We invite you to discover our expertise through a series of real case studies:

- IMMUNOPHENOTYPING
- FUNCTIONAL ASSAYS
- SOLUBLE BIOMARKERS
- DRUG-TARGET INTERACTIONS

Introduction

Conventional ELISA (Enzyme-Linked Immuno-Sorbant Assay), although still largely used, is limited in terms of sensitivity and the number of analytes that can be analyzed. The emergence of ultra-sensitive technologies, such as the single-molecule array (SIMOA™) technology from Quanterix, offers the possibility to detect and quantify several biomarkers with up to a 100-fold gain in sensitivity. These technologies open the path to the monitoring of previously undetectable circulating biomarkers in multiple pathological processes, notably in the field of neurobiology. Neuroinflammatory and neurodegenerative disorders, whatever their origin, lead to the accumulation of specific neuronal proteins in cerebrospinal fluid and blood. Neurofilament-Light chain (NF-L) is one of the 3 subunits, with intermediate (NF-M) and heavy (NF-H) chains, constituting the main components of intermediate filaments in neurons. Neurofilaments are involved in axonal growth and maintenance, as well as electric transmission in the nervous system. While displaying low turnover in physiological non pathological conditions, neurofilaments rise up in cerebrospinal fluid and blood during neuroaxonal injury. NF-L is a blood biomarker of interest in the differential diagnosis of some neurodegenerative disorders such as Amyotrophic Lateral Sclerosis, Parkinsonian disorders, but also of prognostic value for progression and response to therapy in Multiple Sclerosis (MS). Moreover, it is linked to disease activity in MS, Alzheimer's, and Huntington's. In these neurological pathologies, there is a need for targeting early stages of disease and monitoring therapeutic intervention in easily accessible biological fluids. Blood NF-L is a valuable biomarker not only in neurological disorders but also in many other indications including cancer, infectious diseases, autoimmune syndromes, or sport-related concussions. It is then crucial that assays for monitoring this biomarker are robust, accurate, and reproducible, in other terms, reliable.

Our team has qualified the NF-Light Advantage Kit from Quanterix (ref: 102258) on the SIMOA™ HD1 system, evaluating its performance in terms of dynamic range, precision, parallelism and selectivity, lot-to-lot consistency and stability for quantifying NF-L in both plasma and serum matrices.
Our team has qualified the NF-Light Advantage Kit from Quanterix (ref: 102258) on the SIMOA™ HD-1 system, evaluating its performance in terms of dynamic range, precision, parallelism and selectivity, lot-to-lot consistency and stability for quantifying Nf-L in both plasma and serum matrices.

NF-Light Advantage Kit has been qualified with paired serum and EDTA-plasma samples from 8 healthy donors (HD) aged from 24 to 68 (3 > 60 years-old), and with 8 serum samples from MS patients with secondary progressive MS (SPMS) or with relapsing-remitting MS (RRMS) (Figure 8B).

After demonstrating that calibration curve, prepared according to manufacturer’s instructions, met precision (CV≤25%) and recovery (70-130%) acceptance criteria, we were able to extend the dynamic range down to 0.34 pg/mL (Figure 1).

While recombinant Nf-L protein was poorly recovered when spiked in blood matrices (Figure 2A), likely due to differences between recombinant and endogenous protein, parallelism could be demonstrated over 3 to 4 serial dilutions in serum or plasma samples from respectively healthy or MS individuals (Figure 3) and the minimal required dilution (MRD) of 1:4 was confirmed in blood matrix. In addition, selectivity was demonstrated by fractionally admixing high Nf-L with low Nf-L serum samples (Figure 2B). As a consequence, validation parameters, except accuracy, were evaluated on endogenous Nf-L.

**Figure 1 - Calibration curve of Nf-L kit**

**Figure 2 - Selectivity of the method tested on recombinant (Fig.2A) and endogenous Nf-L (Fig. 2B)**

**Figure 2A**: Recovery of recombinant Nf-L, spiked at 180 pg/mL and measured at MRD (1:4) ranges from 34 to 44% in serum and 33 to 39% in plasma, largely below the acceptance criteria (70-130%). Recovery improves with dilutions (1:8 and 1:16), suggesting interference between reference material and blood matrix.

**Figure 2B**: Samples with elevated endogenous Nf-L (MS4 and MS8) were admixed with samples with low endogenous Nf-L (MS2 and MS6) at different ratios. For each ratio, measured values (blue shaded bars with *) are compared to theoretical/expected levels and are highly correlated with a coefficient of correlation $r^2=0.9954$. 
Nf-L was measured in plasma and serum samples from 2 healthy donors in diluted matrix (1:6 to 1:16) to reach 0.5-0.7 pg/mL. Each sample was measured in 10 replicates. Individual values (blue symbols) as well as mean (black horizontal bar) are shown for each donor (1&6) in serum or plasma.

Intra- and inter-run precision were evaluated in plasma and serum from 4 healthy individuals (Donor 1 (D1), 3, 4 & 5). Coefficient of variation (CV), calculated according to “SFSTP commission reports on the harmonization of approaches in the validation of quantitative analytical procedure” is below 25 % for each run.

Serum & plasma from 5 healthy individuals <60 (HD1-5) and 3 >60 (HD6-8) and serum from 8 patients with multiple sclerosis (MS) (Neurobiotec, CRB Hospices Civils de Lyon, France) tested at MRD (1:4), MRD/2 (1:8), MRD/4 (1:16) and MRD/8 (1:32).

The kit has shown a good parallelism on 3 dilutions in HD, and 4 dilutions in MS samples.

This method displayed good precision in both plasma and serum, when tested at the MRD, with coefficient of variation (CV) below 20 % for measured levels in the range of 1-2 pg/mL (Figure 4). Lower Limit of Quantification (LLOQ) was challenged in diluted blood matrix on 10 replicates (Figure 5).

Nf-L levels were measured down to 0.55 and 0.63 pg/mL respectively in plasma (at 1:8 dilution) and serum (1:16 dilution) from HD.

In addition, Nf-L in blood matrices was shown to be stable both upon up to 3 freeze-thaw cycles as well as over a 9-month period at -80°C (Figure 6).

Finally, 2 distinct lots of the kit were evaluated on 3 samples from healthy donors and 8 samples from MS patients, showing good consistency with a recovery ranging from 77 to 91 % (Figure 7).

This kit allowed us to quantify endogenous levels of Nf-L, not only in MS samples, but also in blood samples from healthy individuals, and in individuals over 60 years-old.

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According to results reported by others, Nf-L levels tended to be higher in the serum of MS patients compared to healthy individuals, and in individuals over 60 years-old.

Intra-run variability

Inter-run variability

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<th>CV Intra (%)</th>
<th>Donor 1</th>
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<th>Donor 3</th>
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<table>
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<th>CV Intra (%)</th>
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<tr>
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CONCLUSION

Despite poor recovery of spiked recombinant protein, the assay showed very good sensitivity and precision allowing to measure levels <1 pg/mL of Nf-L in blood matrices (e.g. in healthy donors <60) with good lot-to-lot consistency. Overall this method showed performance characteristics suitable for the monitoring of Nf-L in blood in the context of therapeutic intervention in neurological disorders.

REFERENCES


