NEW HIGH-THROUGHPUT, FULLY AUTOMATED IMMUNOASSAY FOR PLASMA NEUROFILAMENT LIGHT CHAIN

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BACKGROUND

Neurofilament light chain (NfL) is an important non-specific neurological marker. Given its low abundance in blood (picomolar and sub-picomolar levels), improvement in the sensitivity of NfL assays is necessary for accurate detection. Here, we assess the analytical performance characteristics for the high-throughput, fully automated plasma NfL immunoassay currently under development on the Beckman Coulter Access 2 and DxI 9000 Immunoassay Analyzers.

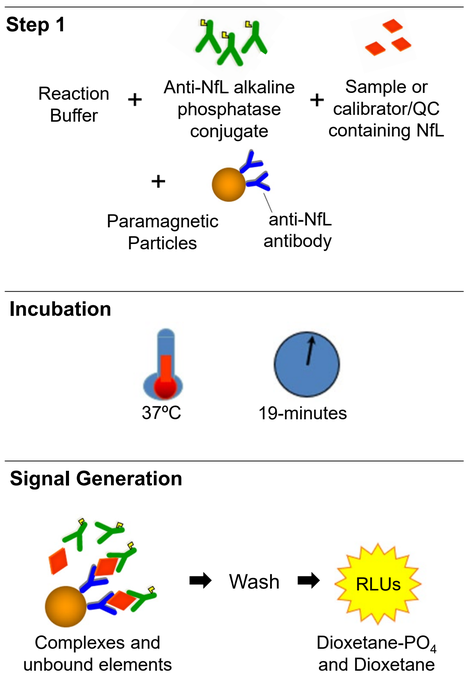
METHODS

Assay Format

The research use only (RUO) NfL assay is a one-step sandwich assay utilizing an anti-NfL monoclonal antibody (MAb)/alkaline phosphatase conjugate, and paramagnetic particles coated with a complementary anti-NfL MAb. Samples and reactants are incubated and washed, then a chemiluminescent substrate is added. The light generated is directly proportional to the NfL concentration in the sample.

Samples were screened on the DxI 9000 and Access 2 Immunoassay Analyzers, capable of analyzing up to 450 and 100 tests/hour, respectively. The assay's time to first result is ~26 minutes on the DxI 9000 and ~31 minutes on the Access 2 Immunoassay Analyzers.

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.

The study was run on a single Access 2 and DxI 9000 Immunoassay Analyzer, with one reagent and calibrator lot. Interference and cross-reactivity were assessed on a single pooled K2 EDTA plasma sample with ~80 pg/mL endogenous NfL. Stock solutions of potential cross-reactants/interferents were spiked into the sample to the target concentrations and control samples were prepared in the same manner but with the solvent and without the potential cross-reactant/interferent analyte. Each sample was analyzed in duplicate.

Comparison Study

A method comparison study was completed to compare the Beckman Coulter RUO NfL assay on the Access 2 and DxI 9000 Immunoassay Analyzers, the Quanterix RUO NF-light™ V2 Advantage Kit (Item #104073) assay on the Simoa HD-X Immunoassay Analyzer, and the Lumipulse NfL Blood (Item #81215) on the LUMIPULSE G1200 analyzer.

K2 EDTA plasma samples containing NfL spanning the analytical measuring range of the assay were tested (N=40). All Samples were tested in replicates of two, on one reagent lot. A Passing-Bablok linear regression was fit between the methods.

Linearity and Dilution Recovery

Studies were performed to assess the linearity and dilution recovery of Beckman Coulter RUO NfL assay on the Access 2 and DxI 9000 Immunoassay Analyzers with samples that cover the full analytical measuring range.

For the linearity study, admixtures were prepared using low and high NfL samples. Two high NfL samples were used: 1) a native K2 EDTA sample with ~100 pg/mL endogenous NfL; or 2) a K2 EDTA sampled spiked with ~11,000 pg/mL NfL antigen. The low NfL sample was prepared by pooling K2 EDTA samples at the low end of the measuring range. These low and high samples were then mixed in pre-defined ratios and analyzed in quadruplicate.

Dilution recovery was performed using a native sample with ~100 pg/mL endogenous NfL or a contrived sample was prepared by spiking NfL antigen into K2 EDTA sample to reach a concentration near the top end of the calibrator curve. These samples were then diluted into sample diluent and analyzed in quadruplicate.

Imprecision

Studies were performed to assess the imprecision of the Beckman Coulter RUO NfL assay. The study was run using a single reagent and calibrator lot, on a single Access 2 or DxI 9000 Immunoassay Analyzer. A combination of native K2 EDTA plasma and control samples spiked with NfL antigen spanning the range of the assay were measured over 6 days, with 5 replicates per run, and 2 runs per day. Within-run, between-run, and within-laboratory (total) variances and CV% were then calculated for each sample.

Endogenous Plasma NfL levels

A study was performed to assess the ability to recognize endogenous NfL in EDTA plasma samples collected from populations with high (population 1) , medium (population 2), and low (population 3) NfL levels. These different populations were then analytically measured on the Beckman Coulter Access 2, Beckman Coulter DxI 9000, and Quanterix Simoa analyzers. The study was run on a single Access 2, DxI 9000, and Simoa immunoassay analyzers, with one reagent lot. Each sample was tested in duplicate.

Sensitivity

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

The LoB study was performed using one Access 2 and one DxI 9000 Immunoassay Analyzers using one reagent lot and one calibrator lot. Two sets of S0 calibrator Matrix and wash buffer were analyzed over three days, with five replicates per sample and one run per day.

Sensitivity Continued

LoD and LoQ analysis was performed by monitoring signal variance of a panel of eight native K2 EDTA samples over 5 days on either an Access 2 or DxI 9000 Immunoassay Analyzers. The study was performed using one reagent and one calibrator lot.

RESULTS

Table 1. Analytical specificity (cross-reactivity)

Analyte	Test Concentration	NfL Control Dose (pg/mL)	NfL Test Dose (pg/mL)	% Dose Change
GFAP	2 ng/mL	80.8	80.4	-0.4%
Vimentin	360 ng/mL	80.8	81.3	0.7%
Keratin	20 ng/mL	80.8	79.8	-1.2%
Peripherin	15 ng/mL	80.8	81.7	1.1%
Internexin	80 ng/mL	80.8	80.9	0.1%
Nf-H	80 ng/mL	80.8	81.6	1.0%
Nf-M	80 ng/mL	80.8	78.7	-2.5%
Desmin	130 ng/mL	80.8	80.9	0.2%
Synemin	100 ng/mL	80.8	78.1	-3.3%
Nestin	100 ng/mL	80.8	77.8	-3.7%

Table 2. Analytical specificity (interfering substances)

Interferent	Test Concentration	NfL Control Dose (pg/mL)	NfL Test Dose (pg/mL)	% Dose Change
Hemoglobin	2.0 mg/mL	27.35	25.85	-5.5%
	2.5 mg/mL	26.48	24.94	-5.8%
	5.0 mg/mL	25.64	23.12	-9.8%
Bilirubin-conjugated	0.2 mg/mL	26.55	26.56	0.0%
	0.4 mg/mL	26.67	25.12	-5.8%
Bilirubin-unconjugated	0.4 mg/mL	27.71	27.88	0.6%
Human Serum Albumin	60 mg/mL	24.68	26.13	5.9%
Triolein	15 mg/mL	25.83	25.77	-0.2%
Acetaminophen	0.156 mg/mL	26.85	26.01	-3.1%
Ibuprofen	0.219 mg/mL	26.79	25.64	-4.3%
Heparin	3.3 U/mL	29.02	29.17	0.5%
Aripiprazole	1800 ng/mL	25.95	26.31	1.4%
Donepezil	300 ng/mL	27.05	27.42	1.4%
Galantamine	500 ng/mL	27.05	26.35	-2.6%
Memantine	450 ng/mL	27.05	26.81	-0.9%

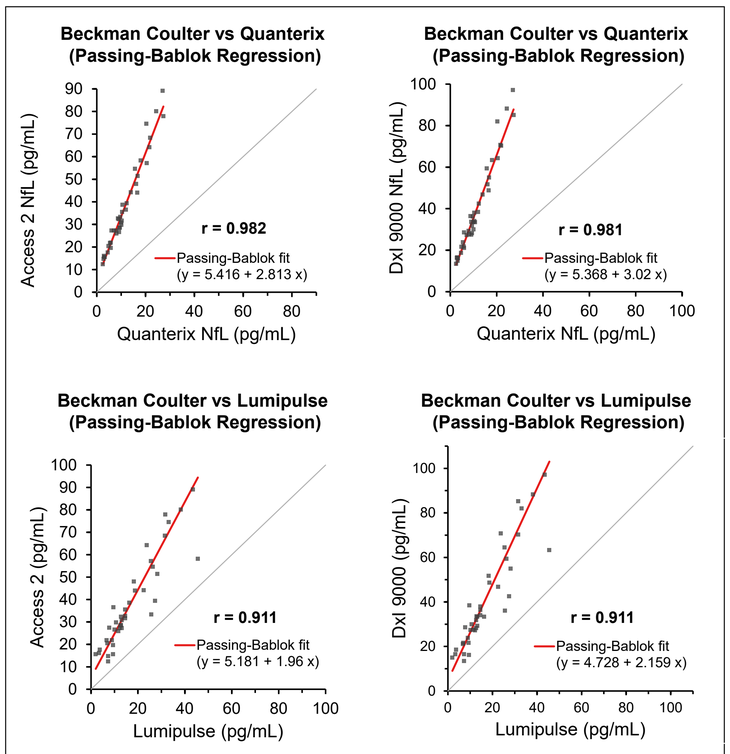


Figure 2. A comparison study between the Beckman Coulter RUO NfL assay on Access 2 or DxI 9000 analyzers versus the Quanterix NfL and Lumipulse NfL assays were evaluated. The Beckman Coulter NfL assay showed a positive bias on both Access 2 and DxI 9000 instruments, slope = 2.8 and slope = 3.0 respectively. Both the Beckman Coulter NfL and Quanterix NfL assays had an excellent correlation, r=0.98.

Table 3. Dilution Recovery

DF	Access 2		DxI 9000	
	Mean Dose (pg/mL)	% Recovery	Mean Dose (pg/mL)	% Recovery
Neat	8308.5	N/A	8585.8	N/A
2	4109.4	99%	4340.0	104%
5	1594.6	96%	1713.7	103%
10	800.4	96%	849.7	102%
20	408.9	98%	431.0	104%

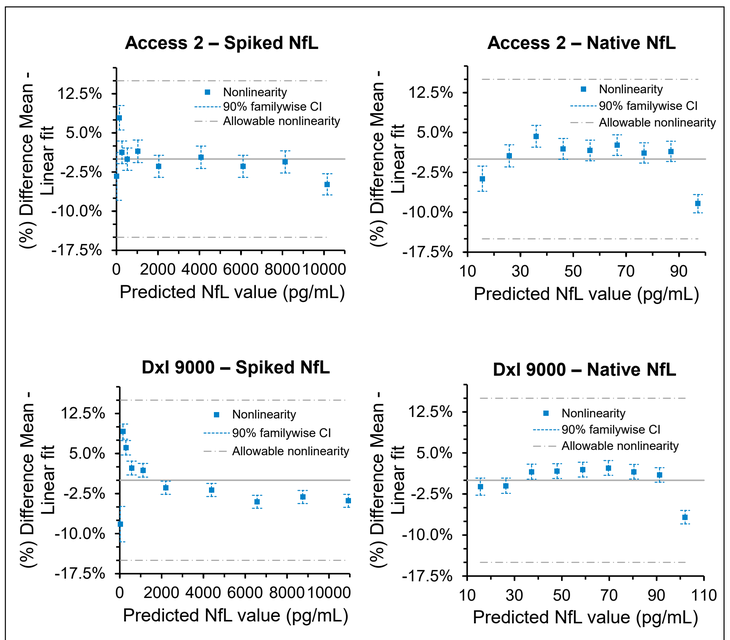
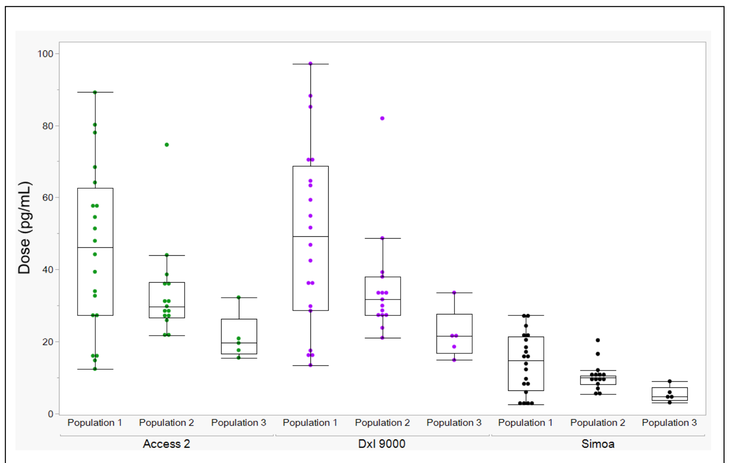


Figure 3. Linearity study was performed using K2 EDTA samples on the Access 2 and DxI 9000 Immunoassay Analyzers. % Non-linearity was <10% across all concentrations evaluated, and for both spiked and native samples.

Table 4. Imprecision

Sample ID	Access 2		DxI 9000	
	Mean Dose (pg/mL)	Total CV (%)	Mean Dose (pg/mL)	Total CV (%)
P1	30.81	6.51	32.08	2.59
P2	43.66	3.60	46.00	2.17
P3	76.16	2.43	67.71	1.72
P4	170.88	2.18	181.86	1.33
P5	540.81	2.32	561.04	1.96
P6	1685.81	2.11	1797.61	1.35



Assay	Population 1 (NfL pg/mL)	Population 2 (NfL pg/mL)	Population 3 (NfL pg/mL)
Access 2	45.63	29.76	19.62
DxI 9000	49.36	31.71	21.58
Simoa	13.97	10.00	4.84

Figure 4. Endogenous NfL levels in EDTA plasma from three different groups with high, medium, and low levels of NfL on Access 2 (green dots), DxI 9000 (purple dots), and Simoa (black dots). Table below shows median doses of NfL in each population group compared across the three different analyzers.

Table 5. Sensitivity

Parameter	Access 2 (pg/mL)	DxI 9000 (pg/mL)
Limit of Blank (LoB)	1.46	0.88
Limit of Detection (LoD)	2.95	2.19
Limit of Quantitation (LoQ)	4.20	3.10

CONCLUSION

The Beckman Coulter RUO NfL 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 NfL in 100% of samples on both analyzers, even the young-healthy controls, and had high correlation with commercially available RUO NfL assays. The data shows that the Beckman Coulter RUO NfL assay may have promise as a blood-based biomarker for research, drug development, diagnosis, disease monitoring, and patient care.