NEW HIGH-THROUGHPUT, FULLY AUTOMATED IMMUNOASSAY FOR PLASMA AND SERUM BRAIN-DERIVED TAU

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BACKGROUND

Brain-derived Tau (BD-Tau) shows promise as a marker of neurodegeneration (N) for differentiation of AD from other dementias in the amyloid, tau, neuro-degeneration (ATN) framework. The availability of high-throughput assays will make BD-Tau tests more accessible and may enable more reliable and widespread use. We describe the performance of the highly-sensitive Access BD-Tau research-use only (RUO) immunoassay* in K2 EDTA plasma and serum on the Beckman Coulter Access 2 Immunoassay Analyzer and DxI 9000 Immunoassay Analyzer.

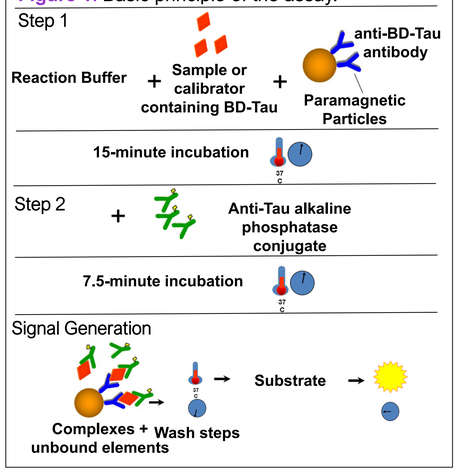
METHODS

Assay Format

Beckman Coulter's Access BD-Tau (RUO) assay is a 2-step sandwich assay using an anti-BD-Tau monoclonal antibody (MAb)/ alkaline phosphatase conjugate along with an anti-BD-Tau MAb bound to paramagnetic particles. 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 before a chemiluminescent substrate is added. The light generated is directly proportional to the sample's BD-Tau concentration (Figure 1).

Samples were screened on the DxI 9000 Immunoassay Analyzer and the Access 2 Immunoassay Analyzer, capable of analyzing up to 450 and 100 tests/hour, respectively. The assay's time to first result is ~32 minutes on the DxI 9000 Immunoassay Analyzer and ~38 minutes on the Access 2 Immunoassay Analyzer.

Figure 1. Basic principle of the assay.



Cross Reactivity and Interfering Substances

Studies were performed to assess analytical specificity through responses to known potential cross-reactants, endogenous interferents, and common drugs for AD. Peripheral Tau cross-reactants included dipeptides with sequences spanning junctions 4-4a & 4a-5 of Tau protein.

The study was run on a single DxI 9000 Immunoassay Analyzer, with a single reagent and calibrator lot. Interference and cross-reactivity were assessed on 2 K2 EDTA plasma samples with ~3 pg/mL endogenous BD-Tau. Stock solutions of potential cross-reactants/ interferents were spiked into 1 of the 2 subject samples to the target concentrations; control samples were prepared in the same manner but with solvent without the potential cross-reactant/interferent. Each sample was analyzed in replicates of 5 (Table 1).

Sample-Type Comparison Study

A sample-type comparison study compared the Beckman Coulter Access BD-Tau (RUO) assay in K2 EDTA plasma versus serum on the DxI 9000 and Access 2 Immunoassay Analyzers.

K2 EDTA plasma and serum samples containing BD-Tau spanning the low end of the assay's analytical measuring range were tested. All samples were tested in replicates of 2 (DxI 9000) or 3 (Access 2), on a single reagent lot. A Passing-Bablok linear regression was fit between the methods (Figure 2).

Platform Comparison Study

A method comparison study compared the Beckman Coulter Access BD-Tau (RUO) assay on the DxI 9000 Immunoassay Analyzer and Access 2 Immunoassay Analyzers.

K2 EDTA plasma and serum samples containing BD-Tau spanning the low end of the analytical measuring range of the assay were tested. All samples were tested in replicates of 2 on the DxI 9000 Immunoassay Analyzer or 3 on the Access 2 Immunoassay Analyzer, on a single reagent lot. A Passing-Bablok linear regression was fit between the methods (Figure 3).

Linearity and Dilution Recovery

Studies were performed to assess the linearity and dilution recovery of the Beckman Coulter Access BD-Tau (RUO) assay on the Access 2 Immunoassay Analyzer and the DxI 9000 Immunoassay Analyzer with samples that cover the full analytical measuring range.

For the linearity study, admixtures were prepared using low and high BD-Tau samples. The high BD-Tau sample included a native K2 EDTA sample spiked with ~250 pg/mL BD-Tau antigen. The low BD-Tau sample was prepared using a K2 EDTA sample from a single subject at the low end of the measuring range. These low and high samples were then mixed in pre-defined ratios and analyzed in replicates of 4 (Figure 4).

Dilution recovery was performed using a K2 EDTA sample spiked with ~250 pg/mL BD-Tau antigen. These samples were then diluted into sample diluent and analyzed in replicates of 4 (Table 2).

Imprecision

Studies were performed to assess the imprecision of the Beckman Coulter Access BD-Tau (RUO) assay. The studies were run on a single Access 2 Immunoassay Analyzer or DxI 9000 Immunoassay Analyzer, on a single reagent and calibrator lot. A combination of native K2 EDTA plasma and control samples spiked with BD-Tau antigen spanning the range of the assay were measured over 5 days, with 5 replicates per run, and 2 runs per day. Within-laboratory (total) variances and CV% were then calculated for each sample (Table 3).

Endogenous Plasma and Serum BD-Tau Levels

A study was performed to assess the ability to recognize endogenous BD-Tau in EDTA Plasma and Serum samples collected from populations with high (population 1) and low (population 2) BD-Tau levels using the Beckman Coulter Access BD-Tau (RUO) assay.

Endogenous Plasma and Serum BD-Tau Levels Continued

These different populations were then analytically measured on the Beckman Coulter Access 2 Immunoassay Analyzer and DxI 9000 Immunoassay Analyzer. The study was run on two Access 2 Immunoassay Analyzers and two DxI 9000 immunoassay analyzers, on a single reagent and calibrator lot. Each samples was tested in replicates of 2 on the DxI 9000 Immunoassay Analyzer or 3 on the Access 2 Immunoassay Analyzer (Figure 5).

Sensitivity

Studies were performed to estimate the limit of blank (LoB), limit of detection (LoD), and limit of quantitation (LoQ) for the Beckman Coulter Access BD-Tau (RUO) assay on the Access 2 Immunoassay Analyzer and DxI 9000 Immunoassay Analyzer.

The LoB study was performed using a single Access 2 Immunoassay Analyzer and a single DxI 9000 Immunoassay Analyzer using a single reagent lot and a single calibrator lot. 2 sets of S0 calibrator matrix and wash buffer were analyzed over 3 days, with 5 replicates per sample and a single run per day (Table 4).

LoD and LoQ analysis was performed by monitoring dose variance of a panel of 9 native K2 EDTA samples over 5 days on either an Access 2 Immunoassay Analyzer or DxI 9000 Immunoassay Analyzer. The study was performed using a single reagent lot and a single calibrator lot. All samples were analyzed over 5 days, with 9 replicates per sample and a single run per day (Table 4).

RESULTS

Table 1. Analytical specificity (cross-reactants and interfering substances)

Interferent/Cross-reactant	Test Concentration	BD-Tau Control Dose (pg/mL)	BD-Tau Test Dose (pg/mL)	% Dose Change
Peripheral Tau, junction 4-4a	500 pg/mL	3.47	3.38	-2.8%
Peripheral Tau, junction 4a-5	500 pg/mL	3.47	3.18	-8.5%
Hemoglobin	2.5 mg/mL	2.89	2.88	-0.2%
	5.0 mg/mL	2.89	2.67	-7.6%
Bilirubin-conjugated	0.4 mg/mL	3.24	3.15	-2.8%
Bilirubin-unconjugated	0.4 mg/mL	3.15	3.09	-1.7%
Human Serum Albumin	60 mg/mL	2.86	2.94	2.5%
Triolein	15 mg/mL	3.34	3.35	0.2%
Acetaminophen	0.156 mg/mL	3.32	3.28	-1.1%
Ibuprofen	0.219 mg/mL	3.28	3.29	0.3%
Heparin	3.3 U/mL	3.33	3.33	-0.1%
Rivastigmine	1200 ng/mL	3.34	3.32	-0.7%
Aripiprazole	1800 ng/mL	3.31	3.34	0.8%
Donepezil	300 ng/mL	3.30	3.34	1.1%
Galantamine	500 ng/mL	3.30	3.34	1.2%
Memantine	450 ng/mL	3.30	3.35	1.7%

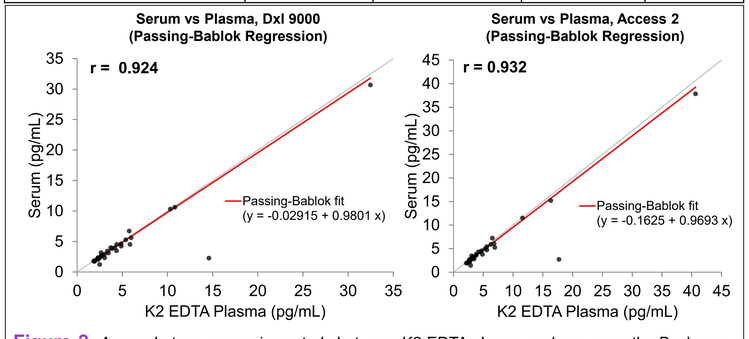


Figure 2. A sample type comparison study between K2 EDTA plasma and serum on the Beckman Coulter Access BD-Tau (RUO) assay on both the DxI 9000 Immunoassay Analyzer (N = 33) and Access 2 Immunoassay Analyzer (N = 33) was evaluated. The Beckman Coulter Access BD-Tau (RUO) assay showed a negative bias on K2 EDTA plasma versus serum on both DxI 9000 and Access 2, slope = 0.98 and slope = 0.97, respectively. The Beckman Coulter Access BD-Tau (RUO) assay had great correlation on both DxI 9000 and Access 2, r = 0.924 and r = 0.932, respectively.

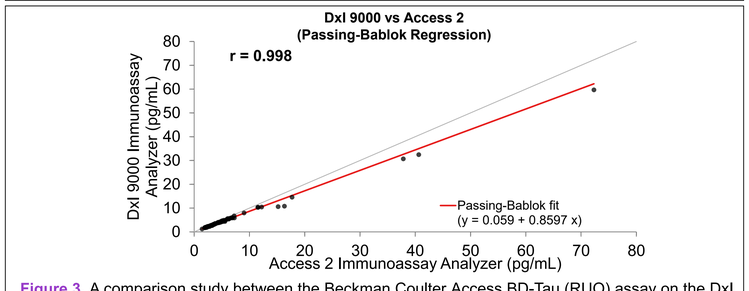


Figure 3. A comparison study between the Beckman Coulter Access BD-Tau (RUO) assay on the DxI 9000 Analyzer versus the Access 2 Analyzer (N = 76) was evaluated. The Beckman Coulter Access BD-Tau (RUO) assay showed a negative bias on the DxI 9000 Analyzer versus the Access 2 Analyzer, slope = 0.86. The Beckman Coulter Access BD-Tau (RUO) assay on the DxI 9000 Analyzer versus the Access 2 Analyzer had excellent correlation, r = 0.998.

Table 2. Dilution Recovery

DF	Access 2 Immunoassay Analyzer				DxI 9000 Immunoassay Analyzer			
	Wash Buffer		Calibrator Matrix		Wash Buffer		Calibrator Matrix	
	Dose (pg/mL)	% Recovery	Dose (pg/mL)	% Recovery	Dose (pg/mL)	% Recovery	Dose (pg/mL)	% Recovery
Neat	281.06	N/A	281.06	N/A	224.73	N/A	224.73	N/A
2	133.20	98	134.28	97	109.90	95	108.61	96
5	50.84	96	51.76	99	43.15	90	44.34	92
10	24.66	98	26.26	101	22.05	88	22.74	93
20	12.97	102	13.50	104	11.52	92	11.71	96
40	7.00	111	7.05	109	6.22	100	6.10	100
80	3.94	121	3.82	114	3.39	112	3.20	109

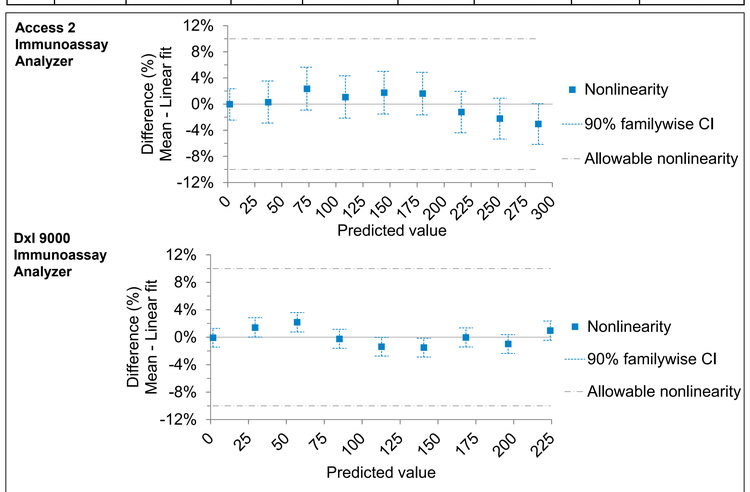
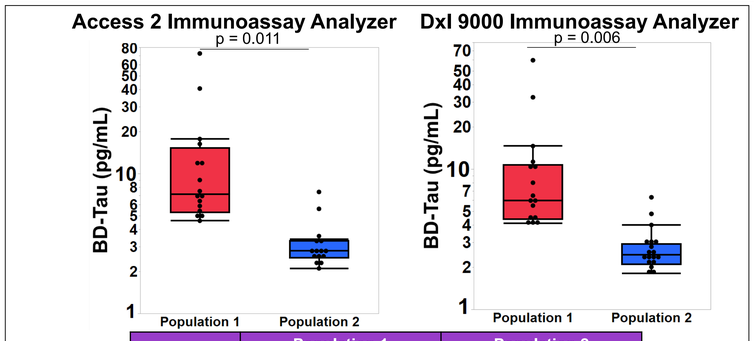


Figure 4. Linearity study was performed using K2 EDTA samples on the Access 2 Immunoassay Analyzer and the DxI 9000 Immunoassay Analyzer. % Non-linearity was <10% across all concentrations evaluated.

Table 3. Imprecision

Sample ID	Access 2 Immunoassay Analyzer		DxI 9000 Immunoassay Analyzer	
	Mean Dose (pg/mL)	Total CV (%)	Mean Dose (pg/mL)	Total CV (%)
P1	3.28	3.42	2.69	3.42
P2	15.59	3.78	12.47	2.18
P3	61.69	2.29	49.28	2.67
P4	224.15	2.83	185.24	2.20



Assay	Population 1 (BD-Tau pg/mL)	Population 2 (BD-Tau pg/mL)
Access 2	7.1	2.8
DxI 9000	5.9	2.4

Figure 5. Endogenous BD-Tau levels in EDTA plasma from two different groups with high and low levels on the Access 2 Immunoassay Analyzer and DxI 9000 Immunoassay Analyzer. Table above shows median doses of BD-Tau in each population group compared across the two different analyzers.

Table 4. Sensitivity

Parameter	Access 2 Immunoassay Analyzer (pg/mL)	DxI 9000 Immunoassay Analyzer (pg/mL)
Limit of Blank (LoB)	0.083	0.034
Limit of Detection (LoD)	0.181	0.057
Limit of Quantitation (LoQ)	0.337	0.131

CONCLUSION

The Beckman Coulter Access BD-Tau (RUO) assay provides fast, highly sensitive, and precise results in an automated immunoassay on the Beckman Coulter DxI 9000 and Access 2 Immunoassay Analyzers. The assay was able to detect BD-Tau in 100% of samples on both analyzers. The endogenous BD-Tau data show that the Beckman Coulter Access BD-Tau (RUO) assay may have promise as a blood-based biomarker in AD research, drug development, diagnosis, disease monitoring, and patient care.