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Laboratory Validation of a Neurofilament Light Chain (NfL) Clinical Trial Assay for Applications in Neurological Diseases Testing Services

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Background:

The identification and quantification of axonal damage in neurological diseases management could be crucial for improved prognosis and diagnostic accuracy. Neurofilament light chain (NfL) is a neuron specific structural protein that can be detected in blood and cerebrospinal fluid (CSF) as a biomarker associated with axonal injury or degeneration.[1]

The role of NfL as a biomarker has been reported in Alzheimer's disease (AD), traumatic brain injury (TBI), and multiple sclerosis (MS).[2] Siemens Healthcare Laboratory (SHL) validated an in-house NfL assay that runs on a Siemens Healthineers high-throughput platform.

Methods:

The SHL NfL Clinical Trial assay (CTA) runs on the Atellica® Immunoassay (IM) Analyzer, which is a part of the Atellica Solution (Figure 1). The assay is an automated two-site sandwich immunoassay using direct chemiluminometric technology. This NfL assay uses two specific non-competing monoclonal antibodies against the conserved rod domain of NfL, without cross-reactivity for neurofilament medium chain (NfM) or neurofilament heavy chain (NfH). In the assay's Lite Reagent, the detection antibody is labeled with a high yield acridinium ester. The SHL NfL CTA was analytically validated in a CLIA-CAP-accredited laboratory for use in clinical trials. Validation studies were performed using serum, lithium heparin (LiH) plasma, EDTA plasma, and cerebrospinal fluid (CSF).

Figure 1. The Atellica Solution.

Results:

Analytical performance

The reproducibility, sensitivity, and linearity of the NfL assay were assessed for serum, plasma, and CSF. The quantitation range was determined to be the range of concentrations that met acceptance criteria for precision (<20%CV) and linearity (R^2 >0.95).

Table 1. NfL assay performance characteristics.

Performance Characteristic	Serum	EDTA Results	LiH Results	CSF Results
Sensitivity	LoB: 0.46 pg/mL LoD: 0.71 pg/mL LLoQ: 3.86 pg/mL	LoB: 0.58 pg/mL LoD: 1.71 pg/mL LLoQ: 4.93 pg/mL	LoB: 1.32 pg/mL LoD: 1.80 pg/mL LLoQ: 2.35 pg/mL	LoB: 32.3 pg/mL LoD: 54.2 pg/mL LLoQ: 85.5 pg/mL
Reproducibility (%CV across assay range)	4.9 to 8.4%	7.7 to 18.1%	3.5 to 16.3%	4.0 to 16.5%
Assay range	3.86 to 500 pg/mL R^2: 0.985	4.93 to 477 pg/mL R^2: 0.997	2.35 to 549 pg/mL R^2: 0.988	85.5 to 25,700 pg/mL R^2: 0.990

Specimen handling and stability

Donor blood and CSF samples were cycled through different short-term storage conditions to determine acceptable sample handling parameters. The samples were also frozen to determine long-term stability. Sample results with a bias of less than 20% were considered acceptable. The results at the time of this publication are shown in Table 2. Long-term frozen stability testing is ongoing.

Table 2. NfL stability results.

Storage Condition	Serum	LiH	EDTA	CSF
Freeze/thaw	Up to 6 freeze/thaw cycles			
Room temperature	Up to 1 week			
Refrigerated 4–8°C	Up to 2 weeks			Up to 1 week
Frozen –15 to –25°C	2 years		3 months	
Frozen –60 to –90°C	2 years			

Interference (endogenous substances)

Donor samples were spiked with endogenous substances at high concentrations. No assay interference was observed in the presence of the substances shown in Table 3. The highest observed result bias was 10%.

Table 3. Endogenous substances.

Interference Test Panel	
Hemoglobin below 500 mg/dL	RF below 193 U/mL
Direct bilirubin below 60 mg/dL	Biotin below 3500 ng/mL
Indirect bilirubin below 40 mg/dL	Glucose 220 mg/dL
Albumin below 6 g/dL	Neurofilament heavy chain
Triglycerides below 2000 mg/dL	Neurofilament medium chain

Drug interference

Donor samples were spiked with common over-the-counter drugs and drugs typically used to treat multiple sclerosis and Alzheimer's disease. No interference was observed at levels equal to or less than three times the C_{max} of the drug. The highest observed result bias was 10%. See Table 4 for the drugs tested for interference.

Table 4. Drug interference.

Drug Interference Test Panel			
Donepezil	Sertraline	Acetaminophen	Dimethyl fumarate
Rivastigmine	Bupropion	Aspirin	Teriflunomide
Memantine	Duloxetine	Beta interferon 1a	Mitoxantrone
Galantamine	Imipramine	Beta interferon 1b	Cladribine
Citalopram	Ibuprofen	Fingolimod	
Mirtazapine	Siponimod		

Method comparison

A panel of 50 serum samples and 51 CSF samples were tested on the SHL NfL CTA and Quanterix SIMOA assays. The results were compared to determine the quantitation differences between the two assays. Deming regression analysis was performed, and quantitation differences were assessed using a Bland Altman plot. See Figure 2 for the serum analysis and Figure 3 for the CSF analysis.

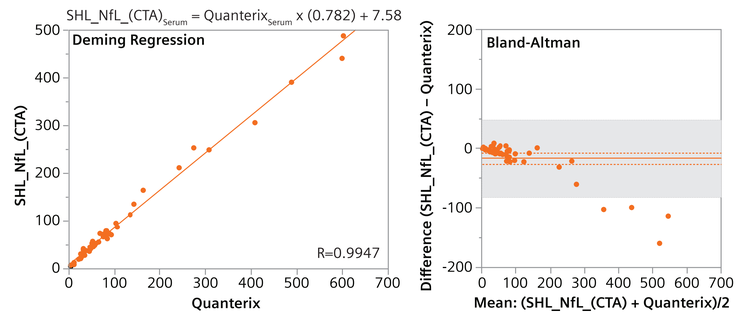

Figure 2. Method comparison with serum (pg/mL)

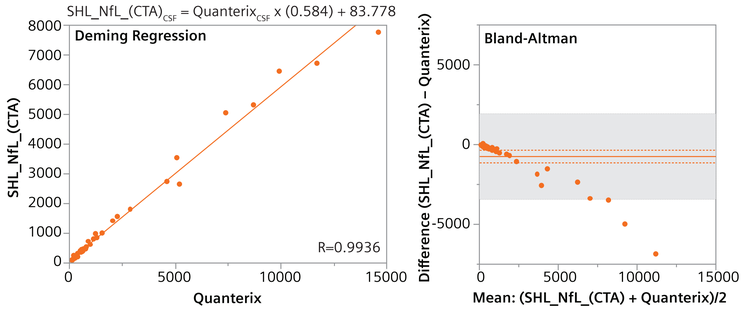

Figure 3. Method comparison with CSF (pg/mL)

The serum results indicate a very close relationship between the SHL NfL CTA and SIMOA assays, with a correlation coefficient of 0.995. There is a slight bias in quantitation values as indicated by a slope of 0.78, with the SIMOA assay yielding higher values at the higher end of the assay range (>200 pg/mL). The average quantitation difference was 8%.

The CSF assays exhibit a similar fit of data, with a correlation coefficient of 0.994. There is a similar quantitation bias as indicated by a slope of 0.58, with the SIMOA assay yielding higher values. The average quantitation difference was 29%.

Conclusions:

Using the SHL NfL CTA, NfL levels in CSF, serum, and plasma samples were detected reproducibly across the reporting range. The assay produces reliable results in the presence of endogenous substances known to cause assay interference, common over-the-counter drugs, and drugs used to treat two neurological conditions. The SHL NfL CTA exhibited good correlation to the SIMOA NfL assay. The stability of NfL in samples exposed to different storage conditions was shown to be practical for the use of NfL detection in clinical trials.

References:
1. Gaetani L, Blennow K, Calabresi P, Di Filippo M, Parnetti L, Zetterberg H. Neurofilament light chain as a biomarker in neurological disorders. J Neurol Neurosurg Psychiatry. 2019;90(8):870-81.
2. Barro C, Chitnis T, Weiner HL. Blood neurofilament light: a critical review of its application to neurologic disease. Ann Clin Transl Neurol. 2020;7(12):2508-23.

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