# Test Definition: ADEVL

Alzheimer Disease Evaluation, Spinal Fluid

## Overview

### Useful For

Assessment of adults with cognitive impairment being evaluated for Alzheimer disease and other causes of cognitive impairment

These assays should **not be used** to predict the development of dementia or other neurologic conditions or to monitor response to therapies.

### Special Instructions

- Spinal Fluid Specimen Collection Instructions for Alzheimer Disease Evaluation

### Highlights

Measurement of beta-amyloid (1-42)(Abeta42), total Tau, and phosphorylated Tau (p-Tau181) in cerebrospinal fluid is useful in the differential diagnosis of Alzheimer disease (AD) and other causes of cognitive impairment.

The p-Tau181/Abeta42 ratio provides excellent concordance with amyloid positron emission tomography (PET) imaging to assess the presence of amyloid deposition in patients with AD.

### Method Name

Electrochemiluminescent Immunoassay (ECLIA)

### NY State Available

Yes

## Specimen

### Specimen Type

CSF

### Specimen Required

**Supplies:**

Alzheimer's Disease Evaluation (ADEVL) Collection Kit (T836)

CSF AD Biomarker Tubes (T833; also included in T836)

**Container/Tube:**

**Preferred:** Sarstedt CSF False Bottom Tube 63.614.625 (2.5 mL)

**Acceptable:** Sarstedt 72.703.600 (1.5 mL) or Sarstedt 72.694.600 (2 mL)

**Specimen Volume:** 1.5 to 2.5 mL

**Collection Instructions:**

1. Perform lumbar puncture and discard the first 1 to 2 mL of cerebrospinal fluid (CSF).
2. Collect CSF directly into one of the listed collection tubes until the tube is at least 50% full.*

3. Send CSF specimen in original collection tube. Do **not aliquot**.
**Note: Polystyrene collection tubes are not acceptable**. Exposure of CSF to polystyrene tubes may result in falsely low Abeta42 concentrations. For more information see Cautions.
*The Alzheimer's Association consensus protocol for handling of CSF for clinical measurements of Abeta42 and tau recommends using the drip method for CSF collection and directly collecting into a low-bind polypropylene tube. Although some clinicians prefer the syringe pull method due to speed of collection, the drip method reduces the risk of Abeta42 binding to the plastic of any syringe used.
4. Collection instructions can also be found on Spinal Fluid Specimen Collection Instructions for Alzheimer Disease Evaluation (T967).

### Forms
If not ordering electronically, complete, print, and send a Neurology Specialty Testing Client Test Request (T732) with the specimen.

### Specimen Minimum Volume
See Specimen Required

### Reject Due To

| | |
|---|---|
| Gross hemolysis | Reject |
| Gross lipemia | OK |
| Gross icterus | Reject |

### Specimen Stability Information

| Specimen Type | Temperature | Time | Special Container |
|---|---|---|---|
| CSF | Refrigerated (preferred) | 14 days | BlueTop SARSTEDT |
| | Ambient | 12 hours | BlueTop SARSTEDT |
| | Frozen | 60 days | BlueTop SARSTEDT |

## Clinical & Interpretive

### Clinical Information
Two neuropathologic features observed in the brain of patients with Alzheimer disease (AD) dementia are the presence of plaques composed of beta-amyloid (Abeta) peptides and intracellular neurofibrillary tangles containing hyperphosphorylated Tau (tubulin-associated unit) proteins. These 2 groups of molecules are the most established biomarkers of the disease used in clinical and research practice. Positron emission tomography (PET) imaging using US Food and Drug Administration approved amyloid radiotracer (amyloid-PET) to visualize the presence of amyloid lesions in the cerebral cortex is available in some specialized centers. Measuring Abeta42 peptides and certain phosphorylated Tau (such as p-Tau181) proteins in cerebrospinal fluid (CSF) may be used as a means to assess the presence of amyloid pathology. In particular, the use of the p-Tau181/Abeta42 ratio has been shown to be an excellent surrogate marker of amyloid plaque burden, caused by increased deposition of beta-amyloid 1-42 in the brain. The use of these 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 and Alzheimer's Association Research Framework.

The CSF assays included in this evaluation are beta-amyloid (1-42; Abeta42), total Tau (t-Tau), and phosphorylated Tau (p-Tau181).

Abeta42 is approximately 4-kDa protein of 42 amino acids that is formed following proteolytic cleavage of a transmembrane protein known as amyloid precursor protein. Due to its hydrophobic nature, Abeta42 has the propensity to form aggregates and oligomers. Oligomers form fibrils that accumulate into amyloid plaques. These pathological changes in Abeta42 are reflected by the decrease in the CSF concentrations of Abeta42 and/or by the increase in the brain uptake of specific tracers during beta-amyloid PET.

Tau is present as six isoforms in human brain tissue. These isoforms are generated by alternative splicing of the pre-messenger RNA. The t-Tau assay measures all these isoforms. The most common post-translational modification of Tau proteins is phosphorylation. During neurodegeneration, abnormal phosphorylation leads to the formation of intracellular neurofibrillary tangles composed of the Tau protein that has undergone hyperphosphorylation and developed aggregates of hyperphosphorylated Tau proteins called p-Tau. The p-Tau assay detects phosphorylated Tau at threonine 181 (p-Tau181).

Pathological changes associated with AD are reflected by an increase in the CSF concentrations of t-Tau and p-Tau. Increases in CSF t-Tau reflect the intensity of the neuronal and axonal damage and degeneration and are associated with a faster progression from MCI to AD. Increases in CSF p-Tau concentrations are also associated with a faster progression from MCI to AD with more rapid cognitive decline in AD patients and in mild AD dementia cases.

The Alzheimer's Association has developed appropriate use criteria to guide safe and optimal use of CSF testing for AD pathology detection in the diagnostic process. The use of CSF biomarker testing may be indicated for the following patient groups:
1. Patients with subjective cognitive decline who are considered at increased risk for AD
2. Patients with MCI that is persistent, progressing, and unexplained
3. Patients with symptoms that suggest possible AD
4. MCI or dementia with an onset at an early age (younger than 65 years)
5. Patients meeting core clinical criteria for probable AD with typical age of onset
6. Patients whose dominant symptom is a change in behavior (eg, Capgras syndrome, paranoid delusions, unexplained delirium, combative symptoms, and depression) and where AD diagnosis is being considered.

## Reference Values

Beta-amyloid (1-42) (Abeta42): >834 pg/mL

Total-Tau: < or =238 pg/mL

Phosphorylated-Tau 181: < or =21.6 pg/mL

p-Tau/Abeta42: < or =0.028

## Interpretation

A beta-amyloid (1-42; Abeta42) result greater than 834 pg/mL is consistent with a negative amyloid positron emission tomography (PET) scan. A negative amyloid PET scan indicates the presence of no or sparse neuritic plaques and is

inconsistent with a neuropathological diagnosis of Alzheimer disease (AD). An Abeta42 result greater than 834 pg/mL is associated with a reduced likelihood that a patient's cognitive impairment is due to AD.

Total Tau (t-Tau) and phosphorylated Tau (p-Tau181) cerebrospinal fluid (CSF) concentrations increase approximately 2 to 3-times as much in patients with mild-moderate AD as compared to age-matched controls. A t-Tau and/or p-Tau181 concentration of less than or equal to 238 pg/mL and less than or equal to 21.6 pg/mL, respectively, reduces the likelihood that a patient's cognitive impairment is due to AD.

The use of p-Tau181/Abeta42 ratio provides better concordance with amyloid PET scan when compared to Abeta42, p-Tau181, and t-Tau individually. The p-Tau/Abeta42 ratio provides better concordance with amyloid PET imaging when compared to Abeta42, phospho-Tau and total-Tau individually. A cut-off of 0.028 provides optimal balance between negative percent agreement (NPA) and positive percent agreement (PPA) when compared to amyloid PET results. A p-Tau/Abeta42 ratio of 0.028 or less has a 92% NPA with normal amyloid PET. A ratio above 0.028 has a 92% PPA with abnormal amyloid PET.

High CSF t-Tau protein concentrations are found in other neurodegenerative diseases such as prion disease or Creutzfeldt-Jakob disease (CJD). In this situation, an elevated t-Tau concentration and an increased t-Tau to p-Tau ratio has a very high specificity for differential diagnoses of CJD.

| **Abnormal (+)/normal (-)** | **Individual comments for AD reporting values** |
|---|---|
| Abeta42 (-)<br>phospho Tau (-)<br>total Tau (-) | Normal concentrations of Abeta42, phospho-Tau, and total-Tau concentrations are present in CSF. These results are not consistent with the presence of pathological changes associated with Alzheimer disease. |
| Abeta42 (+)<br>phospho-Tau (-)<br>total-Tau (-) | Abnormal Abeta42 concentrations are present in CSF.<br>Phospho-Tau and total-Tau concentrations are normal.<br>These results may be consistent with Alzheimer-related pathologic change. |
| Abeta42 (+)<br>phospho-Tau (+)<br>total-Tau (-) | Abnormal Abeta42 and phospho-Tau concentrations are present in CSF.<br>The total-Tau concentration is normal.<br>These results are consistent with the presence of Alzheimer disease. |
| Abeta42 (+)<br>phospho Tau (+)<br>total Tau (+) | Abnormal Abeta42, phospho-Tau and total-Tau concentrations are present in CSF. These results are consistent with the presence of Alzheimer disease. |
| Abeta42 (+)<br>phospho Tau (-)<br>total Tau (+) | Abnormal Abeta42, and total-Tau concentrations are present in CSF.<br>The phospho-Tau concentration is normal.<br>These results may be consistent with Alzheimer-related pathologic change. |
| Abeta42 (-)<br>phospho-Tau (+)<br>total-Tau (-) | Abnormal phospho-Tau concentrations are present in CSF.<br>Abeta42 and total-Tau concentrations are normal.<br>These results are not consistent with the presence of pathological changes associated with Alzheimer disease. |

| | |
|---|---|
| Abeta42 (-)<br>phospho tau (-)<br>total-Tau (+) | Abnormal total-Tau concentrations are present in CSF.<br>The Abeta42 and phospho-Tau concentrations are normal.<br>These results are not consistent with the presence of pathological changes associated with Alzheimer disease. |
| Abeta42 (-)<br>phospho-Tau (+)<br>total-Tau (+) | Abnormal phospho-Tau and total-Tau concentrations are present in CSF.<br>The Abeta42 concentration is normal.<br>These results are not consistent with the presence of pathological changes associated with Alzheimer disease. |

This table and interpretations are based on the National Institute on Aging and Alzheimer's Association research framework diagnostic recommendations.

### Cautions

A positive cerebrospinal fluid (CSF) beta-amyloid 42 (Abeta42), total Tau (t-Tau), or phosphorylated Tau (p-Tau181) result, or p-Tau181/Abeta42 ratio does not establish a diagnosis of Alzheimer disease (AD) or other cognitive disorder. These results should be interpreted in combination with other clinical diagnostic and radiologic evaluations.

An abnormal p-Tau181/Abeta42 ratio in the context of a normal Abeta42 may be observed in some individuals. In some situations, interindividual differences in overall concentration of Abeta peptide production and/or in p-Tau elevation stemming from other neurodegenerative disease may result in an abnormal p-Tau181/Abeta42 ratio.

To achieve the best clinical performance (ie, keep patient misclassification rate at a minimum), it is important that the recommended pre-analytical protocol for sample collection is followed. Based on the Alzheimer's Association international guidelines for consensus handling of CSF for clinical measurements of Abeta42 and Tau, CSF should be collected using the drip method and directly collected into a low bind polypropylene tube. The low bind polypropylene tube should be filled to at least 80% of the tube volume capacity. Failure to adhere to this sample collection recommendation may impact the measured Abeta42 concentration and may influence the interpretation relation to the laboratory used cut-offs.

Additionally, it is recognized that using the recommended Alzheimer's Association drip method may not be feasible for every patient collection scenario. If a different method for CSF collection is used, it is critical that the CSF is collected directly into a low bind polypropylene tube and sent to Mayo Clinic Laboratories per test recommendations.

Improper specimen handling or interindividual differences in overall concentration of Abeta peptide production may yield an abnormally low Abeta42 in the context of a normal p-Tau181/Abeta42 ratio. Results should be interpreted in concordance with other clinical information.

Exposure of CSF to polystyrene tubes can reduce concentrations of the amyloid Abeta42 by as much as 20% to 50% due to adherence of the sticky amyloid protein to polystyrene tube surface material, potentially altering clinical interpretation, including the p-Tau181/Abeta 42 ratio. p-Tau181 and t-Tau protein do not substantially adhere to polystyrene collection tubes.

Failure to adhere to the specimen collection instructions provided may result in falsely low Abeta42 concentrations and potential misdiagnosis of AD.

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

There is no high-dose hook effect at Abeta42 concentrations up to 6000 pg/mL, p-Tau181 concentrations up to 300 pg/mL, and t-Tau concentrations up to 4267 pg/mL.

CSF biotin concentrations up to 1200 ng/mL do not interfere with this assay. Concentrations up to 1200 ng/mL may be present in specimens collected from patients taking extremely high doses of biotin up to 300 mg/day.(1) In a study among 54 healthy volunteers, supplementation with 20 mg/day biotin resulted in a maximum serum biotin concentration of 355 ng/mL one-hour postdose.(2)

In rare cases, some individuals can develop antibodies to mouse or other animal antibodies (often referred to as human anti-mouse antibodies [HAMA] or heterophile antibodies), which may cause interference in some immunoassays. The presence of antibodies to streptavidin or ruthenium can also rarely occur and may interfere in this assay. Caution should be used in interpretation of results, and the laboratory should be alerted if the result does not correlate with the clinical presentation.

### Clinical Reference

1. Peyro Saint Paul L, Debruyne D, Bernard D, Mock DM, Defer GL. Pharmacokinetics and pharmacodynamics of MD1003 (high-dose biotin) in the treatment of progressive multiple sclerosis. Expert Opin Drug Metab Toxicol. 2016;12(3):327-344
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3. van Harten AC, Wiste HJ, Weigand SD, et al. Detection of Alzheimer's disease amyloid beta 1-42, p-tau, and t-tau assays. Alzheimers Dement. 2022;18(4):635-644. doi:10.1002/alz.12406
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6. Leuzy A, Mattsson-Carlgren N, Cullen NC, et al. Robustness of CSF AB42/40 and AB42/P-tau181 measured using fully automated immunoassays to detect AD-related outcomes. Alzheimers Dement. 2023;19(7):2994-3004. doi:10.1002/alz.12897
7. Jack CR Jr, Bennett DA, Blennow K, et al. NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. Alzheimers Dement. 2018;14(4):535-562
8. Lifke V, Kollmorgen G, Manuilova E, et al. Elecsys Total-Tau and Phospho-Tau (181P) CSF assays: Analytical performance of the novel, fully automated immunoassays for quantification of tau proteins in human cerebrospinal fluid. Clin Biochem. 2019;72:30-38
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10. Shaw LM, Arias J, Blennow K, et al. Appropriate use criteria for lumbar puncture and cerebrospinal fluid testing in the diagnosis of Alzheimer's disease. Alzheimers Dement. 2018;14(11):1505-1521
11. Hansson O, Batrla R, Brix B, et al. The Alzheimer's Association international guidelines for handling of cerebrospinal

fluid for routine clinical measurements of amyloid beta and tau. Alzheimers Dement. 2021;17(9):1575-1582. doi:10.1002/alz.12316

## Performance

### Method Description

Beta-amyloid (1-42):
The Roche cobas assay for determining beta-amyloid (1-42) in cerebrospinal fluid (CSF) uses a sandwich assay principle. A biotinylated monoclonal beta-amyloid (1-42) antibody and a monoclonal beta-amyloid (1-42) specific antibody labeled with a ruthenium complex react to form a sandwich complex. Streptavidin-coated microparticles are added, and the interaction between biotin and streptavidin allows the complex to become bound to the solid phase. The reaction mixture is then aspirated into the measuring cell, microparticles are captured onto the electrode, and the application of voltage induces chemiluminescent emission, which is measured by a photomultiplier.(Package insert: Elecsys beta-Amyloid (1-42) CSF II. Roche Diagnostics; Version 1.0, 12/2022)

Total-Tau:
The Roche cobas assay for determining total-Tau in CSF uses a sandwich assay principle. Two biotinylated monoclonal Tau-specific antibodies and a monoclonal Tau-specific antibody labeled with a ruthenium complex react to form a sandwich complex. Streptavidin-coated microparticles are added, and the interaction between biotin and streptavidin allows the complex to become bound to the solid phase. The reaction mixture is then aspirated into the measuring cell, microparticles are captured onto the electrode, and the application of voltage induces chemiluminescent emission, which is measured by a photomultiplier.(Package insert: Elecsys Total-Tau CSF. Roche Diagnostics; Version 2.0, 10/2023)

Phospho-Tau:
The Roche cobas assay for determining phospho-Tau in CSF uses a sandwich assay principle. A biotinylated monoclonal antibody specific for phosphorylation at threonine 181 and a monoclonal Tau-specific antibody labeled with a ruthenium complex react to form a sandwich complex. Streptavidin-coated microparticles are added, and the interaction between biotin and streptavidin allows the complex to become bound to the solid phase. The reaction mixture is then aspirated into the measuring cell, microparticles are captured onto the electrode, and the application of voltage induces chemiluminescent emission, which is measured by a photomultiplier.(Package insert: Elecsys Phospho-Tau (181P) CSF. Roche Diagnostics; Version 1.0, 12/2022)

### PDF Report

No

### Day(s) Performed

Tuesday, Thursday, Friday

### Report Available

1 to 4 days

### Specimen Retention Time

12 months

**Performing Laboratory Location**

Mayo Clinic Laboratories - Rochester Superior Drive

## Fees & Codes

### Fees

- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their account representative. For assistance, contact Customer Service.

### Test Classification

This test has been modified from the manufacturer's instructions. Its performance characteristics were determined by Mayo Clinic in a manner consistent with CLIA requirements. This test has not been cleared or approved by the US Food and Drug Administration.

### CPT Code Information

AB42P-82234
PTAUP-84393
TTAUP-84394

### LOINC® Information

| Test ID | Test Order Name | Order LOINC® Value |
|---|---|---|
| ADEVL | Alzheimer's Disease Evaluation, CSF | 104134-2 |

| Result ID | Test Result Name | Result LOINC® Value |
|---|---|---|
| PTABR | p-Tau/Abeta42 | 41027-4 |
| ADINT | AD Interpretation | 69048-7 |
| AB42P | Abeta42 | 33203-1 |
| PTAUP | Phospho-Tau(181P) | 72260-3 |
| TTAUP | Total-Tau | 30160-6 |