A guide to the cerebrospinal fluid (CSF) test panel for Creutzfeldt-Jakob Disease (CJD)

Preamble

This guide provides a brief description of the test panel assays, and suggestions for interpretation of results. For further information, please contact the Prion Diseases Section at 204-789-6078 or cjd@phac-aspc.gc.ca.

Background

Sub-acute encephalopathies, including Creutzfeldt-Jakob disease (CJD), constitute a large and heterogeneous group of rare invariably fatal diseases also known as prion diseases. They occur most often in humans as sporadic disease, but can also occur as genetic or infectiously acquired disease. Like most neurodegenerative diseases, prion diseases are caused by disease-associated misfolded proteins. In prion diseases the misfolded isoform (PrPd) of the host prion protein (PrPc) [1] is the causative agent. This key biological fact precludes the use of conventional technologies, such as PCR and serology, which are commonly applied to the direct and specific detection of infectious agents. As a result, ante-mortem diagnosis of CJD has relied upon clinical presentation, neurological examination, supporting investigations such as MRI scanning of the brain and cerebrospinal fluid (CSF) testing for the presence of increased amounts of indirect protein markers. The most commonly used markers are 14-3-3ɣ[2] and total microtubule-associated tau (t-tau)[3].

A newer test, known as real-time quaking-induced conversion (RT-QuIC) demonstrated how the pathogenic protein, PrPd, could be detected more directly[4]. To allow for robust clinical testing, the Prion Diseases Section adapted the RT-QuIC test for use in a diagnostic setting. We call our version of the test "end-point" quaking-induced conversion (EP-QuIC) [5,6]. The QuIC assays exploit the natural ability of the disease-associated, misfolded isoform, PrPd, to induce conversion of the normal cellular form of the prion protein, PrPc, into a misfolded form *in vitro*. Recently the Prion Diseases Section published results of a prospective study evaluating the performance of our CSF-based test panel.[7] The positive predictive values of the EP-QuIC, 14-3-3ɣ and t-tau tests in this 623 sample study were 96%*, 68% and 66% respectively.

EP-QuIC analyses of the 15 samples in the cohort identified as having mutations within the PRNP gene produced one false negative EP-QuIC result. The CJD causative mutation in this incorrectly identified sample was D178N with homozygous methionine at the polymorphic codon 129. The patient exhibited weight loss and sleep disturbances characteristic of the disease caused by this genetic profile, known as Fatal Familial Insomnia (FFI). Though the more common forms of genetic CJD are well detected by EP-QuIC,

concurrent biochemical and genetic testing would ensure the detection of genetic CJD cases producing a false negative EP-QuIC result.

Reporting suspected cases to the CJD surveillance system (1-888-489-2999) and the local/provincial public health authority is strongly advised.

Test Availability

The Public Health Agency of Canada's Prion Diseases Section offers a CSF-based test panel consisting of immunoassays for 14-3-3gamma and t-tau proteins as well as detection of the disease-associated form of the prion protein by EP-QuIC. All three assays are accredited under Can-P4E (ISO/IEC 17025).

Principles of the Assays

Commercial solid phase enzyme immunoassays (ELISA) are used for the detection of 14-3-3-gamma (CycLex Co.) and t-tau (Fujirebio). The protein antigen to be measured is bound between a capture antibody (bound to the plate) and a form of detection antibody (conjugated with an enzyme (HRP) or directly labelled with biotin). The combination of HRP and biotin causes a colorimetric reaction which, when applied to standards and references, allows for the determination of the concentration of a target protein from the original sample.

The EP-QuIC assay exploits the natural ability of the disease-associated, misfolded isoform, PrPd, to induce conversion of the normal cellular form of the prion protein (PrPc) into a misfolded form *in vivo*. In EP-QuIC, samples are added to wells containing in-house manufactured recombinant PrP (rPrP). The mixture is heated to 42°C with periodic quaking over a period of 66 hours. Presence of PrPd in the sample will induce the conversion of the rPrP into large insoluble aggregates. The resulting insoluble rPrP aggregates generated by this process then bind a fluorescent dye (thioflavin T), causing a change in the dye's fluorescence emission spectrum that can be measured with a spectrofluorometer.

Test Performance

Definitive diagnosis of CJD requires pathological examination of brain tissue post-mortem.

Prospectively acquired Canadian cerebrospinal fluid samples were used to assess the performance characteristics of the three ante-mortem tests used to support diagnoses of Creutzfeldt–Jakob disease. The patient group of 623 samples included: 98 autopsy confirmed definite CJD cases, 24 probable CJD cases, 222 cases with alternative diagnoses and 279 unknown-but-not-suspect-CJD cases.

14-3-3 gamma assay: Using a cut-off for a positive test of 20,000 arbitrary units per mL[8] (20 000 AU/mL) where 1 AU ≈ 1 pg/mL, positive and negative predictive values of 68% and 96% respectively were obtained[7].

Total tau assay: Using a study-specific intermediate optimal cut-off of 976 pg/mL, the positive and negative predictive values of the t-tau assay in this cohort of patients were 66% and 98%, respectively [7].

NML's EP-QuIC assay: Using the cut-off threshold of a 4-fold relative increase in signal within each of three replicates, an individual sample was scored as positive when at least two of the three replicates were at or above threshold. In instances where only one of the three replicates was above threshold, the sample was reanalyzed using three different dilutions of CSF in the reaction mixture. Samples exhibiting one of three replicate wells as positive in any of the three dilution sets were scored as indeterminate. On average, an indeterminate result occurs once every 100 samples. Samples with all three replicates below the 4-fold threshold were scored as negative for the presence of PrPd.
The positive and negative predictive values using our in-house generated substrate, in the above defined cohort of samples were 97.5% and 99.4% respectively**.

	CJD		non-CJD		Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
	TP (a)	FN (b)	FP (c)	TN (d)	a/(a+b)	d/(d+c)	a/(a+c)	d/(d+b)
EP-QuiC	119	3	3	498	97.5%	99.4%	97.5%	99.4%
					93.0-99.5%	98.3 - 99.9%	92.8 - 99.2%	98.2 - 99.8%
14-3-3	103	19	49	452	84%	90%	68%	96%
					76.8 to 90.4	87.3 to 92.7	61.5 to 73.5	94.0 to 97.3
t-tau	112	10	59	442	92%	88%	66%	98%
					85.4 to 96.0	85.1 to 90.9	59.8 to 70.8	96.1 to 98.8

*using both commercial and in-house manufactured components[7].
** using only in-house manufactured components.

References

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