NEW HIGH-THROUGHPUT, FULLY AUTOMATED IMMUNOASSAY FOR PLASMA GLIAL FIBRILLARY ACIDIC PROTEIN

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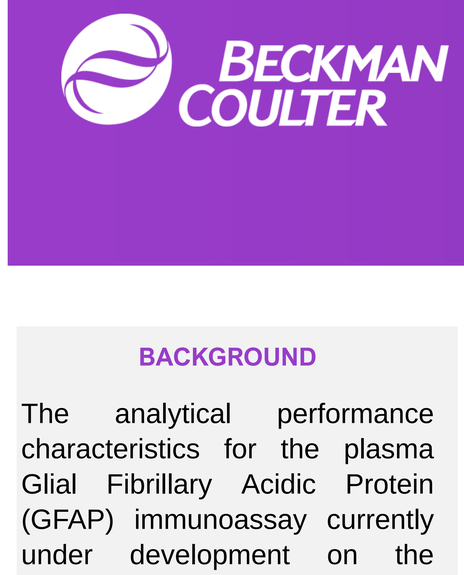
BACKGROUND

The analytical performance characteristics for the plasma Glial Fibrillary Acidic Protein (GFAP) immunoassay currently under development on the Beckman Coulter Access 2 and DxI 9000 Immunoassay Analyzers* are described. GFAP is an important non-specific neurological marker.

METHODS

Assay Format

The prototype GFAP assay is a one-step sandwich assay utilizing an anti-GFAP mouse monoclonal (MAb) antibody/alkaline phosphatase conjugate and paramagnetic particles coated with a complementary anti-GFAP mouse MAb. One hundred microliters of sample are incubated with reactants, sample and reactants are washed, and a chemiluminescent substrate is added. The light that is generated is directly proportional to the GFAP concentration in the sample. The assay time to first result is ~30 minutes.



Cross Reactivity and Interfering Substances

Studies were performed to assess known potential cross-reactants with GFAP. Additionally, common drugs and endogenous interfering substances were tested to assess for potential interference.

Each study was run on one Access 2 and one DxI 9000 Immunoassay Analyzer, one reagent lot and one calibrator lot. K2 EDTA plasma samples containing low levels of analyte were measured. Samples were tested in triplicate per run.

Comparison Study

A method comparison study was completed to compare the prototype GFAP assay on the Access 2 Analyzer to the DxI 9000 Immunoassay Analyzer for K2 EDTA plasma sample types. Method comparison studies were performed on one DxI 9000 Access Immunoassay Analyzer and one Access 2 instrument.

20 K2 EDTA plasma samples containing GFAP concentrations spanning the low end of the analytical measuring range of the assay were tested. All samples were tested in duplicate on a single reagent lot.

Concordance Study

A concordance study was completed to compare the prototype GFAP assay on Access 2 to the Quanterix GFAP RUO assay on the HD-X Simoa Immunoassay Analyzer for K2 EDTA plasma sample types. Method comparison studies were performed on one HD-X Simoa Immunoassay Analyzer and one Access 2 instrument.

EDTA plasma samples were tested. All samples were tested in duplicate on a single reagent lot across the three external sites. The Quanterix HD-X Simoa Immunoassay was tested at an external site.

Linearity and Dilution Recovery

Studies were performed to assess the linearity of the Access GFAP on the Access 2 and DxI 9000 Access Immunoassay Analyzers.

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 high concentration sample was prepared by spiking native CSF sample into the K2 EDTA sample to reach a concentration. The low concentration sample used was calibrator matrix. In addition to the high and low 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 quadruplicate.

For the dilution recovery study, a high sample was prepared by spiking recombinant GFAP antigen into the K2 EDTA sample to reach a concentration near the top end of the calibrator curve. The sample was serially diluted by a dilution factor of 2 in calibrator matrix. This study was run on one Access 2 and one DxI 9000 Immunoassay Analyzer, using one reagent lot and one calibrator lot.

Imprecision

Studies were performed to assess the imprecision of the prototype GFAP assay on the Access 2 and DxI 9000 Immunoassay Analyzers.

Each study was run on two Access 2 and one DxI 9000 Immunoassay Analyzers, two reagent lots, and two calibrator lots. A combination of native K2 EDTA plasma and control samples spiked with recombinant antigen spanning the low end of the analytical measuring range of the assay were measured. Each sample was tested in triplicate per run. Two runs per day were completed over five days on each calibrator lot and reagent lot combination.

Detection Capability

Studies were performed to estimate the Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ) for the Access GFAP assay in development on the Access 2 and DxI 9000 Immunoassay Analyzers.

For the estimation of LoB, two Access 2 and one DxI 9000 Immunoassay Analyzers were used in the study design with two reagent lots and two calibrator lots. One S0 calibrator preparation for each respective assay was used for the LoB determination. Blank samples were tested over five days two runs per day in quadruplicate for each pack lot and calibrator lot.

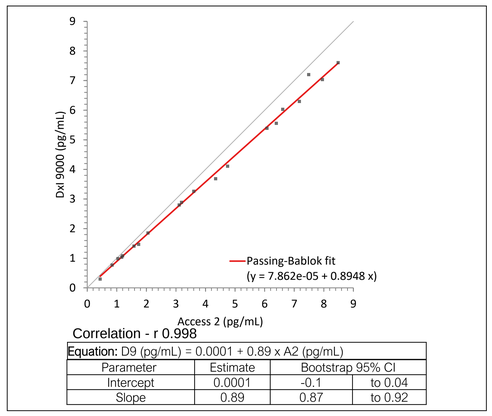
For estimation of LoD and LoQ, two Access 2 and one DxI 9000 Immunoassay Analyzers were used in the study design with two reagent lots and two calibrator lots. A combination of native K2 EDTA plasma healthy control samples and control samples spiked with recombinant antigen spanning the low end of the analytical measuring range of the assay were measured. Samples were tested in triplicate per run with two runs per day and five total days on each pack lot and calibrator lot. This resulted in a minimum of 30 replicates for each sample on each pack/calibrator lot tested.

RESULTS

Cross Reactant	Result
S100-B	Not detected
Amyloid Beta 1-40	
Amyloid Beta 1-42	
Neurofilament Light	
Neurofilament Medium	
Neurofilament Heavy	

Interfering Substances	Result
AD Drugs/Common Drugs	No interference
Proteins and Lipids	

Figure 1 No cross reactivity detected on Access 2 or DxI 9000 by known potential cross-reactants. No interference detected among AD drugs (including Donepezil, Memantine, Aripirazole, Galantamine, and Rivastigamine), common drugs or endogenous protein and lipid-based interferents.

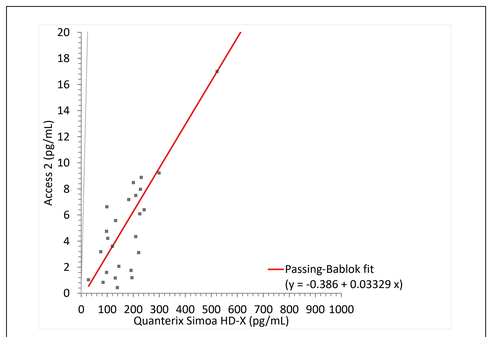


Correlation - r 0.998

Equation: D9 (pg/mL) = 0.0001 + 0.89 x A2 (pg/mL)

Parameter	Estimate	Bootstrap 95% CI	
Intercept	0.0001	-0.1	to 0.04
Slope	0.89	0.87	to 0.92

Figure 2 A comparison study between the GFAP assay in development on the DxI 9000 and Access 2 Immunoassay Analyzers was evaluated. Bias between platforms was acceptable with excellent correlation between platforms.



Correlation - r 0.945

Equation: D9 (pg/mL) = -0.4403 + 0.03373 x Quanterix (pg/mL)

Parameter	Estimate	Bootstrap 95% CI	
Intercept	-0.44	-2.72	to 1.89
Slope	0.034	0.019	to 0.041

Figure 3 A concordance study between the Beckman prototype GFAP assay on the Access 2 and the Quanterix GFAP RUO assay on the HD-X Simoa was evaluated. High correlation between platforms was observed.

Linearity

Low Sample (Cal Matrix)	High Sample (CSF Spike)	Linearity (Dose Recovery %)	
		Access 2	DxI9000
75%	25%	102%	110%
50%	50%	100%	109%
25%	75%	98%	103%
15%	85%	97%	99%

Dilution Recovery

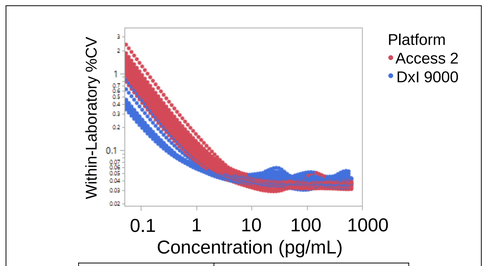
Dilution Factor	Dose Recovery %	
	Access 2	DxI9000
2	108%	110%
4	109%	114%
8	104%	108%
16	106%	109%

Figure 4 Linearity and dilution recovery studies were performed using K2 EDTA samples on Access 2 and DxI 9000 Immunoassay Analyzers. Dose recovery percent was within +/- 10%, meeting design criteria for both platforms.

Access 2		
Sample	Mean Dose (pg/mL)	CV Within-Lab (%)
QC 1	8.6	3.1
QC 2	158.1	2.4
P1	4.2	3.9
P3	18.9	7.7
P5	38.6	3.7

DxI9000		
Sample	Mean Dose (pg/mL)	CV Within-Lab (%)
QC 1	8.1	3.3
QC 2	152.7	2.4
P1	3.7	3.7
P3	17.9	7.8
P5	28.0	3.0

Figure 5 Imprecision was assessed on both the Access 2 and DxI 9000 platforms. Within-lab percent CV was less than 10%, meeting design criteria on both platforms.



Parameter	Dose (pg/mL)	
	Access 2	DxI 9000
LoB	0.16	0.070
LoD	0.34	0.15
LoQ	0.59	0.26

Figure 7 GFAP LoB, LoD and LoQ were evaluated on Access 2 and DxI 9000 Immunoassay Analyzers. A 20% CV cut-off was used for generating an estimate of the LOQ. Assay detection capability was shown to be acceptable on both platforms. Detection capability criteria were met on both platforms.

CONCLUSION

The Beckman Coulter prototype GFAP assay provides highly sensitive results in ~30 minutes, on the DxI 9000 and Access 2 Immunoassay Analyzers. High-throughput and automated precise results demonstrate comparable results to competitive research platforms showing promise for future research studies involving GFAP levels as an indicator for neural injury.

* Full name is DxI 9000 Access Immunoassay Analyzer
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