Validation of an Ultra-sensitive Method for Phospho-Tau 217 (pTau-217) Quantitation in Human Plasma, Serum, and CSF

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ALZHEIMER’s DISEASE AND ASSOCIATED PROTEIN BIOMARKERS

1. Prevalence and Projection

Currently impacts over 6.5 million families in the U.S.Expected to rise to nearly 13 million by 2050, highlighting an urgent need for effective treatments.

2. Pharmaceutical Research Efforts

Major companies like Eisai, Biogen, and Eli Lilly are leading the charge; focused on developing treatments for the early stages of Alzheimer’s, despite the absence of a cure.

3. Advancements in Early-stage Treatment

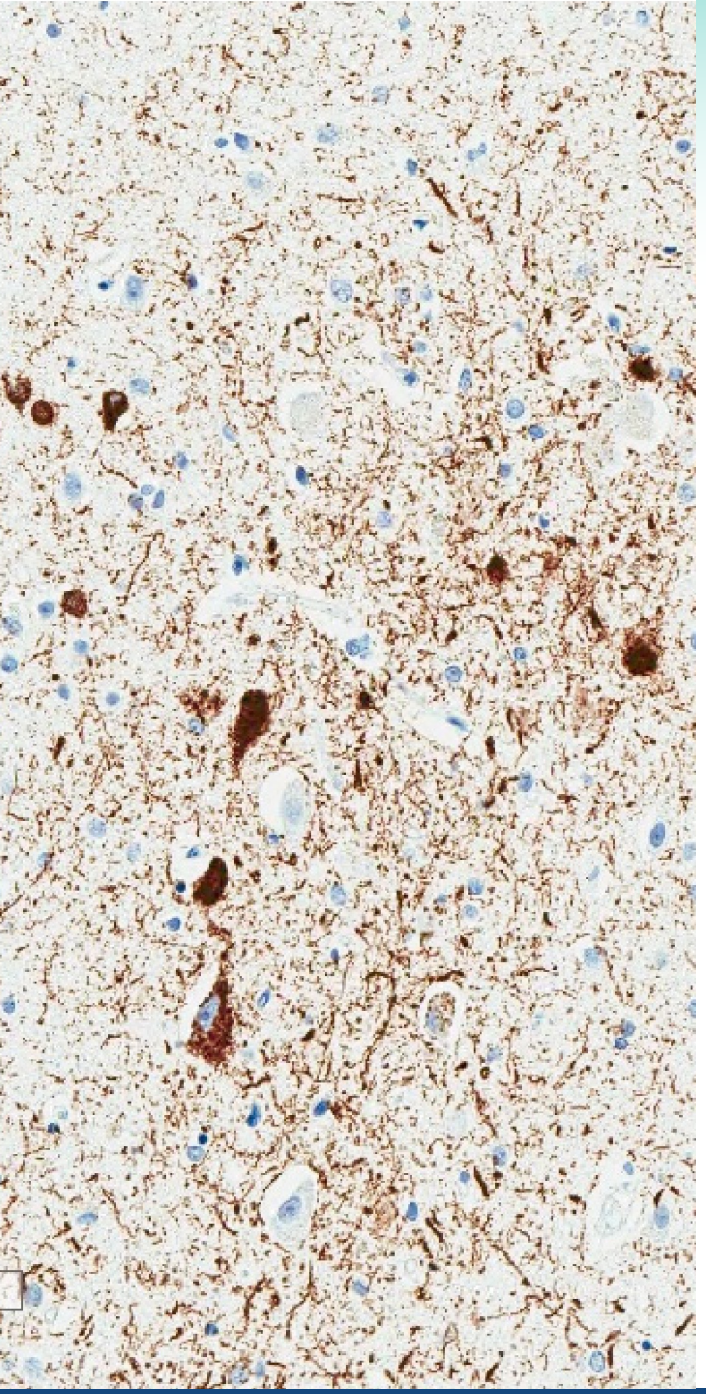
Significant investments into R&D and clinical trials; aimed at slowing the progression of Alzheimer’s in its early stages, potentially improving quality of life.

4. Companion Diagnostics

Innovative approach using biomarkers for monitoring treatment response; enables more personalized treatment plans, increasing the potential for successful outcomes.

5. Impact of Companion Diagnostics on Alzheimer’s Treatment

Could revolutionize the effectiveness of new Alzheimer’s drugs; offers a method to identify patients who are most likely to benefit from specific treatments.



Alzheimer's disease biomarkers

pTau-217, pTau-181 and other phosphorylated tau have been served as biomarkers for AD. Other promising AD biomarkers are Aβ42/Aβ40, GFAP, Nf-L, as well as VAMP-2, SNAP-25, TDP43, APOE4, etc.

Superior Early Detection Capabilities of pTau-217

pTau-217 stands out for its ability to detect Alzheimer's disease (AD) earlier than pTau-181, enabling more prompt interventions. This early detection is critical for differentiating AD from other neurodegenerative conditions.

Enhanced Diagnostic Precision and Clinical Utility of pTau-217

With a higher dynamic range, pTau-217 surpasses pTau-181 in diagnosing and monitoring AD. Its use in blood tests could significantly advance AD research and improve patient care by facilitating the development of targeted treatments.

pTau-217 as a superior AD Biomarker

pTau-217 shows significantly higher levels in CSF for Alzheimer's patients vs. controls

7.3-8.6 fold increase for pTau-217 vs. 3.6-3.7 for pTau-181, This suggests a greater increase in pTau-217 relative to total tau (t-tau) and amyloid-beta (Aβ) pathology

pTau-217 correlates more strongly with PET measures of tau & amyloid pathology

Making it a more accurate biomarker to distinguish AD from other neurodegenerative disorders

pTau-217 shows stronger associations with [18F]flortaucipir retention in AD-affected brain regions

Across various disease stages compared to pTau-181

pTau-217 has a higher dynamic range as a biomarker for Alzheimer's disease due to its stronger correlation with pathological markers

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RATIONALE AND PURPOSE:

- Both blood-based (plasma and serum) and CSF pTau-217 have been shown to be able to discriminate early to mild AD from non-AD neurodegenerative disorders and healthy controls with high sensitivity and specificity.
- Blood-based pTau-217 assay has been reported as accurate as CSF in AD diagnosis.
- The blood-based pTau-217 assay offers several benefits over the CSF method. It is significantly less invasive, more cost-efficient, and provides easier and more accessible sample collection.
- Currently, we are presenting a fully validated, highly sensitive pTau-217 assay. This assay has been compared across three different types of samples—human plasma, serum, and CSF—utilizing ALZpath pTau 217 assay kits on the Quanterix Simoa HD-X platform.

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Representative standard curve data in method development

Assay	STD9	STD8	STD7	STD6	STD5	STD4	STD3	STD2	STD1
Date	0.00244	0.00488	0.00977	0.0391	0.156	0.625	2.5	5	10
	(pg/mL)	(pg/mL)	(pg/mL)	(pg/mL)	(pg/mL)	(pg/mL)	(pg/mL)	(pg/mL)	(pg/mL)
2-Jun-23	0.0031	0.00521	0.00971	0.0377	0.15	0.608	2.53	5.06	10.7
	0.00218	0.00578	0.00945	0.0386	0.159	0.599	2.54	4.95	9.91
Intrarun Mean	0.00264	0.0055	0.00958	0.0382	0.155	0.604	2.54	5.01	10.3
Intrarun SD	0.000651	0.000403	0.000184	0.00064	0.00636	0.00636	0.00707	0.0778	0.559
Intrarun %CV	24.7	7.3	1.9	1.7	4.1	1.1	0.3	1.6	5.4
Intrarun %Bias	8.2	12.7	-1.9	-2.3	-0.6	-3.4	1.6	0.2	3
n	2	2	2	2	2	2	2	2	2

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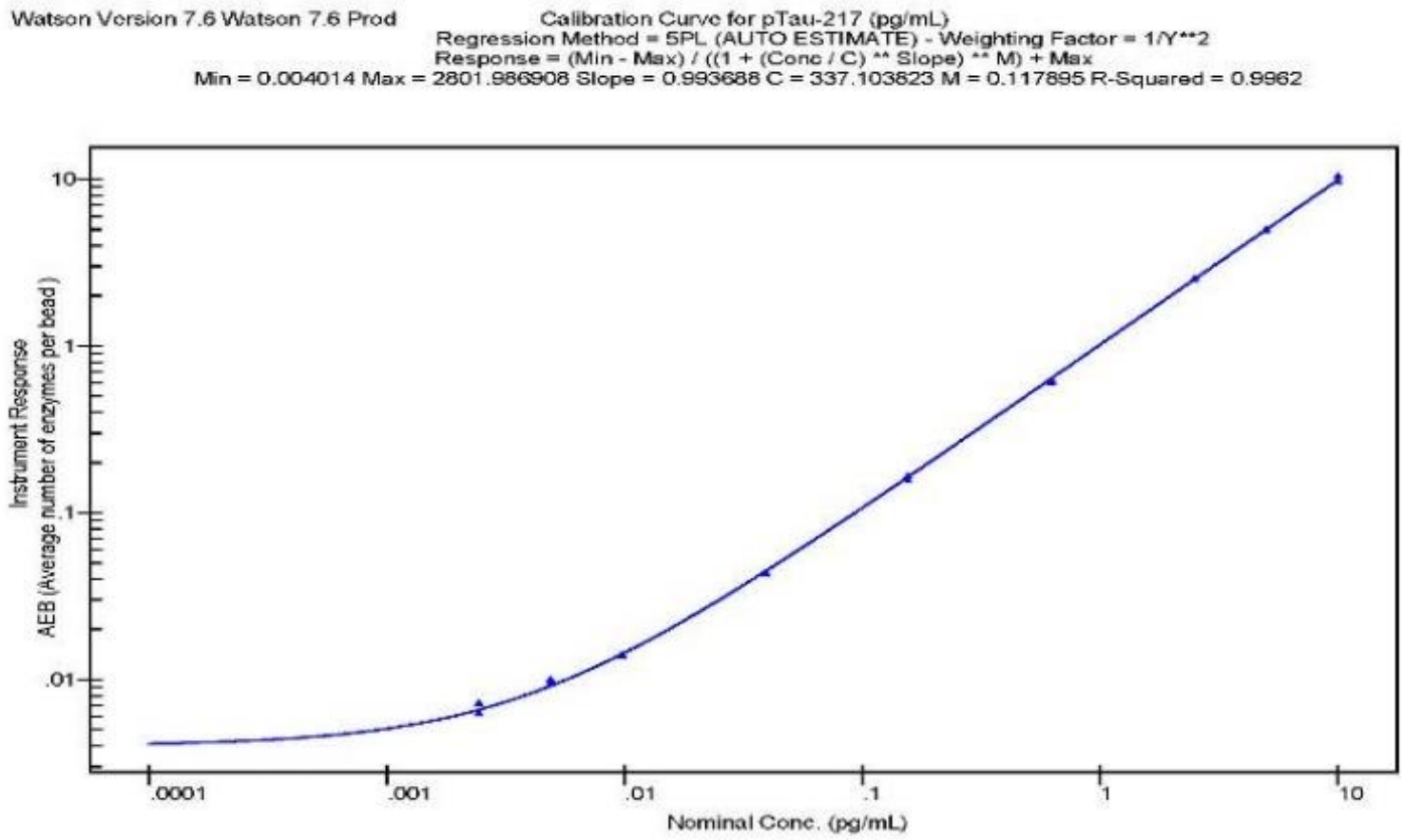
Representative Calibration Curve of pTau-217



Accuracy and Precision (A&P) - all Standards in pTau-217 Validation

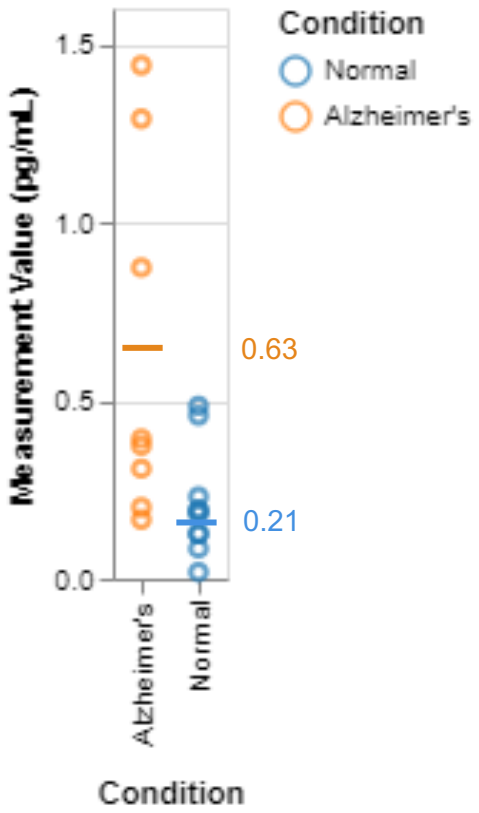
Standard	STD8/0.00977 (pg/mL)	STD7/0.0195 (pg/mL)	STD6/0.0391 (pg/mL)	STD5/0.156 (pg/mL)	STD4/0.625 (pg/mL)	STD3/2.50 (pg/mL)	STD2/5.00 (pg/mL)	STD1/10.0 (pg/mL)
Mean Concentration (pg/mL)	0.00979	0.0195	0.0393	0.158	0.632	2.47	4.96	10.1
Inter-run SD	0.000962	0.00138	0.00205	0.00664	0.0171	0.0809	0.109	0.236
Inter-run %CV	9.8	7.1	5.2	4.2	2.7	3.3	2.2	2.3
Inter-run %Bias	0.2	0.0	0.5	1.3	1.1	-1.2	-0.8	1.0
number	12	10	12	12	12	12	12	12

Intra and Inter-Run A&P of Quality Controls of pTau-217 in Human Plasma, Serum and CSF

QC	Concentrations (pg/mL)	Intra-Run Precision (%CV)	Intra-Run Relative Error (%RE)	Inter-Run Precision (%CV)	Inter-Run Relative Error (%RE)
ULOQ	10.0	2.8	-1.4	4.9	1.5
HQC	2.50	2.8	-2.3	4.7	0.6
MQC (Plasma)	0.504	3.9	-4.3	4.0	-0.1
LQC (Plasma)	0.146	7.8	-4.2	6.8	-0.1
MQC (Serum)	0.342	3.4	2.0	2.9	-0.3
LQC (Serum)	0.130	2.6	-0.1	7.7	1.2
MQC (CSF)	9.62	3.5	1.1	9.0	-0.2
LQC (CSF)	3.45	2.4	1.4	6.3	-0.2
LLOQ	0.00977	6.0	2.4	6.2	-1.3

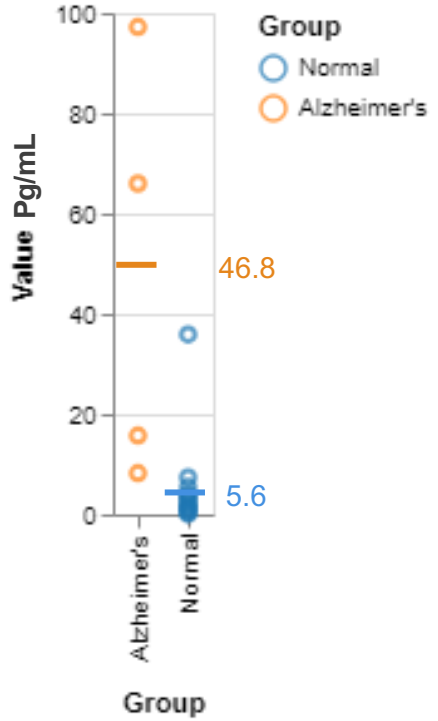
pTau-217 Detectability in Human Plasma

Plasma	Sample ID	Mean	%CV
Normal	P1	0.19	5.2
	P2	0.195*	22.5*
	P3	0.0183*	27.8*
	P4	0.485	15.5
	P5	0.181	0
	P6	0.231	2.8
	P7	0.458	5.4
	P8	0.126	12.4
	P9	0.085	2.7
	P10	0.126	4.5
AD	AD1	0.309	1.4
	AD2	0.373	7.2
	AD3	1.29	3.3
	AD4	0.873	2.3
	AD5	1.44	1
	AD6	0.2	7.4
	AD7	0.166*	44.7*
	AD8	0.393	0.4



pTau-217 Detectability in Human CSF

Human CSF	Sample ID	Mean	%CV
Normal	C1	4.00	4.6
	C2	5.49	1.8
	C3	3.42	4.1
	C4	1.39	11.7
	C5	1.46	3.4
	C6	7.37	9.2
	C7	3.39	7.9
	C8	0.438	11.0
	C9	2.31	2.5
	C10	0.563	4.4
	C11	1.99	1.4
	C12	35.9	2.0
	C13	0.0201*	NA
AD	AD1	8.21	0.3
	AD2	97.2	1.9
	AD3	15.7	2.7
	AD4	66.0	2.7



pTau-217 Assay Platform Comparison

Platform	Method Owner	Matrix	Sensitivity (LLOQ) (pg/mL)	Sensitivity (LLOD) (pg/mL)	Std Curve Dynamic Range (pg/mL)	MRD	Sample Vol. (µl)	Relative Cost	Reference
MSD	Lilly	Plasma/CSF	?	0.48	?	2/4	50	Low	Palmqvist, et al. 2020, JAMA
MSD	Lilly	Plasma	?	0.15	?	2	50	Low	Groot, et al. 2022, Alzheimer's Dis & Res
Simoa	Janssen	CSF	0.040	0.002	0.041-30	8	50	Low	Triana-Baltzer, et al. 2020, J Alz. Dis.
Simoa	Janssen	Plasma/Serum	0.037	0.005	0.037-10	2	172	Low	Triana-Baltzer, et al. 2021, Alz Dement.
Simoa	Janssen	Plasma	?	0.013	?	2.6	50	Low	Groot, et al. 2022, Alzheimer's Dis & Res
Simoa	Janssen	CSF	?	0.013	?	8	50	Low	Groot, et al. 2022, Alzheimer's Dis & Res
IP-LC/MS	C2N	Plasma/CSF	0.05	?	?	?	1000	High	Janelidze, et al., 2023, Brain
MSD	Meso Scale Diagnostics	Plasma/Serum/CSF	5.90	0.880	5.90-2400	Neat	25	Low	MSD website
Simoa	Frontage ALZpath Assay Kit	Plasma/Serum	0.00977	0.00244	0.00977-10.0	3	80	Low	Frontage, Current Presentation
Simoa	Frontage ALZpath Assay Kit	CSF	0.00977	0.00244	0.00977-10.0	20	8	Low	Frontage, Current Presentation

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CONCLUSIONS:

- Validation of the ALZpath pTau 217 assay has been successfully performed using Simoa HD-X platform in human plasma, serum as well as in CSF.
- pTau-217 Simoa assay validation demonstrated that it is a novel approach with ultra sensitivity, high accuracy, and assay robustness.
- Together with the established sensitive pTau-181, GFAP, and Nf-L assays, these assays are available for application in clinical trials and the early diagnosis of neurodegenerative diseases.


FRONTAGE
QUESTIONS?

FRONTAGE

THANK YOU!

FRONTAGE

BACKUP SLIDES

Validation of an Ultra-sensitive Method for Phospho-Tau 217 (pTau-217) Quantitation in Human Plasma, Serum, and CSF

Hemolyzed Blood and Lipid Interference for pTau-217 in Human Plasma, Serum and CSF

Matrix	Condition	Pass/Fail
Plasma	1.0% hemolyzed blood	Pass
	3.0% hemolyzed blood	Pass
	150 mg/DL Lipid	Pass
	300 mg/DL Lipid	Pass
Serum	1.0% hemolyzed blood	Pass
	3.0% hemolyzed blood	Pass
	150 mg/DL Lipid	Pass
	300 mg/DL Lipid	Pass

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Dilution Linearity of pTau-217 in Human Plasma, Serum and CSF

Matrix	MRD	Dilution Factor Pass
Plasma	3X	3X- 48X
Serum	3X	3X- 12X
CSF	20X	20X- 320X

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Short-Term Stability Test of pTau-217 in Human Plasma, Serum and CSF

Matrix	Condition	Pass/Fail
Plasma	Refrigerator (20 hrs)	Pass
	Benchtop (6 hrs)	Pass
	Freeze/Thaw (5X)	Pass
Serum	Refrigerator (20 hrs)	Pass
	Benchtop (6 hrs)	Pass
	Freeze/Thaw (5X)	Pass
CSF	Refrigerator (20 hrs)	Pass
	Benchtop (6 hrs)	Pass
	Freeze/Thaw (5X)	Pass