Development of a Highly Sensitive and Automated Immunoassay for Brain-Derived pTau-217: Analytical Performance in Human Plasma Samples

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Abstract

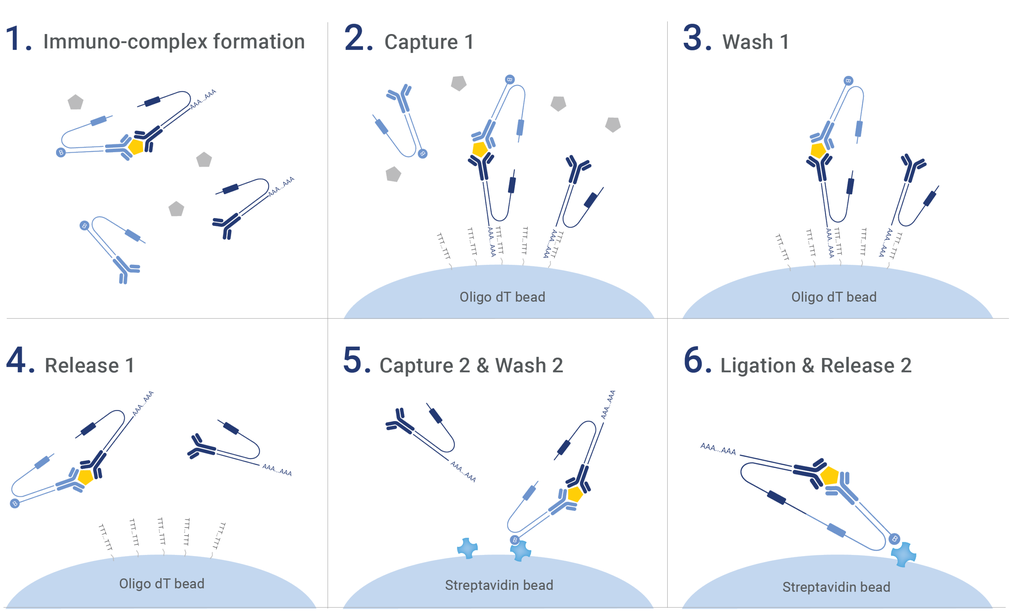
Background: Blood-based biomarkers are increasingly recognized as valuable tools for early detection and longitudinal monitoring of Alzheimer's Disease (AD). Among these, plasma pTau-217 demonstrates high discriminative accuracy for amyloid-PET positivity. However, most circulating pTau-217 originates from peripheral sources, with only ~20% derived from the brain. This highlights the need for a highly sensitive and specific assay to detect brain-derived (BD) pTau-217, a brain-specific isoform that more accurately reflects AD neuropathology.

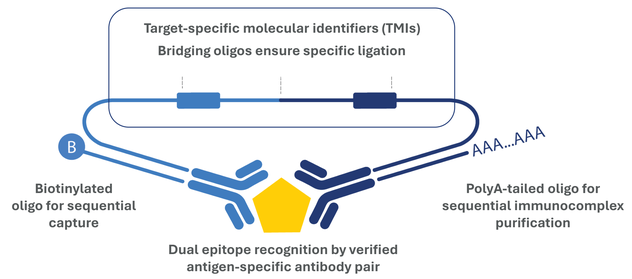
Methods: We previously developed a novel proximity ligation-based NULISA technology, which integrates background suppression steps to enable attomolar sensitivity and automated protein detection across diverse biofluids[1]. Building on our prior development of a 120-plex NULISAseq CNS Disease Panel that enables relative quantification of BD-phosphorylated Tau isoforms along with other markers related to neurodegeneration and inflammation, we have developed a singleplex NULISA assay for absolute quantification of BD-pTau217 with high specificity and sensitivity. The assay was rigorously evaluated for key analytical parameters including dilution linearity, spike recovery, parallelism, accuracy, precision, reproducibility, and specificity.

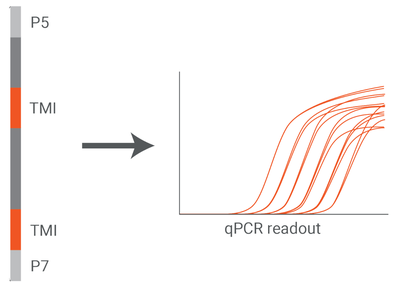
Results: Our novel BD-pTau217 singleplex assay demonstrated strong analytical performance, with inter-plate CVs consistently below 20% across multiple operators, ARGO HT instruments, and days. The assay features a broad dynamic range (0.4–14,655 pg/mL) and a limit of detection of 0.08 pg/mL. In plasma samples from both healthy individuals and AD patients, overall BD-pTau217 detectability was over 95%. Characterization studies such as dilutional linearity, parallelism, and spike recovery confirmed robust assay performance. Additionally, the BD-pTau217 antibody exhibited minimal cross-reactivity with other Tau species, including total Tau, pTau181, pTau205, and pTau212, confirming its high specificity.

Conclusions: The NULISA BD-pTau217 singleplex assay represents a significant advance in blood-based biomarkers for AD, providing brain specificity without the need for cerebrospinal fluid.

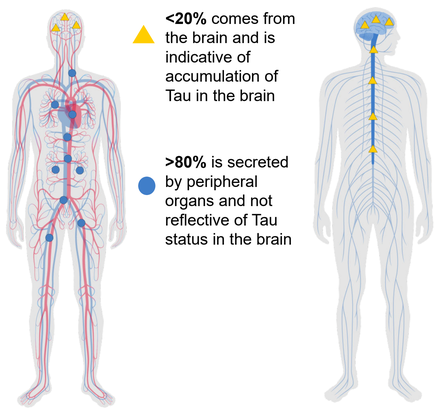
NULISA Automated on the ARGO™ HT Platform Leverages Oligo-Conjugated Antibodies and a Novel Background Suppression Mechanism for High-Throughput Proteomic Analysis with High Specificity in Single-plex and Multiplex Assay Configurations







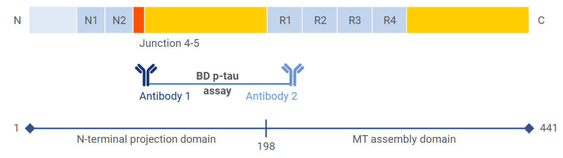
Blood-based pTau217 Assays Need to Distinguish between Peripheral and CNS Sources of pTau217



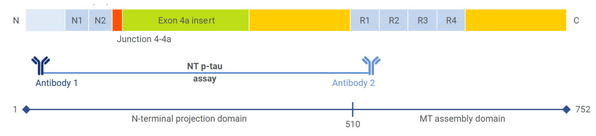
<20% comes from the brain and is indicative of accumulation of Tau in the brain

>80% is secreted by peripheral organs and not reflective of Tau status in the brain

(A) Brain-Derived pTau217 Assay detects the brain-specific pTau217 isoform with an antibody against a unique exon4-5 junction



(B) Amino-Terminal (NT) pTau217 Assay detects both peripheral and brain-derived pTau217 isoforms



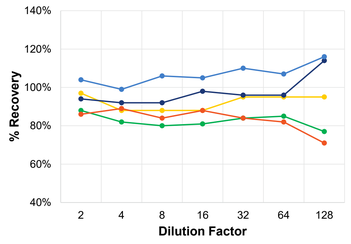
NULISAqpcr™ BD-pTau217 Assay Demonstrates Robust Assay Performance with High Precision, Reproducibility and Recovery with no Significant Interference

Precision and Reproducibility*

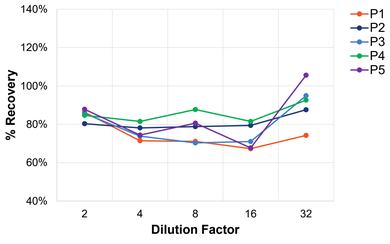
Sample	Avg Conc (pg/mL)	Total % CV	Inter-Assay %CV	Intra-Assay %CV
PR-S1	0.49	15.1	7.6	13.1
PR-S2	0.71	19.8	12.3	15.6
PR-S3	1.21	7.1	4.0	5.9
PR-S4	2.58	8.8	4.7	7.4
PR-S5	14.42	10.3	4.8	9.1
PR-S6	146.33	15.8	12.8	9.4

*Assessed from 6 samples run in 6 replicates across 3 days on 3 ARGOs with 1 reagent lot

Dilutional Linearity



Parallelism



Analytical Specificity and Interference

Substance	K2EDTA Plasma (n=5)	CSF (n=5)
Biotin	3510 ng/mL	3510 ng/mL
Conjugated Bilirubin	475 µM	1 µM
Unconjugated Bilirubin	684 µM	1 µM
Hemoglobin	2 g/L	0.2 g/L
Triglycerides	500 mg/dL	40 mg/dL
HAMA	1200 ng/mL	2.4 ng/mL
Rheumatoid Factor	600 IU/mL	12 IU/mL

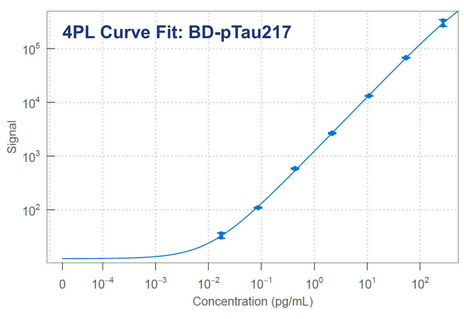
Dil. Linearity	P1	P2	P3	P4	P5
Starting conc (pg/mL)	29.09	30.60	27.27	31.89	31.26
Recovery range	88-97%	92-98%	99-110%	80-88%	82-89%
Parallelism*	**P1***	**P2**	**P3**	**P4**	**P5***
Starting conc (pg/mL)	5.08	4.87	5.52	4.22	3.02
Recovery range (*Excl. <LLoQ)	71-86%	78-88%	70-95%	82-93%	74-106%

Spike and Recovery

		K2EDTA Plasma (n=5)		CSF (n=5)	
Targeted Spike Conc (pg/mL): Plasma	Targeted Spike Conc (pg/mL): CSF	Average Recovery	Recovery Range	Average Recovery	Recovery Range
1	5	108%	99-119%	105%	97-115%
10	50	113%	108-124%	111%	102-120%
100	680	125%	119-136%	101%	95-113%
	All levels	115%	99-136%	106%	95-120%

$$\%\ \text{Recovery} = \frac{\text{Meas.Conc}_{\text{Spiked Donor Sample}} - \text{Meas.Conc}_{\text{Unspiked Donor Sample}}}{\text{Meas.Conc}_{\text{Spiked SD buffer}}} \times 100$$

NULISAqpcr™ BD-pTau217 Assay Detects Brain-Derived pTau217 with High Sensitivity and Specificity



Analytical Sensitivity and Dynamic Range*

	Analytical	Functional
LOD	0.016 pg/mL	0.08 pg/mL
LLoQ	0.06 pg/mL	0.30 pg/mL
ULoQ	2,931 pg/mL	14,655 pg/mL
Dynamic Range	0.06-2,931 pg/mL	0.30-14,655 pg/mL

*Assessed from 30 runs across 5 ARGOs with 1 reagent lot

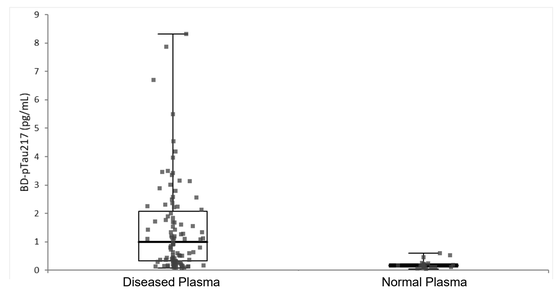
Cross Reactivity with Related Tau Isoforms

	Total Tau	pTau181	pTau205	pTau212	pTau231
Conc (pg/mL)	128	92	92	92	46

High Detectability with the NULISAqpcr™ BD-pTau217 Assay Enables Distinction between Normal and Diseased Plasma Samples

Reference Range and Detectability

	Normal Plasma (n=20)	Diseased Plasma (n=100)
Average (pg/mL)	0.201	1.453
Min (pg/mL)	0.04	0.07
Max (pg/mL)	0.61	8.33
Detectability	99.2%	
	Normal CSF (n=6)	**Diseased CSF (n=36)**
Average (Range, pg/mL)	3.35	3.028
Min (pg/mL)	0.23	0.22
Max (pg/mL)	6.01	22.30
Detectability	74%	



NULISAqpcr™ BD-pTau217 Assay Provides a Valuable Tool for Monitoring Neuropathological Changes in Blood

- Analysis of BD-pTau217 in plasma provides a more accurate reflection of pTau217 pathology in the brain
- The NULISAqpcr BD-pTau217 assay enables highly sensitive and specific detection of BD-pTau217 for early identification and monitoring of AD-related pathology

[1]*Feng, W., Beer, J.C., Hao, Q. 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*

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