Elecsys Phospho-Tau (181P) CSF

cobas®

REF		Σ	SYSTEM
			cobas e 411
08846693190	08846693500	60	**cobas e** 601
			cobas e 602

English

System information

For **cobas e** 411 analyzer: test number 1670
For **cobas e** 601 and **cobas e** 602 analyzers: Application Code Number 179

Please note

The measured phosphorylated Tau (181P) (pTau) value in a given sample, determined with assays from different manufacturers, can vary due to differences in assay methods and reagent. Values determined in samples by different assay methods and on different **cobas e** platforms cannot be used interchangeably.

Please note that due to the sticky properties of the β-Amyloid (1-42) protein, the cut-off for the ratio pTau/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 section "Specimen collection and preparation" of the Elecsys β-Amyloid (1-42) CSF II assay Method Sheet) is strictly followed.

All performance data were generated using frozen cerebrospinal fluid (CSF) material. A positive pTau 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

The Elecsys Phospho-Tau (181P) CSF assay is an in vitro diagnostic immunoassay intended for the quantitative determination of the phosphorylated Tau protein in human CSF.

1. The Elecsys Phospho-Tau (181P) CSF assay is intended to be used alone or in combination with Elecsys β-Amyloid (1-42) CSF II assay as a ratio in adult subjects with mild cognitive impairment (MCI) as an aid to identify subjects who are at lower vs. higher risk of cognitive decline as defined by change in a clinical score within a 2 year period.
2. The Elecsys Phospho-Tau (181P) CSF assay is intended to be used in combination with Elecsys β-Amyloid (1-42) CSF II assay as a ratio in adult subjects with cognitive impairment being evaluated for AD and other causes of cognitive impairment wherein a positive and negative CSF result is concordant with positive and negative amyloid Positron Emission Tomography (PET) scan result, respectively.

Limitations of use

- The Elecsys Phospho-Tau (181P) CSF assay is an adjunct to other clinical diagnostic evaluations.
- A positive Elecsys Phospho-Tau (181P) CSF assay result and/or a positive Elecsys Phospho-Tau (181P) CSF to Elecsys β-Amyloid (1-42) CSF II ratio result does not establish a diagnosis of AD or other cognitive disorder.
- The safety and effectiveness of the Elecsys Phospho-Tau (181P) CSF assay have not been established for monitoring responses to therapies.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on **cobas e** immunoassay analyzers.

Summary

Tau (tubulin-associated unit) protein is one of the two hallmarks of AD, besides β-Amyloid (1-42). Tau is found as 6 molecular isoforms in 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 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 detect the protein or fragments of Tau protein phosphorylated at threonine 181 in human CSF.

Clinical relevance of pTau

In AD, numerous studies show that while CSF β-Amyloid (1-42) levels decrease to around half the level in controls, CSF pTau 181 levels increase around 2-3 fold in mild-moderate AD patients compared to age-matched controls.[3,4] High CSF pTau levels are also associated with a faster progression from MCI to AD with more rapid cognitive decline in AD patients[5] and in mild AD dementia cases.[6]

CSF pTau biomarker might be useful in detecting the likely progression of MCI to AD[7] and has most power when used in combination with CSF β-Amyloid (1-42).

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 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 (11H5V1) and a monoclonal Tau-specific antibody (PC1C6) 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.

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.

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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.

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: all countries: +49-621-7590

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

Specimen collection and preparation

Only CSF collection and sampling tubes made of Polypropylene (PP) material should be used. Do not use tubes made of glass, Polystyrol (PS) or any other material.

In case the ratio pTau/Abeta42 is intended to be used with the provided cut-off, please follow the pre analytical handling procedure for CSF sample collection and measurement, described in section "Specimen collection and preparation" of the Elecsys β-Amyloid (1-42) CSF II assay Method Sheet (REF 08821909190), otherwise the provided cut-off for ratio pTau/Abeta42 is not applicable.

This restriction is not applicable for pTau as a single marker.

Stability of CSF samples: Stable for 8 weeks at -15 to -25 °C (one freeze/thaw cycle), 14 days at 2-8 °C, 5 days at 20-25 °C.

Do not use hemolyzed CSF samples that are visibly colored red.

Centrifuge samples containing precipitates before performing the assay.

Do not use heat-inactivated samples.

Do not use samples and controls stabilized with azide.

Ensure the samples, 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 them capped if not in use.

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 the **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 11298500316, 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.

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Calibration

Traceability: This method has been standardized against a purified reference material Tau(172-205)[pThr181]amide, absolutely quantified via amino acid analysis (AAA). Calibrator values are based on weighted pTau reference material, traceable to 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 **"Guidance: Statistical Quality Control Rule Implementation"** in English language, which is available via navifyportal.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.

Limitations - interference

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	≤ 0.51 µmol/L or ≤ 0.03 mg/dL
Hemoglobin	≤ 0.0031 mmol/L or ≤ 5 mg/dL
Intralipid	≤ 10 mg/dL
Biotin	≤ 4912 nmol/L or ≤ 1200 ng/mL
Rheumatoid factors	≤ 4 IU/mL
IgG	≤ 0.02 g/dL
IgA	≤ 0.002 g/dL
IgM	≤ 0.0005 g/dL
Albumin	≤ 0.05 g/dL

Recovery within ± 3 pg/mL of initial value ≤ 25 pg/mL and within ± 10 % of initial value > 25 pg/mL.

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

Pharmaceutical substances
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 + 1.5	22.5
Metronidazole	123
Phenylbutazone	107
Rifampicin	48
Theophylline	60

Criterion: Recovery within ± 3 pg/mL of initial value ≤ 25 pg/mL and within ± 10 % of initial value > 25 pg/mL.

In addition, the following 15 special drugs were tested. No interference with the assay was found.

Special drugs

Drug	Concentration tested mg/L
Atorvastatin	0.75
Clopidogrel	0.3
Digoxin	0.039
Donepezil	30
Escitalopram	0.192
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: Recovery within ± 3 pg/mL of initial value ≤ 25 pg/mL and within ± 10 % of initial value > 25 pg/mL.

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.

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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.

Limits and ranges

Measuring range

8-120 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 pg/mL. Values above the measuring range are reported as > 120 pg/mL.

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 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 %.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, samples and controls in a protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplicate each for 21 days (n = 84). The following results were obtained:

cobas e 411 analyzer					
		Repeatability		**Intermediate precision**	
Sample	Mean pg/mL	SD pg/mL	CV %	SD pg/mL	CV %
Human CSF 1	15.6	0.262	1.7	0.400	2.6
Human CSF 2	20.5	0.371	1.8	0.662	3.2
Human CSF 3	26.8	0.294	1.1	0.410	1.5
Human CSF 4	31.1	0.322	1.0	0.479	1.5
Human CSF 5	57.8	0.851	1.5	1.61	2.8
Human CSF 6	92.2	0.954	1.0	1.83	2.0
Human CSF 7	104	0.982	0.9	2.07	2.0
PC[c)] pTau 1	12.9	0.143	1.1	0.215	1.7
PC pTau 2	45.9	0.517	1.1	0.777	1.7

c) PC = PreciControl

cobas e 601 and **cobas e** 602 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean pg/mL	SD pg/mL	CV %	SD pg/mL	CV %
Human CSF 1	16.4	0.258	1.6	0.327	2.0
Human CSF 2	21.9	0.359	1.6	0.539	2.5
Human CSF 3	27.9	0.306	1.1	0.478	1.7
Human CSF 4	32.1	0.358	1.1	0.502	1.6
Human CSF 5	59.0	0.626	1.1	1.35	2.3
Human CSF 6	95.1	0.931	1.0	2.09	2.2
Human CSF 7	107	1.27	1.2	2.38	2.2
PC pTau1	14.6	0.171	1.2	0.249	1.7
PC pTau 2	49.7	0.372	0.7	0.945	1.9

Analytical specificity

The test is highly specific for human Phospho-Tau (181P). The following potential cross-reactivity was found:

Cross-reactant	**Concentration tested pg/mL**	**Cross-reactivity %**
Tau(172-205)amide	1300	0.9

Clinical performance

Each laboratory should investigate the transferability of the expected values to its own patient population.

Note: Clinical performance data were generated using the Elecsys Phospho-Tau (181P) CSF assay (REF 07357036190) V1 that highly correlates with V2 of the Elecsys Phospho-Tau (181P) CSF assay (REF 08846693190). In an internal method comparison study (N = 129) the observed Pearson's correlation coefficient was 0.999.

Identification of patients at risk of cognitive decline

The ability of the single biomarker pTau as well as the biomarker ratio pTau/Abeta42 to identify patients at higher vs. lower risk of cognitive decline as defined by change in a clinical score within a 2-year period was assessed in a retrospective study (Roche study RD002530) based on samples from the ADNI1/GO/2 studies measured with the Elecsys Phospho-Tau (181P) CSF assay (REF 07357036190).[13] The primary analysis population included a total of 619 patients from the early (EMCI, 277) and late mild cognitive impairment (LMCI, 342) cohorts with baseline Elecsys CSF assay measurements available. For each of these patients also baseline assessments of the clinical scores Clinical Dementia Rating – sum of boxes (CDR-SB) and Mini-Mental State Examination (MMSE) were available. The average age of the 619 subjects was 72 years (range 54-91 years), 41 %/59 % were female/male, the average education time was 16 years (range 6-20 years) and 51 %/39 %/11 % carried 0/1/2 ApoE4 alleles. The averages (standard deviation, SD) of clinical scores were as follows: CDR-SB, 1.5 (0.9) at baseline, 2.3 (2.1) at 2-year follow-up; MMSE, 27.7 (1.8) at baseline, 26.6 (3.3) at 2-year follow-up. The median (1.48*Median absolute deviation) of the Elecsys CSF marker concentrations at baseline were as follows: pTau, 24.0 (12.0) pg/mL; Abeta42, 837.7 (410.2) pg/mL.

The ability of the biomarkers to separate patients at lower vs. higher risk of cognitive decline (as measured by change in CDR-SB or MMSE) within 2 years was assessed using linear mixed-effects models. The models were adjusted for age, sex, education time and baseline value of the respective clinical score. The cut-off values for pTau and pTau/Abeta42 were defined in study RD002145.

Due to different pre-analytic procedures between BIOFINDER and ADNI, a bridging study RD002475 was used to adjust the cut-offs from Biofinder to ADNI based on optimization for concordance with amyloid PET. Using the indicated cut-offs (see section below), the model-based average change in clinical scores (CDR-SB; MMSE) between baseline and 2 years in the negative group (effect (1)) and the difference in change in clinical scores between biomarker-positive and -negative groups (effect (2)) were as follows:

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Clinical score	Biomarker	Effect (1) Estimate (95 % CI)[d]	Effect (2) Estimate (95 % CI)
CDR-SB	pTau	0.48 (0.34, 0.62)	1.00 (0.78, 1.21)
	pTau/Abeta42	0.17 (0.02, 0.32)	1.42 (1.21, 1.62)
MMSE	pTau	-0.43 (-0.69, -0.18)	-1.80 (-2.20, -1.40)
	pTau/Abeta42	-0.08 (-0.36, 0.20)	-2.17 (-2.56, -1.77)

d) Confidence interval

Both the single marker pTau and the pTau/Abeta42 ratio separated patients at lower vs. higher risk of cognitive decline within 2 years. The pTau/Abeta42 ratio showed superior performance. For instance, change in CDR-SB and MMSE over 2 years between the biomarker-positive and -negative groups according to pTau/Abeta42 ratio differed by more than 1 and -2.5 units (lower confidence limit of effect (2)), respectively. Biomarker-negative patients did not show a change in CDR-SB and MMSE over 2 years more than 0.5 and -0.5 (upper confidence limit of effect (1)), respectively. These results did not change after additional adjustment for ApoE4 genotype (number of E4 alleles).

Model-based time course plot for change in CDR-SB over 2 years for pTau/Abeta42 ratio-based classification (without adjustment for ApoE4 genotype):

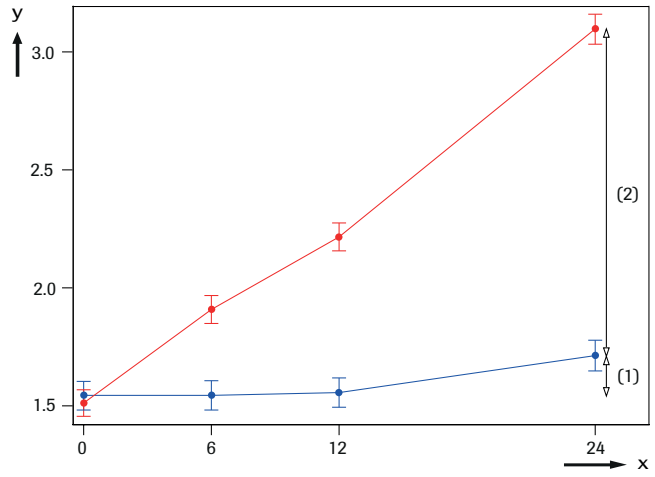

Figure: Model-derived average and standard error of CDR-SB in biomarker-positive (red) and -negative (blue) groups over follow-up time (x-axis; visit time point in months). Effects (1) and (2) as described above are symbolized by arrows.

x: Visit

y: CDR-SB

Concordance with amyloid PET visual read

Concordance with amyloid PET visual read was assessed in a retrospective study (Roche study RD002145) based on samples from the BioFINDER cohort.[14] The primary analysis population consisted of 277 mild cognitive symptoms (MCS) patients for whom CSF measurements and amyloid PET scan results were available (PET tracer: [18F] Flutemetamol). Of the 277 patients, 120 had subjective cognitive decline (SCD), 153 MCI and for 4 patients no assignment was available. The average age was 70 years (range 59-80 years), 42 %/58 % of patients were female/male and 45 %/54 % of patients were ApoE4 carriers/non-carriers. The median (1.48*Median absolute deviation) of the Elecsys markers at baseline was as follows: pTau, 20.0 (9.4) pg/mL; Abeta42, 1048 (593) pg/mL. The amyloid PET scans were read independently by three trained readers and majority voting was used to rate an image as positive or negative, resulting in 110 (40 %) positive and 167 (60 %) negative amyloid PET reads. The cut-offs for Abeta42 and the ratios pTau/Abeta42 and tTau/Abeta42 were established based on the amyloid PET visual read. The agreement rates for the Elecsys CSF markers with amyloid PET visual read were as follows:

	Agreement rates (%) (95 % CI)
Positive percentage agreement (PPA, "sensitivity")	90.9 (83.9, 95.6)
Negative percentage agreement (NPA, "specificity")	89.2 (83.5, 93.5)
Overall percentage agreement	89.9 (85.7, 93.2)

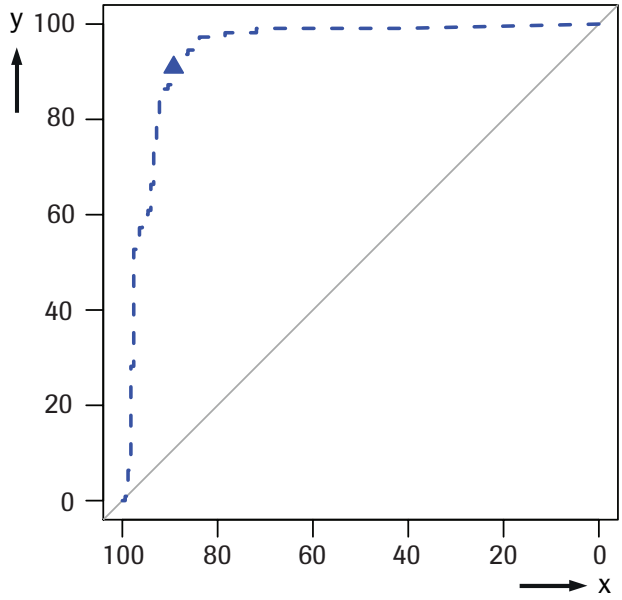

Figure: Receiver-operating characteristic curve of the pTau/Abeta42 ratio with outcome amyloid PET. Triangle denotes PPA and NPA at the cut-off; AUC: 94.4 % (91.5 %, 97.3 %).

x: NPA (specificity) (%)

y: PPA (sensitivity) (%)

Cut-offs for PET concordance and cognitive decline

The influence of pre-analytical handling and assay versions on the measured pTau and Abeta42 levels was investigated in the study RD002842. The pTau values were not affected by the pre-analytical procedure and assay version. For Abeta42, systematic differences between the pre-analytical procedures and assay versions were observed.

The cut-off values for Abeta42 as single biomarker and for pTau/Abeta42 were adjusted according to the observed differences (please see below and method sheet of Elecsys β-Amyloid (1-42) CSF II assay REF 08821909190). Please note, that the provided cut-off value for the pTau/Abeta42 ratio is only valid if the pre-analytical handling procedure described in the section "Specimen collection and preparation" of the Elecsys β-Amyloid (1-42) CSF II assay Method Sheet (REF 08821909190) is used.

The new derived cut-offs for cognitive decline are shown below:

If pTau > 27 pg/mL ⟹ test result positive.

If pTau ≤ 27 pg/mL ⟹ test result negative.

If pTau/Abeta42 ratio* > 0.023 ⟹ test result positive.

If pTau/Abeta42 ratio* ≤ 0.023 ⟹ test result negative.

*The ratio should be rounded to 4 decimal places before comparing against 0.023. If the concentration of one of the analytes is outside the measuring range, the following rules apply:

In cases Abeta42 < 150 pg/mL, Abeta42 > 2500 pg/mL, pTau > 120 pg/mL, pTau < 8 pg/mL ⟹ the value should be set to the respective limit of the measuring range and the ratio should be calculated.

The new derived cut-off for PET concordance is shown below:

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If pTau/Abeta42 ratio* > 0.023 ⟹ test result positive.

If pTau/Abeta42 ratio* ≤ 0.023 ⟹ test result negative.

*The ratio should be rounded to 4 decimal places before comparing against 0.023. If the concentration of one of the analytes is outside the measuring range, the following rules apply:

In cases Abeta42 < 150 pg/mL, Abeta42 > 2500 pg/mL, pTau > 120 pg/mL, pTau < 8 pg/mL ⟹ the value should be set to the respective limit of the measuring range and the ratio should be calculated.

References

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11 EMA/CHMP/SAWP/893622/2011; Committee for Medicinal Products for Human Use (CHMP) ; 17 November 2011; Qualification opinion of Alzheimer's disease novel methodologies/biomarkers for the use of CSF AB 1-42 and t-tau signature and/or PET-amyloid imaging (positive/ negative) as a biomarkers for enrichment, for use in regulatory clinical trials – in mild and moderate of Alzheimer's.

12 EMA/CHMP/539931/2014 2; Committee for Medicinal Products for Human Use (CHMP); 28 January 2016; Draft guideline on the clinical investigation of medicines for the treatment of Alzheimer's disease and other dementias.

13 http://www.adni-info.org/.

14 http://biofinder.se/the_biofinder_study_group/.

For further information, please refer to the appropriate user guide or operator's manual for the analyzer concerned, the respective application sheets and the Method Sheets of all necessary components (if available in your country).

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see navifyportal.roche.com for definition of symbols used):

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume for reconstitution
GTIN	Global Trade Item Number

CE 0123

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