Development and validation of alpha-synuclein aggregation assay using surrogate matrix

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6th EBF YSS_ 24-25 SEP 2020

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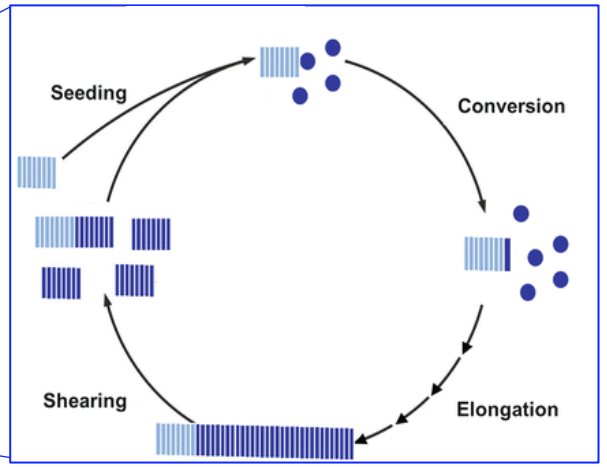
Alpha-synuclein aggregation assay

3 ½ days (90 hrs)

Preloaded silica beads

Shaker and Fluorescent BMG plate reader



Caughey B. et al. (2017)

Human recombinant alpha-synuclein monomers

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Seed (Artificial fibrils or patient CSF or brain homogenate)

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Non-fluorescent ThT

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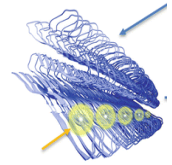
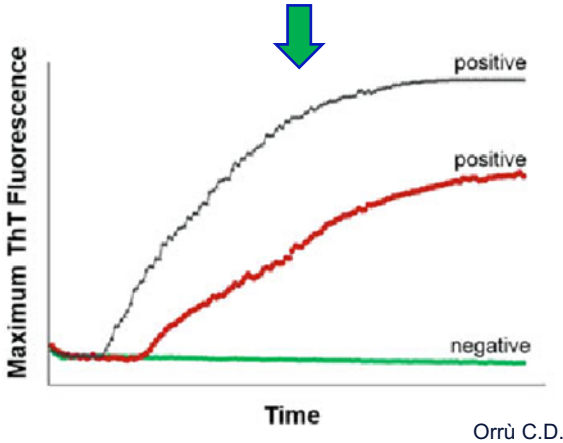
CSF or surrogate matrix

Master mix

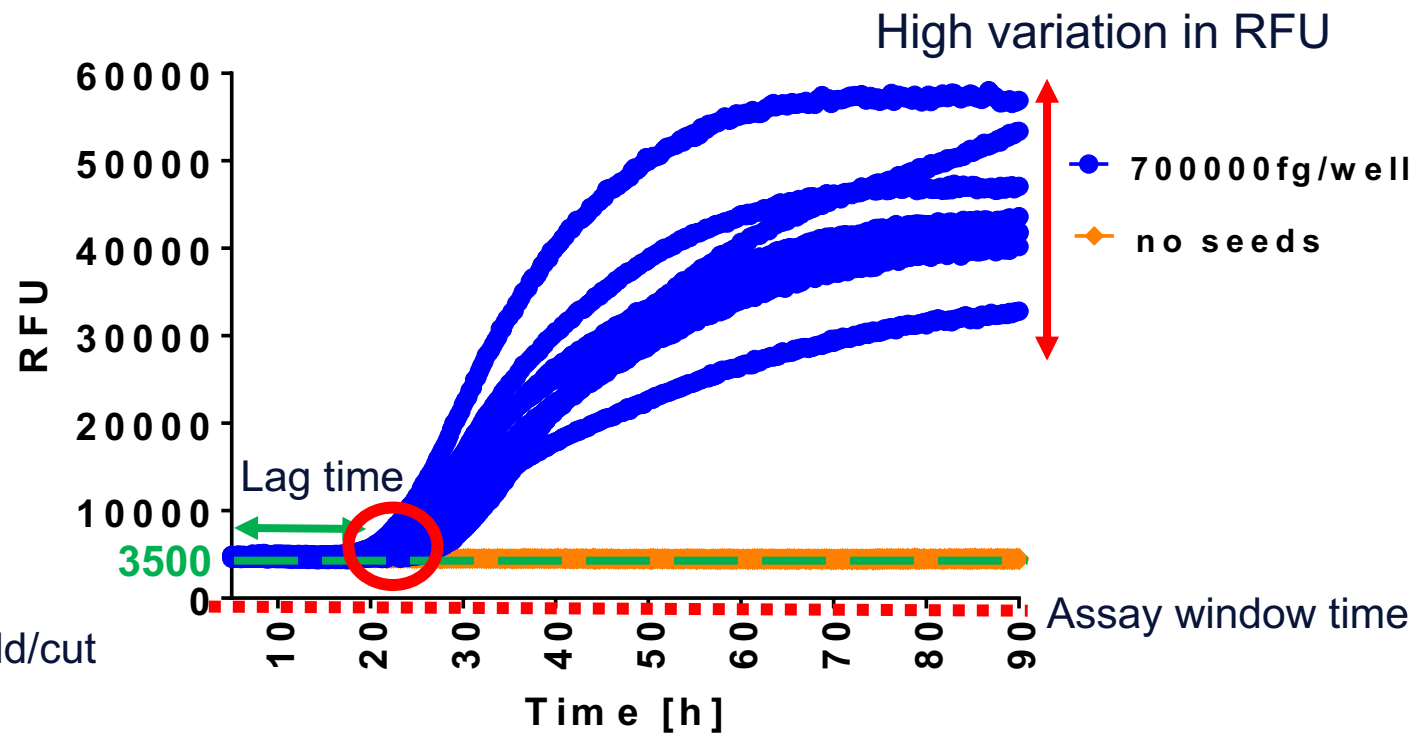


Thioflavin T fluorescence



Orrù C.D. et al. (2017)

Assay parameters



Time to Threshold/cut off signal

- Background signal- Subtract first 4 cycles RFU
- Lag phase/Time to Threshold: Time required to surpass >3500 RFU
- Assay window: 0-90 hrs.
- Assay window is smaller than ligand-binding assays
- Reproducible and consistant time to threshold than RFUs

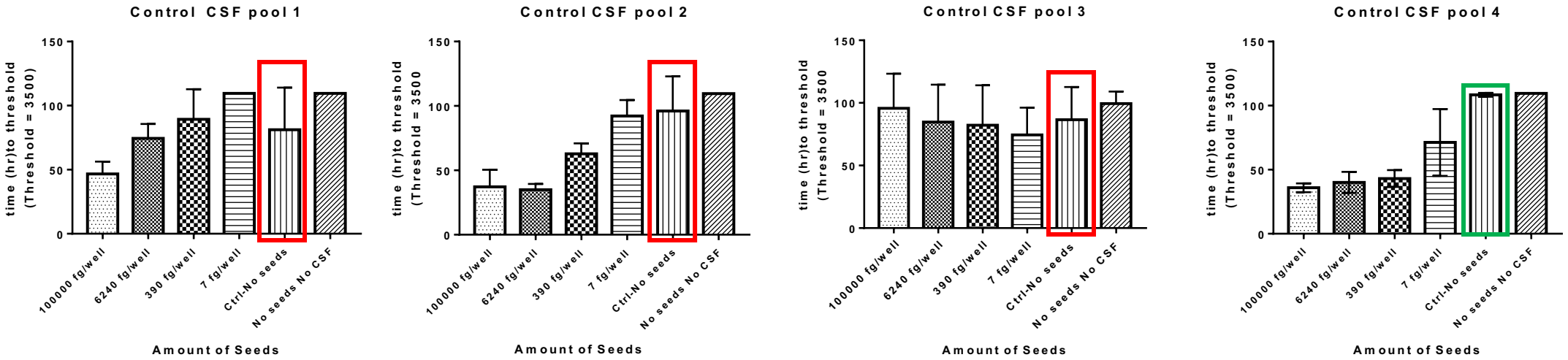
Assay challenges

- Background
- Alpha-synuclein aggregation assay: Reflect alpha-synuclein pathology, promising biomarker for the diagnosis of Parkinson's disease (PD)
- Several labs developed the alpha synuclein aggregation assay (PMCA or RT-QuIC assay)
- Challenges: Reproducibility, standardization, and validation

Assay parameters potentially affecting the reproducibility

- Buffer composition and pH
- Quality of alpha synuclein substrate **Pre-screening of large lots**
- Temperature
- Bead size and material
- Control cerebrospinal fluid (CSF)

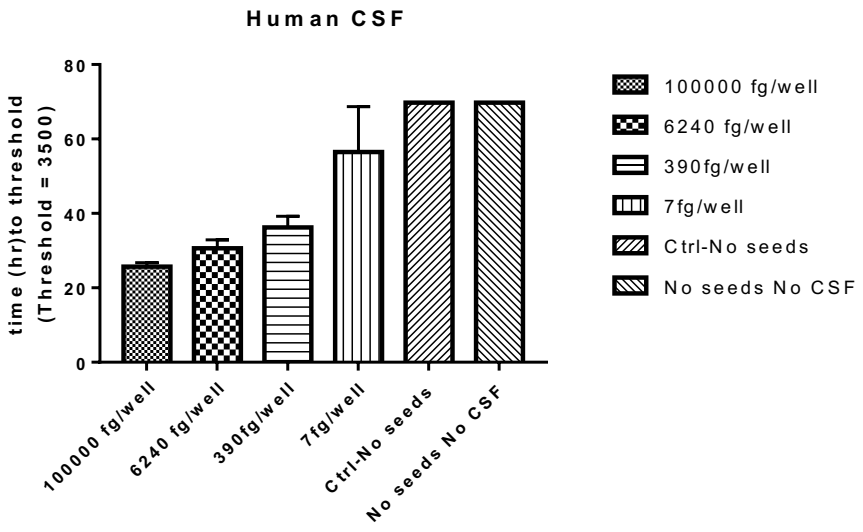
Screening of control CSF pools as spike-in matrix

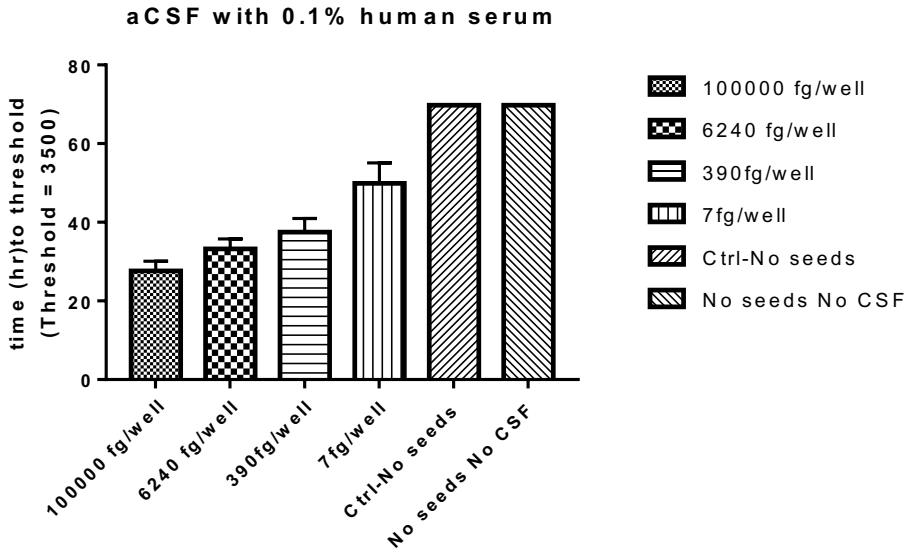


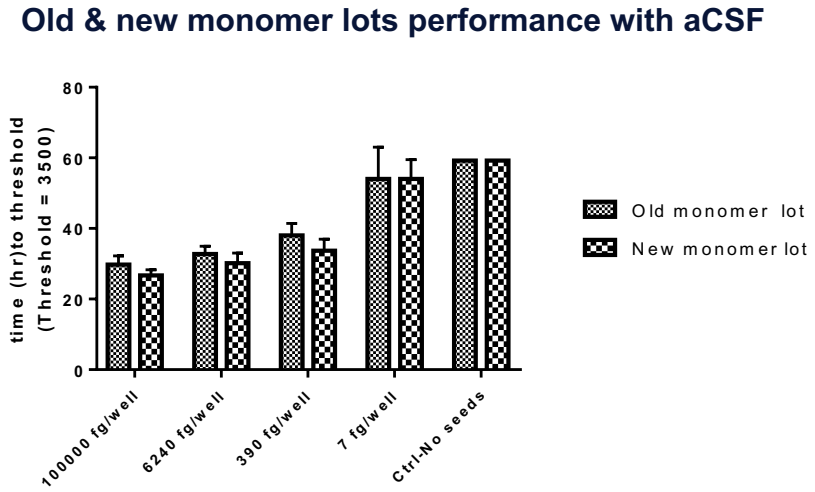
Note:Preliminary assay: assay time >90 hrs.

- Not all Control CSF pools are clean and are free of seeds/aggregation prone
- Very expensive human control CSF (>50,000€/50ml)
- Surrogate matrix: Free of the target analyte (seeds) and mimics the biological sample matrix
- As per EMA bioanalytical method validation guidelines of CSF is a rare and exceptional matrix
- Need of a clean, reliable and cost-effective surrogate matrix i.e artificial CSF to have a reproducible and cosistant assay

Assay performance: artificial CSF vs Human CSF



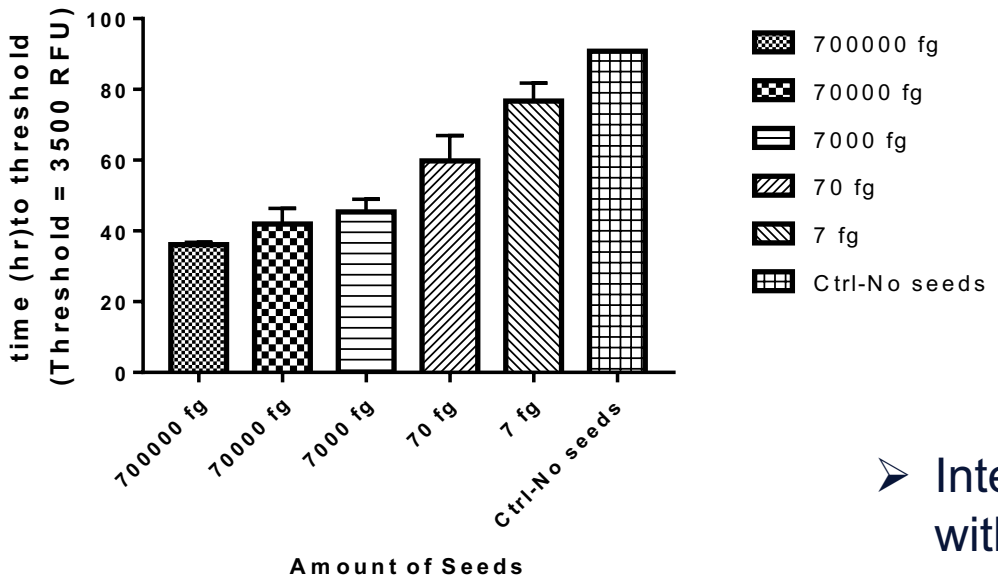




artificial CSF (aCSF): substitutes for CSF with similar ionic composition and no protein content

- Comparable time to threshold with 0.1% Human serum in artificial CSF to ctrl human CSF pool
- Reproducible assay allows lot to lot comparision of monomer

Inter and intra assay precision

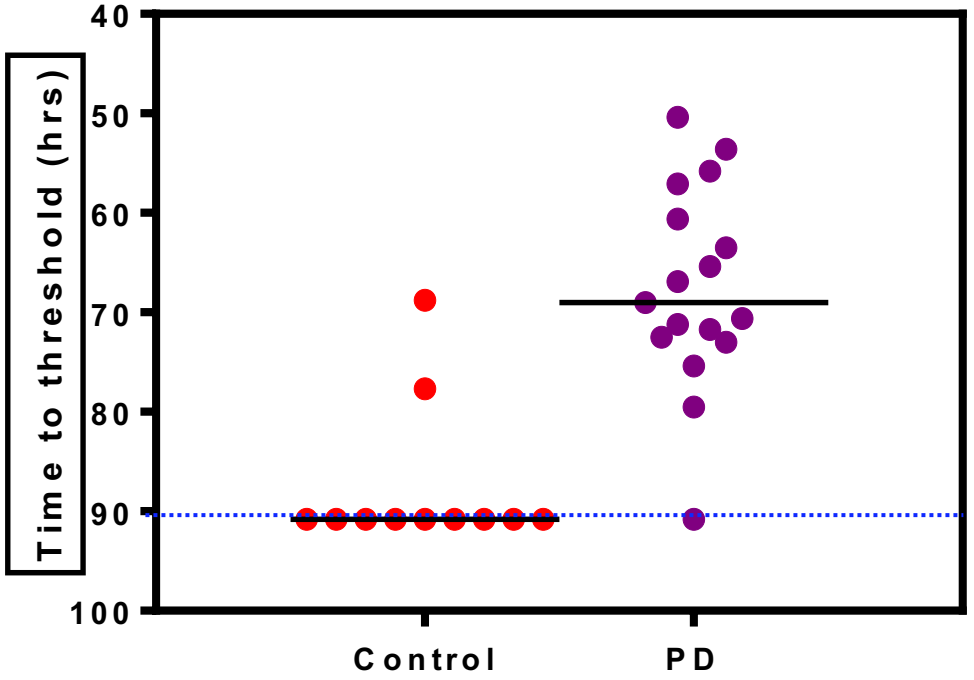


N=6; Data Mean ± SD

- Inter and intra precision of the assay with inter and intra assay CV<21%

Fibril-seed Conc (fg/well)	Avg. Time to threshold (6 runs)	Inter and Intra assay %CV (6 runs)
700000	36	20
70000	42	21
7000	45	19
70	60	20
7	77	17
0	91	0

Alpha synuclein aggregation in PD vs.Ctrl



	Control	PD
Std. Deviation	7.373	10.15
Std. Error of Mean	2.223	2.461

	Control	PD
Number of points		
# Y values analyzed	11	17
Outliers	0	0

- All but one PD samples showed aggregation >90hrs
- Out of 10 control 8 samples are negative for aggregation and 2 of them showed aggregation at >70 hrs.
- Observed fast aggregation in PD vs. Control CSF samples

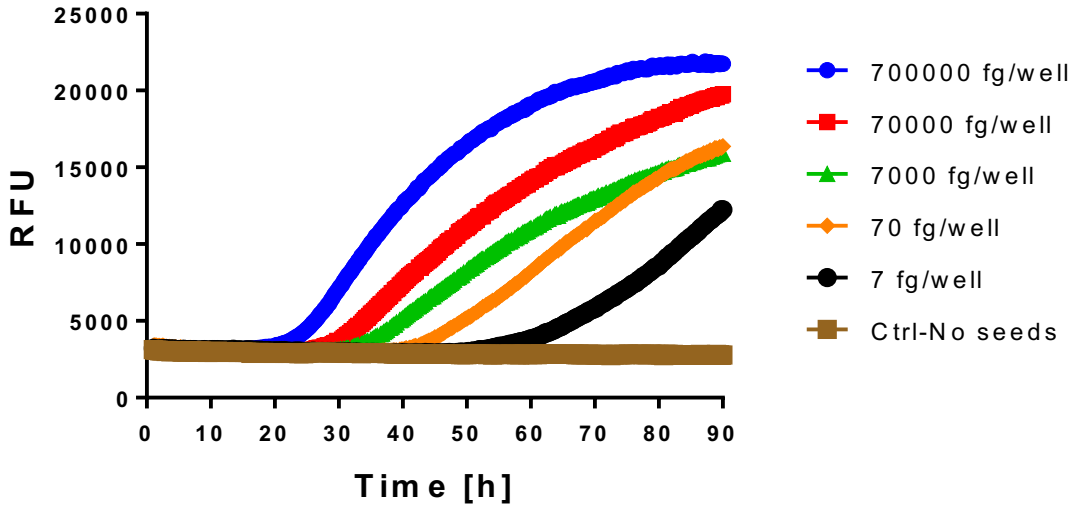
Conclusions and outlook

- Artificial CSF (aCSF) with 0.1% human serum can be used as a surrogante matrix to have reproducible alpha-synuclein aggregation assay
- Assay could discriminate agrregation in Ctrl vs. PD CSF within 90 hrs window
- Testing of larger cohort of samples ongoing to draw conclusions on specificity (descriminating PD vs. Ctrl) of the assay
- Further testing of AD and other synucleinopathy samples is planned to test the ability of the assay to discriminate PD from other synucleinopathies and tauopathies

Thank you

BACKUP

Inter and intra assay precision



N=6; Data Mean ± SD

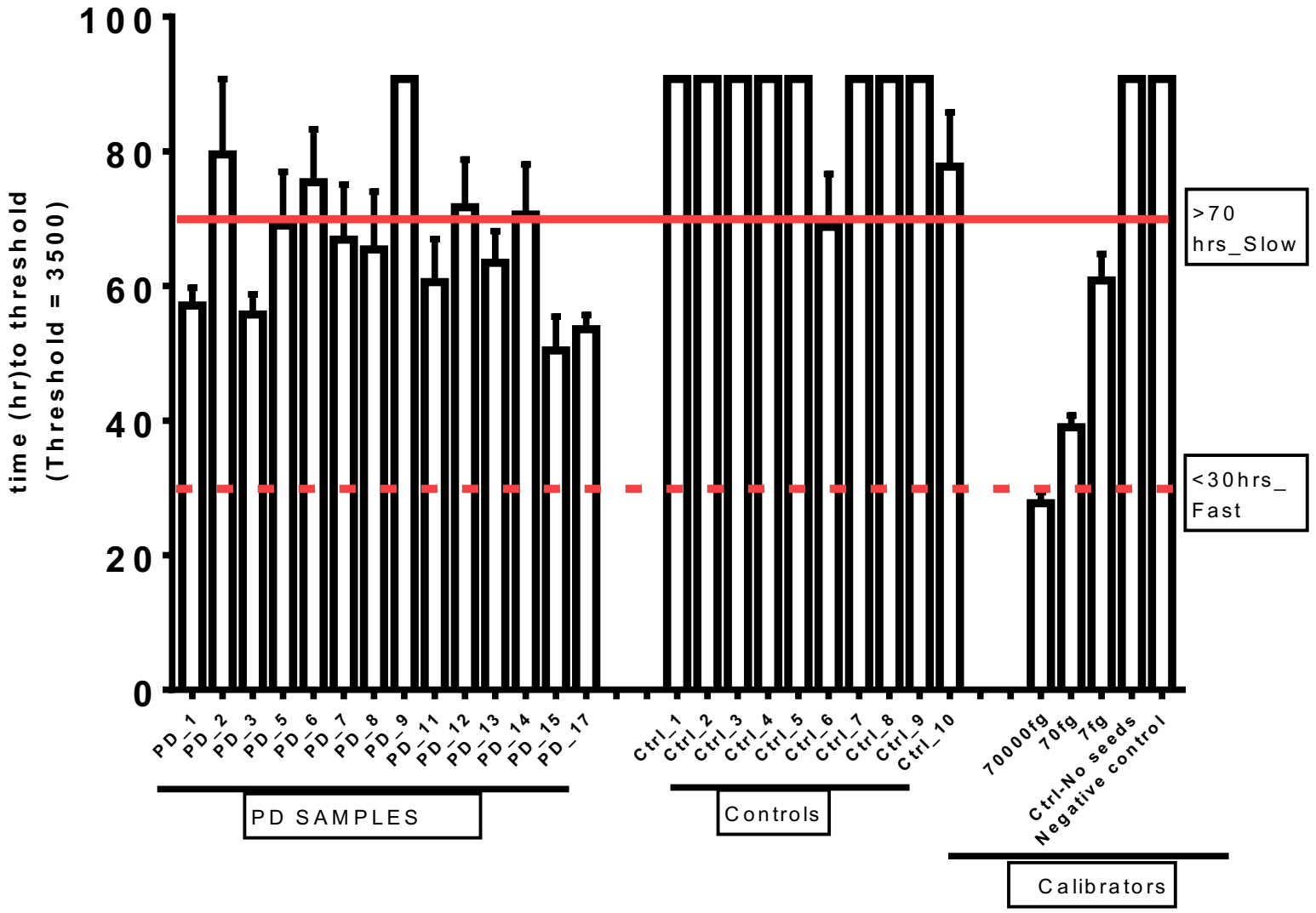
Surrogate matrix

- Perfusion fluid or artificial CSF (aCSF) is a term used for describing commercially available substitutes for CSF with similar ionic composition and no protein content

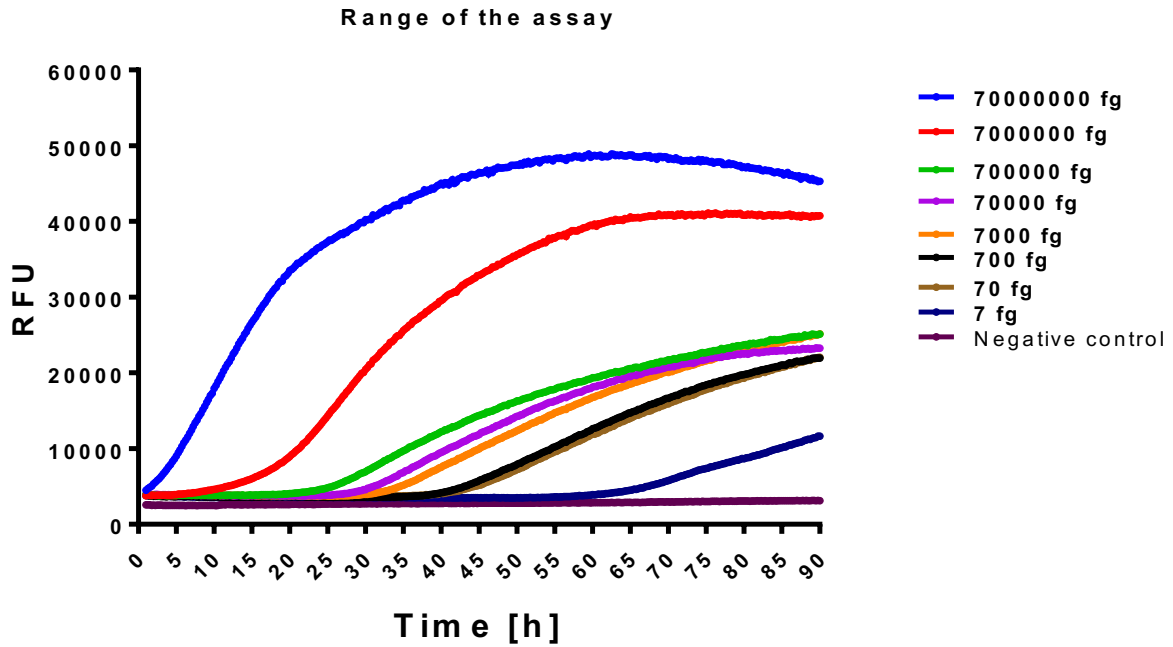
Name	Cost/mL	coment
Human CSF pool	900-1200 €	Often do not have health information of the donors
aCSF or perfusion fluid	20€	~50 times cost effective

Publication	aCSF composition	Application
Lame et al. 2011	5% rat plasma in perfusion fluid	Amyloid peptide quantitation
Dillen et al. 2010	0.15% bovine serum albumin in perfusion fluid	Amyloid peptide quantitation
Hooshfar et al. 2016	0.5–17% rat plasma and perfusion fluid	Small-molecule drug quantitation
Barthélemy et al.2016	0.5% rat serum	Tau protein quantitation
Oe et al. 2006	0.15% human serum albumin in perfusion fluid	Amyloid peptide quantitation
Oeckl et al. 2017	340–1000 µg/mL human serum albumin in perfusion fluid	Quantitation of synuclein species

PD vs. Control



Maximum extension of the assay



- Assy can extend down to below 4hrs time to threshold and RFU up to 50000 RFU
- Due to reproducibility and high variation among the replicates, Time to thresholds has considered as better parameter to define the aggregation
- Assay window is shorter than many classical assays

Definition of postive and negative aggregation

- Each sample measured in quadruplicates
- Positive: More than 2 out of 4 replicates (>50%) are positive
- Negative: None or 1 in 4 wells are positive
- Repetition of Samples: If only 1 or 2 of 4 samples are positive, the analysis was repeated in quadruplicate