Overview

Useful For
Supporting, in conjunction with other tests, a diagnosis of Creutzfeldt-Jakob disease in patients with rapidly progressive dementia when other neurodegenerative conditions have been excluded

Method Name
Immuochemiluminometric Assay (ICMA)

NY State Available
Yes

Specimen

Specimen Type
CSF

Specimen Required
Collection Container/Tube: Sterile vial
Submission Container/Tube: Plastic vial
Specimen Volume: 1 mL

Collection Instructions:
1. Obtain aliquot from second collection vial.
2. Hemolyzed specimens will give false-positive results. Specimens should be centrifuged to remove any red cells before shipping. The test will be canceled if there is any level of hemolysis present.
3. Immediately place aliquot on ice.

Additional Information:
1. Specimens that have not been kept refrigerated or that have been tested for other analytes previously may give a false-positive result.
2. Separate specimens should be submitted when multiple tests are ordered. This will reduce the risk of test cancellation due to stability problems.

Specimen Minimum Volume
0.6 mL

Reject Due To

| Gross hemolysis | Reject |

Specimen Stability Information
Clinical and Interpretive

Clinical Information
The 14-3-3 proteins are a group of highly conserved proteins composed of several isoforms that are involved in the regulation of protein phosphorylation and mitogen-activated protein kinase pathways. They exist in vivo as dimers of the various isoforms with apparent molecular mass of 30 kDa on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and 60 kDa on gel chromatography. Sequence homology among the various isoforms ranges from 22% to 100%. The beta, gamma, and theta isoforms are found in tissues of the nervous system.

Detectable 14-3-3 protein in cerebrospinal fluid (CSF) specimens is indicative of substantial, relatively rapid, neuronal destruction. Increased CSF concentrations of 14-3-3 proteins have been described in patients with various forms of Creutzfeldt-Jakob disease (CJD), some other rapidly progressive dementias, and a large range of other vascular, inflammatory, neoplastic, and metabolic central nervous system (CNS) disorders (see Cautions), which can be associated with significant and rapid neuronal destruction.

The main clinical use of 14-3-3 measurements is in the differential diagnosis of dementia, in particular to distinguish CJD and its variants from other dementias. The most common forms of dementia (progressive multi-infarct dementia and Alzheimer disease) are uncommonly associated with elevated CSF levels of 14-3-3, presumably because of their slow pace of progression.

CJD is an incurable neurodegenerative disease caused by accumulation of self-catalytically misfolded endogenous prion proteins in the CNS. Its cause is most commonly sporadic, but it can be inherited (variations that predispose to misfolding) or acquired (iatrogenic transmission by infected human tissues or tissue extracts, surgical procedures, or by ingestion of some animal products that contain misfolded prion proteins).

The diagnosis of CJD is highly complex and involves clinical history and neurologic examination, electroencephalographs (EEG), magnetic resonance imaging (MRI), and exclusion of other possible causes of dementia, in addition to CSF examination. Several, slightly different scoring systems are in use to integrate these parameters into a final diagnosis of possible, probable, or definite CJD. The most widely accepted of these scoring systems is the WHO set of diagnostic criteria for sporadic CJD from 2018 (see Interpretation).

Reference Values
Normal: < or =2.0 ng/mL
Elevated: >2.0 ng/mL

Interpretation
In cerebrospinal fluid (CSF) specimens, a 14-3-3 protein concentration of 2.0 ng/mL or higher supports the diagnosis of Creutzfeldt-Jakob disease (CJD) in patients who have been carefully preselected based on various diagnostic criteria. CSF 14-3-3 measurement is particularly helpful in sporadic CJD, where it is used as one of several diagnostic criteria.

Sporadic CJD World Health Organization (WHO) diagnostic criteria from 2018:
1. Definitive CJD: Diagnosed by standard neuropathological techniques; and/or immunocytochemically; and/or Western blot confirmed protease-resistant PrP; and/or presence of scrapie-associated fibrils.

2. Probable CJD: Neuropsychiatric disorder plus positive RT-QuIC in cerebrospinal fluid (CSF) or other tissues OR rapidly progressive dementia; and at least 2 of the following clinical features:
   - Myoclonus
   - Visual or cerebellar signs
   - Pyramidal/extrapyramidal signs
   - Akinetic mutism
   AND a positive result on at least 1 of the following laboratory tests
   - A typical EEG (periodic sharp wave complexes) during an illness of any duration
   - A positive 14-3-3 CSF assay in patients with a disease duration of less than 2 years
   - High signal in caudate/putamen on magnetic resonance imaging (MRI) brain scan or at least 2 cortical regions (temporal, parietal, occipital) either on diffusion-weighted imaging (DWI) or fluid attenuated inversion recovery (FLAIR)
   AND without routine investigations indicating an alternative diagnosis.

3. Possible CJD: Progressive dementia; and at least 2 of the following clinical features:
   - Myoclonus
   - Visual or cerebellar signs
   - Pyramidal/extrapyramidal signs
   - Akinetic mutism
   AND the absence of a positive result for any of the 4 tests above that would classify a case as probable; AND duration of illness less than 2 years; AND without routine investigations indicating an alternative diagnosis.


Cautions
Hemolyzed specimens will be rejected. Hemolysis causes falsely elevated 14-3-3 results. The 14-3-3 concentrations in 82 visibly blood-tinged cerebrospinal fluid (CSF) specimens were up to 281 ng/mL, with 74 specimens (90.2%) showing levels above the cutoff.
In addition, specimens may be determined to be unsuitable for testing if any of the following conditions are observed\(^{(1,2)}\):

- Macropscopically hemolyzed
- Xanthochromic
- RBC counts >500 cells per mcL
- WBC counts >10 cells per mcL

The Mayo Clinic 14-3-3 assay is a quantitative assay for 14-3-3 theta/tau isoforms. All other assays are currently based on qualitative or semi-quantitative assessment of 14-3-3 by Western blot of CSF specimens. 14-3-3 results obtained by Western blot can’t be compared directly with the Mayo Clinic 14-3-3 results. However, the published literature suggests comparable sensitivity and specificity ranges between the Mayo assay and Western blot assays.

Regardless of the method used, measurement of 14-3-3 protein in CSF should not be relied upon exclusively to establish the diagnosis of Creutzfeldt-Jakob disease (CJD). Increased concentrations of 14-3-3 protein in CSF have been described in a variety of central nervous system (CNS) diseases other than CJD that are associated with relative rapid (days to months, rather than months to years) destruction of significant amounts of CNS neuronal tissue. Elevation of 14-3-3 could be found in patients with viral encephalitis, paraneoplastic disorder, or a recent stroke. 14-3-3 protein can also be identified in a small subset of patients with diseases that can temporarily mimic CJD, such as Hashimoto encephalitis, metabolic encephalopathy and amyotrophic lateral sclerosis. Furthermore, false-positive 14-3-3 results are also found in patients with other types of dementia that have an unusual rapid evolution of the disease of less than 1 year.

In addition, severe acute CNS episodes of multiple sclerosis, cerebral vasculitides and angiopathies, mitochondrial encephalomyelopathies, CNS storage diseases, widespread or rapidly growing primary or secondary CNS and leptomeningeal tumors might result in 14-3-3 elevations.

**Supportive Data**

A total of 950 cerebrospinal fluid (CSF) specimens, including 14 from patients with definite (autopsy-proven) Creutzfeldt-Jakob disease (CJD), were tested for 14-3-3 protein. Using the cutoff from receiver operating characteristic curve (ROC) analysis, the sensitivity was 78.6% and specificity was 96.7%. This compares to neuron-specific enolase (NSE), which at a cutoff of 43 ng/mL had sensitivity of 78.6% and specificity of 94.0%. In another group of 30 clinically highly possible or probable CJD cases without histological confirmation, NSE was elevated in 25 (83.3%) and 14-3-3 in 21 (70.0%).

In 235 CSF specimens sent in for RBC and WBC counting (CJD was not suspected) the specificity was 94.5%. The 13 specimens that had elevated 14-3-3 results were from patients with disorders known to elevate CSF 14-3-3, such as Guillain-Barre syndrome and viral encephalitis.

**Clinical Reference**


Method Description

The 14-3-3 protein is measured in an immunochemiluminometric assay. The patient specimen is incubated with a monoclonal antibody directed against all 14-3-3 isoform coated on white microtiter plate wells. After washing, a second monoclonal antibody directed against the theta/tau isoforms, labeled with an acridinium ester, is added. The amount of label subsequently bound to the wells is counted in a microtiter plate luminometer that calculates the amount of 14-3-3 present in the specimen. (Unpublished Mayo Method)

Performance

Day(s) and Time(s) Test Performed
Monday, Thursday; 2 p.m.

Analytic Time
2 days

Maximum Laboratory Time
6 days

Specimen Retention Time
3 months

Fees and 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 Regional Manager. For assistance, contact Customer Service.

Test Classification

This test was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. This test has not been cleared or approved by the U.S. Food and Drug Administration.

CPT Code Information
83520

LOINC® Information
# Test Definition: P1433

14-3-3 Protein, CSF

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