HIGH-THROUGHPUT, FULLY AUTOMATED IMMUNOASSAY FOR PLASMA BRAIN-DERIVED P-TAU217

Katharine Hoffmann,[1] Marnie Wallin,[1] Nathan Hoekstra,[1] Laura Mediger,[1] Miklos Szabo,[1] Chris Knutson,[1] Paul Wynveen,[1] Corey Carlson [1].

[1]Beckman Coulter Diagnostics, 250 South Kraemer Boulevard, Brea, CA, USA

BACKGROUND

Significant evidence supports Tau protein phosphorylated at threonine 217 (p-Tau217) as a highly sensitive and specific biomarker for Alzheimer's Disease (AD). Hyper-phosphorylated Tau is associated with brain neurofibrillary tangles, and plasma p-Tau217 is elevated in individuals with amyloid pathology compared with healthy controls. Tau is expressed as distinct isoforms through alternative splicing that are specifically derived from either the brain or periphery.[1,2] Tau from the brain is a smaller species produced via splicing of exon 4 and exon 5, whereas tau from the periphery ("Big Tau") contains an additional exon 4a insert.[3] Further, antibodies enabling specific detection of splicing junctions are possible to distinguish between tau from the brain vs. periphery.[4] Specific detection of plasma brain-derived pTau217 (BD-pTau217) may provide improved specificity for AD pathology via reduction of signal contribution from peripherally expressed Tau isoforms.[5]

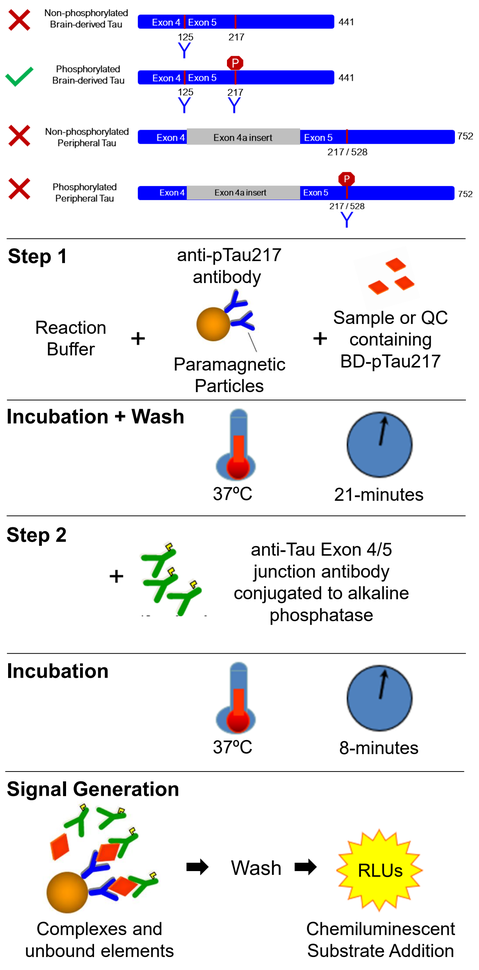
Results are presented herein for a prototype BD-pTau217 immunoassay developed for the Beckman Coulter DxI 9000 Immunoassay Analyzer. The prototype assay was compared to the Access p-Tau217 Research Use Only (RUO) assay, which measures total circulating p-Tau217 regardless of tissue origin, including evaluation of subjects with and without amyloid pathology. Further, CLSI-based analytical studies are presented for the BD-pTau217 prototype assay to assess imprecision, analytical sensitivity, linearity, and analytical specificity.

METHODS

Assay Design

The BD-pTau217 prototype assay is a two-step, two-site immunometric assay for use on the Access DxI 9000 Access Immunoassay Analyzer. The prototype assay uses an antibody with specificity for the Tau Exon 4 and Exon 5 alternative splicing junction in combination with an antibody that recognizes phosphorylated threonine at position 217 on the Tau protein. The antibody specific for Tau phosphorylated at threonine 217 is coupled to paramagnetic particles, while the second antibody specific for a Tau epitope predominantly expressed in the central nervous system is conjugated to alkaline phosphatase.

Figure 1. Basic principle of the assay.



Method Comparison & Differentiation of Amyloid Pathology

A vendor-sourced cohort of N=60 K2 EDTA plasma samples were tested in duplicate using the BD-pTau217 prototype assay and the Access p-Tau217 (RUO) assay. Included within the cohort were 30 samples from subjects with Alzheimer's Disease (AD), 10 samples from subjects with mild cognitive impairment (MCI), and 20 normal samples. Each sample had matched cerebral spinal fluid (CSF) which was tested using the Fujirebio Amyloid 42/40 ratio test to assess the presence of amyloid pathology. Method comparison results are shown in Figure 2 with Passing-Bablok and Bland-Altman analysis. Amyloid pathology differentiation is depicted in Figure 3 with box plots, ratio estimates, and Receiver Operating Characteristic (ROC) analysis.

Imprecision

Imprecision was estimated for the BD-pTau217 prototype assay using an abbreviated study based on CLSI EP05 *Evaluation of Precision of Quantitative Measurement Procedures*. Ten EDTA plasma samples containing concentrations of BD-pTau217 spanning the analytical measuring range were tested in replicates of five with two runs/day over five total days of testing. Within-run, between-run, between-day, and within-laboratory variance components were calculated with coefficient of variation (%CV). Imprecision results are shown in Table 1.

Detection Capability

Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ) were estimated for the BD-pTau217 prototype assay using protocols based on CLSI EP17-A2 *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures*. For LoB estimation, 4 blank samples were tested in replicates of five over 3 days. For LoD and LoQ estimation, 10 EDTA plasma samples were tested in replicates of five with two runs/day over five total days of testing. Detection capability results are depicted in Figure 4 with associated precision profile.

Linearity

Linearity was evaluated using a protocol based on CLSI EP06 *Evaluation of Linearity of Quantitative Measurement Procedures*. Paired low and high EDTA plasma samples covering the full analytical measuring range of the assay were used for linearity determination. In addition to the low and high concentration samples, seven mixtures were tested in this study. These samples were prepared independently by using incrementally larger proportions of the high sample diluted with the low sample, to achieve concentrations that covered the range of the assay. The low sample was run in replicates of eight, and all other samples were run in replicates of four. Linearity results are presented in Figure 5; linear equation, correlation coefficient and non-linearity estimates are provided.

Analytical Specificity

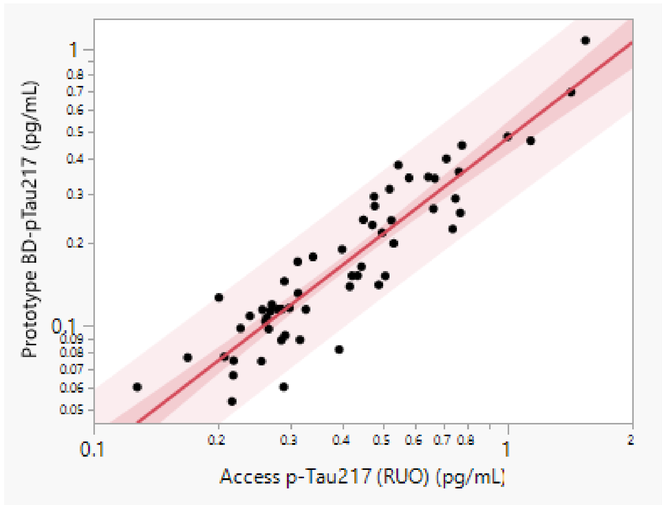
Assay specificity was evaluated for potentially interfering or cross-reacting substances using a protocol based on CLSI EP07 *Interference Testing in Clinical Chemistry*. Each substance was dissolved in an appropriate solvent and spiked into an EDTA plasma sample containing BD-pTau217 analyte. Each substance-spiked sample and a matched solvent-spiked control were tested in replicates of four. The magnitude of interference or cross-reactivity was estimated by comparing the test and corresponding control results. Analytical specificity results are shown in Table 2.

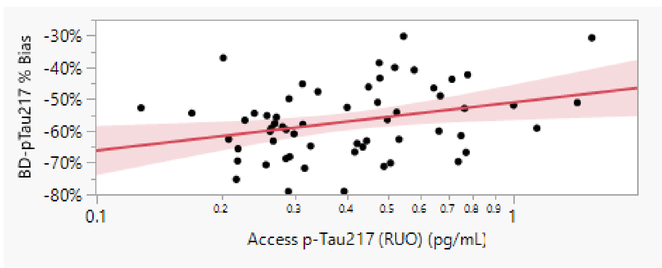
Dilution Recovery

Two EDTA plasma samples containing high concentrations of analyte were diluted 1:2, 1:5, and 1:10 with Access DxI 9000 system wash buffer. Each neat and diluted sample were tested in replicates of four, and percent recovery was calculated for each sample and dilution factor. Dilution recovery results are depicted in Table 3.

RESULTS

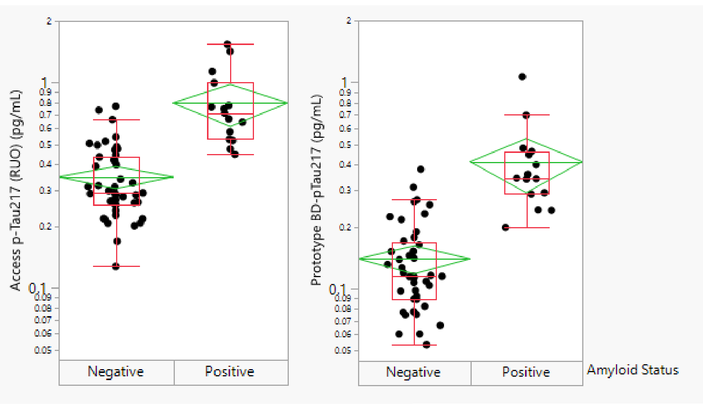
Figure 2. Method Comparison of BD-pTau217 prototype assay vs. Access p-Tau217 (RUO) assay.



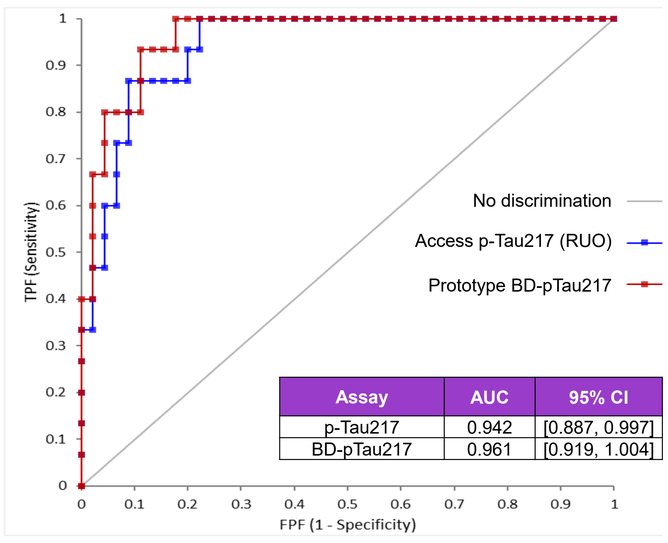


N	R	Passing-Bablok				Bland-Altman	
		Slope	Slope 95% CI	Intercept	Intercept 95% CI	Mean Bias (%)	Bias 95% CI
60	0.93	0.53	[0.45, 0.64]	-0.03	[-0.07, -0.01]	-57	[-79, -35]

Figure 3. Differentiation of samples with and without amyloid pathology as characterized by CSF β-Amyloid 42/40 ratio.



Assay	Amyloid Status	N	Mean (pg/mL)	Ratio	Median (pg/mL)	Ratio
p-Tau217	Negative	45	0.348	2.3	0.290	2.5
	Positive	15	0.798		0.711	
BD-pTau217	Negative	45	0.140	3.0	0.115	3.0
	Positive	15	0.413		0.344	

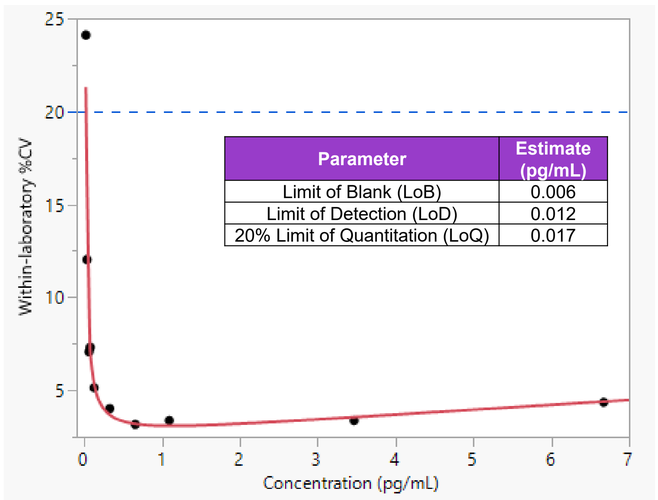


Assay	AUC	95% CI
p-Tau217	0.942	[0.887, 0.997]
BD-pTau217	0.961	[0.919, 1.004]

Table 1. BD-pTau217 prototype assay imprecision

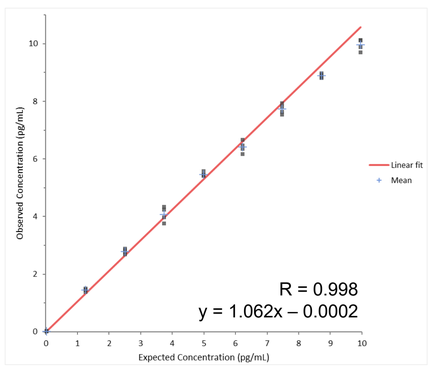
Sample	N	Mean (pg/mL)	Within-Run %CV	Between-Run %CV	Between-Day %CV	Within-Lab %CV
1	50	0.018	20.7	12.3	0.0	24.1
2	50	0.032	11.4	3.7	0.0	12.0
3	50	0.059	6.5	2.9	0.0	7.1
4	50	0.074	6.0	2.3	3.4	7.3
5	50	0.124	4.8	1.7	0.0	5.1
6	49	0.325	3.7	0.0	1.5	4.0
7	50	0.653	3.1	0.0	0.7	3.2
8	50	1.091	2.9	1.2	1.3	3.4
9	50	3.466	3.2	0.0	0.9	3.4
10	50	6.675	4.4	0.0	0.1	4.4

Figure 4. BD-pTau217 prototype assay LoB, LoD, and LoQ.



Parameter	Estimate (pg/mL)
Limit of Blank (LoB)	0.006
Limit of Detection (LoD)	0.012
20% Limit of Quantitation (LoQ)	0.017

Figure 5. BD-pTau217 prototype assay linearity



Concentration (pg/mL)	Non-linearity (%)
0.020	-4.9%
1.262	8.3%
2.505	4.9%
3.747	2.6%
4.989	3.2%
6.232	-3.1%
7.474	-2.5%
8.716	-3.9%
9.959	-5.8%

Table 2. BD-pTau217 prototype assay analytical specificity (interference testing).

Substance	Substance Concentration	Control Concentration (pg/mL)	Test Concentration (pg/mL)	Concentration Change (%)
Galantamine	250 mg/L	0.292	0.289	-1.2%
Memantine	250 mg/L	0.292	0.285	-2.6%
Donepezil	30 mg/L	0.292	0.282	-3.6%
Rivastigmine	45 mg/L	0.292	0.291	-0.3%
Aripiprazole	45 mg/L	0.283	0.284	0.4%
Ibuprofen	0.219 mg/mL	0.268	0.275	2.8%
Acetaminophen	0.156 mg/mL	0.287	0.280	-2.3%
Heparin	3.3 U/mL	0.291	0.279	-4.2%
Hemoglobin	10 mg/mL	0.267	0.274	2.7%
Bilirubin (Conj)	0.4 mg/mL	0.292	0.282	-3.5%
Bilirubin (Unconj)	0.4 mg/mL	0.278	0.278	-0.1%
Triolein	15 mg/mL	0.284	0.281	-1.1%
HAMA	1000 ng/mL	0.362	0.341	-5.8%
RF	50 IU/mL	0.384	0.383	-0.2%
HSA	0.15 g/mL	0.222	0.217	-2.3%
Non-Phospho Tau	100 pg/mL	0.282	0.292	3.6%

Table 3. BD-pTau217 prototype assay dilution recovery.

Sample	Dilution	Observed Concentration (pg/mL)	Expected Concentration (pg/mL)	Recovery (%)
1	Neat	1.149		
	1:2	0.590	0.574	103%
	1:5	0.237	0.230	103%
	1:10	0.119	0.115	104%
2	Neat	3.714		
	1:2	1.887	1.857	102%
	1:5	0.757	0.743	102%
	1:10	0.373	0.371	100%

CONCLUSION

The BD-pTau217 prototype assay on the DxI 9000 Immunoassay Analyzer shows significant correlation to the Access p-Tau217 (RUO) assay with comparable ability to differentiate the presence of amyloid pathology, as determined by CSF β-Amyloid 42/40 ratio, for a small cohort. Greater negative bias at low concentrations and increased fold difference of mean/median values may reflect selective detection of CNS-enriched tau species and potential opportunity to improve differentiation of amyloid positive and negative samples. The BD-pTau217 prototype assay exhibited excellent analytical performance as assessed through studies of imprecision, analytical sensitivity, linearity, analytical specificity, and dilution recovery. Of note, all samples from the cohort tested herein yielded concentrations above the derived LoQ. Further study of larger, well-characterized clinical cohorts is recommended to understand potential additional value for BD-pTau217 as a biomarker for Alzheimer's disease.

References:

1. Georgieff et al. 1991. *J Cell Sci.* 100(Pt 1): 55-60
2. Couchie et al. 1992. *Proc. Natl. Acad. Sci. USA* 89(10): 4378-81
3. Fischer et al. 2020. Trends Neurosci 43(7): 493-504
4. Gonzalez-Ortiz et al. 2023. *Brain* 146(3):1152-65
5. Janelidze et al. 2025. *Alzheimer's & Dementia* 21(4):e70208

2026-15161