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Development of a Sensitive Serum Neurofilament Light Assay on Siemens Routine Immunoassay Platforms

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*At the time of the study

Conclusions

- **The demonstrated sNfL assay performance on a routine automated immunoassay platform provides a possible path for implementation of sNfL testing into drug development and clinical practice in neurological and neurodegenerative diseases.**

Introduction

- Serum neurofilament light chain (sNfL), a marker of neuroaxonal injury, has shown promise as a prognostic and monitoring biomarker in a number of neurological conditions, including multiple sclerosis (MS).[1,2]
- Currently, sNfL is measured with a single-molecule array (SIMOA) assay.
- sNfL integration into clinical practice and drug development would be best supported by a sensitive and standardized assay available on widely accessible routine automated immunoassay analyzers.

Objectives

- To develop a sensitive sNfL assay on an automated routine immunoassay platform.

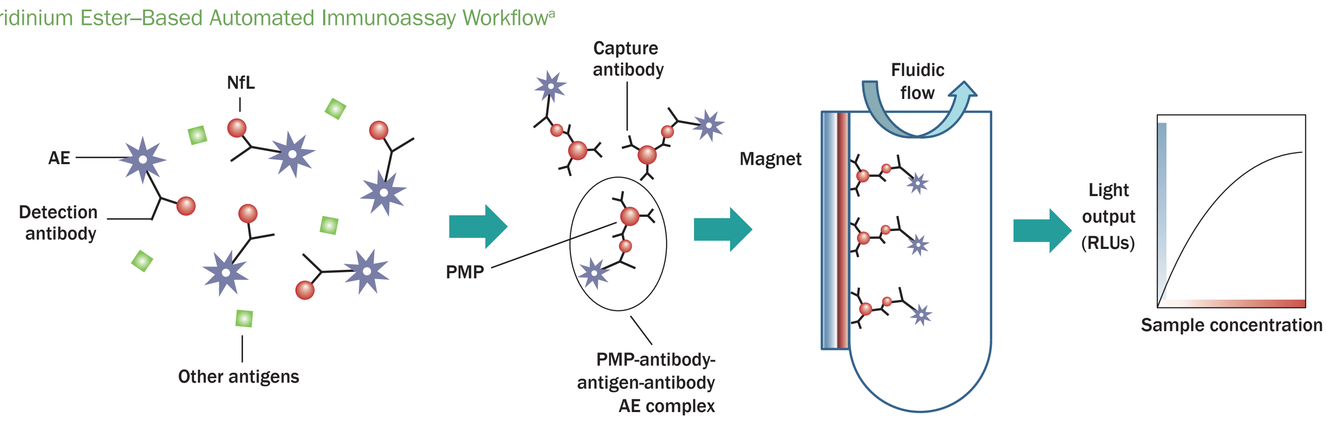
Methods

- Antibodies were screened and selected based on affinity for the NfL antigen.
- Solid-phase paramagnetic bead capture and acridinium ester–based detection reagents were designed for use with Siemens routine immunoassay platforms, the ADVIA Centaur® and Atellica® systems (Siemens Healthineers, Tarrytown, NY), as described in Figure 1.
- Calibrators and control materials were developed to enable reliable highly sensitive quantitative reporting.
- Analytical performance of the assay was evaluated with respect to sensitivity utilizing the precision profile approach for estimating the lower limit of quantitation[3] (LLoQ), linearity, parallelism, repeatability, matrix interference, and reagent stability.
- Correlation between sNfL results generated with the routine immunoassay platform and SIMOA NfL-light® Advantage Kit (Quanterix, Billerica, MA) was performed using spiked and clinical samples.

Results

- A sensitive research assay for sNfL was created using selected antibodies, optimized reagents, and test conditions.
- The analytical sensitivity was estimated to be 1.62 pg/LLoQ at a 20% precision limit using 1 lot of reagents (Figure 2A).
- Reagent lot-to-lot variability was assessed, resulting in a LLoQ of 1.84 and 1.85 pg/mL for 2 additional lots (Figure 2B).
- Linearity of the assay was assessed across a range of 1–646 pg/mL NfL in serum. Linear regression results were $R^2 = 0.996$ with $p < 0.001$.
- Repeatability within runs was evaluated across 20 days using samples that spanned 80% of the assay measurement range. Within-run coefficient of variation results were 1.5–3.4%, and total coefficient of variation results were 2.5–5.1% (Table 1).
- Parallel recovery for NfL was demonstrated using donated serum samples from 10 individuals ranging from 16–35 pg/mL sNfL. The sera were diluted 1:2, 1:4, and 1:8. Recovery at all dilutions was within 20% of expected values (Table 2; Figure 3).
- Analysis of analytical panel samples spiked with NfL antigen (n = 10), and individual serum samples (N = 122) from patients with MS demonstrated high correlation ($R^2 = 0.998$, $p < 0.0001$; and $R^2 = 0.838$, $p < 0.0001$, respectively) between NfL results derived from the Siemens automated analyzer and from the Quanterix SIMOA platform (Figures 4 and 5).
- NfL values generated from the Siemens assay demonstrated association with clinical and radiological disease activity measures in patients with MS, similar to those observed with the comparator assay (Figure 6).

Figure 1. Acridinium Ester–Based Automated Immunoassay Workflow[a]



AE = acridinium ester; NfL = neurofilament light chain; PMP = paramagnetic particle; RLU = relative luminescence unit
[a]As implemented on the Centaur and Atellica testing platforms.

Table 1. Twenty-Day Precision of the Siemens Assay, Assessed for Repeatability (Within-Run) and Total (Within-Lab) Precision[4,a]

NfL Sample n = 80	Mean Dose	Within-Run Variability		Total Variability	
	pg/mL	SD	CV	SD	CV
Endogenous Level 1	7.65	0.26	3.4%	0.39	5.1%
Endogenous Level 2	17.23	0.57	3.3%	0.76	4.4%
Endogenous Level 3	54.18	0.96	1.8%	1.66	3.1%
Recombinant Level 1	17.89	0.39	2.2%	0.58	3.2%
Recombinant Level 2	54.95	0.84	1.5%	1.37	2.5%
Recombinant Level 3	438.03	7.49	1.7%	12.17	2.8%

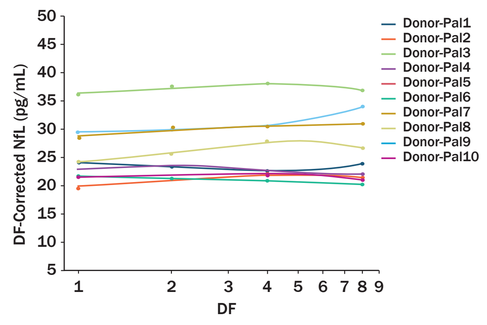
CV = coefficient of variation; NfL = neurofilament light chain
Endogenous refers to NfL from cerebrospinal fluid samples spiked in serum.
[a]Endogenous and recombinant NfL samples were selected to span 80% of the overall assay measurement range (low, medium, and high). Each sample was tested twice per run; 2 runs were completed per day using 1 reagent lot over the course of 20 days for a total of 80 measurements per sample.

Table 2. Parallel Dilution Recovery of 10 Individual Serum Samples With Endogenous NfL Across a Range of 16–35 pg/mL, Tested at 1:2, 1:4, and 1:8 Dilutions[a]

Sample ID	Neat	1:2		1:4		1:8	
	Mean Dose (pg/mL)	Mean Dose (pg/mL)	% Recovery	Mean Dose (pg/mL)	% Recovery	Mean Dose (pg/mL)	% Recovery
Donor-Pal1	21.7	10.4	95.8%	5.0	108.6%	2.7	101.1%
Donor-Pal2	16.7	9.4	88.6%	4.8	86.6%	2.3	89.1%
Donor-Pal3	34.7	18.1	95.7%	9.2	94.4%	4.4	97.8%
Donor-Pal4	19.5	11.2	86.9%	4.9	98.8%	2.4	99.9%
Donor-Pal5	18.8	9.7	96.8%	5.0	94.7%	2.3	100.2%
Donor-Pal6	19.0	9.2	103.1%	4.6	104.4%	2.2	108.8%
Donor-Pal7	26.3	14.2	92.4%	7.1	92.0%	3.6	90.3%
Donor-Pal8	21.8	11.6	93.8%	6.5	84.5%	3.1	88.9%
Donor-Pal9	27.5	14.0	98.2%	7.2	95.7%	4.0	84.9%
Donor-Pal10	18.8	9.8	95.8%	4.9	95.8%	2.3	101.7%

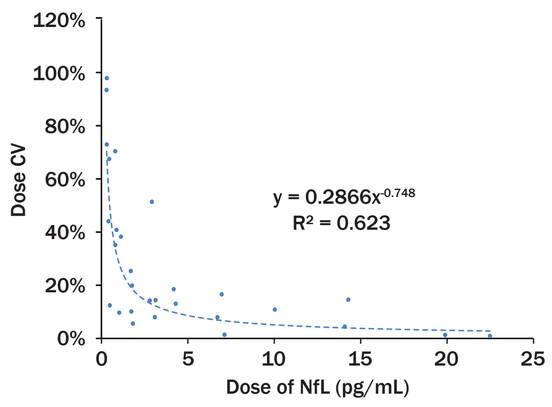
DF = dilution factor
[a]Recovery was within ± 20% of expected values for all samples at all dilutions.

Figure 3. Parallel Dilution Recovery of 10 Individual Serum Samples[a]



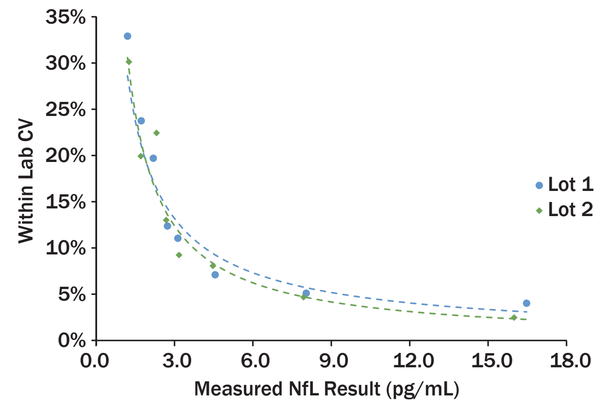
DF = dilution factor; NfL = neurofilament light chain
[a]Each NfL result was corrected for DF.

Figure 2A. LLoQ Was Calculated Using the Precision Profile Method[a,3]



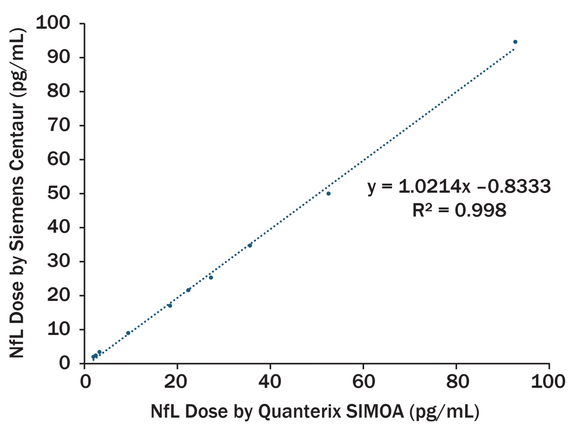
CV = coefficient of variation; LLoQ = lower limit of quantitation; NfL = neurofilament light
[a]One instrument run (Centaur) and 1 lot of reagents were used. LLoQ was determined to be 1.62 pg/mL at the 20% precision limit.

Figure 2B. Analytical Samples at 8 Concentrations Spanning the Assay LoB to 15-Fold Above the LoB, Generated From Donor Serum With Endogenous NfL Diluted in Pooled Donor Serum First Depleted of NfL Using Antibody Immunoabsorption[a]



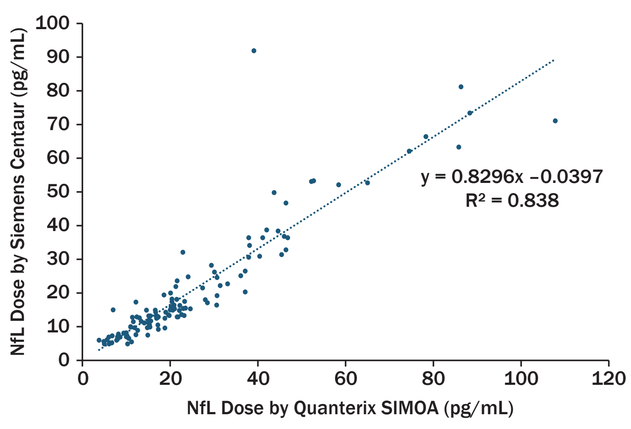
CV = coefficient of variation; LoB = limit of blank; NfL = neurofilament light chain
[a]Inter-run, interday, and interlot variation were tested (4 replicates per sample; 2 runs per day over 5 days; 2 reagent lots). The lower limit of quantitation for lots 1 and 2 were 1.84 and 1.85, respectively, at a precision cutoff of 20% CV.

Figure 4. Analysis of Pooled Serum Samples Spiked With Recombinant NfL Antigen Across the Lower Assay Range (< 100 pg/mL)[a]



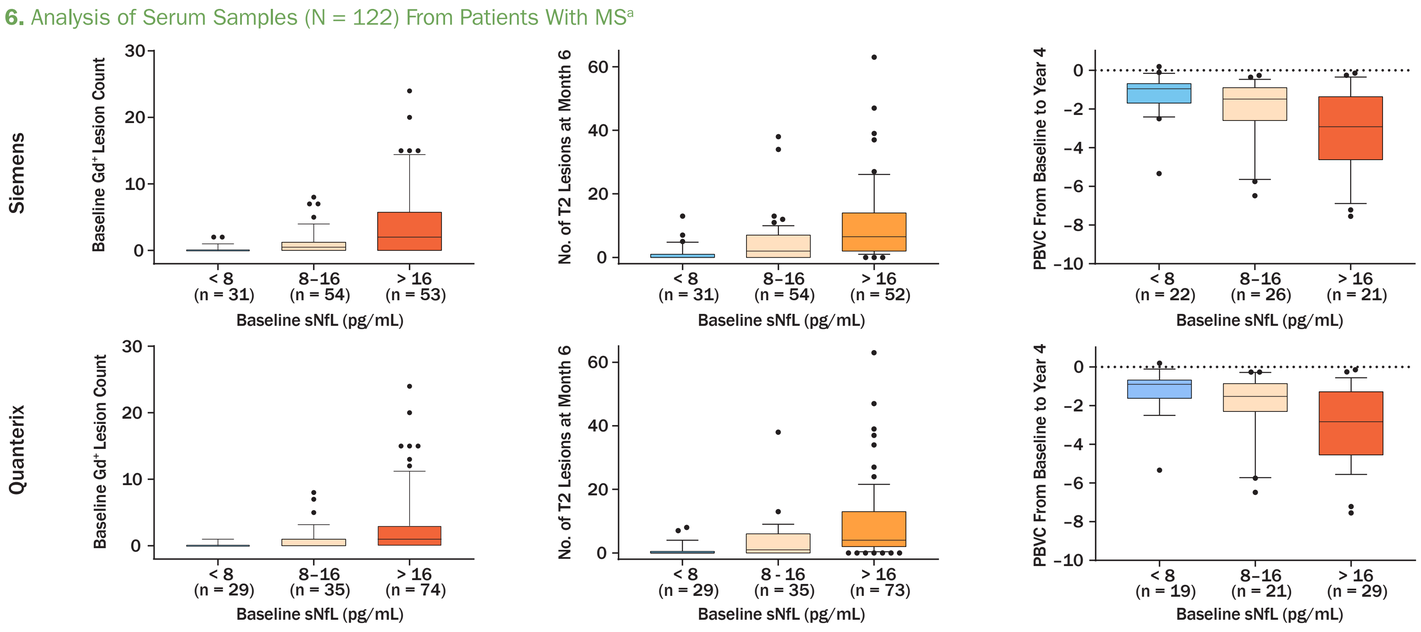
NfL = neurofilament light chain; SIMOA = single-molecule array
[a]Strong correlation was demonstrated ($R^2 = 0.9982$; $p < 0.0001$) between the Siemens and the Quanterix assays.

Figure 5. Analysis of Serum Samples (N = 122) From Patients With MS[a]



MS = multiple sclerosis; NfL = neurofilament light chain; SIMOA = single-molecule array
[a]Moderately strong correlation was demonstrated ($R^2 = 0.838$; $p < 0.0001$) between the Siemens and Quanterix assays. Due to limited sample volumes available, 120 serum samples were diluted 1:3 and 2 serum samples were diluted 1:4 in sample diluent. Reported dose values were adjusted for dilutions.

Figure 6. Analysis of Serum Samples (N = 122) From Patients With MS[a]



Gd⁺ = gadolinium-enhancing; MS = multiple sclerosis; PBVC = percentage brain volume change; sNfL = serum neurofilament light chain
[a]The Siemens and Quanterix assays demonstrated association with clinical and radiological disease activity measures in patients with MS.

References 1. Kuhle J, et al. *Neurology* 2019;92(10):e1007-e1015. 2. Sormani MP, et al. *Ann Clin Transl Neurol*. 2019;6(6):1081-1089. 3. Clinical and Laboratory Standards Institute. *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline–Second Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2012. 4. Clinical and Laboratory Standards Institute. *Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline–Third Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2014. **Disclosures** TP, CMS, and RAR: employees of and hold stock/stock options in Biogen; LS: employee of Biogen at the time the analysis was conducted; PAC: Principal Investigator on grants to Johns Hopkins from Annexon and Biogen; consulting fees from Biogen and Disarm; SL, CG, MM, and AJU: employees of Siemens Healthcare Laboratory, which was contracted by Biogen for this work. **Acknowledgments** This study was sponsored by Biogen (Cambridge, MA, USA) and Siemens Healthcare Laboratory (Berkeley, CA, USA). Writing and editorial support for the preparation of this poster was provided by Excel Scientific Solutions (Fairfield, CT, USA): funding was provided by Biogen.