Improving the sensitivity of CSF RT-QuIC in humans by examining cases with indeterminate results

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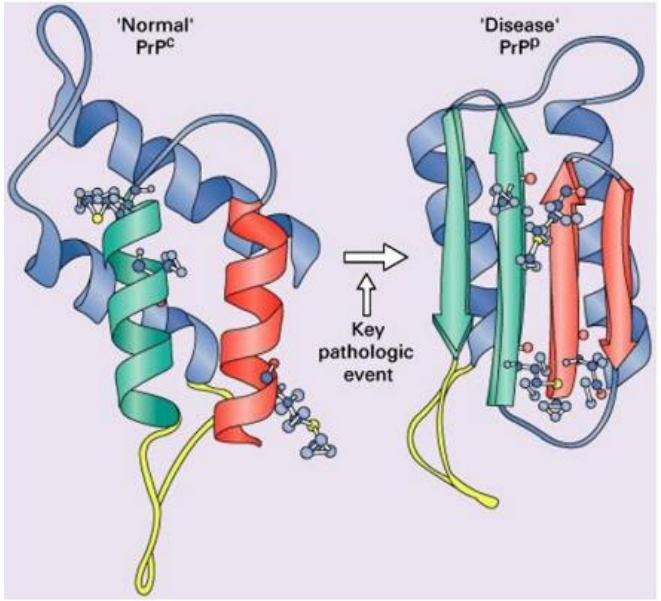
What is Creutzfeldt-Jakob disease (CJD)?

CJD is a fatal neurodegenerative disease caused by the presence of an abnormal (misfolded) protein in the brain tissue, which can induce further misfolding of normal proteins and subsequent formation of aggregates.



Neuropathological examination is the only way to definitely diagnose and determine the cause of prion disease.

PHOTO CREDITS: (A) ELLISON D, LOVE S, CHIMELLI L, ET AL. NEUROPATHOLOGY. 2ND EDITION. EDINBURGH (UK): MOSBY; 2004.
(B) MARTIN BARRAUD
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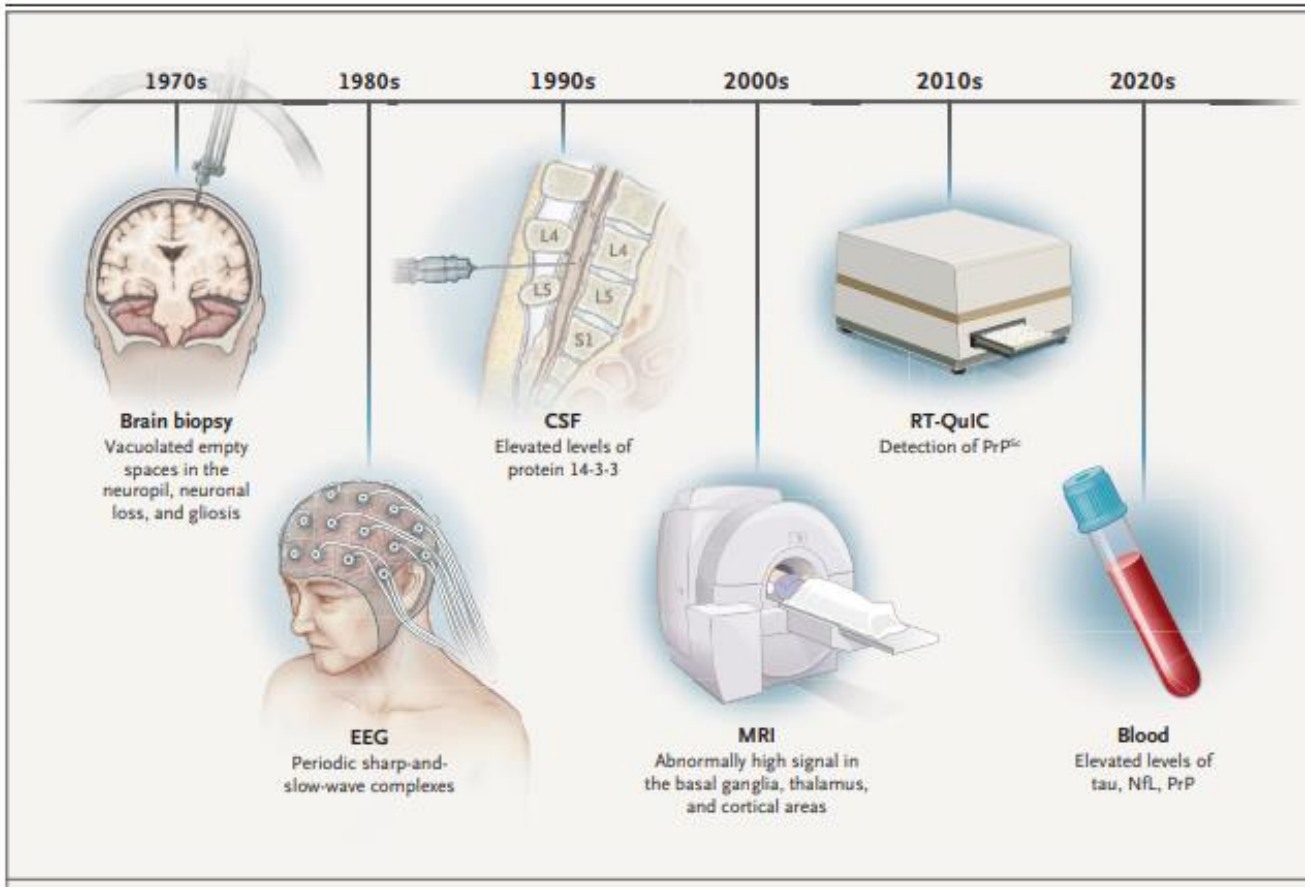
Progress in the Development of Clinical Diagnostic Techniques for CJD



ZERR, I. (2022) N ENGL J MED 386 (14),1345-1350.

Diagnostic Performance of Current Clinical Laboratory Tests for CJD

Clinical laboratory testing for the detection of prion disease is currently limited to the use of CSF and brain biopsy specimens in symptomatic individuals.

Test	Ability to detect CJD (sensitivity, %)	Ability to distinguish CJD from other diseases (specificity, %)
Non-specific CSF biomarkers		
14-3-3	61-95	40-92%
tau	87-90	67-75
Specific biomarkers		
CSF PrP^{Sc} (RT-QuIC)	92-97.2	98.5-100
Brain biopsy PrP^{Sc} Western Blotting	20-60	

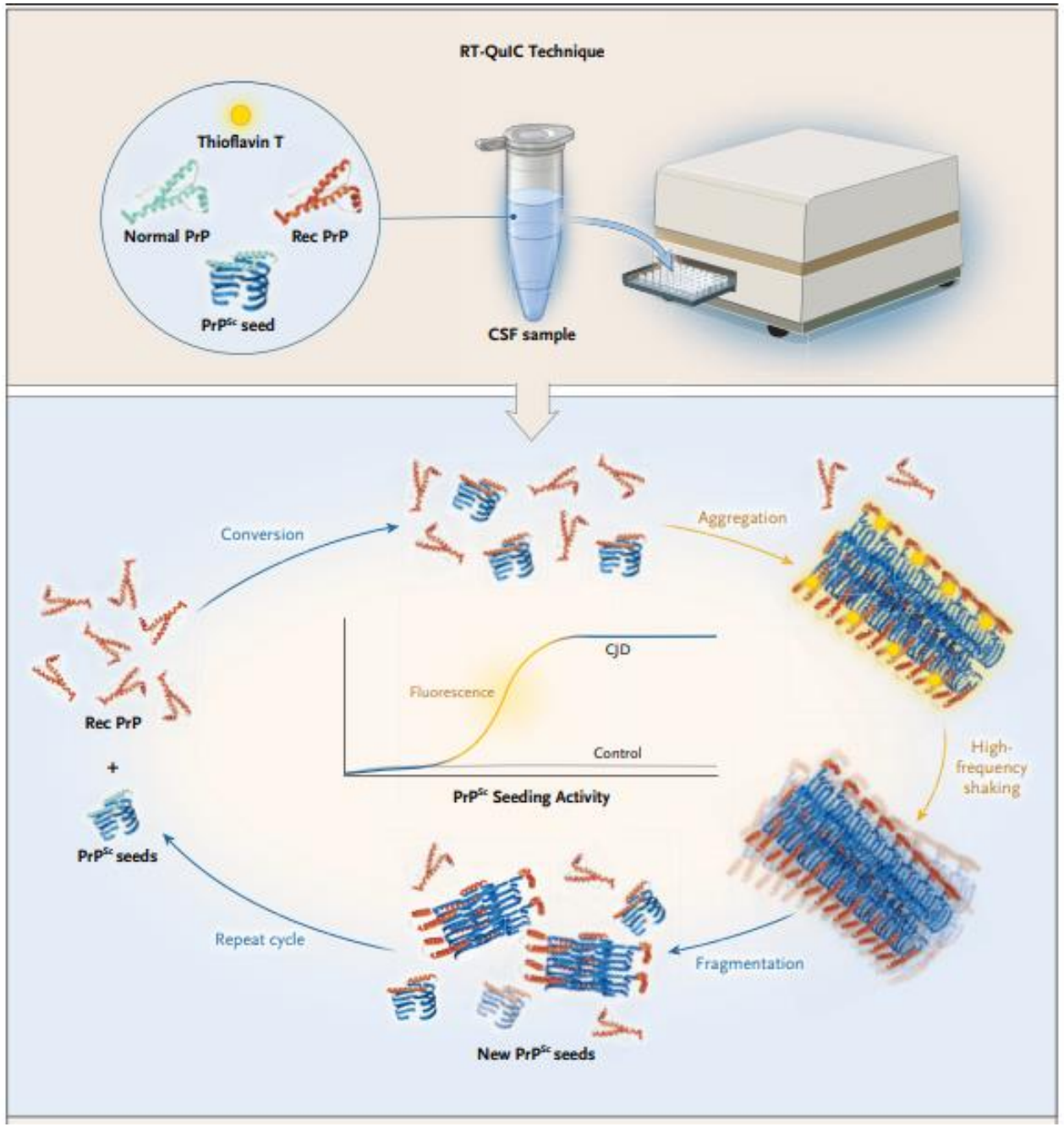
Development of CSF RT-QuIC represents a major diagnostic advance in the field!

TABLE ADAPTED FROM RHOADS, ET AL. J. CLIN. MICROBIOL. (2019) 57(10), E00769-19.

CSF RT-QuIC Technique

RT- QuIC = **R**eal-**T**ime **Qu**aking-**I**nduced **C**onversion

- First clinically available assay that specifically detects prions without requiring analysis of brain tissue
- Available clinically in the US in 2015
- Added to the CDC's diagnostic criteria for CJD in 2018
- 2nd generation currently used for clinical testing
  - Optimized by modifying reagents and incubation conditions
  - ↑ sensitivity and shorter detection time



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Improving the sensitivity of CSF RT-QuIC in humans by examining cases with indeterminate results

- **National Prion Disease Pathology Surveillance Center** has offered CSF RT-QuIC testing since 2015
  - Tested ~7,000 CSF specimens in 2022

- Data indicates:
  - ~10% of prion diseases have a false negative CSF RT-QuIC test
  - ~ 1% of CSF samples examined yield an "indeterminate" CSF RT-QuIC result

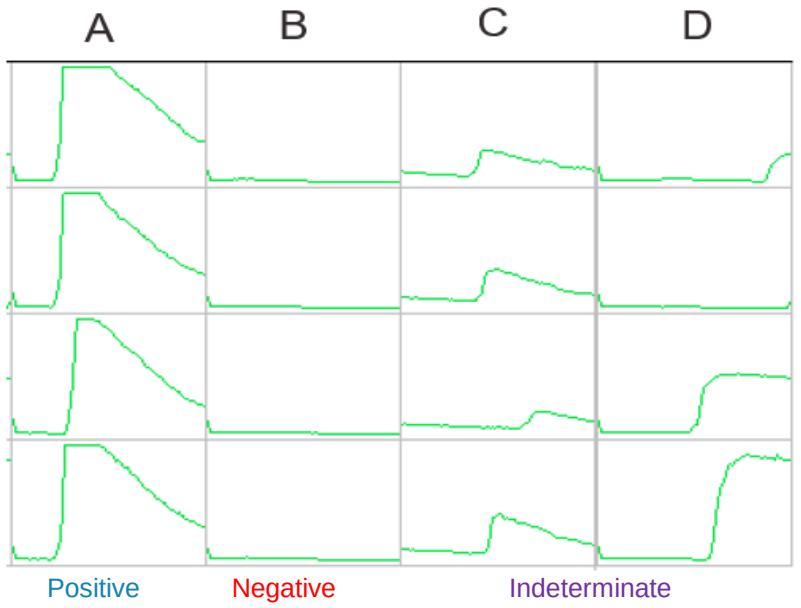
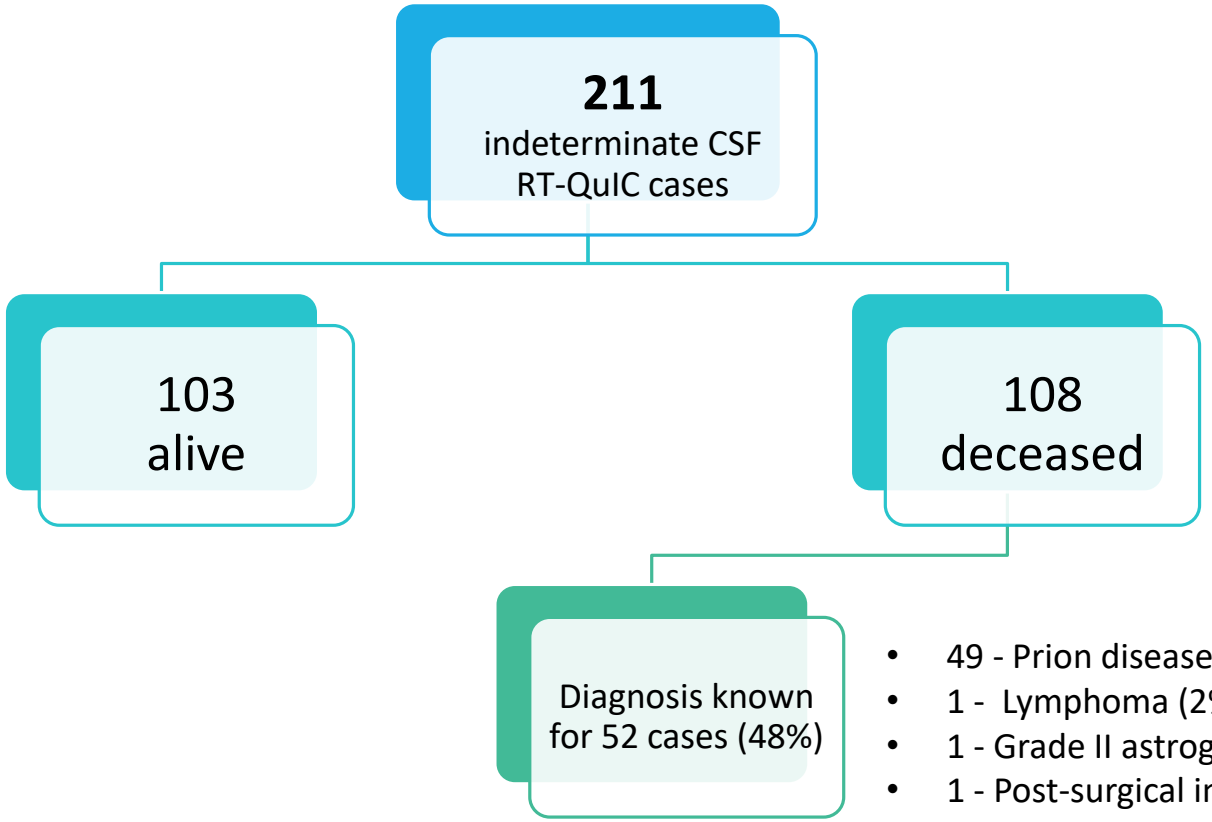


Figure 1. Examples of CSF RT-QuIC curves of quadruplicate well analysis for (A) typical positive result, (B) typical negative result, (C) indeterminate result with abnormal seeding below the threshold established for positive, (D) indeterminate result with an uncharacteristic pattern.

Aim to enhance performance of CSF RT-QuIC to further improve the laboratory diagnosis of human prion disease, especially in genetic and rare sporadic prion disease subtypes that most often result in false negative CSF RT-QuIC results!

Characterization of patient cases with an “indeterminate” CSF RT-QuIC result (2016-2021)



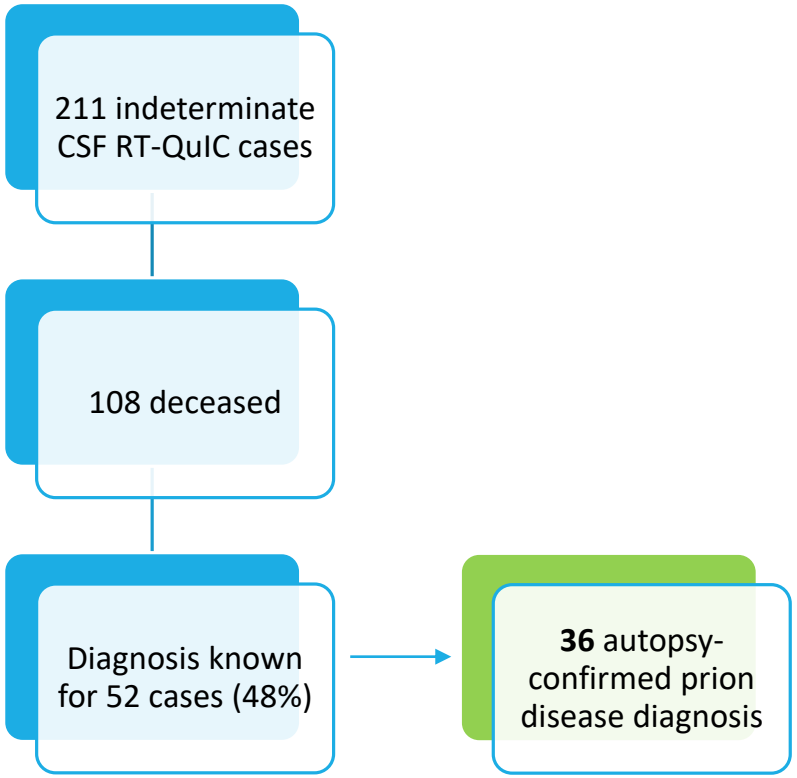
- 49 - Prion disease (94%)
- 1 - Lymphoma (2%)
- 1 - Grade II astroglioma (2%)
- 1 - Post-surgical infection (2%)

Distribution by sex	
Male	92 (44%)
Female	119 (56%)

Distribution by race	
Unknown	122 (57.8%)
White	75 (35.5%)
Hispanic	8 (3.8%)
Asian	3 (1.4%)
Black	2 (0.9%)
Indian	1 (0.5%)

Distribution by age (years)			
	Mean	Median	Range
Alive	65	66	14-93
Deceased	66	67	22-88

Characterization of patient cases with an "indeterminate" CSF RT-QuIC result



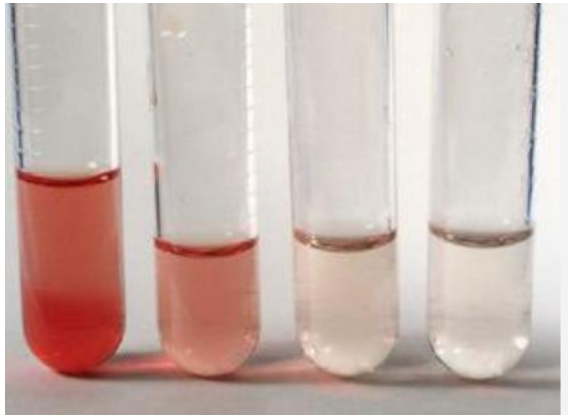
Prion Subtype	# indeterm CSF RT-QuIC cases (n=36)
MM1-2	9 (25.0 %)
MM1	7 (19.4 %)
MV1-2	5 (13.9 %)
MV2	4 (11.1 %)
VV1	2 (5.6 %)
VV2	2 (5.6 %)
MV1	2 (5.6 %)
VPSPr	2 (5.6 %)
MM2	1 (2.8%)
VV1-2	1 (2.8%)
T188R-129V	1 (2.8%)

CSF RT-QuIC Curve Analysis for Cases with Indeterminate Results

Most samples were of good specimen quality (not bloody).

CSF specimen quality	# of samples
Clear	186 (88%)
Slightly bloody	24 (11.5%)
Thawed	1 (0.5%)

A single CSF RT-QuIC test ordered for most cases. A second test order was most commonly placed after 1 month.



# RT-QuIC tests ordered	# of cases
1	183 (87%)
2	26 (12%)
3	2 (1%)

	# of days between test orders
Average	50
Median	35
Range	5-217

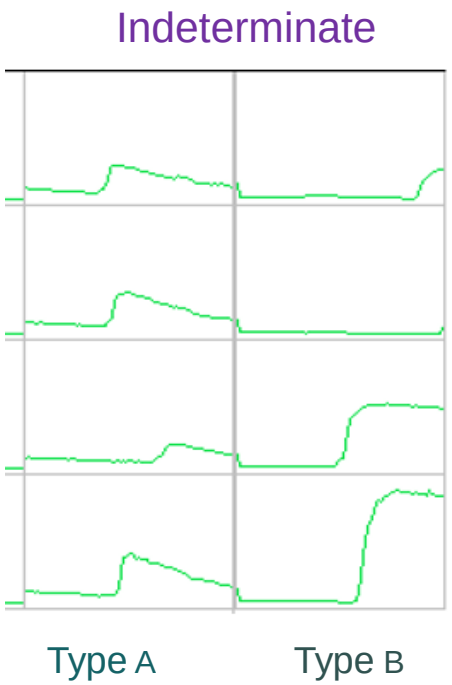
CSF RT-QuIC Curve Analysis for Cases with Indeterminate Results

For >2/3 of cases, patients with an initial indeterminate CSF RT-QuIC result eventually had a positive result when re-tested.

Initial vs Final CSF RT-QuIC test result	# of cases (n=28)
Indeterm → Positive	12 (43%)
Indeterm → Negative	8 (29%)
Indeterm → Indeterm	1 (3.5%)
Negative → Indeterm	5 (17.5%)
Positive → Indeterm	2 (7%)

CSF RT-QuIC Curve Analysis for Cases with Indeterminate Results

Characteristics of Type A Indeterminate Curves	
Number of cases	100 (49%)
# specimens slightly bloody	9
# of wells with seeding activity	0/4 17 1/4 19 2/4 27 3/4 13 4/4 24
Average fluorescence intensity	31211
Average lag time	22

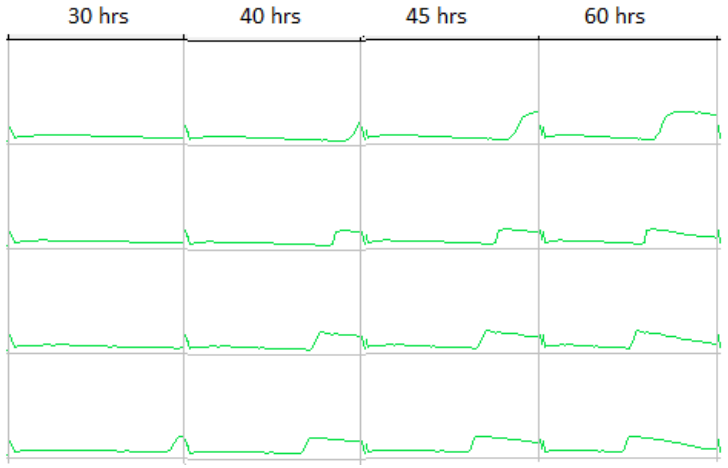


Characteristics of Type B Indeterminate Curves	
Number of cases	105 (51%)
# specimens slightly bloody	15
# of wells with seeding activity	0/4 22 1/4 29 2/4 37 3/4 9 4/4 8
Average fluorescence intensity	32667
Average lag time	31

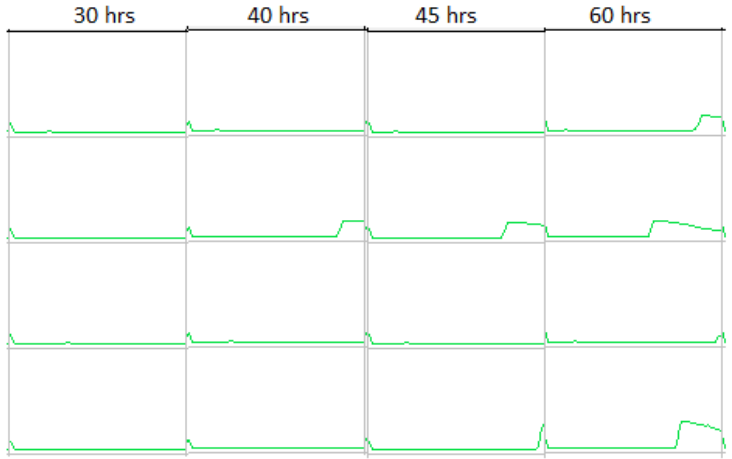
CSF RT-QuIC Curve Analysis for Cases with Indeterminate Results

	RT-QuIC Result					Hits/Well				Max as %PBH			
Case #	**60 HR**	**Case Dx subtype**	**30 HR**	**40 HR**	**45 HR**	**60 HR**	**30 HR**	**40 HR**	**45 HR**	**60 HR**	**30 HR**	**40 HR**	**45 HR**
Case A	Indeterminate	Sporadic MM1	Negative	Indeterminate	Indeterminate	4/4	1/4	4/4	4/4	15	4	13	15
Case B	Indeterminate	Negative	Negative	Negative	Indeterminate	3/4	0/4	1/4	2/4	11	1	1	8
Case C	Indeterminate	Negative	Negative	Negative	Negative	2/4	0/4	0/4	0/4	8	2	2	2

Case A



Case B



Case C

Preliminary analysis of data for shortening CSF RT-QuIC test run time suggests that it may successfully eliminate a subset of indeterminate results!

Investigating variable detection of prion disease subtypes in CSF RT-QuIC

1.) Reviewed original CSF RT-QuIC validation data for autopsy-confirmed prion disease cases to better understand the variability in detection rates for the different subtypes

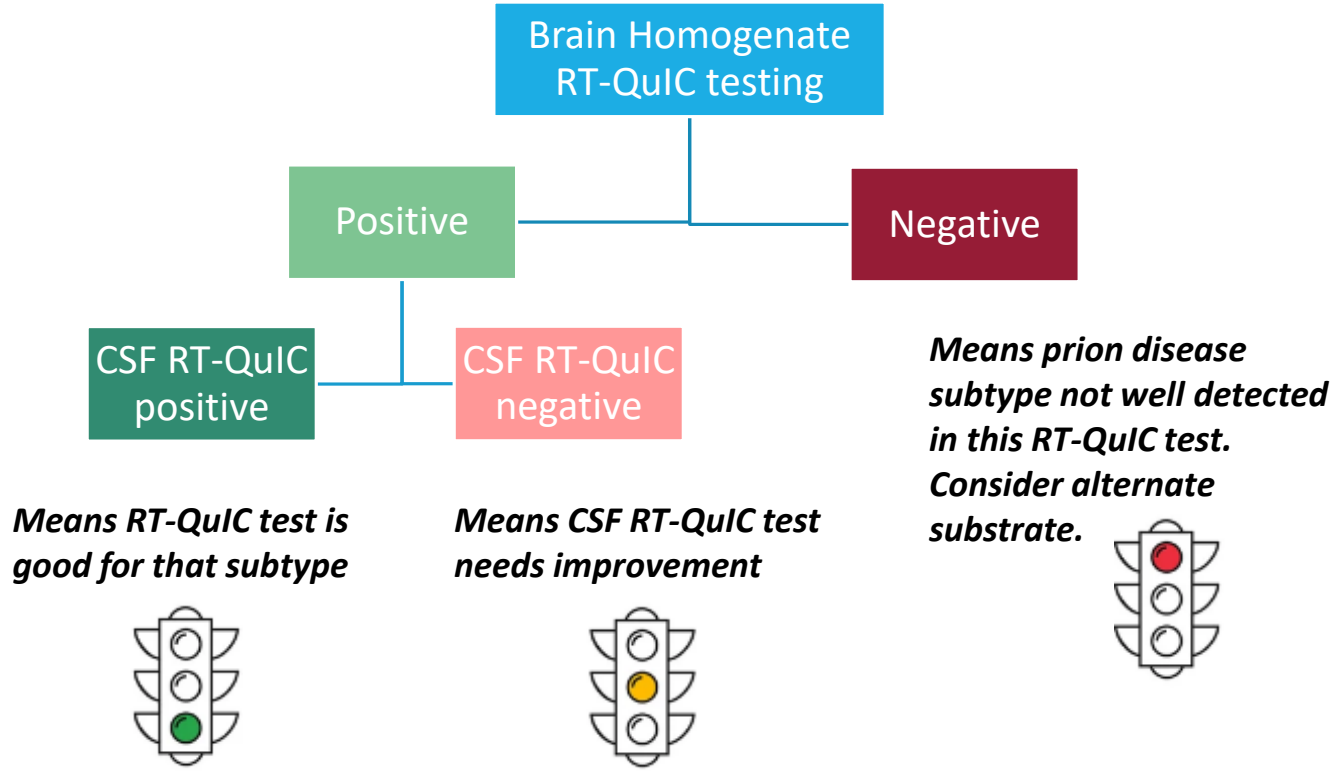
Summary of CSF RT-QuIC hits from test validation data

Prion Subtype	# samples tested	# that would be called positive by RT-QuIC criteria (%)
VV2	14	14 (100%)
MM1	41	38 (93%)
MV1	10	9 (90%)
MV2	9	6 (67%)
VV1	8	3 (38%)
MM2	9	3 (33%)

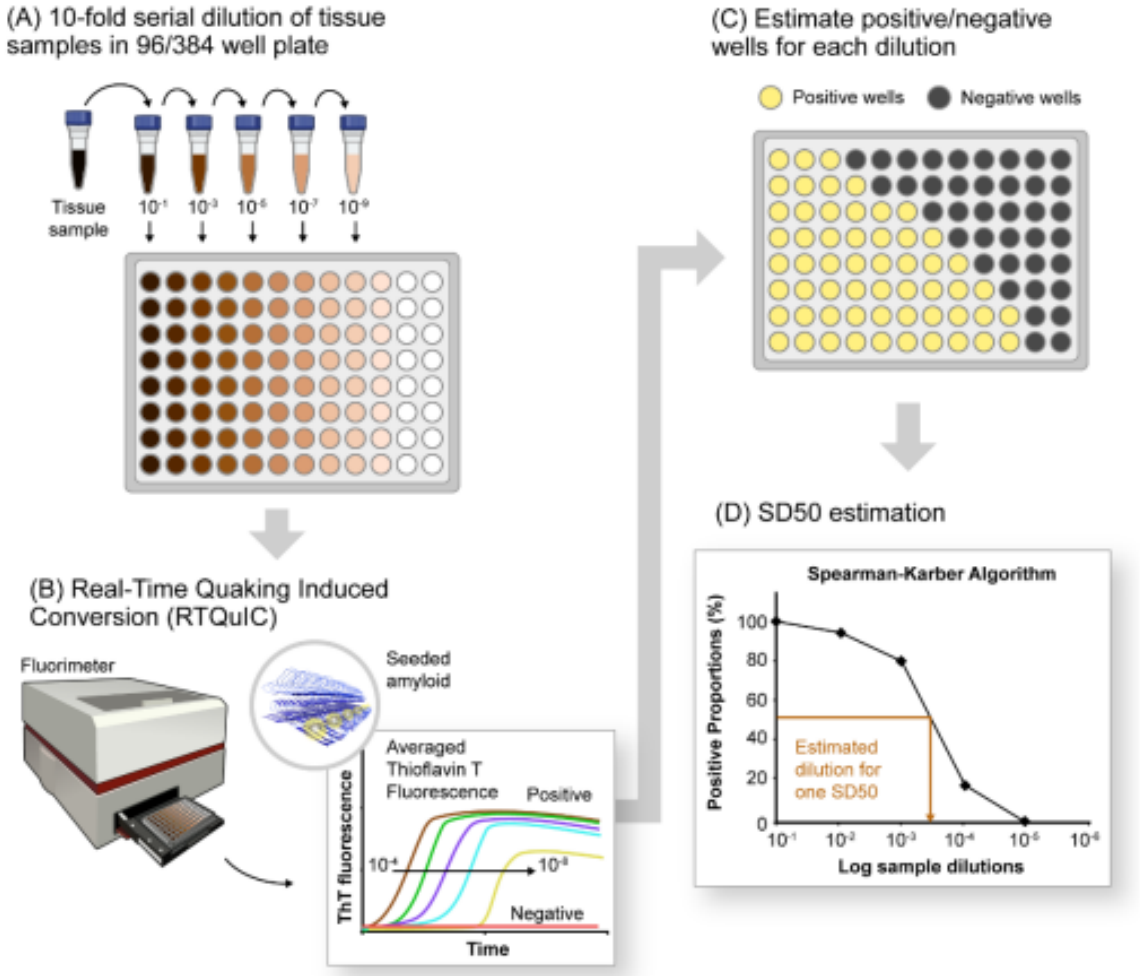
Investigating variable detection of prion disease subtypes in CSF RT-QuIC

2.) Performed additional RT-QuIC testing on brain tissue homogenate of autopsy-confirmed prion disease cases that did not generate clearly positive CSF RT-QuIC results

Interpretation of paired BH and CSF RT-QuIC results



BH RT-QuIC testing and endpoint dilution analysis



The 50% seeding dose (SD_{50}) is defined as the amount of sample giving positive reactions in 50% of replicate reaction wells.

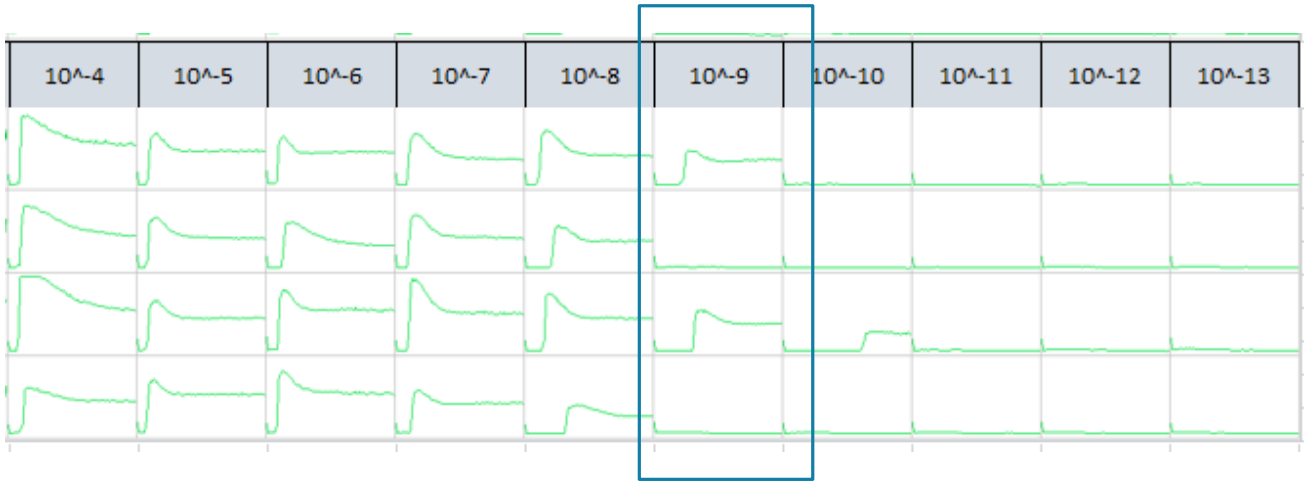


IMAGE FROM: CAUGHEY B., ET AL (2022), BIOMOLECULES, 12, 576.

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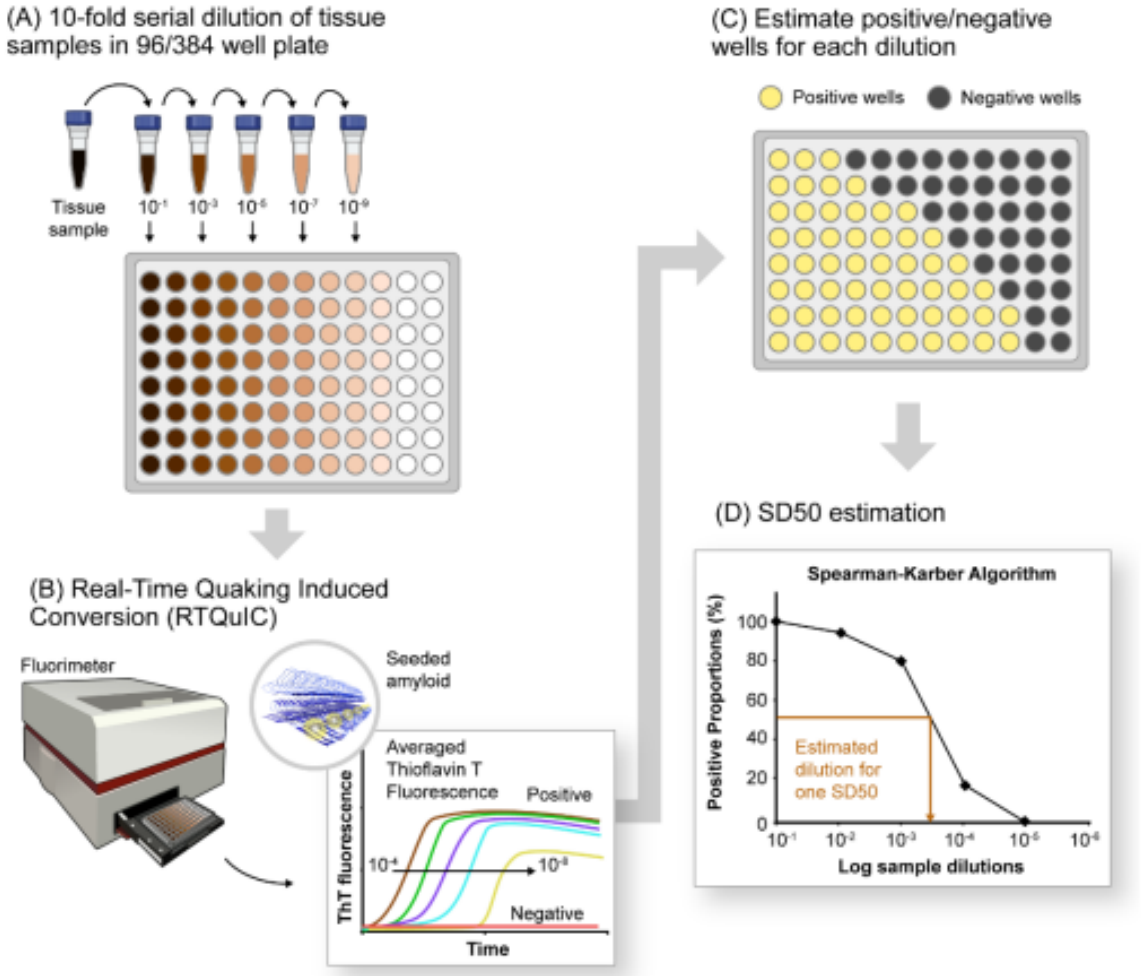


Prion Subtype	Calculated SD50
MM1-2	-10.25
MM1	-10.00
MV1	-9.50
MV2	-9.50
MV1-2	-8.75
VV2	-8.75
VV1-2	-8.63
VV1	-7.50
MM2	-7.38
VPSPr	-7.13

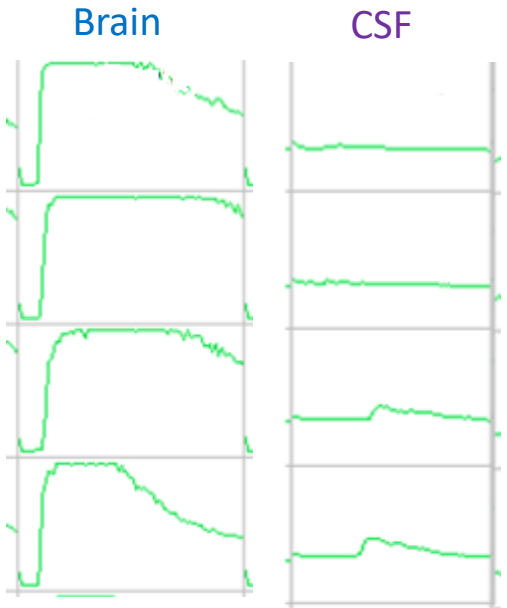
BH RT-QuIC data suggests that all prion subtypes are detected in our method but the minimum concentration needed to generate positive seeding activity varies.

IMAGE FROM: CAUGHEY B., ET AL (2022), BIOMOLECULES, 12, 576.

BH RT-QuIC testing on autopsy-confirmed prion disease cases with indeterminate CSF RT-QuIC results

Seeding activity was detected in the brain homogenate RT-QuIC testing for all autopsy-confirmed prion disease patients with an indeterminate CSF RT-QuIC result!

This suggests that the differential performance of the various sCJD subtypes in the current clinical CSF RT-QuIC assay may be due to either the circulating concentration of PrP^{Sc} or an interfering substance in the CSF vs prion strains.



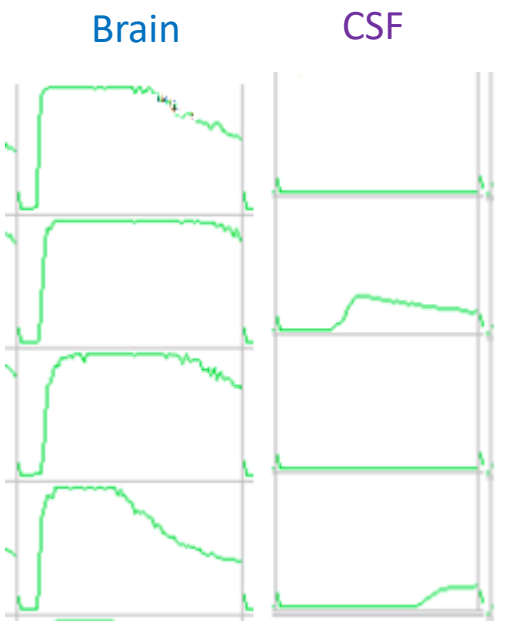


IMAGE ADAPTED FROM: ORRU, C.D. ET AL (2012), PRION, 6:2, 147-152.

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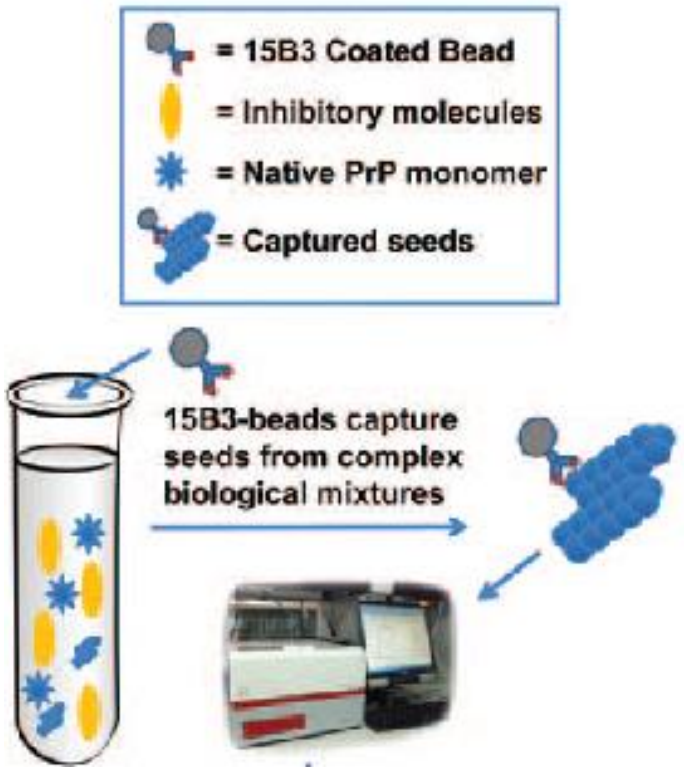

Future studies include exploring an immunoprecipitation step prior to CSF RT-QuIC analysis to help determine cause of indeterminate results (low concentration of PrP^{Sc} vs interference)

IMAGE ADAPTED FROM: ORRU, C.D. ET AL (2012), PRION, 6:2, 147-152.

Summary and Conclusions

CSF RT-QuIC is a very good clinical test for detecting prion disease but there is still room for improvement, especially in prion disease subtypes that often yield false negative results.

From 2016-2021, the National Prion Disease Pathology Surveillance Center reported an “indeterminate” CSF RT-QuIC result for 211 patient cases.

The demographic data, clinical characteristics, and lab test results for these 211 cases were examined to identify trends, patterns, and relationships. Paired with additional lab testing, the data suggests that there are different causes for indeterminate CSF RT-QuIC results.

Our findings have provided important information to guide future experiments and possible testing protocol adjustments that can improve the clinical diagnosis of human prion disease.

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