Equivalence of Serum and Plasma Neurofilament Light Chain Levels Using a Highly Sensitive Automated Immunoassay

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Background:

Neurofilament light chain (NfL) in blood is a promising new biomarker for neurodegenerative disease[1] and clinical progression in presymptomatic Alzheimer's disease.[2] Well-established standardization of assays and collection methods is necessary before acceptance for clinical use. Data supporting equivalence of plasma and serum NfL testing are not yet widely published. In this study, we compared three blood-collection specimen types, two for plasma and one for serum.

Methods:

An automated NfL immunoassay was developed at Siemens Healthcare Laboratory, LLC,[3] using Siemens Healthineers high sensitivity acridinium ester technology (Figure 1).

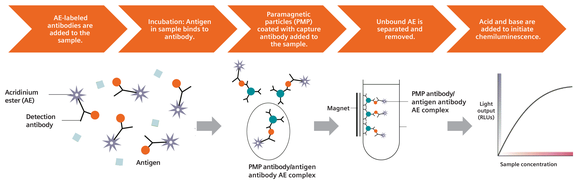

Figure 1. Assay principle. The Siemens Healthineers Atellica IM (AIM) NfL Assay* is an automated two-site sandwich immunoassay using direct chemiluminometric technology. The Solid Phase (SP) reagent contains a biotinylated anti-NfL monoclonal antibody coupled to a paramagnetic particle conjugated with streptavidin. The Lite Reagent (LR) contains a monoclonal anti-NfL antibody conjugated with acridinium ester (AE) for chemiluminescent detection.

Matched serum, heparin-treated plasma, and EDTA-treated plasma from 40 individual donors were acquired and tested for NfL concentration. Sample equivalence was analyzed with Deming regression. Five individual samples of each specimen type were tested neat and at four diluted levels. Recovery was calculated to assess sample parallelism.

Results:

A LLoQ of 1.39 pg/mL was established from two different reagent lots for both serum and plasma (Figure 2). Assay range was determined to be 2–500 pg/mL. The assay was linear across the assay range (Figure 3) and reproducible with %CV ≤6% in all tested conditions (Table 1). The NfL assay was able to tolerate hemolyzed, lipemic, and icteric samples as well as samples with high cholesterol, protein albumin, rheumatoid factor, and high biotin content (Table 2).

All two-way comparisons resulted in strong correlation as shown in Figure 4, including NfL concentrations in serum and lithium heparin plasma (slope = 1.004, r = 0.971), serum and K2EDTA plasma (slope = 1.065, r = 0.961), and lithium heparin plasma and K2EDTA plasma (slope = 1.059, r = 0.979).

NfL concentrations in all diluted serum, K2EDTA plasma, and lithium heparin plasma showed a recovery between 80 to 120% up to 10-fold, the highest dilution factor tested (Figure 5).

Specimen	Reagent Lot	LoB	LoD	LLoQ
Serum	1	0.49	0.96	0.96
	2	0.57	1.03	1.25
K2EDTA plasma	1	0.65	0.97	0.97
	2	0.58	1.04	1.39

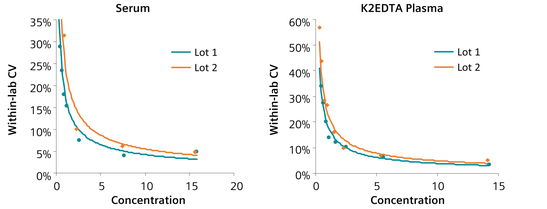

Figure 2. Assay sensitivity. 60 measurements per specimen type per reagent lot for LoB, and 40 measurements per concentration, per specimen type, per lot for LoD and LoQ was collected. LLoQ was determined using the precision profile approach with 20% CV as cutoff. A maximum of one outlier, using 3SD as cutoff, was removed from each data point. The highest number among the two specimen types AND two reagent lots was chosen. It was determined that LoB = 0.65 pg/mL, LoD = 1.04 pg/mL, LLoQ = 1.39 pg/mL.

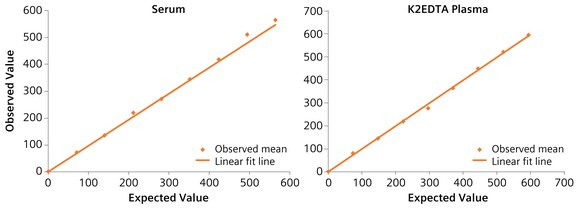

Figure 3. Assay linearity. For each sample type, a 9-level panel evenly spaced across the measuring interval was prepared and tested. Linearity was established from 0.4–596.0 pg/mL NfL in serum and 0.4–594.3 pg/mL in K2EDTA plasma.

Table 1. Assay reproducibility. Five-day precision from two independent reagent lots was assessed for within-run repeatability and total within-lab precision. %CV was ≤6% in all conditions tested.

Specimen Type	Reagent Lot	NfL level	# Days	# Runs	# Reps	Mean (pg/mL)	Within-run Repeatability		Within-lab Precision	
							SD (pg/mL)	CV%	SD (pg/mL)	CV%
Serum	Lot 1	Low	5	10	20	7.1	0.2	3.3	0.3	3.7
		Medium	5	10	20	43.9	1.8	4.2	1.9	4.4
		High	5	10	20	351.1	15.3	4.4	18.0	5.1
	Lot 2	Low	5	10	20	6.9	0.2	3.5	0.3	4.6
		Medium	5	10	20	43.0	1.8	4.2	2.4	5.5
		High	5	10	20	337.2	18.6	5.5	20.3	6.0
K2EDTA Plasma	Lot 1	Low	5	10	20	10.1	0.3	2.8	0.4	3.9
		Medium	5	10	20	45.7	2.1	4.7	2.4	5.3
		High	5	10	20	346.3	16.4	4.7	19.8	5.7
	Lot 2	Low	5	10	20	9.9	0.2	2.4	0.4	4.2
		Medium	5	10	20	46.0	2.4	5.2	2.7	5.8
		High	5	10	20	325.0	14.0	4.3	17.0	5.2

Table 2. Interference study. AIM NfL assay was not affected by common interfering substance, including high biotin content.

Interferent	Level	Control NfL Dose (pg/mL)	Sample NfL Dose (pg/mL)	%Bias	Pass/ Fail	Control NfL Dose (pg/mL)	Sample NfL Dose (pg/mL)	%Bias	Pass/ Fail
		Serum				K2EDTA Plasma			
2000 mg/dL INTRALIPID	Low	14.84	14.20	–4%	Pass	17.73	15.99	–10%	Pass
	High	270.18	259.57	–4%		292.03	275.36	–6%	
750 U/mL RF serum	Low	14.83	15.78	6%	Pass	15.50	16.13	4%	Pass
	High	287.68	260.71	–9%		308.18	314.76	2%	
500 mg/dL cholesterol	Low	14.83	15.09	2%	Pass	15.50	16.75	8%	Pass
	High	287.68	278.15	–3%		308.18	339.83	10%	
6 g/dL human serum albumin	Low	14.83	15.43	4%	Pass	15.50	16.95	9%	Pass
	High	287.68	287.99	0%		308.18	336.63	9%	
500 mg/dL human hemoglobin	Low	17.74	18.45	8%	Pass	19.12	19.21	1%	Pass
	High	333.09	341.60	3%		322.68	330.69	2%	
60 mg/dL direct bilirubin	Low	17.74	17.35	2%	Pass	19.12	18.83	–2%	Pass
	High	333.09	316.33	–5%		322.68	320.32	–1%	
40 mg/dL indirect bilirubin	Low	17.74	17.12	0%	Pass	19.12	19.72	3%	Pass
	High	333.09	314.83	–5%		322.68	327.77	2%	
3500 ng/mL biotin	Low	17.74	17.93	5%	Pass	19.12	19.64	3%	Pass
	High	333.09	362.84	9%		322.68	342.55	6%	

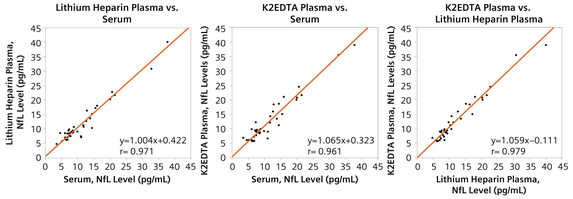

Figure 4. Serum and plasma equivalence. Matched serum, heparin plasma, and EDTA plasma from 40 individual donors were tested. NfL concentrations in plasma and serum were determined to be equivalent.

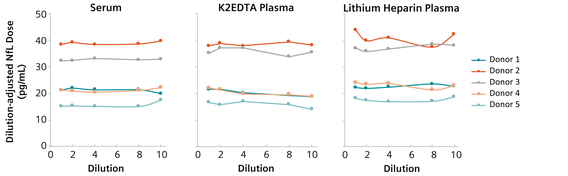

Figure 5. Parallelism. Five individual samples of each specimen type with endogenous NfL levels from 15 to 45 pg/mL were tested neat and 2, 4, 8, and 10-fold diluted. Recovery from all conditions was between 80 and 120%. Therefore, parallelism was established for the AIM NfL assay in serum, K2EDTA plasma, and lithium heparin plasma. Samples could be diluted up to 10-fold and yield expected dose results.

Conclusion:

A sensitive and automated NfL assay was developed. With a well-optimized automated immunoassay, NfL concentrations in plasma and serum were equivalent.

References:

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2. Preische O, et al. Serum neurofilament dynamics predicts neurodegeneration and clinical progression in presymptomatic Alzheimer's disease. *Nat Med*. 2019 Feb;25(2):277-83. doi: 10.1038/s41591-018-0304-3
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*This test was developed and its analytical performance characteristics were determined by the Siemens Healthcare Laboratory. It has not been cleared or approved by the U.S. Food and Drug Administration.

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