NEW HIGH-THROUGHPUT, FULLY AUTOMATED IMMUNOASSAY FOR PLASMA P-TAU217

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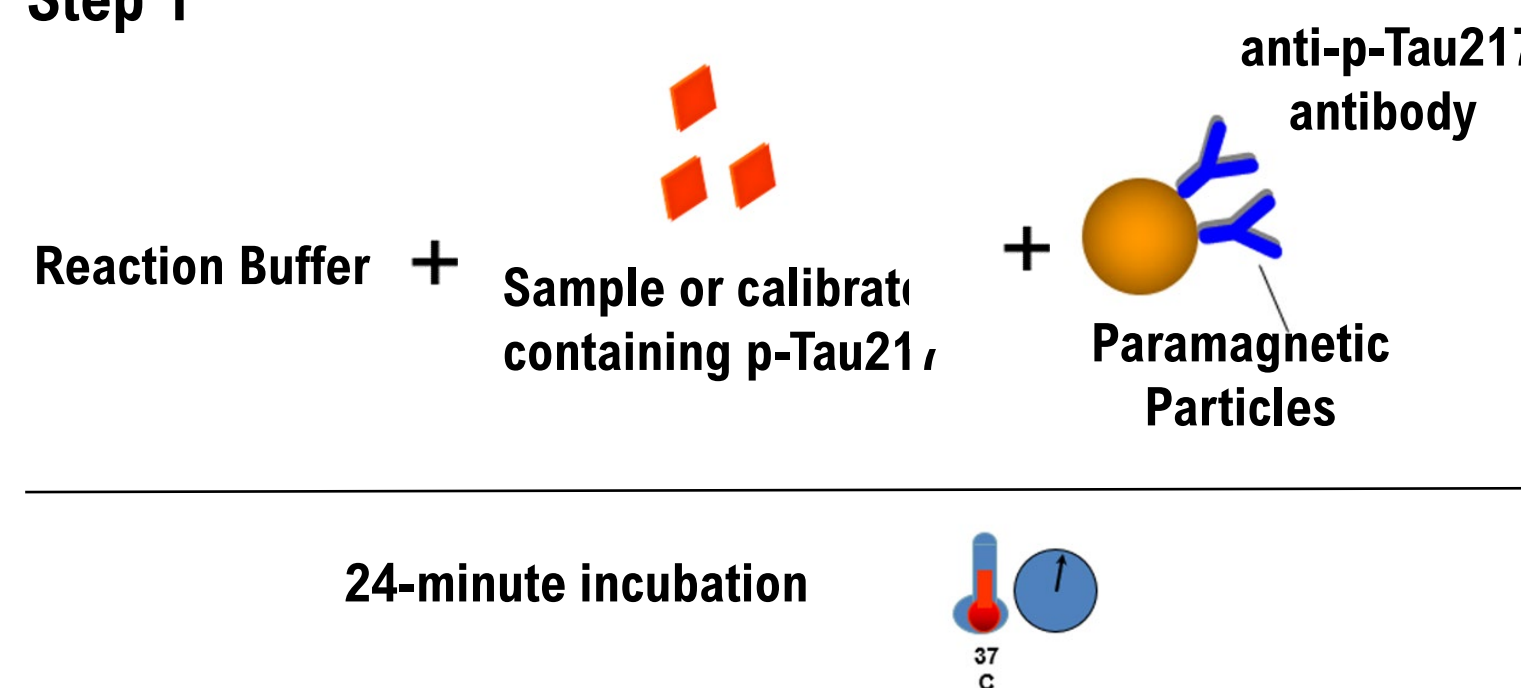Beckman Coulter, Inc., Brea, CA USA

BACKGROUND

Hyperphosphorylated Tau is a pathologic hallmark of the neurofibrillary tangles in Alzheimer's Disease (AD). Plasma p-Tau217 levels have shown correlation with amyloid pathology, having implications for the diagnosis and prognosis of neurodegenerative diseases such as AD.[1] Several assays for p-Tau217 in plasma have been previously described; however, there is a need to make this test more accessible to clinical laboratories around the world.[2] We describe the development to date of a precise and highly sensitive plasma p-Tau217 assay developed for the high-throughput, fully automated, Beckman Coulter DxI 9000 Immunoassay Analyzer*.
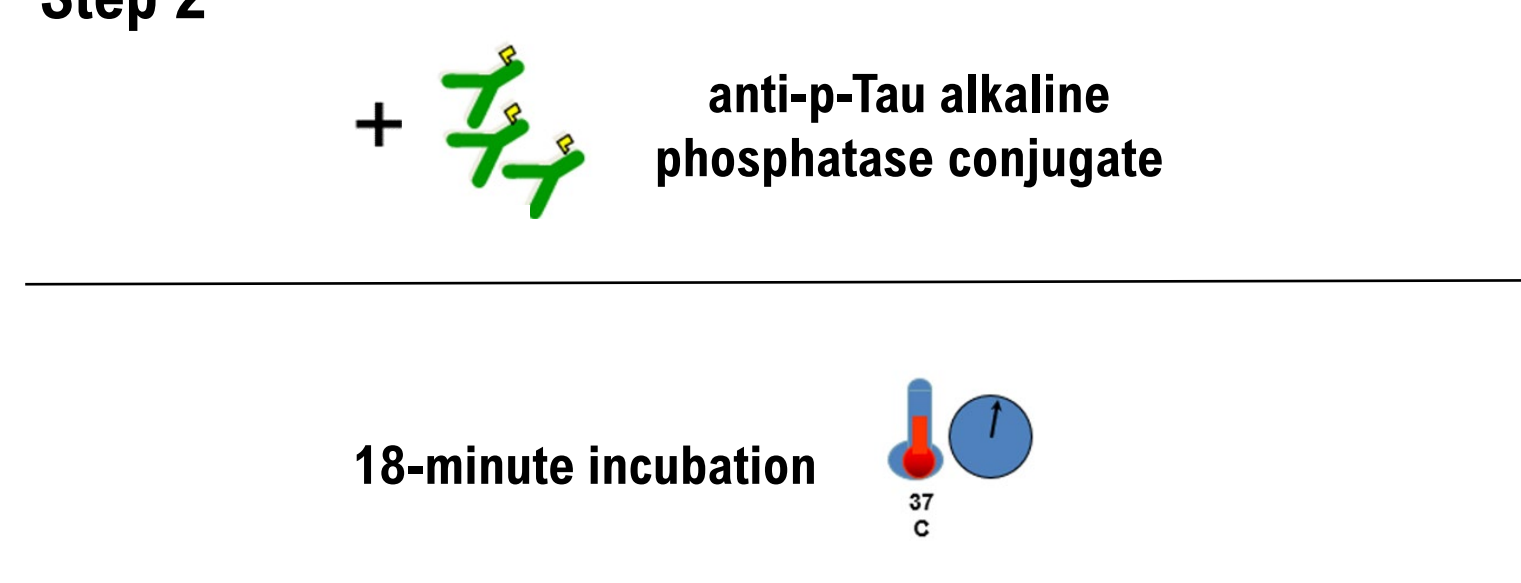
METHODS

Assay Format

The prototype p-Tau217 assay is a two-step sandwich assay utilizing an anti-tau monoclonal (MAb) antibody/alkaline phosphatase conjugate along with an anti-p-Tau217 MAb bound to paramagnetic particles. EDTA plasma sample, reaction buffer, and MAb-coated particles are incubated and washed. This is followed by addition of the ALP-conjugate with another incubation and wash step before a chemiluminescent substrate is added. The light that is generated is directly proportional to the p-Tau217 concentration in the sample. The assay time to first results is ~48 minutes.
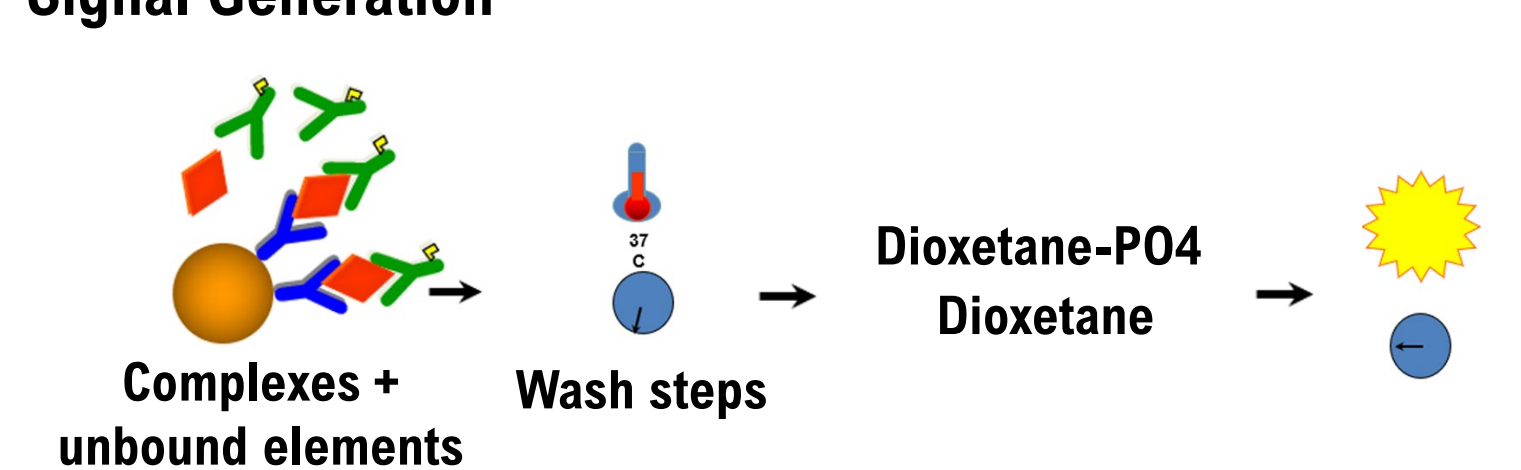

Step 1

anti-p-Tau217 antibody

Reaction Buffer + Sample or calibrator containing p-Tau217 + Paramagnetic Particles

24-minute incubation

Step 2

+ anti-p-Tau alkaline phosphatase conjugate

18-minute incubation

Signal Generation

Complexes + unbound elements → Wash steps → Dioxetane-PO4 Dioxetane →

Cross Reactivity and Interfering Substances

Studies were performed to assess analytical specificity through responses to known potential immunoassay endogenous interferents, common drugs for Alzheimer's Disease, and non-phosphorylated Tau.

The study was run on a single DxI 9000 Analyzer, with one reagent and calibrator lot. Interference and cross reactivity was assessed on a single pooled K2 EDTA plasma sample with ~80 fg/mL endogenous p-Tau217. Stock solutions of potential interferents were spiked into the patient sample to the target concentrations and control samples were prepared in the same manner using the solvent without the potential interferent. Each sample was analyzed in triplicate.

Comparison Study

A method comparison study was completed to compare the Beckman Coulter prototype p-Tau217 assay on DxI 9000 Analyzer to the Quanterix Simoa ALZpath p-Tau217 Research Use Only (RUO) assay for K2 EDTA plasma sample types. Method comparison studies were performed on a single DxI 9000 Analyzer and a single Quanterix Simoa instrument.

38 K2 EDTA plasma samples containing p-Tau217 concentrations spanning the analytical measuring range of the assay were tested. All samples were tested in replicates of two, on one reagent and calibrator lot. A Passing-Bablok linear regression was fit between the 2 methods and corresponding Bland-Altman % bias plot was generated to model the alignment between methods.

Linearity

A study was performed to assess the linearity of the Beckman Coulter p-Tau217 assay on the DxI 9000 Analyzer.

Samples covering the full analytical measuring range of each assay were used for the linearity determination. K2 EDTA plasma sample types were evaluated. A native K2 EDTA sample containing a concentration at the low end of the measuring interval was obtained.

For the linearity study, a pool of high p-Tau217 concentration K2 EDTA Alzheimer's Disease positive samples was generated along with a pool of low concentration K2 EDTA plasma from a panel of cognitively normal patients. These low and high concentration pools were then mixed in 3 additional pre-defined ratios and all 5 samples then analyzed in quadruplicate on a single reagent and calibrator lot on a single DxI 9000 Analyzer.

The data were analyzed based on CLSI EP06-ED2 guidance. The observed concentrations from the low and high concentration base pools were used to determine the expected concentration based on the known mixture ratios. A weighted linear model was fit to a plot of the observed measured concentration relative to the expected linear response concentrations. The percent non-linearity was calculated as the percent difference between the mean observed concentration verses the estimated concentrations from the linear fit.

Imprecision

A study was performed to assess the imprecision of the Beckman Coulter prototype p-Tau217 assay on the Access 2 and DxI 9000 Analyzers. The study was run on a single reagent and calibrator lot, on a single DxI 9000 Analyzer. Native K2 EDTA plasma samples with endogenous p-Tau217 concentrations spanning the range of the assay were measured across each of 3 days, with 2 runs per day and 5 replicates per run yielding a total of 30 replicates per sample.

Variances and CV% were calculated for Within-run, Between-run, Day-to-Day, and Within-laboratory (Total) variance components for each sample.

Discrimination

A study was performed to assess the discrimination between amyloid positive and negative populations within a cohort of cognitively impaired individuals. Amyloid status was determined by the β-Amyloid 1-42 / 40 ratio, as measured on the Meso Scale Diagnostic Aβ42 and Aβ40 assays using a cutoff of 0.065 as described in previously published study.[3]

The study was run on one DxI 9000 Analyzer, with one reagent and calibrator lot. Thirty-eight (38) K2 EDTA plasma samples, consisting of 28 Alzheimer's Disease (AD) diagnosed patient samples (MMSE score range 18-25) and 10 patients with mild cognitive impairment (MCI) (MMSE range 24-27) were evaluated using the Beckman Coulter prototype p-Tau217 assay in replicates of two.

Detection Capability

Studies were performed to estimate the Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ) for the Beckman Coulter prototype p-Tau217 assay on the DxI 9000 Analyzer system.

Analysis was performed on data generated from 11 individual calibrations. The observed variance of the zero-analyte calibrator was used to model the potential LoB relative to each calibration. LoD and LoQ estimates utilized the observed signal variance of a panel of native K2 EDTA patient samples which was applied to model the expected concentration variance at pre-determined concentrations across each of the calibrations.

RESULTS†

Analytical Specificity (Interference and Cross-reactivity)

Substance	Test Concentration	% Difference from Control
Acetaminophen	0.156 mg/mL	<5%
Bilirubin (conjugated)	0.4 mg/mL	<5%
Bilirubin (unconjugated)	0.4 mg/mL	<5%
Hemoglobin	10 mg/mL	<5%
Heparin	3.3 U/mL	<5%
Human Serum Albumin	150 mg/mL	<5%
Ibuprofen	0.219 mg/mL	<5%
Triolein	15 mg/mL	<5%
Aripiprazole	1800 ng/mL	<5%
Donepezil	300 ng/mL	<5%
Galantamine	500 ng/mL	<5%
Memantine	450 ng/mL	<5%
Non-phosphorylated Tau	5 ng/mL	<5%

Figure 1 No cross reactivity to non-phosphorylated Tau detected. No significant interference detected among AD drugs (including Donepezil, Memantine, Aripiprazole, Galantamine, and Rivastigmine), common drugs or endogenous protein and lipid-based interferents.
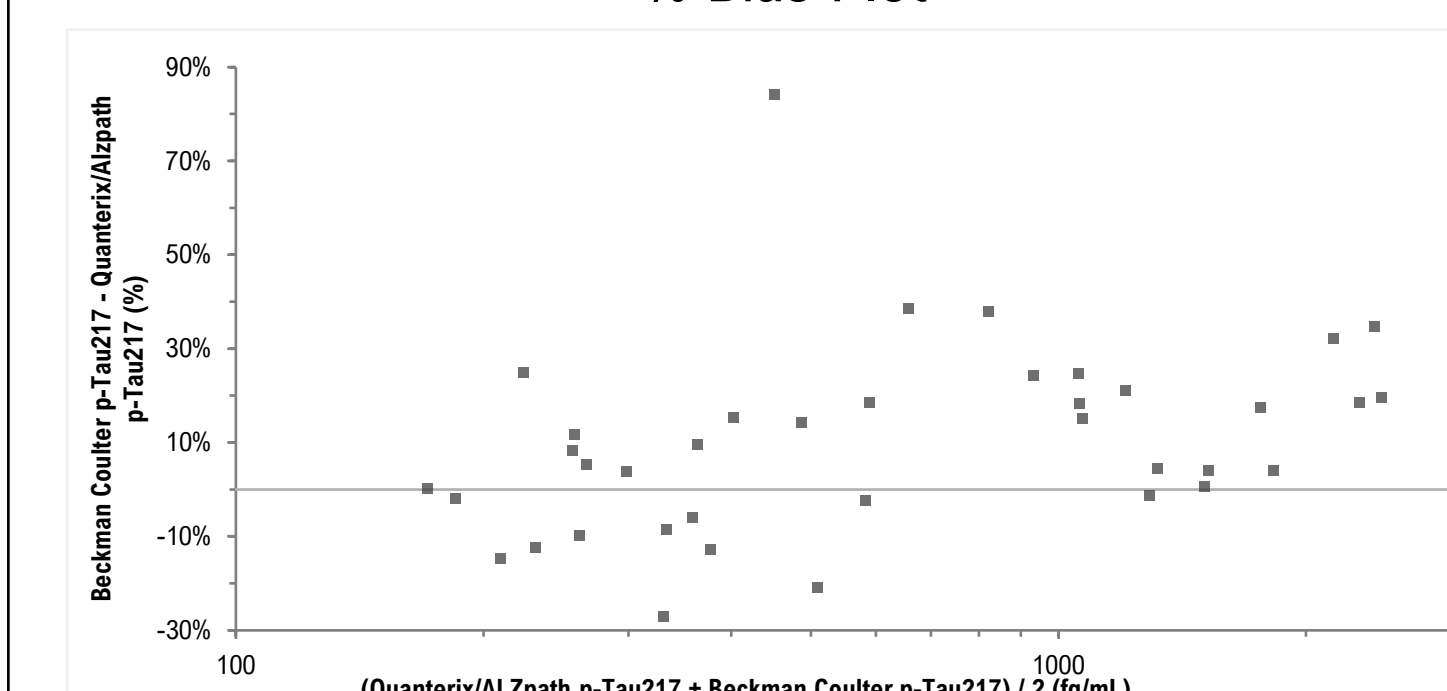
Method Comparison

Passing-Bablok Regression
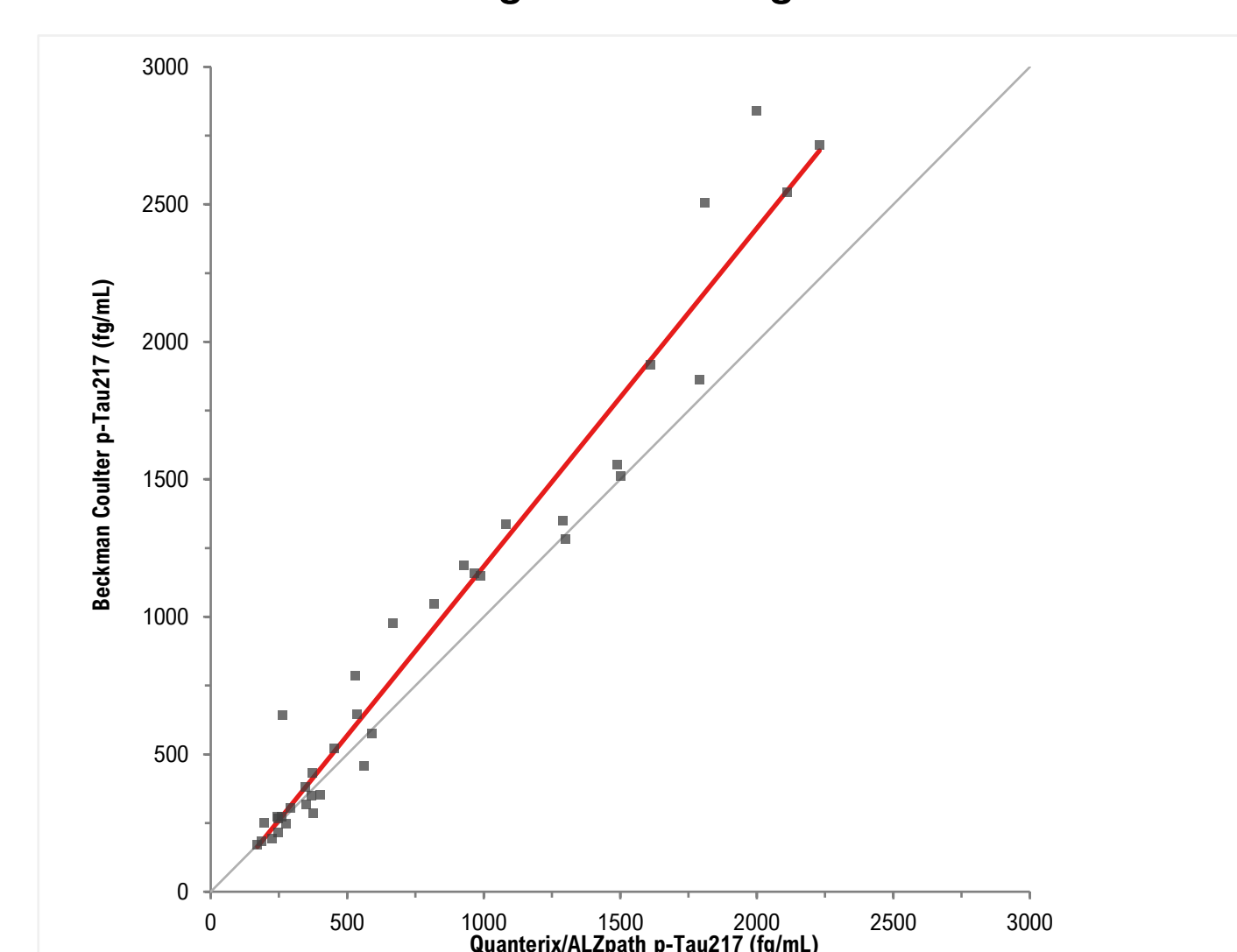
Equation: Beckman Coulter p-Tau217 (fg/mL) = -46.13 + 1.229 Quanterix/Alzpath p-Tau217 (fg/mL)

% Bias Plot

Correlation – r: 0.978

Parameter	Estimate	Bootstrap 95% CI	
Intercept	-46.13	-98.70	to -10.67
Slope	1.229	1.085	to 1.344

Figure 2 A comparison study between the Beckman Coulter prototype p-Tau217 assay on DxI 9000 Analyzer and the Quanterix/ALZpath Simoa RUO p-Tau217 assay was performed. A slight positive bias was observed with good correlation between assays.

Linearity

| Observed Concentration (fg/mL)	Expected Concentration (fg/mL)	Linear Fit (fg/mL)	% Non-Linearity
86.1	86.1	86.1	0.0%
533.8	513.8	537.0	-0.6%
1068.0	941.6	988.0	8.1%
1429.2	1359.3	1438.9	-0.7%
1797.1	1797.1	1889.9	-4.9%

Figure 3 Linearity study was performed using K2 EDTA samples on the DxI 9000 Analyzer. % Non-linearity was <10% across all concentrations evaluated.

Imprecision

Sample	N	Mean Concentration (fg/mL)	CV Within-Lab (%)
1	30	65.5	11.9
2	30	212.1	7.7
3	30	573.5	7.2
4	30	694.7	4.3

Figure 4 Imprecision was assessed on 4 native K2 EDTA plasma samples on the DxI 9000 Analyzer across 3 days and 2 runs per day. Within-lab percent CV was less than 10% for samples >200 fg/mL and <12% for a low concentration sample at ~66 fg/mL.

Clinical Discrimination
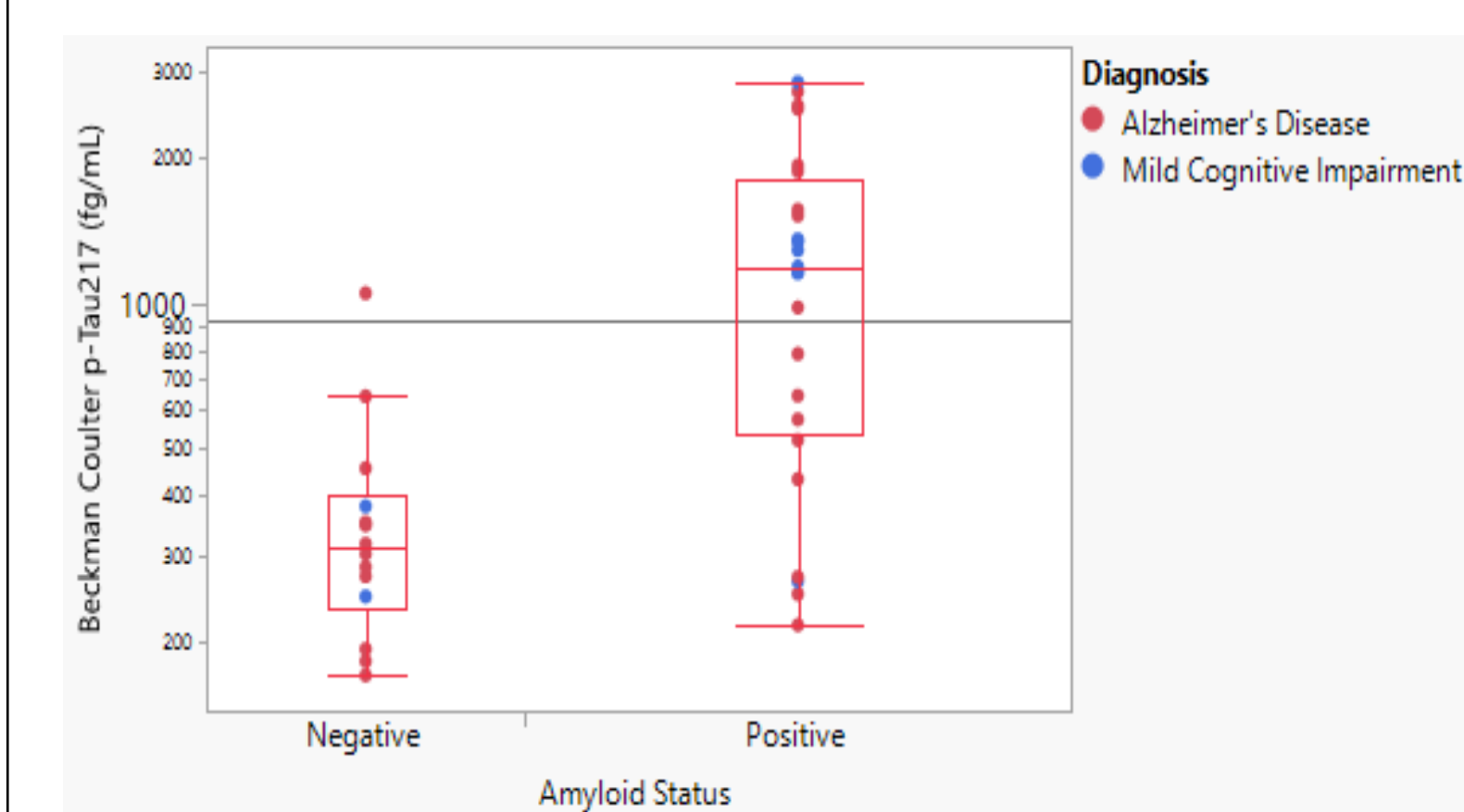
Parameter	Result
Median Amyloid Positive (fg/mL)	1172
Median Amyloid Negative (fg/mL)	312
Ratio (Positive : Negative)	3.8

Figure 5 Discrimination between amyloid positive and amyloid negative patient samples in a population of cognitively impaired patients. Study demonstrates discrimination comparable to other p-Tau217 assays in literature.

Analytical Sensitivity
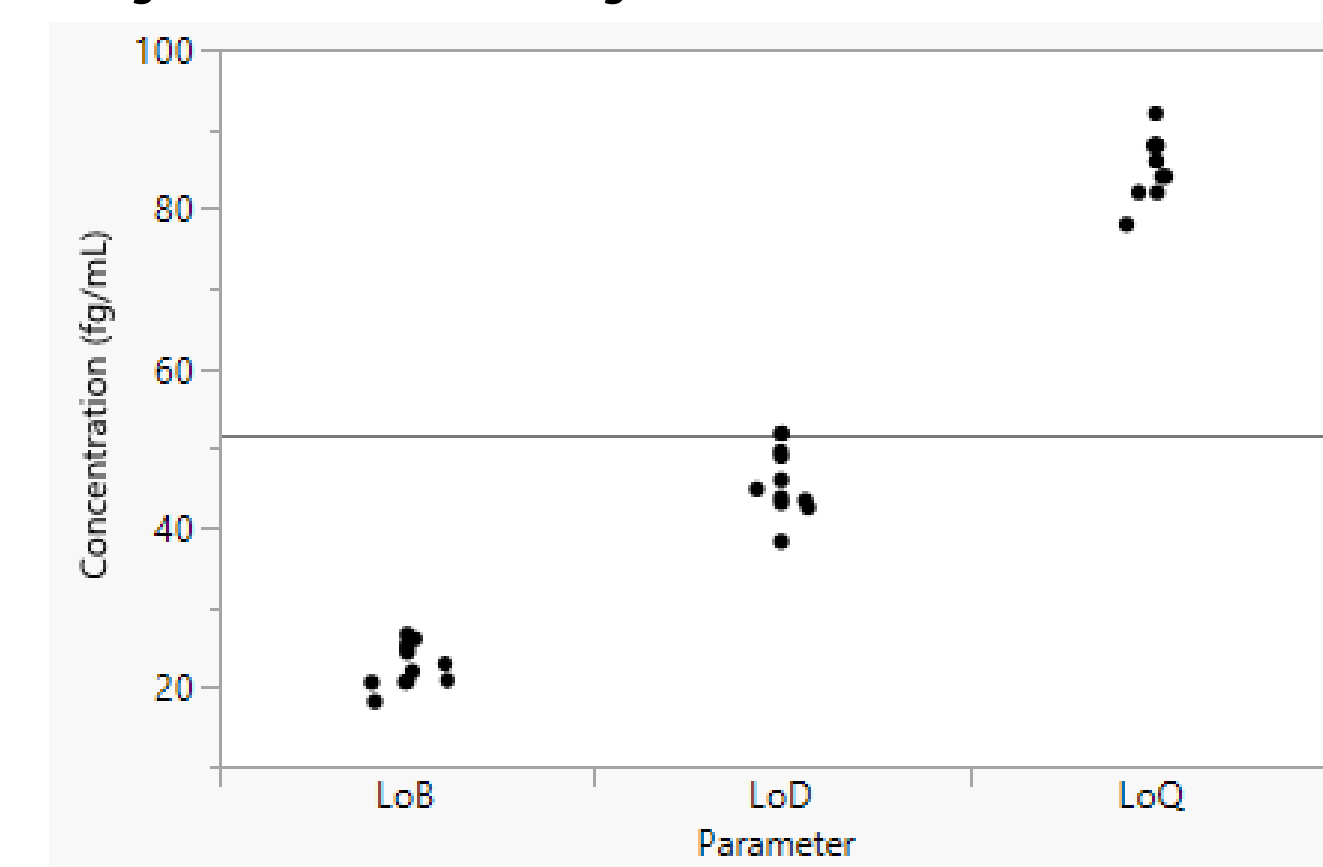
Parameter	Maximum Estimate (fg/mL)
Limit of Blank	27
Limit of Detection	52
Limit of Quantitation	92

Figure 6 The Beckman Coulter prototype p-Tau217 assay LoB, LoD and LoQ were estimated. This low-end precision demonstrates analytical sensitivity capable of detecting and quantifying p-Tau217 concentrations in cognitively healthy and amyloid negative populations.

CONCLUSION

Beckman Coulter is in development of a highly sensitive plasma p-Tau217 assay, a promising blood-based biomarker for neurodegenerative research applications. The fully automated Beckman Coulter DxI 9000 Immunoassay Analyzer provides high throughput, precise results, showing promise to expand research opportunities for this assay in neurodegenerative research.

REFERENCES

1. Ferreira, Pamela CL, et al. "Plasma p-tau231 and p-tau217 inform on tau tangles aggregation in cognitively impaired individuals." Alzheimer's & Dementia 19.10 (2023): 4463-4474.
2. Therriault, Joseph, et al. "Comparison of two plasma p-tau217 assays to detect and monitor Alzheimer's pathology." EBioMedicine 102 (2024).
3. Hertze J, Minthon L, Zetterberg H, Vanmechelen E, Blennow K, Hansson O. Evaluation of CSF biomarkers as predictors of Alzheimer's disease: a clinical follow-up study of 4.7 years. J Alzheimers Dis. 2010;21(4):1119-1128. doi:10.3233/jad-2010-100207