 (583/646) (87.7 - 92.3)[l)]
PPV[o)]	93.3 (306/328) (90.3 - 95.4)[p)]
NPV[q)]	87.1 (277/318) (83.5 - 90.0)[p)]
Likelihood ratios (95% CI)	
LR+[r)]	12.0 (8.0 - 18.0)[s)]
LR-[t)]	0.13 (0.10 - 0.17)[s)]

k) PPA = positive percent agreement

l) 95 % CI are calculated using a Wilson score method for binomial proportions

m) NPA = negative percent agreement

n) OPA = overall percent agreement

o) PPV = positive predictive value

p) 95 % CI are calculated using 95 % CI for the corresponding likelihood ratio and prevalence

q) NPV = negative predictive value

r) LR+ = positive likelihood ratio

s) 95% CI are calculated using an asymptotic method for ratios of two independent binomial proportions

t) LR- = negative likelihood ratio

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

Concordance with amyloid PET classification across clinical cohorts is shown in the table below.

Table 5: Agreement rate percentages by clinical cohort

Cohort	PPA [%] (n/N) (95 % CI)	NPA [%] (n/N) (95 % CI)	OPA [%] (n/N) (95 % CI)
SMC (N = 94)	66.7 (16/24) (46.7 – 82.0)	92.9 (65/70) (84.3 – 96.9)	86.2 (81/94) (77.8 – 91.7)
EMCI (N = 272)	79.4 (85/107) (70.8 – 86.0)	94.5 (156/165) (90.0 -97.1)	88.6 (241/272) (84.3 – 91.9)
LMCI (N = 152)	90.2 (92/102) (82.9 – 94.6)	88.0 (44/50) (76.2 – 94.4)	89.5 (136/152) (83.6 – 93.4)
AD (N = 128)	99.1 (113/114) (95.2 – 99.8)	85.7 (12/14) (60.1 – 96.0)	97.7 (125/128) (93.3 – 99.2)

Analyses of clinical performance by gender and age are shown in the tables below.

Table 6: Agreement with visual amyloid PET by gender

Gender	PET Positive	PET Negative	PPA [%] (n/N) (95 % CI)	NPA [%] (n/N) (95 % CI)	OPA [%] (n/N) (95 % CI)
Male (N = 348)	N = 193	N = 155	87.05 (168/193) (81.6 – 91.1)	92.26 (143/155) (87.0 – 95.5)	89.37 (311/348) (85.7 – 92.2)
Female (N = 298)	N = 154	N = 144	89.61 (138/154) (83.8 – 93.5)	93.06 (134/144) (87.7 – 96.2)	91.28 (272/298) (87.5 – 94.0)

Table 7: Agreement with visual amyloid PET by age

Age [years]	PET Positive	PET Negative	PPA [%] (95 % CI)	NPA [%] (95 % CI)	OPA [%] (95 % CI)
≥ 55 to 59 (N = 32)	N = 14	N = 18	92.86 (13/14) (68.5 – 98.7)	94.44 (17/18) (74.2 – 99.0)	93.75 (30/32) (79.9 – 98.3)
≥ 60 to 69 (N = 221)	N = 86	N = 135	89.53 (77/86) (81.3 – 94.4)	94.07 (127/135) (88.7 – 97.0)	92.31 (204/221) (88.0 – 95.1)
≥ 70 to 79 (N = 296)	N = 185	N = 111	87.03 (161/185) (81.4 – 91.1)	90.99 (101/111) (84.2 – 95.0)	88.51 (262/296) (84.4 – 91.97)
≥ 80 (N = 97)	N = 62	N = 35	88.71 (55/62) (78.5 – 94.4)	91.43 (32/35) (77.6 – 97.0)	89.69 (87/97) (82.1 – 94.3)

There was little variation in PPA and NPA between males and females and with increasing age. These data indicate that gender and age differences did not translate into meaningful differences in assay performance.

The validation study was conducted in cohorts that are primarily caucasian. Sample sizes from other races are not sufficient to report clinical performance characteristics by race.

iv) Adjustment of the defined ratio cutoff due to assay re-standardization and adoption of routine-use pre-analytical protocol

Compared with the corresponding first-generation assay, the Elecsys β-Amyloid (1-42) CSF II assay was re-standardized using certified reference materials (CRMs) ERM®-DA480/-481/-482/IFCC.[19,20] Additionally, a new routine-use pre-analytical protocol for CSF handling (as described in the **Specimen collection and preparation** section) was adopted for use with the Elecsys β-Amyloid (1-42) CSF II and Elecsys Phospho-Tau (181P) CSF assays. Consequently, because of the changes in assay standardization and pre-analytical handling protocol, a second bridging study using CSF samples from subjects undergoing diagnostic lumbar puncture due to suspicion of normal pressure hydrocephalus (N = 25 for Abeta42 and N = 22 for pTau181) was performed to address systematic differences between results generated with the first and second assay version.

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

Assay performance with respect to NPA and PPA at the adjusted ratio cutoff of 0.023 is expected to be similar to the performance of the original cutoff defined in BioFINDER1.

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