

CSF Diagnostic Testing for CJD at the NDDL / ANCJDR

The Australian National Creutzfeldt-Jakob Disease Registry (ANCJDR) with the National Dementia Diagnostics Laboratory (NDDL) provides routine diagnostic testing services to aid the assessment of suspected Creutzfeldt-Jakob Disease (CJD) cases nationwide and as required, to New Zealand and several countries throughout Asia. Services provided include biomarker analyses of cerebrospinal fluid (CSF) as detailed below:

- **CSF 14-3-3 protein test (NATA accredited):**
14-3-3 protein in CSF, is a non-specific marker of neuronal injury or death in the central nervous system. 14-3-3 protein is detected by an Elisa immunoassay. 14-3-3 detection is quantitative and a positive CSF 14-3-3 protein result has approximately 90% sensitivity and specificity for sporadic CJD*.
- **CSF Total Tau protein test (NATA accredited):**
Similar to 14-3-3, an elevated concentration of total tau protein in CSF is a non-specific marker of neuro-axonal injury. The estimation of total tau in CSF is complementary to 14-3-3 protein detection for supporting the diagnosis of sporadic CJD. Performed by Elecsys® immunoassay, total tau testing is quantitative, and a positive result (>536 ng/L) has sensitivity and specificity of 88% and 91% respectively, for sporadic CJD*.
- **CSF RT-QuIC assay (NATA accredited):**
The RT-QuIC assay specifically identifies the presence of misfolded prion protein, a definitive biomarker of CJD. RT-QuIC is a protein-misfolding amplification technique that, following "seeding" with a patient's CSF, increases the concentration of misfolded prion protein within the assay up to detectable quantities. Test results are reported as positive (misfolded prion protein has been detected) or negative (not detected), with specificity of approximately 99% and sensitivity of 92.7% for sporadic CJD.

Collection and Preparation of CSF Specimens for CJD Diagnostic Testing

Note: Adhering to these instructions will enable the most accurate and comprehensive biomarker testing for CJD and Alzheimer's disease (AD). When collected this way, both the CJD and AD biomarker testing can be carried out on a single CSF sample.

CSF collection:

- CSF must be clear and non-haemolysed
- Collect CSF by gravity feed at lumbar puncture directly into a blue capped, low protein-binding tube - **SARSTEDT, Order Number 63.614.625** (pictured)
- A total of 2.5 ml (minimum volume) is required
- No further sample processing

Guidelines for the Collection, Handling and Transport of CSF specimens
for CJD and Alzheimer's Disease Diagnostic Analysis

- **Do not spin**
- **Do not transfer CSF into any other tubes**
- Store specimens in the refrigerator at 2-8°C, **DO NOT FREEZE**



CSF specimens that are collected and/or stored into traditional yellow capped polypropylene tubes (or other tubes), as well as refrigerated or frozen CSF samples, are still suitable for 14-3-3, total-tau protein and RT-QUIC testing.

CSF samples that do not meet the above collection criteria, can still be tested but may be reported with caveats. This applies especially where a sample is referred for both CJD and AD screening as the collection criteria are most relevant for estimation of the beta-amyloid protein concentrations in CSF for determining an AD diagnosis.

Unsuitable samples:

The following test suitability criteria apply to the CSF CJD biomarker testing. All specimens are screened upon receipt and those that do not meet the test criteria are considered unsuitable and will not be tested. Specimens that are unsuitable for 14-3-3 protein testing may be suitable for all other CJD and AD biomarker tests.

- **14-3-3 test**
 - Red blood cell count must be less than $500 \times 10^6/L$ for 14-3-3 Protein testing**
 - White blood cell count must be less than $10 \times 10^6/L$ for 14-3-3**
 - CSF must be clear and colourless (not macroscopically haemorrhagic or xanthochromic)
 - CSF must not be centrifuged
- **T-tau test**
 - Red blood cell count must be less than $5000 \times 10^6/L$
 - CSF must be non-haemolysed
- **RT-QuIC Assay**
 - Red blood cell count must be less than $1250 \times 10^6/L$
 - White blood cell count must be less than $10 \times 10^6/L$
 - Total CSF protein concentration must be less than 1.0 g/L

National Dementia Diagnostics Laboratory (CJD)

CSF Collection Protocol

for Referring Clinicians and Laboratories



Guidelines for the Collection, Handling and Transport of CSF specimens
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Referrals and Shipment of CSFs to the ANCJDR

Delivery address:

Australian National CJD Registry

The Florey
Kenneth Myer Building
30 Royal Parade, corner Genetics Lane
Gate 11, Rear loading Dock
The University of Melbourne, Parkville, VIC 3052

Please ensure the following:

- The specimen is double bagged and packed securely (tube intact and firmly sealed)
- Ship at 2-8°C (wet-ice / cold gel-pack) within 7 days of collection date. (The referring laboratory is responsible for organizing a suitable courier service to pick up and deliver the sample to the ANCJDR)
- A copy of the original doctor's request slip is provided. This MUST accompany the specimen
- Please fill out a copy of the NDDL(CJD) CSF Specimen Data Sheet
 - downloadable from ANCJDR website
<https://florey.edu.au/our-research/scientific-facilities-and-research-services/australian-national-cjd-registry/>
 - This form can be used when both the CJD and AD screens are requested on a single CSF sample.
 - Alternatively, the NDDL AD CSF specimen datasheet may be completed (downloadable from NDDL website <https://florey.edu.au/our-research/scientific-facilities-and-research-services/national-dementia-diagnostics-laboratory/>)
 - Completion of one form is sufficient for both screens, it is not necessary to fill out two forms
- The routine CSF microbiology (red blood cell and white blood cell counts) and biochemistry results (protein and glucose levels) are provided.

Please contact the ANCJDR to notify of incoming samples prior to sending

Tel: +61 3 8344 1949

Fax: +61 3 9349 5105

Email: ancjd-reg@unimelb.edu.au

Information about CSF diagnostic testing and reporting

- The CSF 14-3-3 Protein test is performed weekly by the ANCJDR
- The CSF Total Tau assay is performed weekly by the NDDL.
- RT-QuIC is performed weekly by the ANCJDR.
- Interim reports will be sent by email to the referring laboratory as results become available with a final report issued following completion of all testing.

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- Positive results will be verbally reported to the requesting clinician within 48 hours of the test result being called
- A researcher from the Registry will contact the requesting clinician directly to clarify all salient clinical details as required.
- Unsuitable samples will not be tested (see above). Upon receipt of such samples the referring laboratory will be contacted and advised. A report outlining the reason for the sample not being tested will be issued.

Delays in reporting

- Please directly contact your referring laboratory to check for results.
- Technical difficulties may cause delays in reporting. These delays cannot be predetermined and the test will continue to be repeated until a satisfactory result is obtained.

Cost for diagnostic testing (effective from 01/07/2022)

- CJD test: Domestic Referrals – No Charge
International - \$500
- AD test - \$400 (effective from 01/07/2023)

*Based on international experience, in carefully selected patients. False positive 14-3-3 protein results may be recognized in several other diseases such as encephalitis (especially Herpes Simplex) and recent cerebral infarcts. T-Tau False positives are recognized in various disease processes; for example encephalitis, encephalopathies and recent cerebral infarcts, and possibly some tauopathies and AD.

**Based on cumulative experience and published results, these samples are deemed unsuitable due to the 14-3-3 protein existing in erythrocytes, platelets and plasma. Lysis of these cells releases the 14-3-3 protein into the CSF thus contaminating the sample and causing a false positive result. (Day I.N.M and Thompson R.J. *Clinica Chimica Acta* 1984; 136: 219-228, Collins et al, *J of Clin Neurosci* 2000; 7:203-208).