

Detectability of neurodegeneration biomarkers in tear fluid using NULISA

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Background

- Blood-based biomarkers offer the potential to improve access to Alzheimer's disease diagnostic testing; however, they remain limited by logistical and pre-analytical challenges.
- Basal tear fluid (BTF) may represent a viable alternative biofluid, offering lower costs and a simplified workflow. Our previous work demonstrated that phosphorylated tau (p-tau) isoforms are measurable in BTF, and other studies reported the presence of additional neurodegeneration markers (e.g., A β , NFL, α -synuclein).
- However, these findings were generated using different immunoassay platforms and across multiple centers, limiting comparability.

Objective

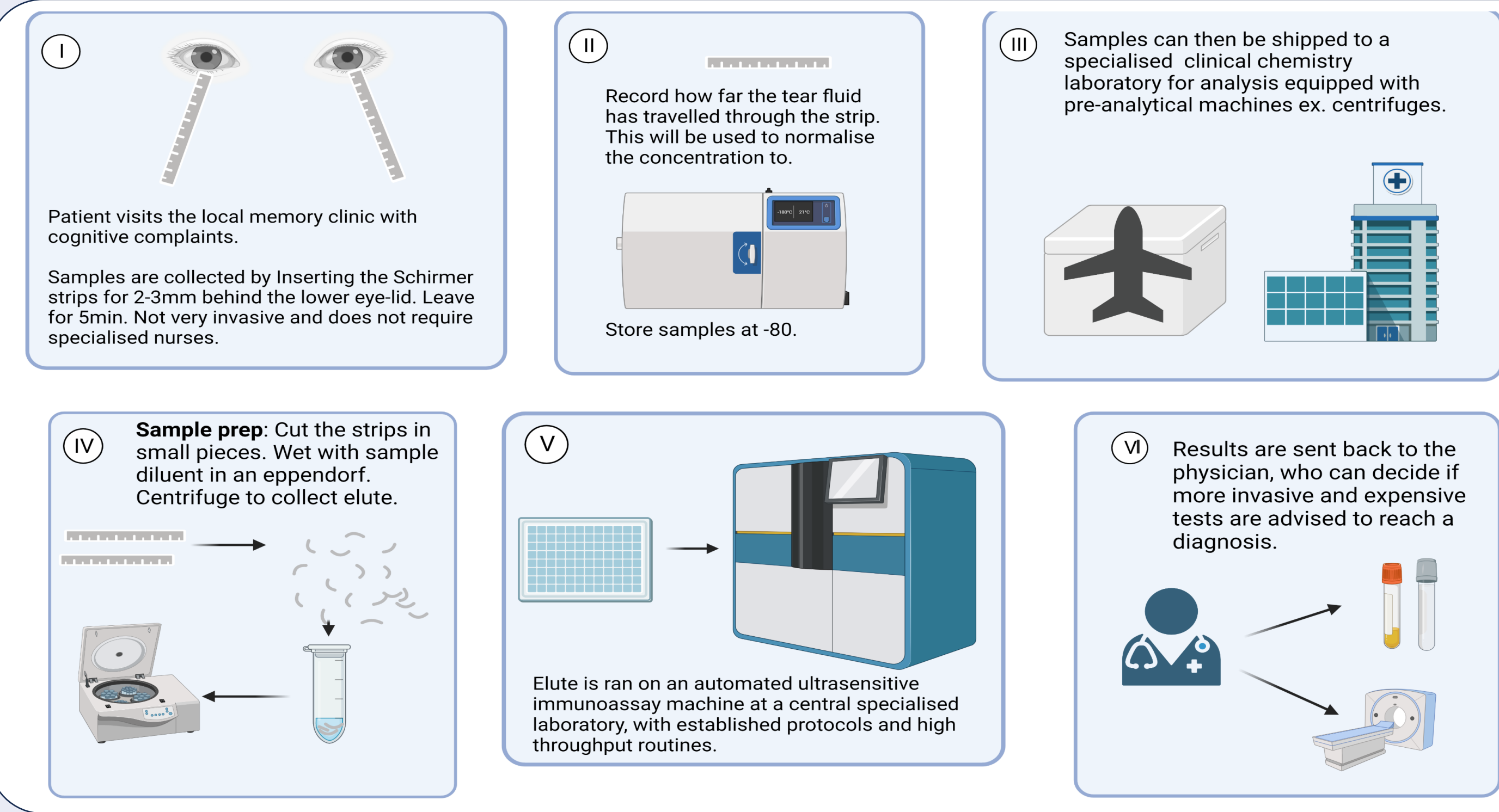
Leveraging the multiplex NULISA platform, we aimed to:

- Measure neurodegeneration biomarkers in BTF using the NULISAseq CNS panel, with the aim of assessing their detectability in a target screening population recruited in a tertiary care setting.

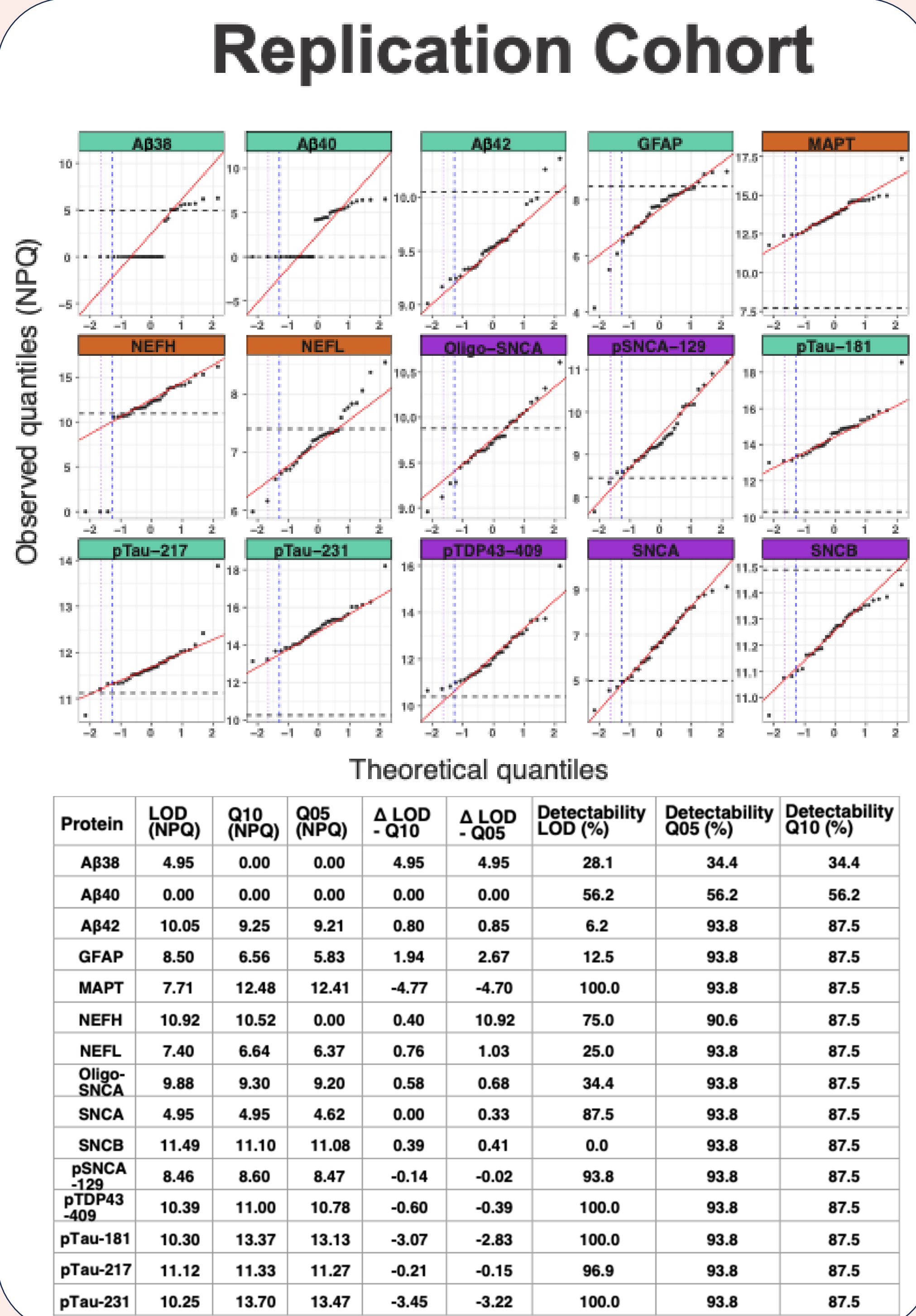
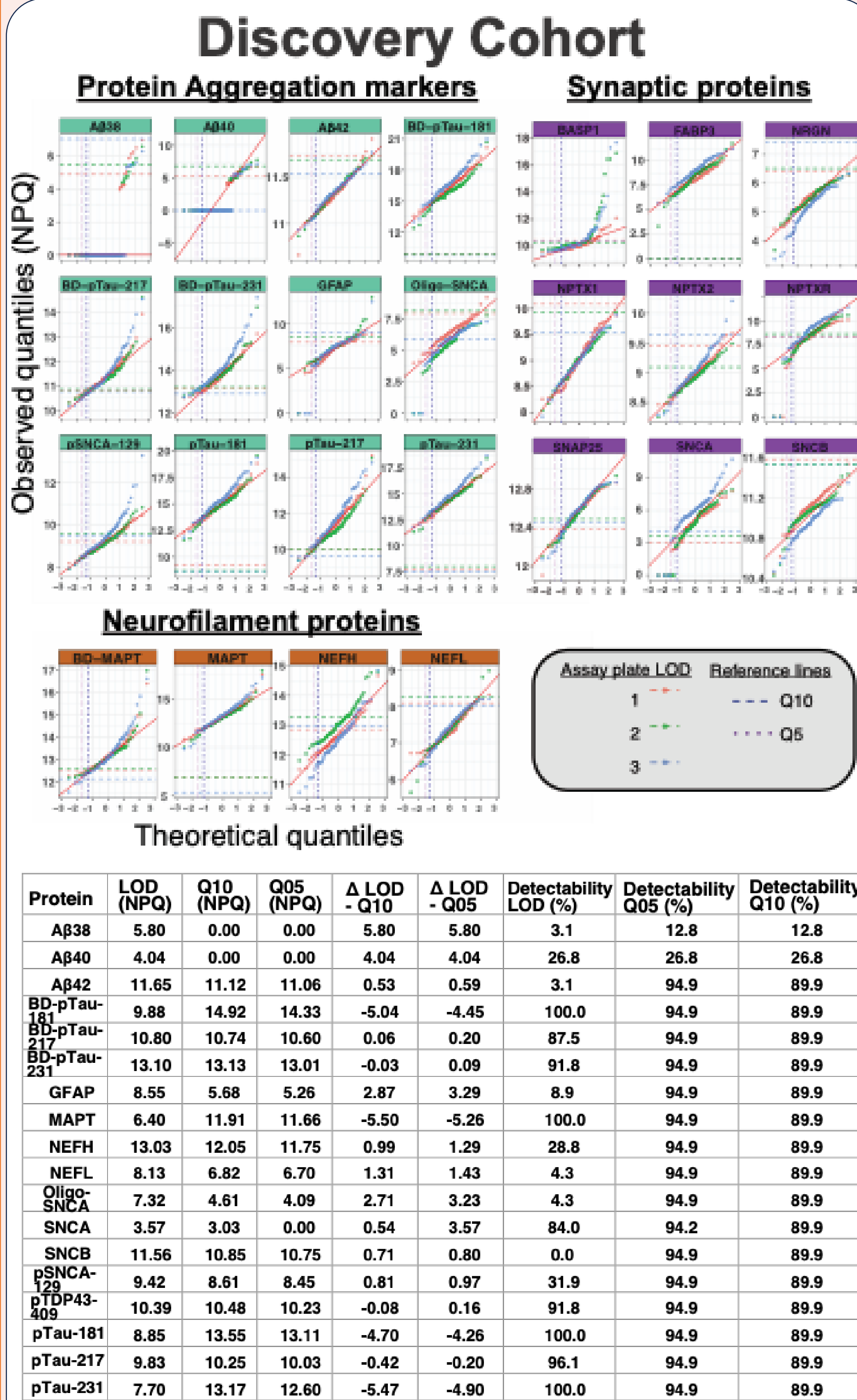
Methods

Discovery Cohort				Replication Cohort			
Characteristic	Non-AD N = 129 ¹	AD N = 92 ¹	p-value ²	Characteristic	CTRL N = 15 ¹	AD N = 15 ¹	NA N = 21 ¹
Sex			0.005	Sex			
Female	44 (36%)	47 (51%)		Female	8 (53%)	8 (53%)	0 (0%)
Male	79 (64%)	45 (49%)		Male	7 (47%)	7 (47%)	2 (100%)
Age (years)	74.0 $\pm$ 7.4	71.8 $\pm$ 7.3	0.000	Age (years)	64.7 (8.3)	71.8 (8.2)	68.8 (2.1)
MMSE (total score)	26.5 $\pm$ 3.1	24.5 $\pm$ 4.0	<0.001	MMSE (total score)	26.0 (2.7)	25.0 (3.8)	26.8 (2.7)
CSF Amyloid status			<0.001	CSF (global score)			
Negative	92 (75%)	6 (6.4%)		CSF	2 (13%)	2 (13%)	0 (0%)
Positive	30 (25%)	87 (93%)		CSF	11 (73%)	8 (53%)	2 (100%)
Diagnosis			<0.001	CSF Amyloid status			
AD	0 (0%)	92 (100%)		Negative	10 (67%)	0 (0%)	0 (0%)
FTD	5 (4.1%)	0 (0%)		Positive	8 (53%)	18 (100%)	0 (0%)
LBD	4 (3.3%)	0 (0%)		NA	0 (0%)	0 (0%)	2 (100%)
Mixed	6 (4.9%)	0 (0%)		CSF Tau status			
none	5 (4.1%)	0 (0%)		Negative	18 (100%)	0 (0%)	8 (38%)
NPH	50 (41%)	0 (0%)		Positive	0 (0%)	18 (100%)	8 (38%)
Other	40 (35%)	0 (0%)		Unknown	0	0	2
Valid	16 (13%)	0 (0%)		AD			
CSF tau (ng/L)	173.8 $\pm$ 82.2	319.8 $\pm$ 119.9	<0.001	A.T.	19 (93%)	6 (40%)	0 (0%)
CSF p-tau (ng/L)	17.7 $\pm$ 15.0	33.8 $\pm$ 16.6	<0.001	A.T.	5 (26%)	6 (40%)	0 (0%)
CSF Amyloid-Beta 42 (ng/L)	666.6 $\pm$ 561.7	665.9 $\pm$ 242.2	0.002	A.T.	0 (0%)	18 (100%)	0 (0%)
Total run length (mm)	15.6 $\pm$ 14.7	16.5 $\pm$ 14.0	0.6	NA	0 (0%)	6 (40%)	2 (100%)
Mean $\pm$ SD; n (%)				CSF p-tau (ng/L)	26.8 (2.4)	62.7 (27.2)	NA (NA)
Wilcoxon Two Sample Test; Pearson's Chi-squared test				CSF Amyloid-Beta 42 (ng/L)	621.7 (436.2)	541.7 (179.5)	NA (NA)
				Total run length (mm)	22.9 (16.5)	20.3 (16.9)	26.5 (29.0)
				Mean $\pm$ SD; n (%)			
				Wilcoxon Two Sample Test; Pearson's Chi-squared test			

- Samples were collected at two different memory clinics University of Copenhagen (Discovery cohort) and University of Gothenburg (Replication cohort) using a standardized protocol.
- Samples were measured using the NulisaSeq 120-plex CNS panel [V1: Replication; V2: Discovery cohort) on the NULISA platform. Counts were normalized to internal controls and then log transformed into NULISA Protein quantifications (NPQ).
- Detectability was established as per manufacturer guidelines (mean blank +3SD) and QQ plots were used to assess distributional properties and lower-tail deviations for each target.

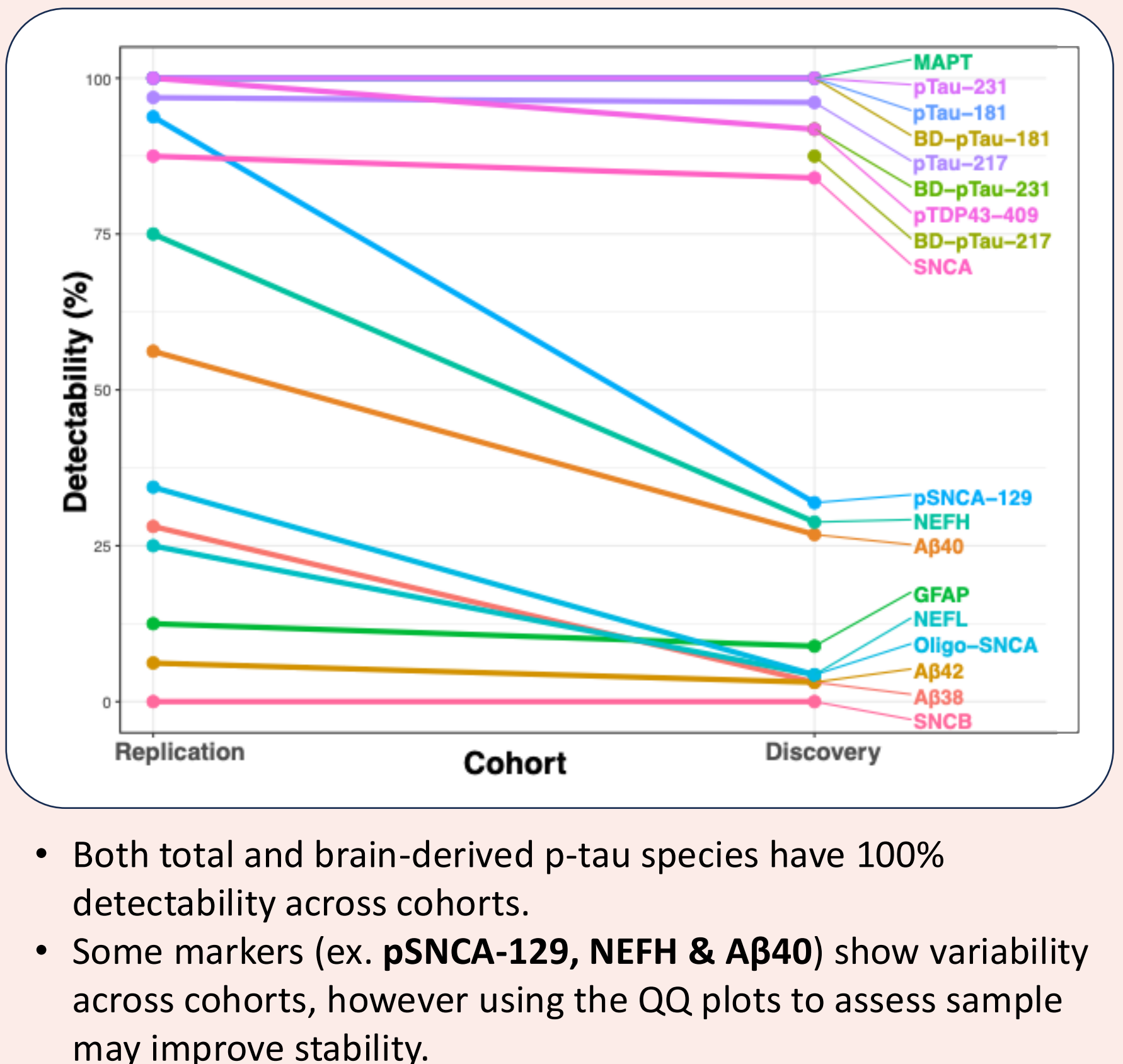


Results I: Markers of amyloid and tau pathology show good detectability in tear fluid



- Relying on the manufacturer's detectability can lead to excluding markers that do not show substantial left-tail censoring (ex. NEFL, SNCB, NEFH, NPTXR, NPTX2, A β 42, GFAP).
- Detectability of proteins was comparable in both independent cohorts.
- Samples with minimal tear fluid collected (assessed by running length) did not exhibit sharp drops in detectability (data not shown).

Results II: Manufacturer detectability cutoff is varies for some proteins across cohorts



- Both total and brain-derived p-tau species have 100% detectability across cohorts.
- Some markers (ex. pSNCA-129, NEFH & A β 40) show variability across cohorts, however using the QQ plots to assess sample may improve stability.

Take home points

- Several neurodegeneration biomarkers are measurable in tear fluid samples collected and handled in a memory clinic setting.
- QQ plots can aid in the inclusion and exclusion of targets in samples due to poor detectability.
- P-tau species were detectable in 100% of samples in both cohorts, whereas β -amyloid showed low detectability.
- Therefore, while currently the diagnostic potential is limited, CNS-derived proteins and neurodegeneration markers are measurable in BTF, warranting further research in its connection to the CNS.