A guide to Creutzfeldt-Jakob Disease (CJD) Cerebrospinal fluid (CSF) End-point Quaking-Induced Conversion (EP-QuIC) Test Interpretation

Preamble

This guide describes the EP-QuIC test in the assessment of CJD or other human prion diseases in patients. For further information, please contact the Prion Diseases Section at 204-789-6078 or cjd@phac-aspc.gc.ca.

Background

Creutzfeldt-Jakob disease (CJD) and other human prion diseases are rare neurodegenerative disorders that are always fatal. Most commonly CJD presents as a rapidly progressive dementia, often accompanied by ataxia and myoclonus, however, symptoms can be heterogenous. The disease is caused by the misfolding of normal cellular prion protein to an amyloid form that is aggregated and transmissible. Divergent clinical presentations are partly attributed to different subtypes of CJD that occur when the prion protein misfolds into different, distinct conformations. They occur most often in humans as sporadic disease, but can also occur as genetic or acquired disease, either through surgery or infection.

Historically, diagnosis has relied upon the detection of surrogate protein biomarkers in patient cerebrospinal fluid (CSF) that are raised in rapidly progressive dementia. However, the recent development of the quaking-induced conversion test (QuIC) that directly detects the disease-causing agent, has altered the landscape of CJD testing (1). The NML offers the “end-point” quaking-induced conversion (EP-QuIC) test that has proven to be robust and reproducible, obviating the need to rely on the detection of non-specific markers of neurodegeneration (2). The sensitivity and specificity of the test is greater or equal to 96% and greater than or equal to 99% respectively (2,3).

Test Availability

As of August 1, 2025, the National Microbiology Laboratory in Winnipeg will only offer ante-mortem testing of CSF by the EP-QuIC test (accredited under (ISO/IEC 17025). Tests are performed twice weekly and reports are available 3 to 8 days following receipt of CSF in most cases.

NML will cease to offer routine reporting of the CSF biomarkers 14-3-3 and total tau proteins. Total tau analysis will continue to be performed and results can be

available upon request to NML's Prion Diseases Section using the contact methods listed below

Principles of the Test

The EP-QuIC test exploits the natural ability of the disease-associated, misfolded prion protein to act as a template for the conversion of the normal form. The CSF sample is added to wells containing in-house manufactured recombinant full-length prion protein as substrate. The mixture is heated to 42°C in 96-well plate with vigorous, periodic, shaking for 66 hours. Refolded recombinant prion protein forms amyloid fibrils that are detected by binding to the fluorescent dye, (thioflavin T), that can be measured with a spectrofluorometer. Samples are run in triplicate and both positive and negative controls are run on each plate. A 4-fold relative increase in fluorescent signal over the 66 hour incubation for all three replicates is required for a **Positive** test result. Occasionally, only one or two of the three replicates meets the threshold. In this case the test is repeated using higher and lower concentrations of CSF. In most cases a definitive test result is obtained following this step, however, if the test continues to produce inconsistent results across any dilution set, the EP-QuIC result is reported as **Indeterminate**. On average, an indeterminate result occurs once every 100 samples. A **Negative** result is reported when fluorescence in all three wells does not reach the threshold.

Test Performance

The positive and negative predictive values of the EP-QuIC test, have been calculated as > 96% and >99 % respectively (2,3).

Interpretation

A positive test result from EP-QuIC supports a probable diagnosis of a human prion disease in the clinical context of progressive neuropsychiatric syndrome (4).

Although the test has a high degree of accuracy, there are conditions that have been reported to yield false-positive results on rare occasions. Most of these are in patients subsequently diagnosed with inflammatory brain diseases including autoimmune encephalitis, paraneoplastic neurological encephalopathies, and stroke with acute infarction (5, 6).

A small number of false negative tests also occur, estimated at less than 1 in 200 negative tests. This has been reported particularly at very early stages of disease (7). If there is a high suspicion of prion disease, repeated testing may be useful. False negative tests are also occasionally encountered in genetic prion diseases, notably Gerstman-Straussler-Scheinker disease and fatal familial insomnia, as well as some atypical cases of sporadic disease characterized by rare biochemical subtypes of prions. The NML is currently trialing an updated EP-QuIC test that has improved performance in these patients.

Definitive diagnosis of sporadic prion disease can be confirmed through neuropathological evaluation of brain tissues following autopsy. The Canadian CJD Surveillance System (CJDSS) coordinates autopsies and neuropathologic examination on suspected cases of human prion disease and can be contacted by phone at 1-888-489-2999, or by email at cjdsurveillance@phac-aspc.gc.ca.

A positive test result from EP-QuIC supports a probable diagnosis of a human prion disease in the clinical context of progressive neuropsychiatric syndrome (4). This testing is for a provincially reportable, and nationally notifiable disease and is therefore of public health concern. Please inform your appropriate provincial public health laboratory and provincial public health authorities of a positive test result.

References

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