

A SEED AMPLIFICATION ASSAY DETECTING PATHOLOGICAL ALPHA-SYNUCLEIN IN PARKINSON'S DISEASE AND DEMENTIA WITH LEWY BODIES



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Background

Seed amplification assays (SAA) for detecting Parkinson's disease (PD) and dementia with Lewy bodies (DLB) would benefit from further analytical improvements.

Methods

Optimizations: Sample prep. and α -synuclein (asyn) substrate sequence.
Trueness: Analysis of quality control (QC) material across 9 independent runs.
Analytical sensitivity: Serial dilution of asyn pre-formed amyloid fibrils (PFF).
Performance: Blinded case-control study.

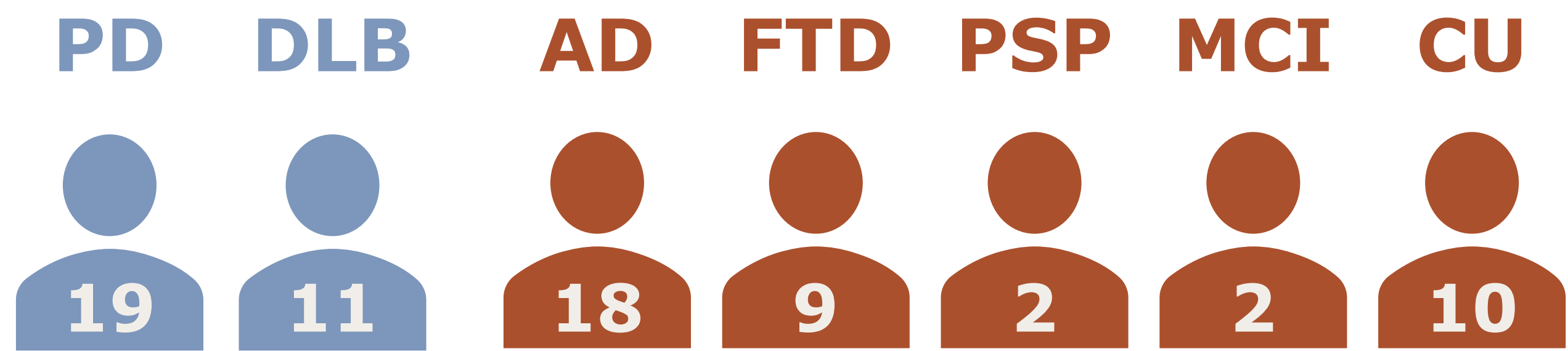


Fig. 1: Cohort composition (n=71) for SAA performance evaluation. (AD: Alzheimer's disease; FTD: frontotemporal dementia; PSP: progressive supranuclear palsy; MCI: mild cognitive impairment; CU: cognitively unimpaired).

Results

Optimizations: Slow rotation vs. vortexing samples and use of K23Q vs. WT asyn substrate improved asyn+ and asyn- differentiation.

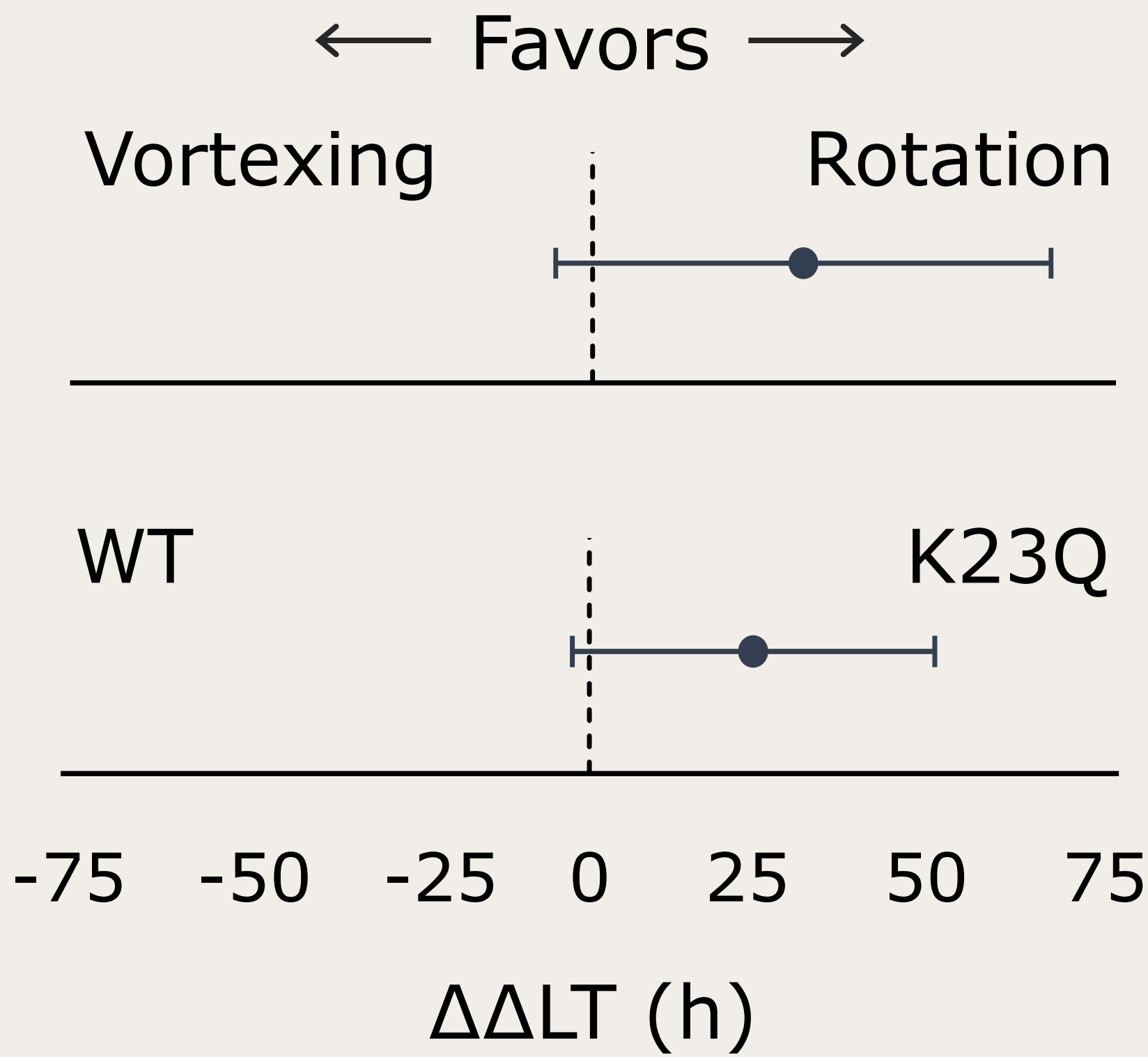


Fig. 2: Difference in lag time differences ($\Delta\Delta LT$) between asyn+ and asyn- QCs.

Trueness: asyn+ and asyn- QC were accurately classified 100% of the time.

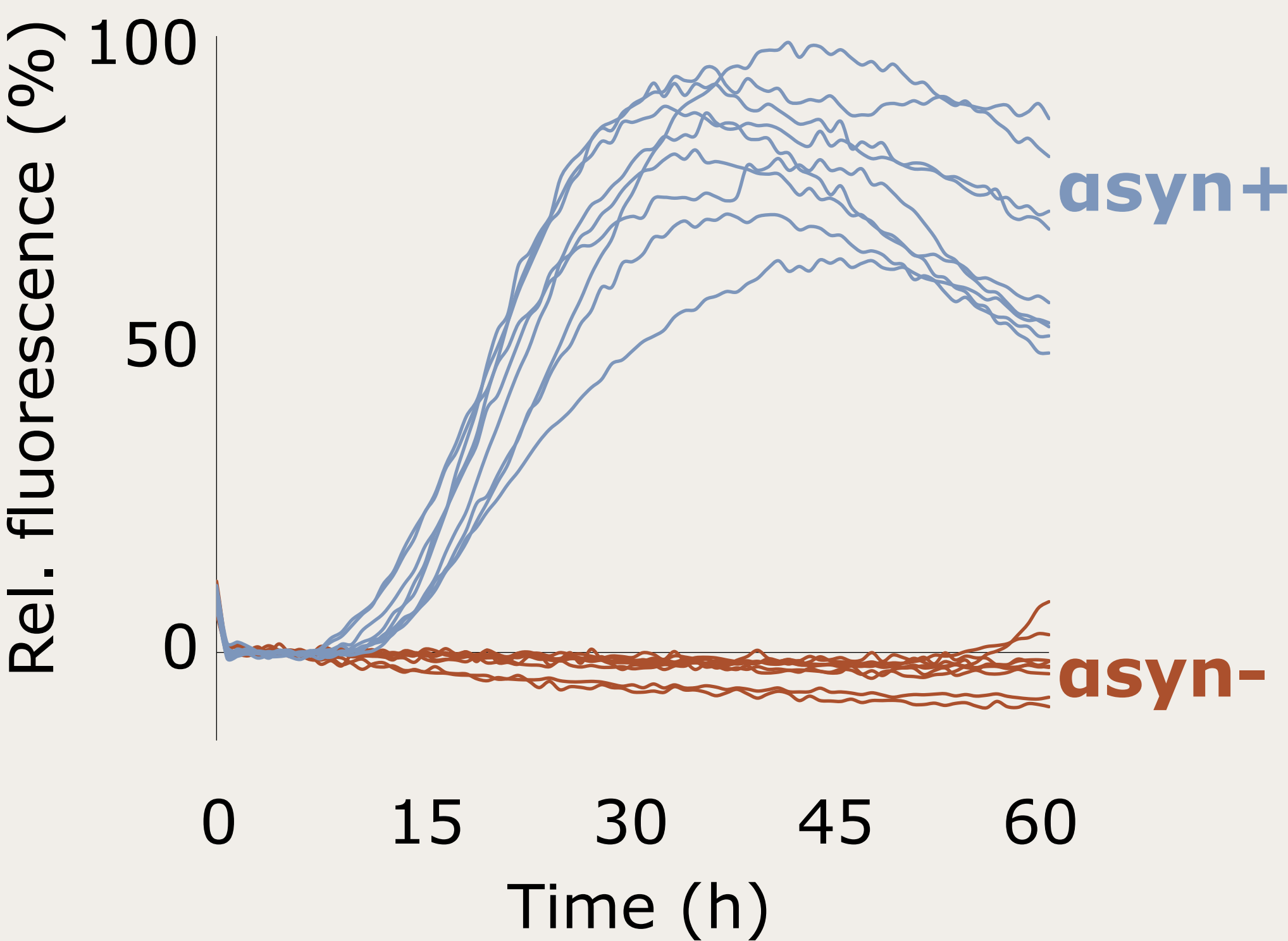


Fig. 3: QC SAA results asyn+ and asyn- QCs. (curves = average of 4 replicates).

Analytical sensitivity: LLOD = 250 fg/mL of asyn PFF.

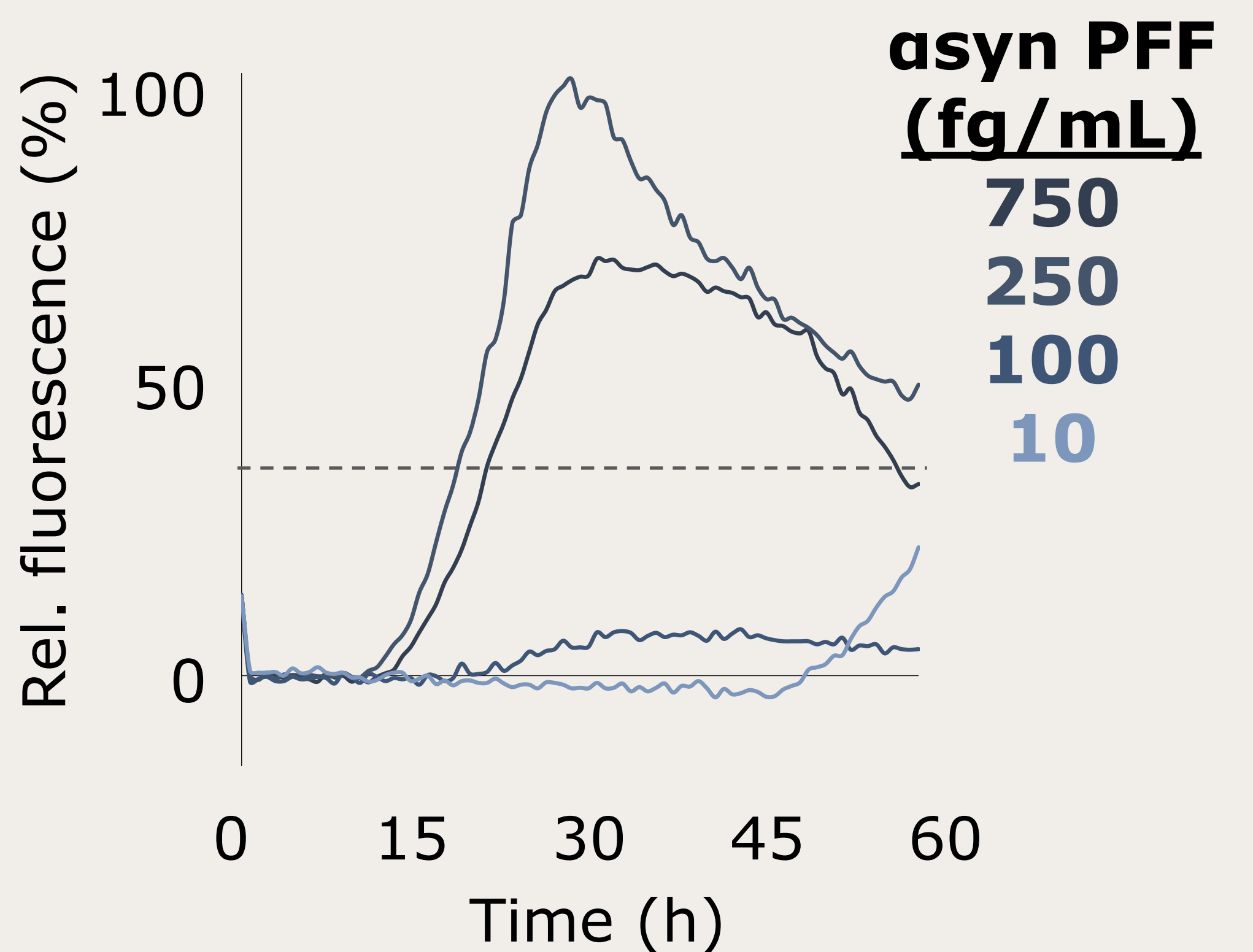


Fig. 4: asyn PFFs at varying concentrations (solid lines: average of 4 replicates; dotted line: positivity threshold).

Diagnostic performance:

Table 1: Concordance

		Clinical diagnosis	
		PD & DLB	Control
SAA	Positive	29	7
	Negative	1	34

Table 2: Diagnostic metrics

Sensitivity	96.7%
Specificity	82.9%
Diagnostic accuracy	88.7%

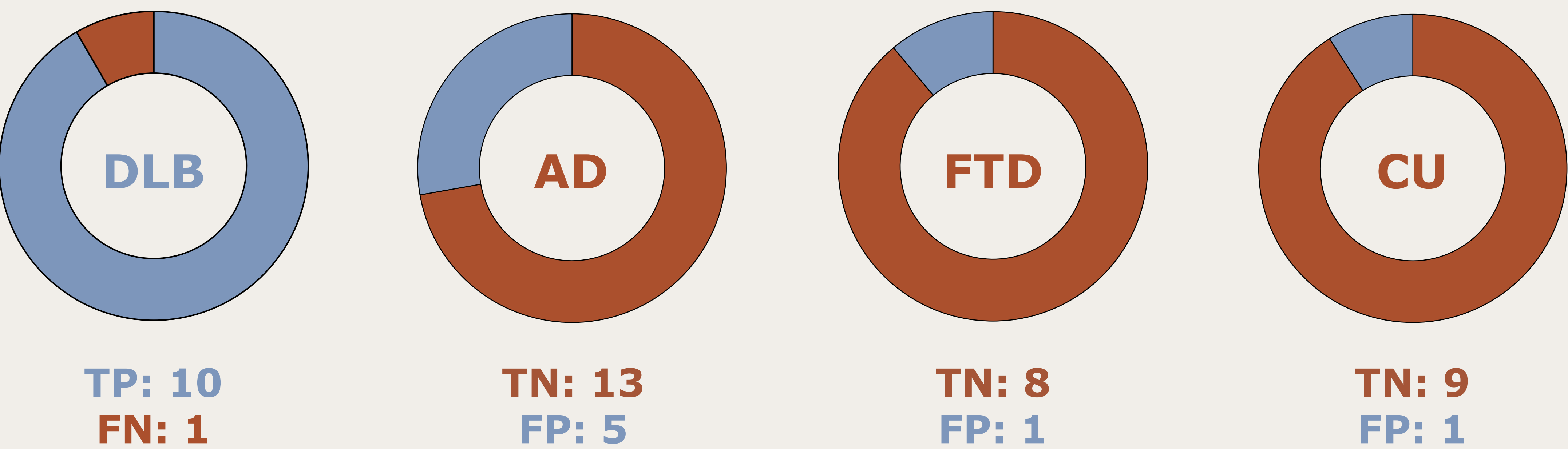


Fig. 5: SAA asyn+ and asyn- results by diagnostic groupings. True positive (TP) and negative (TN), and false positive (FP) and negative (FN) counts noted.

Conclusion

Optimized sample preparation improved reproducibility and combined with K23Q substrate, improved assay performance.

In a case control study, the assay achieved high accuracy for detection of asyn pathology.

