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Sensitive Serum/Plasma Neurofilament Light Immunoassay

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1 Abstract

Increased concentration of neurofilament light protein (NF-L) in CSF is a proven marker for neuronal injury. Obtaining a CSF sample is invasive; therefore measurement of this biomarker in blood would be preferred. However, use of blood is complicated by the extremely low NF-L concentrations and the presence of many potential assay interferents.

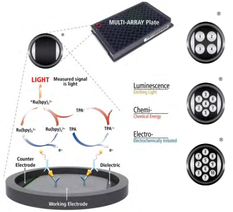
Using a next generation assay format based on MULTI-ARRAY® technology, MSD developed and characterized a highly sensitive immunoassay for NF-L in serum and plasma. Limit of blank (LOB) was determined by running 20 replicates of zero calibrator per plate and calculating the concentration corresponding to the average zero calibrator signal plus 2.5 standard deviations. Lower and upper limits of quantitation (LLOQ and ULOQ, respectively) were determined by running six plates on two days with four replicates per plate. LLOQ was defined as the lowest concentration with a total CV of 20% or less. The reference range in the normal population was established per CLSI EP28-A3c by testing serum samples from 80 apparently healthy individuals. Association of serum/plasma NF-L with neurocognitive status was evaluated by testing 120 samples from individuals with Alzheimer's, dementia, traumatic brain injury (TBI), or HIV with well-characterized neurocognitive status.

The LOB, LLOQ, and ULOQ for the assay were 0.7 pg/mL, 3.4 pg/mL, and 1,700 pg/mL, respectively. Total CVs of four QC samples (15 plates, 5 days) were 8%–14%. Serum and plasma samples diluted linearly. NF-L concentrations in the reference (healthy) sample set (5th, 25th, 50th, 75th, and 95th percentiles) were 10 pg/mL, 15 pg/mL, 19 pg/mL, 26 pg/mL, and 51 pg/mL, respectively. Median concentrations for mild and severe HIV-Associated Neurocognitive Disorder samples were 35 pg/mL and 56 pg/mL. Median concentration in Alzheimer's, dementia, and TBI samples were 220 pg/mL, 154 pg/mL, and 135 pg/mL, respectively. Matched serum and plasma concentrations correlated well (R^2 = 0.73). NF-L concentrations in the normal population correlated with age (p=0.03).

The assay enables quantitation of low NF-L levels in serum and plasma samples and should prove useful for studying neuronal injury caused by diseases such as Alzheimer's or HIV.

2 Methods

MSD's electrochemiluminescence (ECL) detection technology uses SULFO-TAG™ labels that emit light upon electrochemical stimulation initiated at the electrode surfaces of MULTI-ARRAY and MULTI-SPOT® microplates.
A next-generation MULTI-ARRAY technology with significantly higher sensitivity was used for the data presented in this poster.

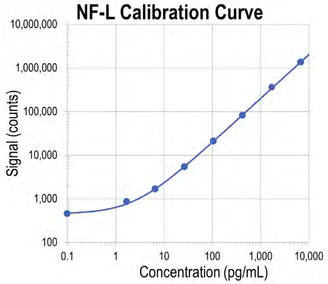


Electrochemiluminescence Technology

- Minimal non-specific background and strong responses to analyte yield high signal-to-background ratios.
- The stimulation mechanism (electricity) is decoupled from the response (light signal), minimizing matrix interference.
- Only labels bound near the electrode surface are excited, enabling non-washed assays.
- Labels are stable, non-radioactive, and directly conjugated to biological molecules.
- Emission at ~620 nm eliminates problems with color quenching.
- Multiple rounds of label excitation and emission enhance light levels and improve sensitivity.
- Carbon electrode surface has 10X greater binding capacity than polystyrene wells.
- Surface coatings can be customized.

3 Calibration Curve

Native purified NF-L from bovine spinal cord was used to calibrate the assay. The figure to the right shows a typical calibration curve. ECL technology enables a wide dynamic range (four orders of magnitude).



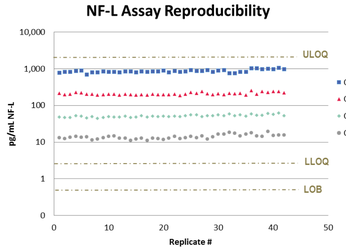
4 Assay Range

	Sample	Signal (counts)	pg/mL	Conc. %CV
ULOQ	ULOQ1	436,589	1,700	8
	ULOQ2	215,338	850	9
	ULOQ3	104,305	425	3
LLOQ	LLOQ1	25,689	106	7
	LLOQ2	13,419	53	9
	LLOQ3	7,021	27	7
	LLOQ4	3,804	13	7
	LLOQ5	2,268	7	8
	LLOQ6	1,342	3.4	13
	LLOQ7	962	1.7	29

The LOB was determined by running 20 replicates of zero calibrator per plate and calculating the concentration corresponding to the average zero calibrator signal plus 2.5 standard deviations. The LLOQ and ULOQ were determined by running six plates on two days with four replicates per plate. LLOQ was defined as the lowest concentration with a total CV of 20% or less. ULOQ was defined as the highest concentration with a total CV of 20% or less.

LOB	0.7 pg/mL
LLOQ	3.4 pg/mL
ULOQ	1,700 pg/mL

5 Reproducibility



	QC 01	QC 02	QC 03	QC 04
NF-L (pg/mL)	862	207	51	14
CV (21 plates, 7 days)	9%	8%	8%	14%

The reproducibility of the NF-L assay was determined by including two replicates each of four QC samples spanning the assay range in each run.
The assay had good reproducibility: Total CVs over 21 plates run on 7 days were between 8 and 14%.

6 Clinical Samples

Reference range samples: 80 serum samples from apparently healthy individuals were tested to determine the NF-L reference range. Samples reflected a representative adult U.S. population with respect to race, age, and gender.

CHARTER samples: 120 well-characterized serum and EDTA plasma samples from the CNS HIV Anti-Retroviral Therapy Effects Research (CHARTER) cohort were used for this study. This sample set included 11 individuals with HIV-Associated Dementia (HAD), 25 individuals with mild neurocognitive disorder (MND), and 30 individuals with no or asymptomatic neurocognitive impairment (ANI).

Remnant serum, EDTA plasma, and heparin plasma samples from apparently healthy individuals and from individuals with Alzheimer's, dementia, and TBI were obtained from commercial sample vendors. Only limited information such as age and gender was provided for most of these samples.

Acknowledgements

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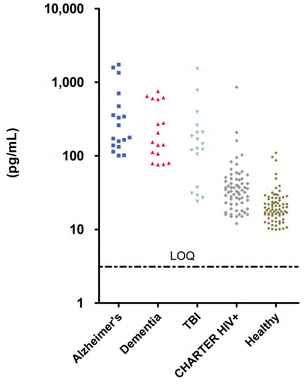
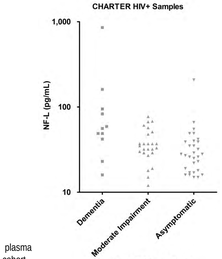
7 NF-L Concentrations in Serum and Plasma Samples



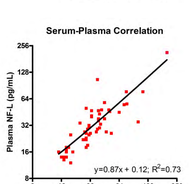
The figure to the left summarizes testing of commercial samples from individuals with Alzheimer's, dementia, and TBI; from HIV positive individuals from the CHARTER cohort, and from apparently healthy individuals. NF-L concentrations in the reference (healthy) sample set (5th, 25th, 50th, 75th, and 95th percentiles) were 10 pg/mL, 15 pg/mL, 19 pg/mL, 26 pg/mL, and 51 pg/mL, respectively. Median concentrations for mild and severe HIV-Associated Neurocognitive Disorder samples were 35 pg/mL and 56 pg/mL. Median concentration in Alzheimer's, dementia, and TBI samples were 220 pg/mL, 154 pg/mL, and 135 pg/mL, respectively.



The figure to the right shows dependence of NF-L serum or plasma concentrations on neurocognitive status in samples from the CHARTER cohort.

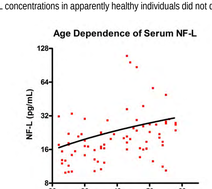
8 Serum Plasma Correlation

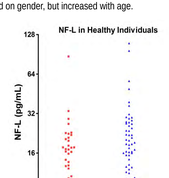
The CHARTER cohort included matched serum and EDTA plasma samples. Plasma and serum correlated well.



9 Dependence on Age and Gender

Serum/plasma NF-L concentrations in apparently healthy individuals did not depend on gender, but increased with age.





10 Dilution Linearity; Parallelism

	Dilution Factor	EDTA Plasma Signal (counts)	EDTA Plasma pg/mL (dilution corrected)	EDTA Plasma Recovery		Serum Signal (counts)	Serum pg/mL (dilution corrected)	Serum Recovery
Normal Donor 1	neat	4,627	11.3	100%	Normal Donor 9	10,477	27.9	100%
	2	3,106	13.8	122%		5,502	27.6	99%
	4	1,844	12.8	114%		3,352	30.4	109%
	8	1,360	14.2	126%		2,037	30.2	108%
Normal Donor 2	n/a	n/a	n/a	n/a	Normal Donor 10	6,442	16.5	100%
	2	7,116	36.8	100%		3,775	17.6	107%
	4	4,249	40.7	111%		2,374	19	116%
	8	2,525	41.6	113%		1,479	17	103%
Normal Donor 3	neat	6,314	16.3	100%	Normal Donor 11	7,569	19.9	100%
	2	3,096	14.2	87%		4,083	19.9	100%
	4	1,917	14.8	91%		2,622	22.9	115%
	8	1,336	16.3	100%		1,580	21.9	110%
Normal Donor 4	neat	3,846	9.25	100%	Normal Donor 12	24,565	68.7	100%
	2	2,311	9.68	105%		14,172	77.8	113%
	4	1,388	8.75	95%		7,375	77.5	113%
	8	999	8.56	93%		4,286	84.1	122%
TBI Donor 5	neat	21,081	57.6	100%	TBI Donor 13	24,707	67.7	100%
	2	10,941	58.4	101%		13,946	75.3	111%
	4	5,934	60.1	104%		7,062	72.9	108%
	8	3,398	61.9	107%		4,363	84.1	124%
TBI Donor 6	neat	22,263	60.9	100%	TBI Donor 14	63,231	174	100%
	2	12,579	67.7	111%		30,801	169	97%
	4	6,911	71.2	117%		15,686	170	98%
	8	3,562	65.6	108%		8,032	168	97%
TBI Donor 7	neat	46,606	132	100%	TBI Donor 15	10,255	27.6	100%
	2	24,679	138	105%		5,613	28.6	104%
	4	14,752	162	123%		3,046	27.8	101%
	8	7,563	159	121%		1,948	30.4	110%
TBI Donor 8	neat	38,141	108	100%	TBI Donor 16	67,595	192	100%
	2	19,826	110	102%		35,272	199	103%
	4	10,539	114	106%		16,925	187	97%
	8	5,510	112	104%		9,096	195	101%

Serum and plasma samples from apparently healthy individuals and from TBI individuals were diluted two-, four-, and eight-fold. The table to the left shows that nearly all samples diluted linearly. The average recovery of diluted samples was 107%.
This indicates that the assay can accurately measure NF-L in serum and plasma samples with little or no matrix effect.
The data also indicate that the NF-L assay is sufficiently sensitive to dilute samples (e.g. to save precious sample volume).

11 Spike Recovery

	Spike Conc. (pg/mL)	EDTA Plasma Signal (counts)	EDTA Plasma Conc. (pg/mL)	EDTA Plasma Recovery (%)		Heparin Plasma Signal (counts)	Heparin Plasma Conc. (pg/mL)	Heparin Plasma Recovery (%)		Serum Signal (counts)	Serum Conc. (pg/mL)	Serum Recovery (%)
Donor 1	5,000	149,113	5,525	109	Donor 4	91,586	3,466	68	Donor 7	106,977	4,022	79
	2,500	61,072	2,350	91		36,722	1,441	55		47,451	1,845	71
	1,250	33,533	1,320	99		22,359	892	66		28,779	1,139	85
	0	2,208	81	Ave. = 100		1,852	65	Ave. = 63		1,986	71	Ave. = 78
Donor 2	5,000	129,315	4,822	96	Donor 5	85,490	3,245	64	Donor 8	123,716	4,621	92
	2,500	55,282	2,137	84		39,001	1,528	60		51,649	2,001	79
	1,250	34,122	1,343	104		22,113	882	68		27,029	1,072	82
	0	1,278	39	Ave. = 95		1,054	29	Ave. = 64		1,403	45	Ave. = 84
Donor 3	5,000	123,001	4,596	91	Donor 6	97,547	3,682	73	Donor 9	103,581	3,899	77
	2,500	49,047	1,904	75		43,238	1,687	66		50,438	1,956	77
	1,250	26,876	1,066	82		25,066	996	76		26,491	1,051	82
	0	1,263	39	Ave. = 83		1,332	42	Ave. = 72		1,090	31	Ave. = 79
Control Spike (Calibr. Diluent)	5,000	126,647	4,726	94								
	2,500	57,026	2,201	88								
	1,250	29,017	1,148	92								
	0	440	0	Ave. = 91								

Serum and plasma samples from apparently healthy individuals were spiked with NF-L. The average recovery of spiked samples was 93% for EDTA plasma, 66% for heparin plasma, and 80% for serum. This indicates that the assay can accurately measure NF-L in serum and EDTA plasma samples with little or no matrix effect.

12 Conclusion

Using a next generation assay format based on MULTI-ARRAY technology, MSD developed and characterized a highly sensitive immunoassay for NF-L in serum and plasma.

The sensitivity of this assay is an order of magnitude better than typical concentrations in normal human serum or plasma.

The assay should prove useful for studying neuronal injury caused by diseases such as Alzheimer's or HIV.

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