# Elecsys β-Amyloid (1-42) CSF II

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| REF | | Σ | SYSTEM |
|---|---|---|---|
| 08821941190 | 08821941500 | 100 | **cobas e** 402<br>**cobas e** 801 |

**English**

**System information**

| Short name | ACN (application code number) |
|---|---|
| AB42 2 | 10097 |

**Please note**

The measured β-amyloid (1-42) value in a given sample, determined with assays from different manufacturers, can vary due to differences in assay methods and reagent. Values determined on 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 protein, the Elecsys assay cut-off provided in this document is only valid if the below described pre-analytical handling procedure (see section "Specimen collection and preparation") is strictly followed.**

All performance data were generated using frozen cerebrospinal fluid (CSF) material. A positive β-amyloid (1-42) 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 is an in vitro diagnostic immunoassay intended for the quantitative determination of the β-amyloid (1-42) protein concentration in human cerebrospinal fluid (CSF).

1. The Elecsys β-Amyloid (1-42) CSF II assay is intended to be used in adult subjects with cognitive impairment being evaluated for Alzheimer disease (AD) and other causes of cognitive impairment. Result above the cutoff is consistent with a negative amyloid positron emission tomography (PET) scan. Negative β-amyloid PET scans indicate sparse to no neuritic plaques and are inconsistent with a neuropathological diagnosis of AD at the time of image acquisition; a negative scan result reduces the likelihood that a patient's cognitive impairment is due to AD.
2. The Elecsys β-Amyloid (1-42) CSF II assay is intended to be used in combination with Elecsys Phospho-Tau (181P) CSF or Elecsys Total-Tau CSF 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 are concordant with positive and negative amyloid Positron Emission Tomography (PET) scan result, respectively.
3. Elecsys β-Amyloid (1-42) CSF II assay is intended to be used alone or in combination with Elecsys Phospho-Tau (181P) CSF or Elecsys Total-Tau CSF 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.

Limitations of use

- Elecsys β-Amyloid (1-42) CSF II assay is an adjunct to other clinical diagnostic evaluations.
- A positive Elecsys β-Amyloid (1-42) CSF II assay result and/or a positive Elecsys Phospho-Tau (181P) CSF or Elecsys Total-Tau 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 β-Amyloid (1-42) CSF II 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**

The Elecsys β-Amyloid (1-42) CSF II assay is designed to detect the β-amyloid (1-42) peptide, a small, 4 kDa protein of about 40 amino acids that is formed following proteolytic cleavage of a transmembrane protein known as amyloid precursor protein (APP). Cleavage of APP occurs via 2 events: cleavage by β-secretase within the extracellular domain and cleavage by γ-secretase in the transmembrane region. Due to its hydrophobic nature, the β-amyloid (1-42) peptide has the property to form aggregates and oligomers. Oligomers of higher order form fibrils that accumulate into β-amyloid plaques.[1] A Clinical relevance of β-amyloid (1-42) peptide deposition in the brain as 1 of the 2 hallmarks of AD, besides neurofibrillary tangles, can be detected by several methods: (a) histopathological staining of β-amyloid (1-42) deposits in post mortem brain tissue; (b) use of radiolabeled tracers that bind to β-amyloid deposits in the brain and can then be detected in vivo using PET scan; (c) measuring the β-amyloid 42 level in CSF because lower titers in CSF are believed to reflect accumulation of this molecule in the brain.[2,3]

Pathological changes in the β-amyloid metabolism are the earliest alterations during AD development known so far that can be utilized diagnostically. They are reflected by the decrease in the CSF concentrations of β-amyloid (1-42) as well as by the increase in the brain uptake of the specific tracers on the β-amyloid PET.[4] Current clinical diagnostic criteria for AD require a patient to have dementia before a diagnosis of AD can be made, and are largely based on the exclusion of other disorders. No clinical method is available for identifying prodromal AD in patients with MCI, as such individuals have only mild disturbances in episodic memory.[5]

Numerous studies show that while CSF β-Amyloid (1-42) levels decrease to around half the level in controls, CSF tTau and CSF pTau 181 levels increase around 2-3 fold in mild-moderate AD patients compared to age-matched controls.[6,7] CSF tTau has been shown to reflect the intensity of the neuronal and axonal damage and degeneration. High CSF tTau is also associated with a faster progression from MCI to AD.[8] CSF pTau 181 levels are also associated with a faster progression from MCI to AD with more rapid cognitive decline in AD patients[9] as well as in very mild AD dementia cases.

CSF pTau and CSF tTau biomarkers have the highest power when used in combination with CSF β-Amyloid (1-42) for detecting the likely progression of subjects with MCI to AD.[10]

The use of AD biomarkers has been included in the new consensus research diagnostic criteria for AD, mild cognitive impairment (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.[11,12] The new IWG-2 (International Working Group 2) criteria recommend the use of either CSF biomarker or PET imaging for evaluation of AD patients.[13] In Europe, the CHMP (Committee for Medicinal Products for Human Use) published a number of positive opinions on the use of biomarkers in the context of AD for enrichment of clinical trials in pre-dementia and mild- to-moderate AD.[14,15]

**Test principle**

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 30 µL of sample, a biotinylated monoclonal β-amyloid (1-42)-specific antibody (21F12), and a monoclonal β-amyloid (1-42)-specific antibody (3D6) 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 II 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 **cobas** link.

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

**Reagents - working solutions**

The **cobas e** pack is labeled as AB42 2.

# Elecsys β-Amyloid (1-42) CSF II

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M Streptavidin-coated microparticles, 1 bottle, 6.1 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-β-amyloid (1-42)-Ab~biotin, 1 bottle, 6.8 mL: Biotinylated monoclonal anti-β-amyloid (1-42) antibody 21F12 (mouse) 2.0 mg/L; phosphate buffer > 100 mmol/L, pH 7.2; preservative.

R2 Anti-β-amyloid (1-42)-Ab~Ru(bpy)$_3^{2+}$, 1 bottle, 6.8 mL: Monoclonal anti-β-amyloid antibody 3D6 (mouse) labeled with ruthenium complex 1.75 mg/L; phosphate buffer > 100 mmol/L, pH 7.2; preservative.

**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.

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 dust/fume/gas/mist/vapours/spray.

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 available via the **cobas** link.

**Storage and stability**

Store at 2-8 °C.

Do not freeze.

Store the **cobas e** pack **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 |
| on the analyzers | 16 weeks |

**Specimen collection and preparation**

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.<br>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.<br>Do not use CSF samples which appear reddish for measurement of Elecsys AD biomarkers.<br>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 mixing/inverting, no tube transfers, no aliquoting, no freezing and normally no centrifugation) until measurement. | It is strongly recommended that the sample be kept at 2-8 °C during transport and storage up to the time of measurement. Samples can be stored at 2-8 °C for up to 14 days. If transport and storage at 2-8 °C is not feasible, the sample can be transported/stored at room temperature (20-25 °C). If this is the case, measurement is to be performed within 5 days after sample draw. |
| 5. Measurement on the **cobas e** systems: 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: 14 days at 2-8 °C and 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 samples and controls stabilized with azide.

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

Due to possible evaporation effects, samples and calibrators 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 08821976190, CalSet β-Amyloid (1-42) II, for 4 x 1.0 mL
- REF 08821968190, PreciControl β-Amyloid (1-42) II, 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

# Elecsys β-Amyloid (1-42) CSF II

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- **cobas e** analyzer

Additional materials for **cobas e** 402 and **cobas e** 801 analyzers:

- REF 06908799190, ProCell II M, 2 x 2 L system solution
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 07485409001, Reservoir Cup, 8 cups to supply ProCell II M and CleanCell M
- REF 06908853190, PreClean II M, 2 x 2 L wash solution
- REF 05694302001, Assay Tip/Assay Cup tray, 6 magazines x 6 magazine stacks x 105 assay tips and 105 assay cups, 3 wasteliners
- REF 07485425001, Liquid Flow Cleaning Cup, 2 adaptor cups to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning Detection Unit
- REF 07485433001, PreWash Liquid Flow Cleaning Cup, 1 adaptor cup to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning PreWash Unit
- 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.

Place the cooled (stored at 2-8 °C) **cobas e** pack on the reagent manager. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the **cobas e** pack.

**Calibration**

Traceability: This method has been standardized against the 3 certified reference materials (CRMs), ERM®-DA480/IFCC, ERM®-DA481/IFCC and ERM®-DA482/IFCC.

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 **cobas e** pack 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 12 weeks when using the same reagent lot
- after 28 days when using the same **cobas e** pack on the analyzer
- as required: e.g. quality control findings outside the defined limits

**Quality control**

For quality control, use PreciControl β-Amyloid (1-42) II.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per **cobas e** pack, 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 β-Amyloid (1-42) II 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 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.

**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 |

Criterion: Recovery within ± 48 pg/mL of initial value ≤ 480 pg/mL and within ± 10 % of initial value > 480 pg/mL.

There is no high-dose hook effect at β-amyloid (1-42) concentrations up to 6000 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 | 22.5 |
| Metronidazole | 123 |
| Phenylbutazone | 107 |
| Rifampicin | 48 |
| Theophylline | 60 |

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 |

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| Drug | Concentration tested mg/L |
|---|---|
| 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 ± 48 pg/mL of initial value ≤ 480 pg/mL and within ± 10 % of initial value > 480 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.

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

150-2500 pg/mL (defined by the Limit of Quantitation and the maximum of the master curve). Values below the Limit of Quantitation are reported as < 150 pg/mL. Values above the measuring range are reported as > 2500 pg/mL.

### Lower limits of measurement

*Limit of Blank, Limit of Detection and Limit of Quantitation*

Limit of Blank = 50 pg/mL

Limit of Detection = 100 pg/mL

Limit of Quantitation = 150 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 95$^{th}$ 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 ≤ 30 %.

### 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** 402 and **cobas e** 801 analyzers | | | | | |
|---|---|---|---|---|---|
| | | **Repeatability** | | **Intermediate precision** | |
| Sample | Mean pg/mL | SD pg/mL | CV % | SD pg/mL | CV % |
| Human CSF 1 | 157 | 1.53 | 1.0 | 2.44 | 1.6 |
| Human CSF 2 | 200 | 1.83 | 0.9 | 3.17 | 1.6 |
| Human CSF 3 | 764 | 15.2 | 2.0 | 21.3 | 2.8 |
| Human CSF 4 | 973 | 20.9 | 2.1 | 27.2 | 2.8 |
| Human CSF 5 | 1042 | 21.2 | 2.0 | 30.1 | 2.9 |
| Human CSF 6 | 1186 | 22.5 | 1.9 | 32.6 | 2.7 |
| Human CSF 7 | 1243 | 27.7 | 2.2 | 37.3 | 3.0 |
| Human CSF 8 | 2124 | 84.3 | 4.0 | 126 | 5.9 |
| Human CSF 9 | 2290 | 21.6 | 0.9 | 28.6 | 1.2 |
| PC[b)] β-Amyloid (1-42) II 1 | 616 | 4.86 | 0.8 | 6.76 | 1.1 |
| PC β-Amyloid (1-42) II 2 | 1689 | 12.6 | 0.7 | 18.2 | 1.1 |

b) PC = PreciControl

### Analytical specificity

The test is highly specific for human β-amyloid (1-42). The following potential cross-reactivity was found.[16]

| Cross-reactant | Concentration tested pg/mL | Cross-reactivity % |
|---|---|---|
| β-Amyloid (1-38) | 10000 | < 0.9 |
| β-Amyloid (1-40) | 10000 | < 1.6 |

### Method comparison

A comparison of the Elecsys ß-Amyloid (1-42) CSF II assay, REF 08821941190 (**cobas e** 402 analyzer; y) with the Elecsys ß-Amyloid (1-42) CSF II assay, REF 08821941190 (**cobas e** 801 analyzer; x) gave the following correlations (pg/mL):

Number of samples measured: 133

| Passing/Bablok[17] | Linear regression |
|---|---|
| y = 1.04x - 6.70 | y = 1.03x -1.85 |
| τ = 0.982 | r = 0.999 |

The sample concentrations were between 168 and 2464 pg/mL.

### 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 first generation Elecsys ß-Amyloid (1-42) CSF (REF 06986811190) that highly correlate with Elecsys ß-Amyloid (1-42) CSF II. In an internal method comparison study (N = 103) the observed Pearson's correlation coefficient was 0.999. The Elecsys ß-Amyloid (1-42) CSF II was re-standardized leading to systematic differences between the first and second version. These differences were taken into account by definition of the clinical decision thresholds.

### Concordance with amyloid PET visual read

Concordance with PET visual read was assessed in a retrospective study (Roche study RD002145) based on samples from the BioFINDER cohort.[18] The primary analysis population consisted of 277 mild cognitive symptoms (MCS) patients for whom banked CSF samples and 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: Abeta42, 1048 (593) pg/mL measured with the first generation Elecsys

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ß-Amyloid (1-42) CSF; pTau, 20.0 (9.4) pg/mL; tTau 240 (100) pg/mL. The amyloid PET scans were read independently by 3 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:

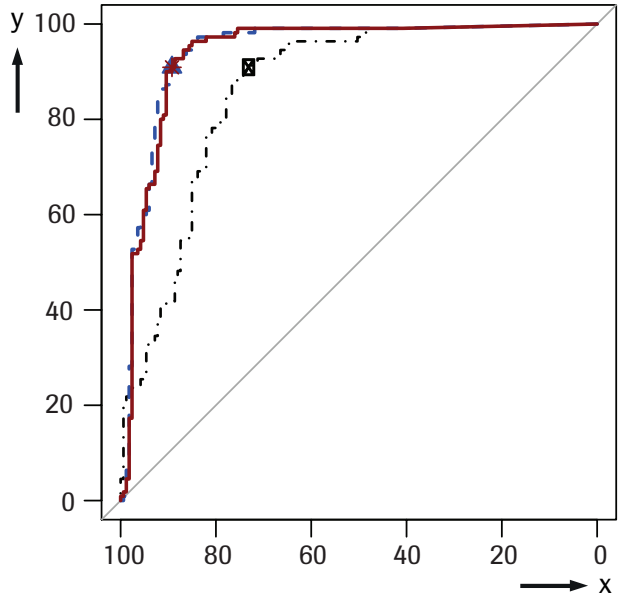
| Agreement rates [%] (95 % CI)[c] | | | |
|---|---|---|---|
| | Abeta42 | pTau/Abeta42 | tTau/Abeta42 |
| PPA[d] | 90.9 (83.9, 95.6) | 90.9 (83.9, 95.6) | 90.9 (83.9, 95.6) |
| NPA[e] | 72.5 (65.0, 79.1) | 89.2 (83.5, 93.5) | 89.2 (83.5, 93.5) |
| OPA[f] | 79.8 (74.6, 84.4) | 89.9 (85.7, 93.2) | 89.9 (85.7, 93.2) |

c) Confidence interval

d) PPA = Positive percentage agreement (sensitivity)

e) NPA = Negative percentage agreement (specificity)

f) OPA = Overall percentage agreement




x: NPA (Specificity) (%) y: PPA (Sensitivity) (%)

Abeta42 ratio tTau/Abeta42 ratio pTau/Abeta42

Figure: Receiver-operating characteristic curves for Abeta42 and ratios pTau/Abeta42 and tTau/Abeta42 with outcome amyloid PET. The Circle/Triangle/asterisk denotes PPA and NPA at the cut-offs for the 3 biomarkers, respectively; Abeta42, AUC: 86.5 % (82.3 %, 90.7 %); pTau/Abeta42, AUC: 94.4 % (91.5 %, 97.3 %); tTau/Abeta42, AUC: 94.0 % (91.0 %, 97.0 %).

### Identification of patients at risk of cognitive decline

The ability of the single marker Abeta42 as well as the biomarker ratios pTau/Abeta42 or tTau/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.[19] 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 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: Abeta42, 838 (410) pg/mL; pTau, 24.0 (12.0) pg/mL; tTau, 257 (107) 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.

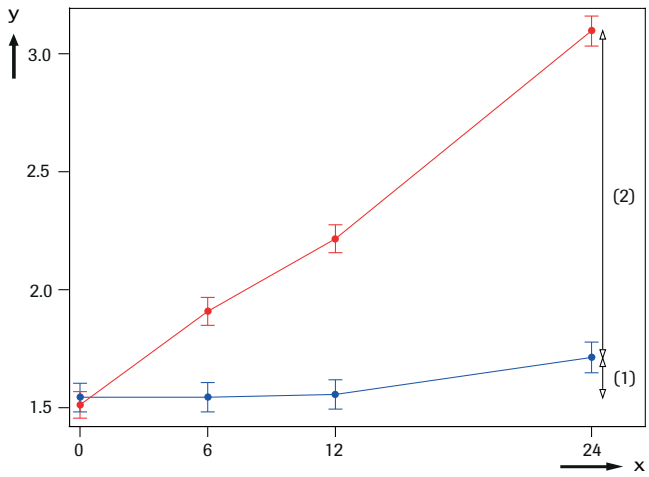
Due to different preanalytic handling procedures between BIOFINDER and ADNI, a bridging study RD002475 was used to adjust the cut offs from Biofinder to ADNI based on optimization for concordance with amyloid PET.

Using these cut-offs for cognitive decline analysis, the model-based average change in clinical scores (CDR-SB; MMSE) between baseline and 2 years in the biomarker-negative group (effect (1)) and the difference in change in clinical scores between biomarker-positive and -negative groups (effect (2)) were as follows:

| Clinical score | Biomarker | Effect (1) Estimate (95 % CI) | Effect (2) Estimate (95 % CI) |
|---|---|---|---|
| CDR-SB | Abeta42 | 0.31 (0.16, 0.46) | 1.10 (0.89, 1.31) |
| | pTau/Abeta42 | 0.17 (0.02, 0.32) | 1.42 (1.21, 1.62) |
| | tTau/Abeta42 | 0.21 (0.07, 0.35) | 1.41 (1.20, 1.62) |
| MMSE | Abeta42 | -0.25 (-0.53, 0.04) | -1.79 (-2.19, -1.40) |
| | pTau/Abeta42 | -0.08 (-0.36, 0.20) | -2.17 (-2.56, -1.77) |
| | tTau/Abeta42 | -0.13 (-0.40, 0.14) | -2.19 (-2.58, -1.79) |

All 3 biomarkers separated patients at lower vs. higher risk of cognitive decline within 2 years. The ratios showed superior performance compared to the single marker Abeta42. For instance, change in CDR-SB and MMSE over 2 years between the biomarker-positive and -negative groups according to pTau/Abeta42 or tTau/Abeta42 ratio differed by more than 1 and -2.5 units (lower confidence limit of effect (2)), respectively. Biomarker ratio-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):




x: Visit y: CDR-SB

Figure: Model-derived average and standard error of CDR-SB in pTau/Abeta42 ratio-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.

### Cut-offs for PET concordance and cognitive decline

As the BioFINDER study used a different pre-analytical handling procedure compared to that described in the section “Specimen collection and

# Elecsys β-Amyloid (1-42) CSF II

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preparation", an adjustment factor was determined in study RD002842 to adjust the cut offs for the new preanalytical protocol measured with the Elecsys β-Amyloid (1-42) CSF II assay. The adjustment factor was generated by bridging between the Biofinder preanalytical handling procedure, measured with Elecsys β-Amyloid (1-42) CSF first generation and the new preanalytical procedure measured with the Elecsys ß-Amyloid (1-42) CSF II assay.

The new derived cut-offs for PET concordance and cognitive decline using the pre-analytical procedure described in the section "Specimen collection and preparation" are shown below:

If Abeta42 ≤ 1030 pg/mL ⟹ test result positive.

If Abeta42 > 1030 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.

If tTau/Abeta42 ratio* > 0.28 ⟹ test result positive.

If tTau/Abeta42 ratio* ≤ 0.28 ⟹ test result negative.

*The ratio should be rounded to 3 decimal places before comparing against 0.28. 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, tTau > 1300 pg/mL, tTau < 80 pg/mL, the value should be set to the respective limit of the measuring range and the ratio should be calculated.

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18 http://biofinder.se/the_biofinder_study_group/

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


For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets, the product information 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 dialog.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 |



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