Appendix

Alpha-synuclein misfolding as fluid biomarker for Parkinson's disease measured with the iRS platform

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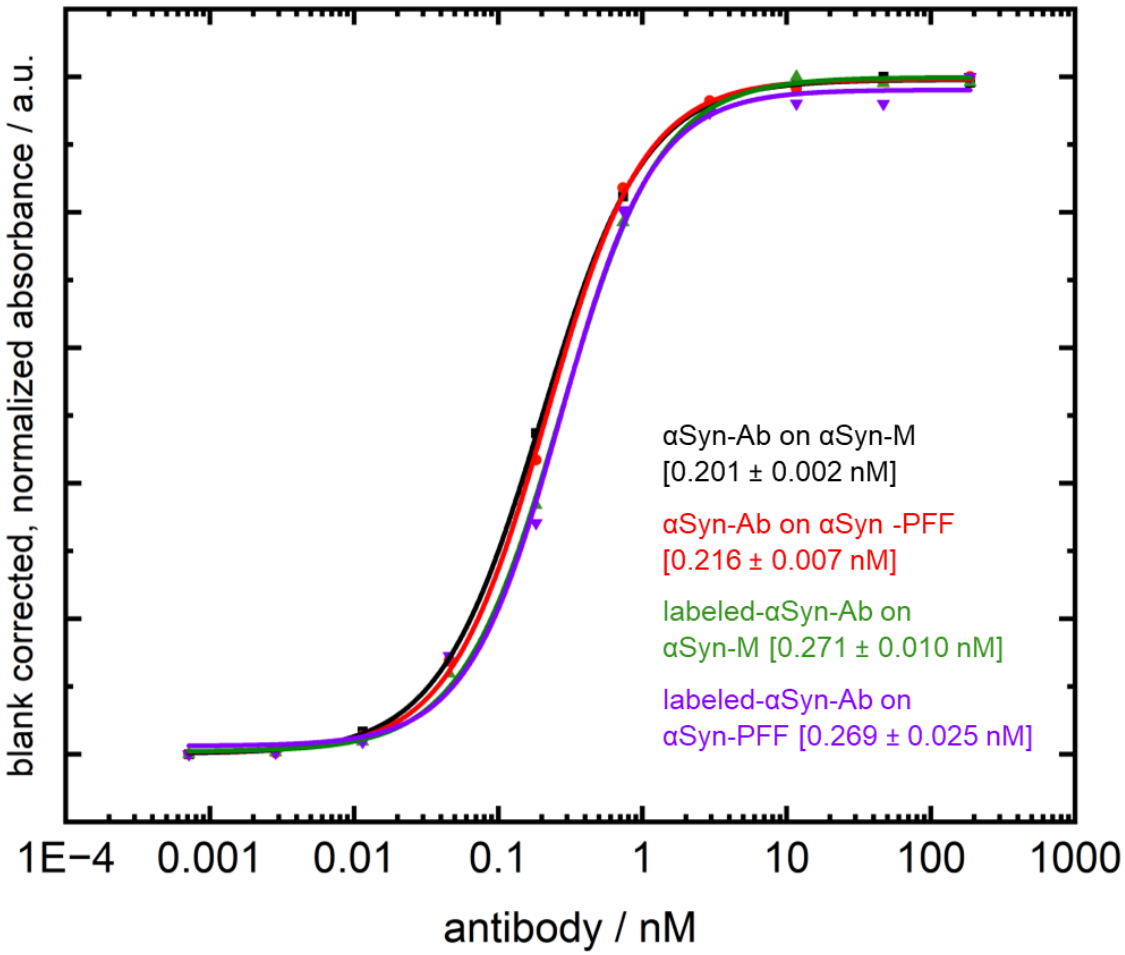
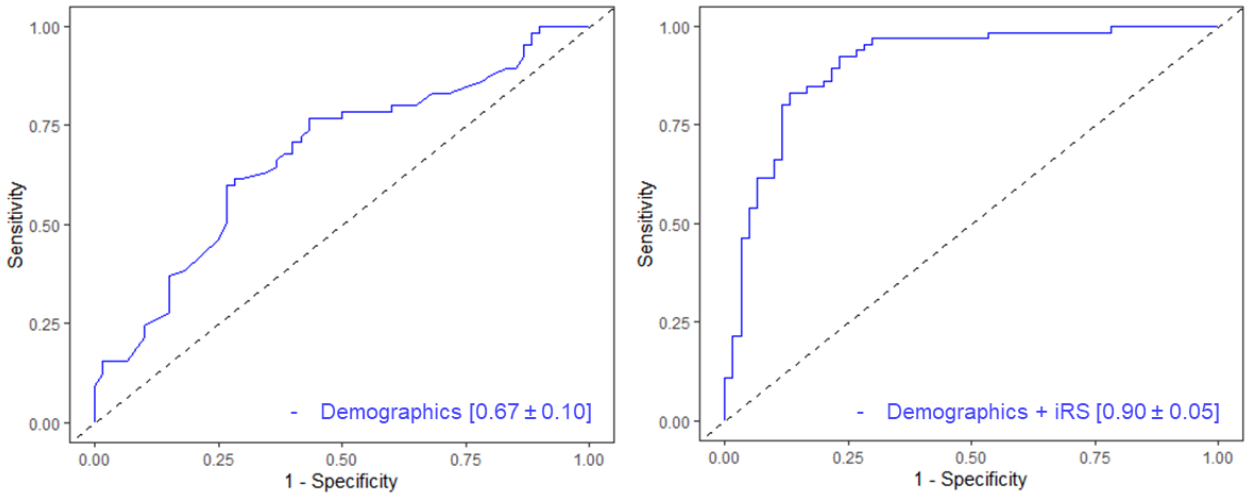


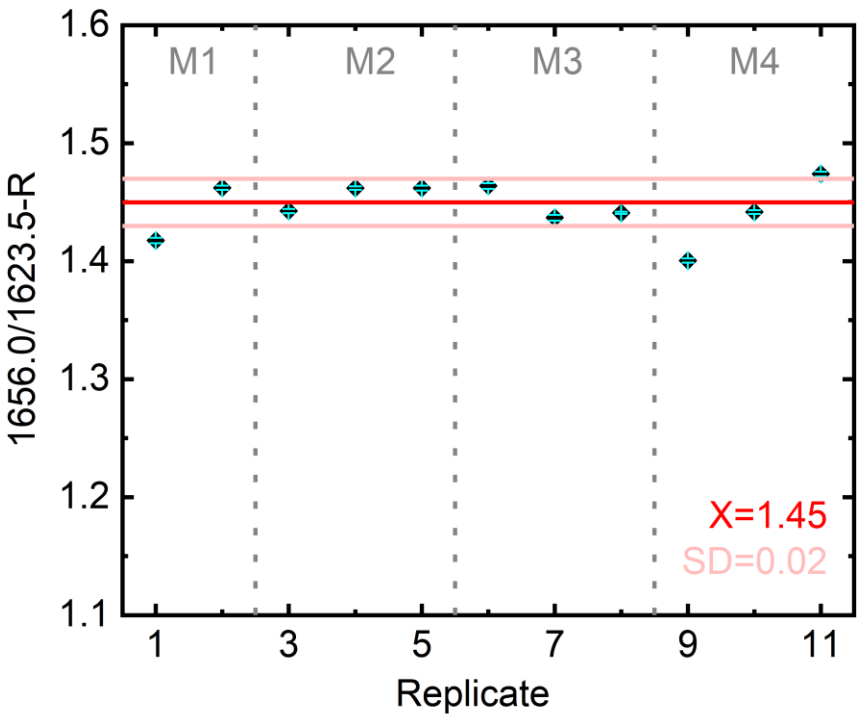
Appendix Figure S1: Indirect ELISA for EC_{50}-value determination of native and labeled capture antibody according to given protocol (EV Materials). EC_{50}-values were determined from blank corrected absorbance difference at 450 - 620nm using a 4-parameter logistic fit model on normalized data. Values demonstrate the fit results of the native and labeled antibody towards αSyn-M (Stressmarq Bioscience INC. SPR-321) and αSyn-PFF (Stressmarq Bioscience INC. SPR-322), as used in the ThT and ATR-FTIR experiments. EC_{50} values from the 4-parameter logistic fit model are 0.20 ± 0.002 nM and 0.22 ± 0.007 nM for native antibody, while labeled antibody shows EC_{50} values of 0.27 ± 0.010 nM and 0.27 ± 0.025 nM.

	Demographics			Demographics + iRS		
Characteristic	**OR**[1]	**95% CI**[1]	**p-value**	**OR**[1]	**95% CI**[1]	**p-value**
Age	1.020	0.988, 1.055	0.2	1.027	0.986, 1.076	0.2
Gender						
m	—	—		—	—	
w	0.264	0.119, 0.564	$7.5 \cdot 10^{-4}$	0.298	0.105, 0.817	0.020
readout10				0.030	0.006, 0.101	$5.6 \cdot 10^{-7}$

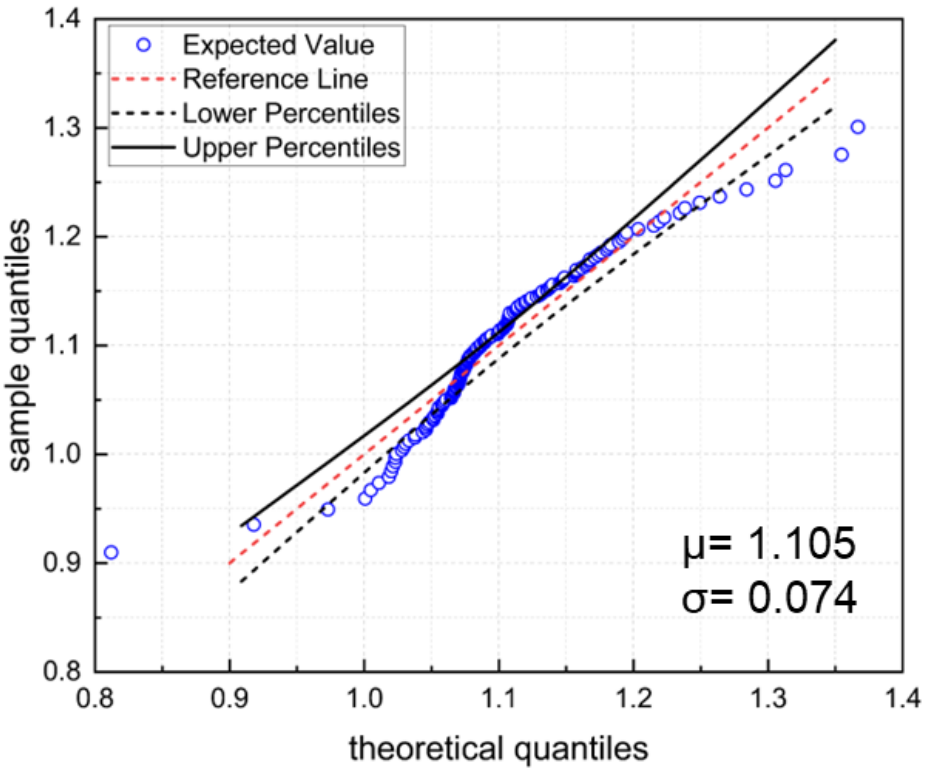
[1]OR = Odds Ratio, CI = Confidence Interval



Appendix Figure S2: Logistic regression models for the combined dataset. The first model, "Demographics" accounts for age and sex only, while the second model "Demographics + iRS," also accounts for the iRS assay read-out. The clinical diagnosis is always used as the dependent variable. Calculated models with characteristics in the upper table were utilized to calculate ROC-AUC curves for both models. The demographics-only model showed an AUC of 0.67 ± 0.10 (p-value $7.5 \cdot 10^{-4}$), while with additional iRS read-out, the AUC increased to 0.90 ± 0.05 (p-value $5.6 \cdot 10^{-7}$). The shown p-values were calculated using a standard Wald test.



Appendix Figure S3: Reproducibility measures with a pooled control CSF sample measured on the αSyn capture antibody surface. The eleven replicates were measured in four measurements (M1-M4). Routine spectra processing (WV and baseline correction, averaging spectra and reference channel subtraction, Fourier self-deconvolution (FSD), and smoothing) was performed. Each replicate is depicted with an error bar (cyan) obtained from applying a smoothing variation by the in-house MATLAB script for data analysis. The mean 1656.0/1623.5-ratio of all measurements was $\bar{X}$=1.45 with a standard deviation of SD=0.02.



Appendix Figure S4: Dataset distribution and conformity to normal distribution by normal Q-Q-Plot of 1656.0/1623.5-Ratios. The mean of the normal distribution is μ=1.105 with a standard deviation of σ=0.074 for the normal distribution. Lower and upper percentiles (black line and dashed black line) mark 95 %-confidence bands, while the red dashed line shows the reference line. Since a considerable amount of data points of the combined data set (n=134) are not located within the confidence borders, the dataset is not normally distributed. Thus, non-parametric models were applied to test the significance of group separations.