

CSF Diagnostic Testing for CJD at the ANCJDR

The Australian National Creutzfeldt-Jakob Disease Registry (ANCJDR) 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 ELISA. 14-3-3 detection is quantitative and a positive CSF 14-3-3 protein result (>23,194 AU/ml) has 81.3% sensitivity and 84.4% specificity for sporadic CJD^{1*}.
- **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 CJD¹.

Collection and Preparation of CSF Specimens for CJD Diagnostic Testing

CSF collection:

- CSF must be clear and non-haemolysed.
- A minimum of 2.5 ml is requested.
- Freezing of samples is recommended. Specimens can be stored in the refrigerator at 2-8°C for up to 4 days only.

CSF samples that do not meet the above collection criteria can still be tested but may be reported with caveats.

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.

- **14-3-3 test**
 - CSF must be clear and colourless (not macroscopically haemorrhagic or xanthochromic).^{**}
 - CSF must not be centrifuged.

Guidelines for the Collection, Handling and Transport of CSF specimens
for CJD Diagnostic Analysis

- **RT-QuIC Assay**
 - White blood cell count must be less than $10 \times 10^6/L$.
 - CSF must be clear and colourless (not macroscopically haemorrhagic) or xanthochromic.

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 sample frozen on dry ice (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/>
- 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 and RT-QuIC are 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.
- 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.

Guidelines for the Collection, Handling and Transport of CSF specimens
for CJD Diagnostic Analysis

- 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

- CJD test: Domestic Referrals – No Charge
International - \$500

1. Senesi et al. *Frontiers in Neurology* 2023; 14: 1072952

*"False" (non prion disease specific) positive results may be recognized in several other diseases, including - encephalitis (especially Herpes Simplex), invasive CNS malignancy and recent cerebral infarcts. Based on analysis of 165 pathologically proven sporadic CJD cases; total CSF protein >1.0g/L is rarely seen in uncomplicated CJD (<2%) and total CSF white cell count is never >10 cells/ μ L in uncomplicated cases (ANCJDR personal communication).

**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).