An Automated Assay for Precise and Sensitive Quantification of pTau-217 in Plasma and Cerebrospinal Fluid

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Summary

Accurate quantification of fluid biomarkers in blood and cerebrospinal fluid is becoming increasingly important for diagnosis, prognosis, and monitoring of neurodegenerative disease. Growing evidence suggests a strong diagnostic utility for a phosphorylated isoform of the Tau protein (pTau-217) as a specific biomarker for Alzheimer's disease. However, many existing methods for detecting and quantifying pTau-217 in CSF and blood are time-consuming, expensive, and/or technically complex.

This study describes the analytical validation of a novel and fully automated immunoassay for measuring pTau-217 in plasma and CSF. The assay utilizes a microfluidics approach (Ella™) and highly specific pTau antibodies to measure up to 72 samples within 90 minutes, using 25 ul sample volume or less. The limit of detection (defined as three standard deviations above the blank) was determined as 0.09 pg/mL, and the limits of quantitation were determined as 0.31-1,200 pg/mL. Intra-assay precision was measured at less than 5% CV using both high- and low-level controls (n=16 replicates per control), and inter-assay precision averaged at 6.3% and 10.2% CV, respectively (n=46 per control). Dilutional linearity experiments demonstrated good assay parallelism with recovery rates of 97-101% for plasma and 92-99% for CSF samples. Spike/recovery experiments demonstrated acceptable accuracy with mean recovery values of 86-94% (plasma) and 112-137% (CSF). To assess the biomarker utility of the assay, endogenous plasma levels of pTau-217 were measured in a cohort of healthy control donors and Alzheimer's disease patients. Results showed strong elevation of both plasma and CSF pTau-217 in ATN positive AD samples (p<0.001, Mann-Whitney test), consistent with previous reports.

In conclusion, this study supports the utility of an automated assay for fast and sensitive pTau-217 quantification in plasma and CSF samples of AD patients, while demonstrating robust analytical performance characteristics.

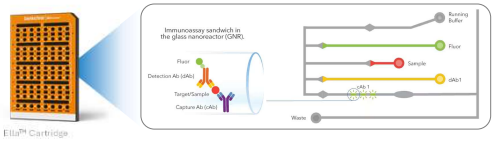
The Simple Plex pTau-217 ALZpath assay

Miniaturization and automation of a pTau-217 assay within a closed cartridge

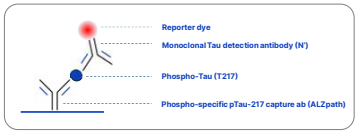
Automated workflow

- Sandwich immunoassay occurs within a microfluidic circuit
- Hands-free
- 25 ul volume required per sample
- 4-log dynamic range
- Results in <90 minutes
- Triplicate results per sample
- Automatic fitting to a built-in standard curve
- Can be plexed with additional analytes

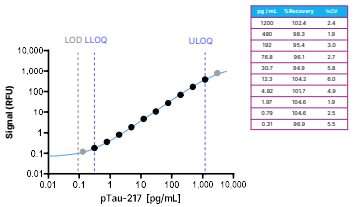
Microfluidics circuit design



Phospho-specific immunoassay configuration



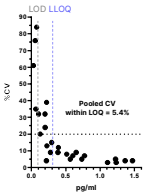
Dynamic range/ Limits of Quantitation



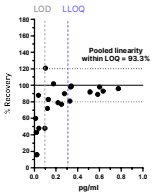
pg/mL	%Recovery	%CV
1200	102.4	2.4
480	98.3	1.9
192	95.4	3.0
76.8	98.1	2.7
30.7	94.9	5.8
12.3	104.2	6.0
4.92	101.7	4.9
1.97	104.6	1.9
0.79	104.6	2.5
0.31	96.9	5.5

Standard curve and dynamic range of the Simple Plex pTau-217 ALZpath assay. Upon running curves on multiple cartridges and cartridge lots, all points within the Limit of Quantitation (LOQ) recovered 80-120% to the target and had a coefficient of variance (CV) lower than 20%. Curve was derived using a 4PL fitting. The Limit of Detection (LOD) is calculated as three standard deviation above the mean of the background signal.

Precision around LLOQ



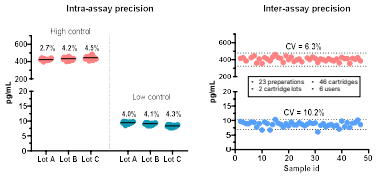
Parallelism around LLOQ



Further confirmation of the lower limit of quantitation (LLOQ) was carried out by serially diulting plasma samples containing the pTau-217 antigen at levels above and below the LLOQ threshold. All samples within the LOQ range had a recovery of 80-120% and CV of <20%.

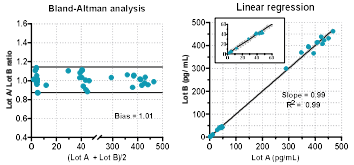
Analytical performance

Precision



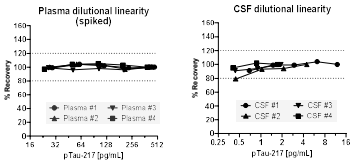
Left: low and high control samples were loaded onto Ella cartridges in replicates (n=16 wells per condition) and assessed across three separate cartridge lots. All conditions yielded CV<5%, indicating high intra-assay precision. Right: high (red) and low (blue) control preparations were made and run by multiple users, across multiple cartridges, cartridge lots, and Ella analyzers. The overall results demonstrate high inter-assay precision (CV<12%). Dotted lines represent 80-120% recovery limits from the mean concentration value.

Lot to lot reproducibility



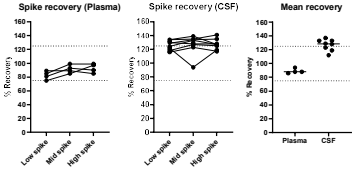
Cartridge lot-to-lot reproducibility was assessed by running samples with concentration values spanning the dynamic range of the assay across two independent cartridge lots. The results demonstrate acceptable equivalence of concentrations values as evident by Bland-Altman (left) and linear regression analysis (right).

Dilutional linearity



Dilutional linearity was assessed by serially diluting samples at a 2-fold dilution scheme. All plasma and CSF samples exhibited good parallelism properties with mean recovery rates within an acceptable range of 80-120%. The mean recovery rates were 100% for plasma, and 94% for CSF.

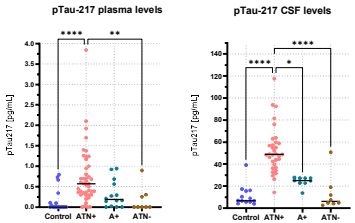
Accuracy



Accuracy was assessed by a spike/recovery experiment at three different spiked analyte concentrations in plasma and CSF. Recovery of spiked plasma (EDTA) samples was at 89%, and recovery of spiked CSF samples averaged at 127%.

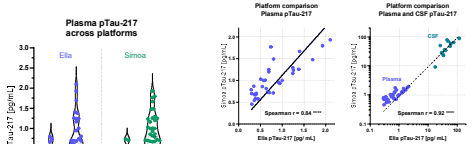
pTau-217 measurement in a clinical cohort

Plasma and CSF pTau-217 in healthy controls and AD patients



Plasma (left) and CSF (right) pTau-217 levels were compared between healthy control donors and clinically diagnosed Alzheimer's disease patients. ATN positive patients (ATN+) had significantly elevated plasma and CSF pTau-217 levels when compared to both healthy controls as well as ATN negative patients. Significant difference in CSF pTau levels was also found upon comparison of ATN+ and Amyloid positive (A+) patient groups. Statistical analysis was performed using the Kruskal-Wallis test, followed by Dunn's multiple comparisons post-hoc test.

Correlation with a bead-based platform



Left: comparison of pTau-217 plasma levels as measured side-by-side using the Simple Plex pTau-217 ALZpath assay and a bead-based platform (Simoa pTau-217 ALZpath assay). Both platforms demonstrated significant (p<0.001) elevation in AD patient samples (ATN+). Right: correlation analysis across the two platforms.

Conclusions

- Utilizing well-characterized and specific antibodies, we describe the validation of a novel pTau-217 assay on a microfluidic platform, enabling its automated analysis in both plasma and CSF.
- The novel Simple Plex pTau-217 ALZpath assay possesses high degree of precision (single digit %CV), robust performance, and good correlation with an existing commercial assay.
- In accordance with published literature, elevated pTau-217 levels were found in plasma and CSF of Alzheimer's disease patients, supporting a strong biomarker utility for the assay.
- Taken together, these results support the applicability of this novel immunoassay as a sensitive, precise, fast, and simple-to-use benchtop research strategy for measuring pTau-217 protein in biofluids.

References

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