

Ultrasensitive 5-plex Blood-Based Assay for Early Alzheimer's Disease Detection Using NULISA™ Technology

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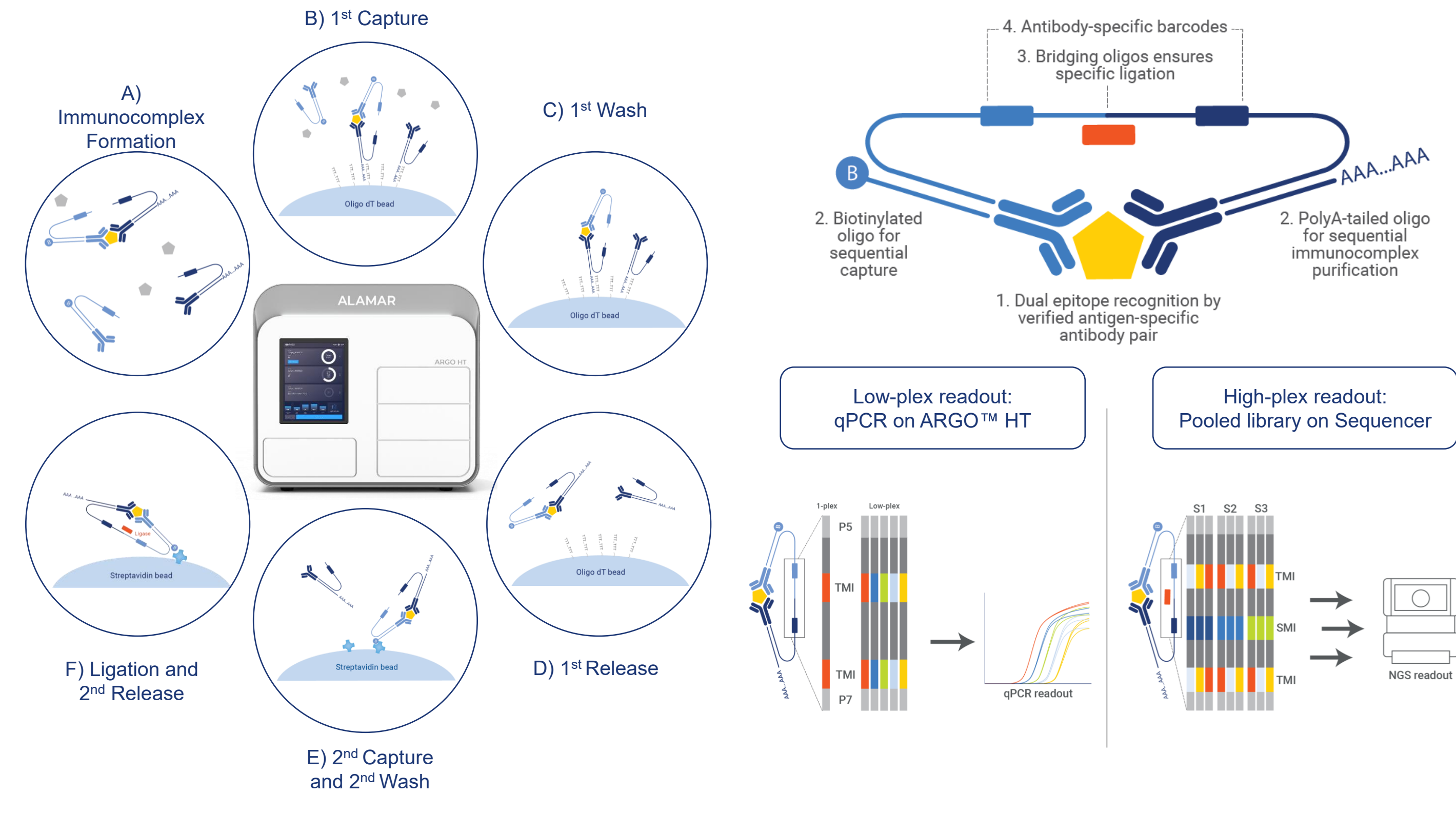


Background

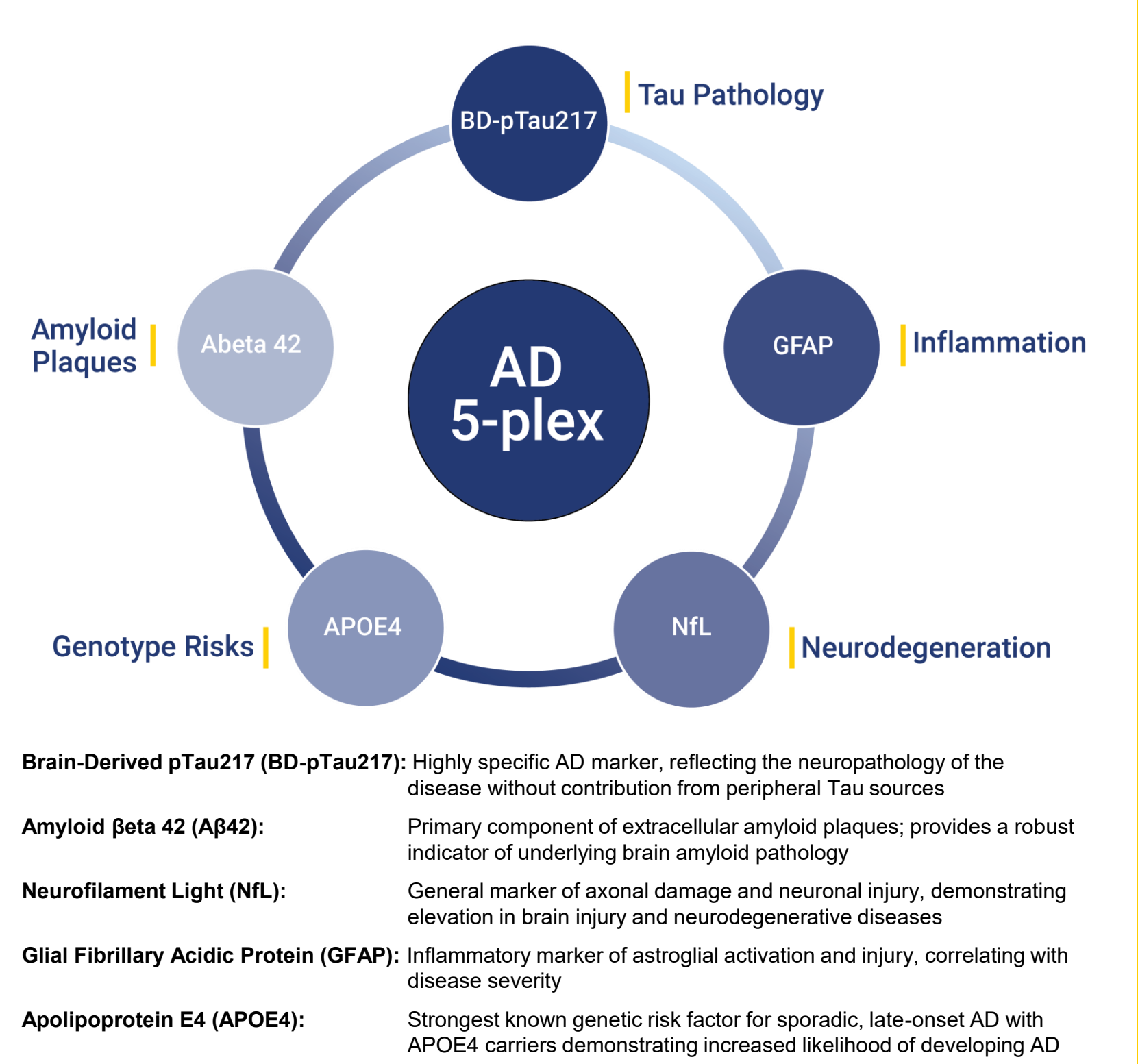
The early and accurate detection of Alzheimer's Disease (AD) remains a significant challenge, particularly with respect to blood-based biomarkers. While certain markers — such as brain-derived isoforms of Tau — have demonstrated promise for distinguishing AD from other dementias, there is a pressing need for accessible, cost-effective assays that combine multiple key markers with high sensitivity. To address this gap, we developed an ultrasensitive 5-plex AD panel using the novel NUCleic-acid Linked Immuno-Sandwich Assay (NULISA) technology¹.

Our qPCR-based NULISA 5-plex AD assay targets five critical biomarkers: **brain-derived pTau-217 (BD-pTau-217)**, **NfL**, **GFAP**, **Ab42**, and **APOE4**. The first four markers, which have been demonstrated to have strong associations with various phenotypes of AD, are detected quantitatively with ultra-high sensitivity. APOE4, an emerging new marker and the strongest generic risk factor for the late onset of AD, is assessed using a qualitative genotyping assay to distinguish carriers from non-carriers. Analytical performance was evaluated in plasma samples to assess detectability, spike-recovery, dilutional linearity, and parallelism. Assay correlations were established with both the NULISA single-plex assay and Neuro 220 Panel.

ARGO™ HT for Fully Automated Ultrasensitive Proteomic Analysis



NULISAqpcr™ AD 5-plex Assay



NULISAqpcr™ AD 5-plex Assay Demonstrates High Sensitivity and Specificity

Analytical Sensitivity and Dynamic Range*								
	BD-pTau217		NfL		GFAP		Aβ42	
	Functional	Analytical	Functional	Analytical	Functional	Analytical	Functional	Analytical
LOD	0.03 pg/mL	0.006 pg/mL	0.04 pg/mL	0.008 pg/mL	0.05 pg/mL	0.010 pg/mL	1.55 pg/mL	0.310 pg/mL
LLoQ	0.14 pg/mL	0.028 pg/mL	0.13 pg/mL	0.026 pg/mL	0.15 pg/mL	0.030 pg/mL	1.93 pg/mL	0.386 pg/mL
ULoQ	35,763 pg/mL	7,153 pg/mL	68,000 pg/mL	13,600 pg/mL	77,844 pg/mL	15,569 pg/mL	989 pg/mL	198 pg/mL
Dynamic Range	5.4 Log ₁₀		5.7 Log ₁₀		5.7 Log ₁₀		2.7 Log ₁₀	

*Assessed from 50 independent runs across 4 ARGOs with 1 reagent lot

Cross Reactivity with Related Tau Isoforms									
	pTau181	pTau205	pTau212	pTau231	Total Tau	NfM	Peripherin	Vimentin	Aβ40
Tested Conc (pM)	2	2	2	2	2	200	88	9000	70
Observed Cross Reactivity	None	0.8% with BD-pTau217	2.4% with BD-pTau217	6.7% with BD-pTau217	None	None	None	None	None

NULISAqpcr™ AD 5-plex Assay Demonstrates High Detectability and Differential Abundance between Normal and Diseased Plasma Samples

Detectability and Reference Range												
	BD-pTau217			NfL			GFAP			Aβ42		
	Normal	Diseased	Total	Normal	Diseased	Total	Normal	Diseased	Total	Normal	Diseased	Total
Detectability	97.10%	100%	99.50%	100%	100%	100%	100%	100%	100%	100%	100%	100%
Quantifiability	20.80%	70.30%	59.60%	98.50%	100%	99.80%	100%	100%	100%	87.50%	99.70%	98.90%
Average	0.07 pg/mL	0.91 pg/mL	0.77 pg/mL	5.21 pg/mL	13.32 pg/mL	11.97 pg/mL	5.97 pg/mL	24.73 pg/mL	21.60 pg/mL	18.80 pg/mL	28.99 pg/mL	28.20 pg/mL
Median	0.05 pg/mL	0.38 pg/mL	0.23 pg/mL	2.89 pg/mL	9.17 pg/mL	8.17 pg/mL	4.79 pg/mL	19.37 pg/mL	17.16 pg/mL	19.80 pg/mL	21.53 pg/mL	21.48 pg/mL
Min	<LoD	0.01 pg/mL	<LoD	0.07 pg/mL	1.09 pg/mL	0.07 pg/mL	0.18 pg/mL	2.39 pg/mL	0.18 pg/mL	0.90 pg/mL	1.55 pg/mL	0.90 pg/mL
Max	0.55 pg/mL	86.71 pg/mL	86.71 pg/mL	101.96 pg/mL	270.41 pg/mL	270.41 pg/mL	30.69 pg/mL	261.37 pg/mL	261.37 pg/mL	40.10 pg/mL	191.70 pg/mL	191.70 pg/mL

NULISAqpcr™ AD 5-plex Assay Demonstrates Robust Assay Performance with High Precision, Reproducibility and Recovery with no Significant Interference

Precision and Reproducibility*												
CV (%)	BD-pTau217			Aβ42			NfL			GFAP		
	Intra-Assay	Inter-Assay	Total	Intra-Assay	Inter-Assay	Total	Intra-Assay	Inter-Assay	Total	Intra-Assay	Inter-Assay	Total
Average	8.9	5.9	15.3	8.1	9.1	13.1	6.0	7.5	13.1	6.2	7.3	12.2
Median	7.2	6.5	13.6	7.9	9.5	12.9	5.9	7.8	12.9	6.4	7.5	12.3
Min	6.0	0.0	11.8	6.5	7.0	11.2	4.3	6.3	11.4	4.8	4.8	10.6
Max	22.1	7.7	24.5	10.0	10.6	15.3	7.6	9.1	14.7	7.5	9.2	14.1

*Assessed from 20 independent runs with 9 K2EDTA plasma samples tested across 5 days with 3 reagent lots

Parallelism*					Dilution Linearity*				
Recovery (%)	BD-pTau217	Aβ42	NfL	GFAP		BD-pTau217	Aβ42	NfL	GFAP
	Intra-Assay	Intra-Assay	Intra-Assay	Intra-Assay					
Average	98%	98%	110%	109%	Starting Spiked Concentration	50 pg/mL	100 pg/mL	200 pg/mL	300 pg/mL
Median	99%	100%	107%	106%	Average Recovery	93%	118%	113%	105%
Min	71%	76%	97%	89%	Median Recovery	94%	111%	97%	103%
Max	129%	125%	138%	134%					

*Assessed with 11 K2EDTA plasma samples serially diluted 2x with Sample Diluent Buffer. At least 80% of the samples displayed good parallelism with 70-130% recovery across all dilution levels above the assay LLoQ.

*Assessed with 5 normal K2EDTA plasma samples spiked with each of the targets and 2x up to 128X. At least 80% of samples showed good recovery within 70-130%.

Spike Recovery*					Interference*				
	BD-pTau217	Aβ42	NfL	GFAP	Highest Tolerable Conc.	BD-pTau217	Aβ42	NfL	GFAP
Spike Concentration (High)	50 pg/mL	100 pg/mL	200 pg/mL	300 pg/mL	Unconj. Bilirubin	684 μM	684 μM	684 μM	684 μM
Spike Concentration (Medium)	10 pg/mL	20 pg/mL	40 pg/mL	60 pg/mL	Conj. Bilirubin	475 μM	475 μM	475 μM	475 μM
Spike Concentration (Low)	1 pg/mL	2 pg/mL	4 pg/mL	6 pg/mL	Biotin	3,510 ng/mL	3,510 ng/mL	3,510 ng/mL	3,510 ng/mL
Average Recovery	107%	89%	80%	76%	HAMA	1,200 ng/mL	1,200 ng/mL	1,200 ng/mL	1,200 ng/mL
Median Recovery	105%	87%	86%	77%	Hemoglobin	5 mg/mL	2 mg/mL	5 mg/mL	10 mg/mL
					Rheumatoid Factor	600 IU/mL	600 IU/mL	600 IU/mL	600 IU/mL
					Triglycerides	500 mg/dL	250 mg/dL	250 mg/dL	1,000 mg/dL

*Assessed with 5 K2EDTA plasma samples spiked with three concentrations of each analyte and compared to spiked Sample Diluent. *Recovery = (Mean Conc. Spiked Donor Samples) / (Mean Conc. Unspiked Donor Samples) x 100. *At least 80% of samples showed good recovery within 70-130%.

Conclusions

- The NULISA 5-plex AD assay demonstrated high detectability for BD-pTau-217, NfL, GFAP, and Ab42 in plasma samples from individuals across the AD continuum.
- Additionally, APOE4 measured with the AD 5-plex assay showed 100% concordance with genotyping records for APOE4 carrier status.
- All assays exhibited strong performance characteristics, and correlations with existing single-plex and multi-plex NULISA assays exceeded R > 0.9.
- Overall, the NULISA 5-plex AD assay offers a highly specific, sensitive and reliable solution for AD biomarker detection in plasma.

References

- Feng, W. et al. NULISA: a proteomic liquid biopsy platform with attomolar sensitivity and high multiplexing. Nat Commun 14, 7238 (2023). <https://doi.org/10.1038/s41467-023-42834-x>

