

ORIGINAL ARTICLE

Concordance of cerebrospinal fluid real-time quaking-induced conversion across the European Creutzfeldt–Jakob Disease Surveillance Network

Neil McKenzie¹ | Gabriele Piconi¹ | Audrey Culeux² | Anna-Lena Hammarin³ | Christos Stergiou⁴ | Socrates Tzartos⁴ | Alexandra A. M. Versleijen⁵ | Jacqueline van de Geer⁵ | Patrick Cras⁶ | Franco Cardone⁷ | Anna Ladogana⁷ | Angela Mannana⁸ | Marcello Rossi⁸ | Matilde Bongiani⁹ | Daniela Perra⁹ | Guenther Regelsberger¹⁰ | Sigrid Klotz¹⁰ | Simone Hornemann¹¹ | Adriano Aguzzi¹¹ | Matthias Schmitz¹² | Mary Andrews¹ | Kimberley Burns¹ | Stéphane Haïk² | Raquel Ruiz-García^{13,14} | Jenny Verner-Carlsson³ | John Tzartos⁴  | Marcel M. Verbeek⁵ | Bart De Vil⁶  | Anna Poggi⁷ | Piero Parchi^{8,15}  | Gianluigi Zanusso⁹ | Ellen Gelpi¹⁰ | Karl Frontzek¹¹ | Regina Reimann¹¹ | Peter Hermann¹²  | Inga Zerr¹² | Suvankar Pal¹ | Alison Green¹ 

¹National CJD Research and Surveillance Unit, Centre for Clinical Brain Sciences, University of Edinburgh, Edinburgh, UK

²Sorbonne Université, Institut du Cerveau—Paris Brain Institute, ICM, Inserm, CNRS, APHP, Hôpital de la Pitié Salpêtrière, Paris, France

³Public Health Agency of Sweden, Solna, Sweden

⁴Tzartos NeuroDiagnostics, Athens, Greece

⁵Neurochemistry Laboratory—Translational Metabolic Laboratory, Department of Laboratory Medicine, Radboud University Medical Centre, Nijmegen, The Netherlands

⁶Laboratory of Neurology, University of Antwerp, Antwerp, Belgium

⁷Department of Neuroscience, Istituto Superiore di Sanità, Rome, Italy

⁸Laboratory of Neuropathology, IRCCS Istituto delle Scienze Neurologiche, Bologna, Italy

⁹Neuropathology Laboratory, University of Verona, Verona, Italy

¹⁰Division of Neuropathology and Neurochemistry, Department of Neurology, Medical University of Vienna, Vienna, Austria

¹¹Institute of Neuropathology, University Hospital of Zurich, Zürich, Switzerland

¹²National Reference Center for Transmissible Spongiform Encephalopathies, Göttingen, Germany

¹³Immunology Department, Biomedical Diagnostic Centre, Hospital Clinic de Barcelona, Barcelona, Spain

¹⁴Neuroimmunology Program, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain

¹⁵Department of Experimental, Diagnostic, and Specialty Medicine (DIMES), University of Bologna, Bologna, Italy

Correspondence

Alison Green, National CJD Research and Surveillance Unit, Centre for Clinical Brain Sciences, University of Edinburgh, 49 Little France Crescent, Edinburgh EH16 4 SB, UK.
Email: alison.green@ed.ac.uk

Abstract

Background and purpose: Cerebrospinal fluid (CSF) real-time quaking-induced conversion (RT-QuIC) has a high degree of sensitivity and specificity for the diagnosis of sporadic Creutzfeldt–Jakob disease (sCJD) and this has led to its being included in revised European CJD Surveillance Network diagnostic criteria for sCJD. As CSF RT-QuIC

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becomes more widely established, it is crucial that the analytical performance of individual laboratories is consistent. The aim of this ring-trial was to ascertain the degree of concordance between European countries undertaking CSF RT-QuIC.

Methods: Ten identical CSF samples, seven from probable or neuropathologically confirmed sCJD and three from non-CJD cases, were sent to 13 laboratories from 11 countries for RT-QuIC analysis. A range of instrumentation and different recombinant prion protein substrates were used. Each laboratory analysed the CSF samples blinded to the diagnosis and reported the results as positive or negative.

Results: All 13 laboratories correctly identified five of the seven sCJD cases and the remaining two sCJD cases were identified by 92% of laboratories. Of the two sCJD cases that were not identified by all laboratories, one had a disease duration >26 months with a negative 14-3-3, whilst the remaining case had a 4-month disease duration and a positive 14-3-3. A single false positive CSF RT-QuIC result was observed in this study.

Conclusions: This study shows that CSF RT-QuIC demonstrates an excellent concordance between centres, even when using a variety of instrumentation, recombinant prion protein substrates and CSF volumes. The adoption of CSF RT-QuIC by all CJD surveillance centres is recommended.

KEYWORDS

cerebrospinal fluid, real-time quaking-induced conversion, Creutzfeldt–Jakob disease, prion

INTRODUCTION

The development of cerebrospinal fluid (CSF) real-time quaking-induced conversion (RT-QuIC) has been a major advance in the timely ante-mortem diagnosis of sporadic Creutzfeldt–Jakob disease (sCJD) [1, 2]. RT-QuIC exploits the ability of small amounts of the disease-associated form of prion protein (PrP^{Sc}) found in CSF and other tissues such as olfactory mucosa to convert native PrP into an aggregated beta-sheet enriched form.

This technique uses recombinant PrP (rPrP) as a substrate that misfolds and aggregates on the addition of CSF or other tissues containing PrP^{Sc}. The addition of Thioflavin T allows the reaction to be monitored in real time, due to a change in the Thioflavin T emission spectrum on binding to aggregated PrP^{Sc} [3].

Cerebrospinal fluid RT-QuIC has a high degree of sensitivity (69%–93%) and specificity (99%–100%) for the diagnosis of sCJD in both retrospective and prospective studies [1, 4–11]. This high degree of specificity has led to CSF RT-QuIC being introduced into the revised European CJD Surveillance Network diagnostic criteria for sCJD [12–14]. A single study from Germany has shown that the inclusion of CSF RT-QuIC into the clinical criteria increases the assessed incidence of CJD from 1.7 to 2.2 per million [13]. As CSF RT-QuIC becomes more widely established it is crucial that the analytical performance of individual laboratories is closely monitored to ensure consistency between laboratories. This will also ensure that sCJD prevalence data from countries performing RT-QuIC analysis are not affected by differences in CSF RT-QuIC performance.

As CSF RT-QuIC becomes more widely established there are resultant changes in methodology, notably in the choice of rPrP

substrate. A small number of CSF RT-QuIC ring-trials have been undertaken which have shown a high degree of concordance between centres; however, these studies have been undertaken before the inclusion of CSF RT-QuIC into the diagnostic criteria or have only included a small number of participants [15–17].

With an increasing number of laboratories undertaking CSF RT-QuIC, there is a need to ensure that analytical performance is optimal, that there is high concordance between laboratories to maintain diagnostic accuracy and that subsequent prevalence data are comparable between centres. The aim of this study was to undertake a CSF RT-QuIC ring-trial that includes all laboratories performing CSF RT-QuIC within the European Creutzfeldt–Jakob Disease Surveillance Network to assess the degree of concordance between centres.

MATERIALS AND METHODS

Participants

A total of 13 laboratories from 11 countries participated in this study. Participating centres were the Institut du Cerveau et de la Moelle épinière, Salpêtrière Hospital, Paris, France (Laboratory 1); Immunoneurology Laboratory, Centro de Diagnóstico Biomédico, Hospital Clínic de Barcelona, Barcelona, Spain (Laboratory 2); Tzartos NeuroDiagnostics, Athens, Greece (Laboratory 3); Public Health Agency of Sweden, Solna, Sweden (Laboratory 4); Neurochemistry Laboratory—Translational Metabolic Laboratory, Department of Laboratory Medicine, Radboud University Medical Centre, Nijmegen, The Netherlands (Laboratory 5); Laboratory

of Neuropathology, IRCCS Istituto delle Scienze Neurologiche, Bologna, Italy (Laboratory 6); Department of Neuroscience, Istituto Superiore di Sanità, Rome, Italy (Laboratory 7); Division of Neuropathology and Neurochemistry, Department of Neurology, Medical University of Vienna, Vienna, Austria (Laboratory 8); Laboratory of Neurology, University of Antwerp, Antwerp, Belgium (Laboratory 9); Neuropathology Laboratory, University of Verona, Verona, Italy (Laboratory 10); National Reference Center for Transmissible Spongiform Encephalopathies, Göttingen, Germany (Laboratory 11); Institute of Neuropathology, Zürich, Switzerland (Laboratory 12); National CJD Research and Surveillance Unit, University of Edinburgh, Edinburgh, UK (Laboratory 13).

Cerebrospinal fluid samples

The CSF samples were provided by the National CJD Research and Surveillance Unit (NCJDRSU) BioResource tissue bank, UK, and had ethical approval for use in research (North-West Haydock REC, 19/NW/0403; Scotland A REC, 18/SS/0041). The inclusion criteria included the presence of appropriate consent for research and a CSF volume of greater than 6 ml. Ten CSF samples were selected; of these seven were from neuropathologically confirmed or clinically probable sporadic CJD [13, 14] and three were from patients on whom the diagnosis of CJD was excluded on clinical grounds (Table 1). One CSF sample (CSF 2) was from a patient with dementia and ataxia and in whom the diagnosis of sCJD was ruled out on the basis of lack of clinical progression and no supportive investigations. The CSF 14-3-3 was negative, the magnetic resonance imaging (MRI) was normal and the electroencephalography was not supportive. One CSF sample (CSF 5) was from a patient with frontotemporal dementia and the remaining CSF sample (CSF 6) was from a patient with delirium secondary to a urinary tract infection who subsequently recovered. CSF samples from sCJD cases with either PRNP codon 129 methionine/methionine (MM) or methionine/valine (MV) genotype were included. No known sCJD-VV cases with sufficient CSF volume were available for selection. The CSF panel also included sCJD cases with both short and long disease durations. CSF samples were coded and aliquoted into polypropylene tubes with sufficient sample for each laboratory to undertake the RT-QuIC analysis twice. The coded CSF samples were sent to each laboratory on dry ice.

Real-time QuIC analysis

Cerebrospinal fluid RT-QuIC analysis was undertaken as previously described [4, 15, 17], using either a FLUOstar Omega or FLUOstar Optima microtitre plate reader (BMG LabTech). Eleven of the 13 laboratories used hamster full-length (23-231aa) rPrP as a substrate: six laboratories produced this in-house and five laboratories used a hamster full-length (Ha FL) rPrP produced by the NCJDRSU, UK. In addition two laboratories used a truncated form of hamster (Ha Tr) rPrP (90-231aa) produced in-house according to a previously

TABLE 1 Clinical and neuropathological details of the CSF samples sent to the participating laboratories

CSF ID	Diagnosis	Age (years), sex	PRNP codon 129/ PrP ^{Sc} isotype	Disease duration (months)	MRI	CSF 14-3-3
1	Neuropathologically confirmed sCJD	57, F	MV, 2A	18	No information	Positive
2	Not CJD (dementia, ataxia)	70, M	nd		Normal	Negative
3	Probable sCJD	71, F	MM, nd	2	Basal ganglia hyperintensity, plus cortical ribboning	Positive
4	Probable sCJD	74, M	MV, nd	12	Basal ganglia hyperintensity	Negative
5	Not CJD (FTD)	50, M	nd		Frontal lobe atrophy	Negative
6	Not CJD (delirium, secondary to UTI)	74, M	nd		No information	Negative
7	Probable sCJD	72, F	nd	5	Basal ganglia hyperintensity, plus cortical ribboning	Weak positive
8	Probable sCJD	68, F	MM, nd	2	Basal ganglia hyperintensity, plus cortical ribboning	Positive
9	Probable sCJD	62, M	MV, nd	Still alive, >26 months	Cortical ribboning only	Negative
10	Probable sCJD	61, F	nd	4	Basal ganglia hyperintensity, plus cortical ribboning	Positive

Abbreviations: CJD, Creutzfeldt-Jakob disease; CSF, cerebrospinal fluid; F, female; FTD, frontotemporal dementia; M, male; MM, methionine/methionine; MRI, magnetic resonance imaging; MV, methionine/valine; nd, not done; PRNP, gene coding for prion protein; PrP^{Sc}, scrapie isoform of the prion protein; sCJD, sporadic Creutzfeldt-Jakob disease; UTI, urinary tract infection.

TABLE 2 Details of CSF RT-QuIC substrate, instrumentation, CSF volume and number of replicates used by each laboratory

Lab ID	Type and source rPrP	Instrument used	Volume CSF used (µl)	Number of replicates used	Positive criteria
1	Ha FL rPrP, in-house	FLUOstar Omega	30	4	90h, cut-off >38,400 RFU in at least 2/4 wells
2	Ha FL rPrP, UoE	FLUOstar Omega	30	4	96h, cut-off >50,000 RFU in at least 2/4 wells
3	Ha FL rPrP, in-house	FLUOstar Omega	30	4	96h, cut-off >25,000 RFU in at least 2/4 (obligatory: sigmoid growth curve)
4	Ha FL rPrP, UoE	FLUOstar Omega	30 and 15	4	80h, cut-off >25,000 RFU in at least 2/4 wells
5	Ha FL rPrP, UoE	FLUOstar Optima	30 and 15	4	96h, >mean signal +5SD of the negative control in at least 2/4 wells (obligatory: sigmoid growth curve)
6	Ha FL rPrP, UoE	FLUOstar Optima	15	4	90h, >10,344 RFU in at least 2/4 wells
	Ha Tr rPrP (Caughey)	FLUOstar Omega	20	4	40h, cut-off >13,233 RFU in at least 2/4 wells
7	Ha FL rPrP, in-house	FLUOstar Omega	30 and 15	4	90h, cut-off >32,000 maximum RFU in at least 2/4 wells
8	Ha FL rPrP, in-house	FLUOstar Omega	30	4	90h, cut-off >50,000 RFU in at least 2/4 wells
9	Ha FL rPrP, UoE	FLUOstar Omega	20	4	90h, cut-off >38,000 RFU in at least 2/4 wells
10	Ha Tr rPrP, in-house	FLUOstar Omega	20	4	55h, >1/4 wells exceed minimum reading plate +10% maximum plate reading [17]
11	Sheep-Ha chimeric	FLUOstar Optima	15	3	80h, >10,000 RFU in >1/3 wells
12	Ha FL rPrP, in-house	FLUOstar Omega	28	3 or 4	90h, cut-off >40,000 RFU in at least 2/4 wells
13	Ha FL rPrP, UoE	FLUOstar Omega	30 and 15	4	90h, >25,000 RFU in at least 2/4 wells (obligatory: sigmoid growth curve)

Abbreviations: CSF, cerebrospinal fluid; FL, full length; Ha, hamster (*Mesocricetus auratus*); RFU, relative fluorescence units; rPrP, recombinant prion protein; RT-QuIC, real-time quaking-induced conversion assay; Tr, truncated; UoE, University of Edinburgh.

CSF ID	Diagnosis	CSF RT-QuIC positive	Concordance
1	Neuropathologically confirmed sCJD	13/13	100%
2	Not CJD (dementia, ataxia)	1/13	92%
3	Probable sCJD	13/13	100%
4	Probable sCJD	13/13	100%
5	Not CJD (FTD)	0/13	100%
6	Not CJD (delirium)	0/13	100%
7	Probable sCJD	13/13	100%
8	Probable sCJD	13/13	100%
9	Probable sCJD	11/12 ^a	92%
10	Probable sCJD	11/12 ^a	92%

Abbreviations: CJD, Creutzfeldt–Jakob disease; CSF, cerebrospinal fluid; FTD, frontotemporal dementia; RT-QuIC, real-time quaking-induced conversion assay; sCJD, sporadic Creutzfeldt–Jakob disease.

^aOne laboratory did not return results on CSF 9 and CSF 10.

TABLE 3 Summary of the CSF RT-QuIC results obtained

reported methodology [5]. One laboratory used a chimeric rPrP composed of the Syrian hamster residues 14–128 followed by sheep residues 141–234 of the R154 Q171 polymorphic haplotype [15]. The volume of CSF used, the number of replicates analysed and the

criteria for a positive RT-QuIC result for each laboratory are given in Table 2.

Each laboratory returned results to laboratory 13 and included a positive, negative, equivocal interpretation, the number of replicates

that were positive and the relative fluorescent units at the decision point for each replicate.

RESULTS

All 13 laboratories correctly identified seven of the 10 CSF samples, which included five sCJD cases and two non-CJD cases. One laboratory did not return results for two of the CSF samples and insufficient CSF was available for repeating the analysis (Table 3). In this specific case, CSF 9 and 10 showed unclear results (one replicate out of four positive in one run, two or three replicates above the cut-off but with unusual aspects of the curves in a second run) and a third analysis would have been necessary to conclude. CSF samples 2, 9 and 10 were identified correctly by 92% of laboratories. CSF 2 (classified as 'Not CJD') was reported as positive by one laboratory which used a sheep-hamster chimeric PrP as substrate for the RT-QuIC assay. The MRI was reported as normal and the CSF 14-3-3 was negative in this patient. CSF 9 was reported as an inconclusive result by one laboratory as only one of the four replicates was positive. This laboratory used 20 µl to seed the reaction. It is interesting to note that, of the four other laboratories that used both 30 and 15 µl, three reported negative results when using 15 µl and only one obtained positive results when using 30 µl. This CSF sample was from a sCJD-MV patient with disease duration of >2 years. The sensitivity of CSF RT-QuIC has been reported to be lower in longer disease duration cases [15] and in PRNP codon 129 MV cases [9, 18]. Based on the above it is conceivable that the lower CSF volume (either 20 or 15 µl) may be the cause of the inconclusive result in this case. CSF 10 was reported as RT-QuIC negative by one laboratory using 28 µl CSF to seed the reaction. This was an sCJD case (classified as 'probable sCJD') with a disease duration of 4 months with a positive CSF 14-3-3 and MRI showing both basal ganglia changes and cortical ribboning in more than two regions.

Of the 11 laboratories that used Ha FL as substrate, five used both 30 and 15 µl CSF to seed the RT-QuIC analysis. Of these, four used the FLUOstar Omega and one the FLUOstar Optima. Of the four laboratories using the FLUOstar Omega, three only obtained positive RT-QuIC results when using 30 µl. For these laboratories using 15 µl gave negative results or only one positive replicate out of four when investigating CSF 4 and CSF 9. This suggests that using 30 µl may be more sensitive than 15 µl when using the FLUOstar Omega, particularly in long disease duration cases. In contrast, the one laboratory that used the FLUOstar Optima and seeded the RT-QuIC reaction with both 30 and 15 µl CSF obtained positive results with 15 µl for CSF 7 and CSF 8 but only one of the four replicates were positive when 30 µl of these same samples was used (Table 4). This suggests that the optimal volume of CSF to seed the RT-QuIC reaction may depend on the instrumentation used.

Two laboratories used the FLUOstar Omega and Ha Tr rPrP as substrate. One laboratory used 15 µl CSF and the other 20 µl CSF to seed the RT-QuIC reaction. Both of these laboratories identified all 10 CSF samples correctly with all replicates being positive for all sCJD cases, including CSF 9 (Table 5).

TABLE 4 Number of positive replicates for each CSF sample sent to those laboratories that used full-length hamster rPrP as substrate

CSF volume	Instrument	Diagnosis	Lab 1	Lab 2	Lab 3	Lab 3	Lab 3	Lab 4	Lab 4	Lab 4	Lab 5	Lab 5	Lab 5	Lab 6	Lab 7	Lab 7	Lab 8	Lab 9	Lab 12	Lab 13	Lab 13	Lab 13
			30 µl	30 µl	30 µl	30 µl	30 µl	30 µl	30 µl	30 µl	30 µl	30 µl	30 µl	30 µl	30 µl	30 µl	30 µl	20 µl	28 µl	30 µl	30 µl	15 µl
			Omega	Omega	Omega	Omega	Omega	Omega	Omega	Omega	Optima	Optima	Optima	Optima	Optima	Omega	Omega	Omega	Omega	Omega	Omega	Omega
CSF 1	sCJD	4/4	4/4	4/4	4/4	3/4	3/4	4/4	3/4	3/4	4/4	4/4	4/4	3/4	4/4	2/4	3/4	4/4	3/3	4/4	4/4	3/4
CSF 2	Not CJD	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
CSF 3	sCJD	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4
CSF 4	sCJD	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	3/4	4/4	4/4	3/4	3/4
CSF 5	Not CJD	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
CSF 6	Not CJD	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
CSF 7	sCJD	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	3/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	3/4
CSF 8	sCJD	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	3/4	4/4	4/4	4/4	4/4	4/4	4/4	3/4	4/4
CSF 9	sCJD	Nd	4/4	4/4	3/4	1/4	4/4	1/4	2/4	2/4	2/4	2/4	2/4	2/4	3/4	3/4	3/4	1/4 ^a	3/3	2/4	2/4	0/4
CSF 10	sCJD	Nd	4/4	4/4	2/4	3/4	4/4	4/4	3/4	3/4	3/4	3/4	3/4	2/4	3/4	3/4	4/4	3/4	0/4	4/4	4/4	3/4

Note: Figures in bold illustrate a discrepancy between results obtained using two different volumes of CSF.

Abbreviations: CJD, Creutzfeldt-Jakob disease; CSF, cerebrospinal fluid; Nd, not done; rPrP, recombinant prion protein; sCJD, sporadic Creutzfeldt-Jakob disease.

^aReported as inconclusive.

TABLE 5 Number of positive replicates for each CSF sample sent to those laboratories that used an alternative rPrP as substrate

CSF vol/instrument/rPrP	Diagnosis	Laboratory 6	Laboratory 10	Laboratory 11
		15 µl/Omega/truncated Ha rPrP	20 µl/Omega/truncated Ha rPrP	15 µl/Optima/Sh-Ha chimeric rPrP
CSF 1	sCJD	4/4	4/4	3/3
CSF 2	Not CJD	0/4	0/4	2/3
CSF 3	sCJD	4/4	4/4	3/3
CSF 4	sCJD	4/4	4/4	3/3
CSF 5	Not CJD	0/4	0/4	0/3
CSF 6	Not CJD	0/4	0/4	1/3 ^a
CSF 7	sCJD	4/4	4/4	3/3
CSF 8	sCJD	4/4	4/4	3/3
CSF 9	sCJD	4/4	4/4	2/3
CSF 10	sCJD	4/4	4/4	3/3

Abbreviations: CJD, Creutzfeldt–Jakob disease; CSF, cerebrospinal fluid; rPrP, recombinant prion protein; sCJD, sporadic Creutzfeldt–Jakob disease; Sh-Ha, sheep-hamster.

^aReported as negative.

Laboratory 6 undertook RT-QuIC analysis using both FLUOstar Optima and FLUOstar Omega with Ha FL rPrP and Ha Tr rPrP, respectively, as substrates. Using the FLUOstar Optima, Ha FL rPrP and 15 µl CSF this laboratory failed to identify CSF 4 correctly, but did so when using the FLUOstar Omega, Ha Tr rPrP and 20 µl CSF.

DISCUSSION

The harmonization of analytical performance and interpretation of CSF RT-QuIC between centres is vital for CJD surveillance, as a positive CSF RT-QuIC has the ability to classify a patient with a progressive neurological disorder as probable sCJD in the absence of any other clinical features or supportive investigations. The high degree of concordance between all 13 laboratories that participated in this study is encouraging and helps to ensure that sCJD surveillance data between countries can be compared in a meaningful manner. These results also support the findings of previous ring-trials that found CSF RT-QuIC to be a robust and reliable test with a high degree of concordance between laboratories [15, 17, 19].

One laboratory reported a positive CSF RT-QuIC result with CSF 2 from a control patient. This was the only laboratory that used a commercially available chimeric sheep-hamster PrP. The probability that this case was sCJD is extremely low. None of the other laboratories reported a positive CSF RT-QuIC in this patient and the CSF 14-3-3 and MRI were negative. Ideally, ring-trial studies would use CSF samples from patients who had subsequent post-mortem neuropathological examination; however, with the falling autopsy rate this is becoming increasingly difficult.

The results of this ring-trial also highlight the importance of optimization of the CSF RT-QuIC assay. For those laboratories using FLUOstar Omega, Ha FL rPrP and 30 µl CSF to seed the reaction gave more accurate results than using 15 µl. Interestingly those laboratories using FLUOstar Omega, Ha FL rPrP and <30 µl CSF to seed the

reaction resulted in not identifying all sCJD cases. The converse was true for the laboratory using FLUOstar Optima and Ha FL rPrP where seeding the reaction with 15 µl CSF rather than 30 µl resulted in better sensitivity. Two laboratories used Ha Tr rPrP as substrate and correctly identified all CSF samples. Interestingly all replicates were positive in the sCJD cases, supporting a previous study that showed higher sensitivity using Ha Tr rPrP compared to Ha FL rPrP [9–11].

With increasing numbers of laboratories introducing CSF RT-QuIC and producing rPrP substrates, standardized protocols for production and evaluation of rPrP substrates are required. The success of RT-QuIC depends on the performance of the rPrP used as a substrate. It must be stable enough not to spontaneously aggregate whilst being continuously shaken, but also able to readily aggregate in the presence of abnormal PrP found in brain or CSF. To date the most suitable rPrP substrates for RT-QuIC have been hamster PrP, full length, truncated or in a chimera. Human rPrP has been used [1]; however, it is more prone to spontaneous aggregation than hamster PrP.

There were nine different forms of hamster rPrP used in this ring-trial and despite this variation the results of the CSF RT-QuIC were comparable across all laboratories. The strength of this study is the large number of sites involved; however, the lack of post-mortem confirmation of both sCJD and non-CJD diseases is a major limitation.

CONCLUSIONS

Cerebrospinal fluid RT-QuIC has been incorporated in the diagnostic criteria since January 2017 but has not been uniformly adopted by all surveillance centres. Many studies have shown that CSF RT-QuIC is a very accurate test for sCJD with a high degree of sensitivity and specificity. This study shows that CSF RT-QuIC demonstrates an excellent concordance between centres, even when using a variety of

instrumentation, different rPrP substrates and a range of CSF volumes to seed the reaction. The adoption of CSF RT-QuIC by all CJD surveillance centres is recommended.

AUTHOR CONTRIBUTIONS

Alison J Green: Conceptualization (lead); writing – original draft (lead). **Neil McKenzie:** Conceptualization (equal); writing – original draft (equal). **Gabriele Ellen Piconi:** Conceptualization (equal); writing – original draft (equal). **Audrey Culeux:** Validation (equal); writing – review and editing (equal). **Anna-Lena Hammarin:** Validation (equal); writing – review and editing (equal). **Christos Stergiou:** Validation (equal); writing – review and editing (equal). **Socrates Tzartos:** Validation (equal); writing – review and editing (equal). **Alexandra Versleijen:** Validation (equal); writing – review and editing (equal). **Jacqueline Van de Geer:** Validation (equal); writing – review and editing (equal). **Patrick Cras:** Validation (equal); writing – review and editing (equal). **Franco Cardone:** Validation (equal); writing – review and editing (equal). **Anna Ladogana:** Validation (equal); writing – review and editing (equal). **Angela Mannana:** Validation (equal); writing – review and editing (equal). **Marcello Rossi:** Validation (equal); writing – review and editing (equal). **Matilde Bongiani:** Validation (equal); writing – review and editing (equal). **Daniela Perra:** Validation (equal); writing – review and editing (equal). **Guenther Regelsberger:** Validation (equal); writing – review and editing (equal). **Sigrid Klotz:** Validation (equal); writing – review and editing (equal). **Simone Hornemann:** Validation (equal); writing – review and editing (equal). **Adriano Aguzzi:** Writing – review and editing (equal). **Schmitz Matthias:** Validation (equal); writing – review and editing (equal). **Mary Andrews:** Validation (equal); writing – review and editing (equal). **Kimberley Burns:** Validation (equal); writing – review and editing (equal). **Stephane Haik:** Writing – review and editing (equal). **Raquel Ruiz-Garcia:** Validation (equal); writing – review and editing (equal). **Jenny Verner-Carlsson:** Validation (equal); writing – review and editing (equal). **John Tzartos:** Validation (equal); writing – review and editing (equal). **Marcel Verbeek:** Validation (equal); writing – review and editing (equal). **Bart De Vil:** Validation (equal); writing – review and editing (equal). **Anna Poleggi:** Validation (equal); writing – review and editing (equal). **Piero Parchi:** Validation (equal); writing – review and editing (equal). **Gianluigi Zanusso:** Validation (equal); writing – review and editing (equal). **Ellen Gelpi:** Validation (equal); writing – review and editing (equal). **Karl Frontzek:** Validation (equal); writing – review and editing (equal). **Regina Reimann:** Validation (equal); writing – review and editing (equal). **Peter Hermann:** Validation (equal); writing – review and editing (equal). **Inga Zerr:** Writing – review and editing (equal). **Suvankar Pal:** Writing – review and editing (equal).

CONFLICT OF INTEREST

None.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

John Tzartos  <https://orcid.org/0000-0001-7948-034X>
 Bart De Vil  <https://orcid.org/0000-0003-1924-2029>
 Piero Parchi  <https://orcid.org/0000-0002-9444-9524>
 Peter Hermann  <https://orcid.org/0000-0003-1889-8325>
 Alison Green  <https://orcid.org/0000-0003-1858-6751>

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