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Research Paper

A French multicenter analytical evaluation of the automated Lumipulse G sNfL blood assay (Fujirebio®) and its comparison to four other immunoassays for serum neurofilament light chain assessment in clinical settings

Etienne Mondésert [a,b,*], Susanna Schraen-Maschke [c], Isabelle Quadrio [d,e], Olivier Bousiges [f], Damien Bouvier [g], Constance Delaby [a,h], Aurélie Bedel [i,j], Sylvain Lehmann [a], Anthony Fourier [d,e]

[a] *Laboratoire de Biochimie Protéomique Clinique (LBPC), Université de Montpellier, CHU de Montpellier, Institut des Neurosciences de Montpellier (INM), INSERM, Montpellier, France*
[b] *Département de Biochimie, Université de Montpellier, CHU de Montpellier, Montpellier, France*
[c] *Univ. Lille, Inserm, CHU Lille, UMR-S-U1172, LiCEND, Lille Neuroscience & Cognition, LabEx DISTALZ, Lille, France*
[d] *Biochimie et Biologie Moléculaire-LBMMS, Unité de diagnostic des pathologies dégénératives, Centre de Biologie et Pathologie Est, Groupement Hospitalier Est, Lyon, France*
[e] *Equipe BIORAN, Centre de Recherche en Neurosciences de Lyon, CNRS UMR 5292, INSERM U1028, Université Lyon 1, Bron, France*
[f] *Laboratoire de biochimie et biologie moléculaire (LBBM), Pôle de biologie Hôpital de Hautepierre-CHU de Strasbourg, CNRS, laboratoire ICube UMR 7357 et FMTS (Fédération de Médecine Translationnelle de Strasbourg), équipe IMIS, Strasbourg, France*
[g] *Service de Biochimie et Génétique Moléculaire, CHU de Clermont-Ferrand, Clermont-Ferrand, France*
[h] *Hospital de la Santa Creu i Sant Pau, Biomedical Research Institute Sant Pau, Universitat Autònoma de Barcelona, Barcelona, Spain*
[i] *Université de Bordeaux, INSERM BRIC U1312, Eq 8 BioGO, France*
[j] *Service de Biochimie, CHU Bordeaux, France*

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ABSTRACT

Objectives: Measurement of serum neurofilament light chain (sNfL) protein is becoming a key biomarker for many neurological diseases. Several immunoassays have been developed to meet these clinical needs, revealing significant differences in terms of variability and results. Here, we propose a French multicenter comparison of 5 sNfL assays.

Methods: 6 replicates of 3 pools with low (10 pg/mL), medium (30 pg/mL) and high (100 pg/mL) sNfL values and one replicate of 12 samples with growing sNfL values were analyzed by six independent French clinical laboratories. The analytical performances of the sNfL blood assay (Fujirebio®) on Lumipulse G were first evaluated then compared to four other immunoassays: NF-light V2 (Quanterix®) on SiMOA HD-X, Human NF-L (Biotechne®) on Ella, R-Plex Human Neurofilament L (MSD®) on Sector 2400; manual ELISA test using Uman Diagnostic/Quanterix®.

Results: Inter-center comparison of the Lumipulse blood assay revealed limited but significant differences in the mean sNfL values across low, medium, and high pools between each city ($p < 0.001$) and between the two different batches used. Coefficients of variation of pools ranged from 2.0 to 16.9 %. Z-score of sNfL results of the 12 samples ranged from −1.70 to +1.71. Inter-technique comparison showed a systematic difference of sNfL values, with a overestimation of MSD and Ella over other tests. Nonetheless, results were all significantly correlated ($p < 0.001$).

Conclusion: The automated Lumipulse assay produced comparable sNfL values across laboratories; but further adjustments are needed to harmonize sNfL results. Biologists and physicians should be aware of the variability in results between different immunoassay suppliers.

* Corresponding author at: Department of Biochemistry, Lapeyronie Hospital, F34295 Montpellier, France.
E-mail address: e-mondesert@chu-montpellier.fr (E. Mondésert).

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1. Introduction

Serum neurofilament light chain (sNfL) protein emerged as a key biomarker in neurological disorders. Being a component of the neuronal skeleton, an increase in its soluble form in fluids like cerebrospinal fluid (CSF) or blood indicates axonal damage, making it a specific biomarker of neuronal injury. [1,2]. As non-etiological biomarker of neurological diseases, the determination of sNfL was assessed and appeared to be relevant in many indications [3–6]. The latter includes diagnostic strategies for degenerative disorders [7,8] or the presence of brain metastases [9]. Prognostic performances of sNfL were also assessed in various conditions including amyotrophic lateral sclerosis [10], multiple sclerosis [11,12] and ischemic stroke [13]. Finally, treatment response was also evaluated by sNfL determination in different diseases [14,15], promising personalized treatment decisions for patients.

Pending complete implementation of sNfL in clinical laboratory practice, different problematics need to be resolved. The final step will be the determination of a range of specific normal and pathological values concerning the multiple contexts of use of sNfL determination [16]. The determination of such cut-off values relies on a good understanding of pre-analytical factors (i.e. type of biological sample, sampling procedures, conservation), analytical factors and post-analytical factors that may modify sNfL concentrations leading to possible misinterpretations of results. Laboratories performing sNfL assays can currently rely on a several international studies to answer some of these questions [17–22]. Most sNfL measurements are based on immunoassays [23], although other modes of quantification have also been described such as mass-spectrometry [24]. Ultra-sensitive single-molecule array (SiMOA) remains the most popular technique to quantify serum sNfL amongst immunoassays in publications since it provided the first proofs of the utility of this circulating biomarker in neurological conditions [12–25]. There are currently a number of other immunoassay suppliers for the analysis of this biomarker, each with its own specific analytical and technical characteristics. When blood sNfL will enter into clinical practice in the near future, each laboratory will have to adopt the technique that best meets its needs in terms of organization, patient recruitment and contexts of use. Importantly, it will be crucial for medical biologists and clinicians to fully know and control analytical characteristics and limits of the chosen method.

The present multi-center study evaluates the analytical performances of the on-demand automated Lumipulse G sNfL blood assay (Fujirebio®), comparing the within-run variability and correlations between six French clinical laboratories, using a set of samples with various sNfL concentrations. On a second part, these performances are compared to four other immunoassays available in these laboratories using the same set of samples: NF-light V2 SIMOA (Quanterix®), Human NF-L (Ella®), NF-light Serum ELISA (UmanDiagnostics®) and R-Plex Human Neurofilament L (MSD®). Results show intra- and inter-technique variability, highlighting the need of results interpretation with caution and standardization.

2. Material and methods

2.1. Experimental design

Six French university hospital clinical laboratories were involved in this study (Fig. 1), located in cities of Montpellier (MTP), Bordeaux (BDX), Strasbourg (SBG), Lille (LIL), Lyon (LYO) and Clermont-Ferrand (CMF). Assays were carried out in 2023 on five analytical systems (Table 1). The same samples were analysed independently on six different Lumipulse® (Fujirebio, Tokyo, Japan) (LMP) apparatus currently implemented in each laboratory and once with three other reagents on three apparatus and with one manual ELISA test.

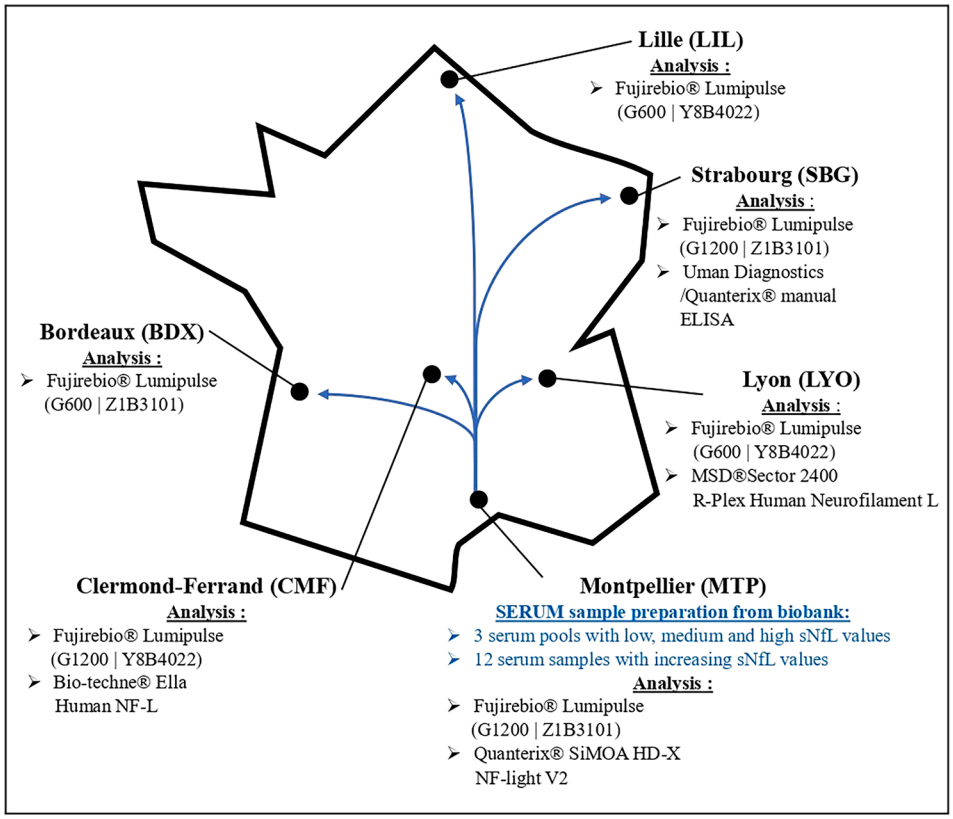

Fig. 1. Experimental design of the study. All centers performed analysis on Fujirebio® Lumipulse either instrument model G600 or G1200 and with either G sNfL blood reagent batch Z1B3101 or Y8B4022. kit was used on Lumipulse analyzer. Other assays were performed once, center location, equipment / supplier and kits are precised. Main characteristics of equipments are detailed Table 1.

2.2. Sample preparation

Samples were all prepared in MTP and conserved in biobank sera stored at −80 °C of patients followed in neurology departments of Montpellier university hospital with a concomitant routine sNfL determination on SIMOA. Written informed consent was obtained for all patients, and the study was part of the approved protocol on sNfL use approved by the local institutional review board (number of declarations: IRB-MTP_2021_04_202100783). This study was conducted in accordance with the declaration of Helsinki as revised in 2013. Samples were pooled into three serum pools with low, medium and high values as well as 12 serum samples with increasing sNfL values were divided into aliquots and sent in each laboratory for analysis. Pools were designed to have mean values on SIM around 10 pg/mL (Pool Low), 30 pg/mL (Pool Medium) and 100 pg/mL (Pool High). Six aliquots of each pool and one aliquot of each sample were assayed on the ten apparatus.

2.3. Pre-analytical and analytical steps

Blood samples were collected on BD® vacutainer SST II tubes with clot activator additive (silica particles) without gel separator. After centrifugation at 10000g for 5 min, serum was separated into 500 μL aliquots in Eppendorf® protein lobind tubes and immediately stored at −80 °C in a storage facility with continuous temperature management. The whole biobanking activity is accredited by an independent organism according to the ISO20387 quality norm. A first defrost/freeze cycle was needed for the constitution of the three pools. A subcontractor company assured samples transportation in dry ice at −80 °C. Before analysis, tubes were thawed on ice, gently homogenized and centrifuged again following the established protocol in each laboratory. Briefly, all assays consisted in immunoassay involving one capture and one detection antibody with various detection principles (Table 1). BDX, LIL and LYO used G600 LMP model while MTP, SBG and CMF used the G1200 model. Two LMP sNfL Blood reagent batches were studied: Z1B3101 batch for MTP, BDX and SBG and Y8B4022 batch for LIL, LYO and CMF. Three other used reagents were used on semi-automated apparatus: NF-light V2® (Quanterix, Billerica, USA) on SiMOA HD-X® (SIM) in MTP, Human NF-L® (Biotechne, Minneapolis, USA) on Ella (ELL) in CMF, R-Plex Human Neurofilament L® (MSD, Rockville, USA) on Sector 2400 (MSD) in LYO. At last, one the fully manual ELISA assay Uman Diagnostic/Quanterix® (Quanterix, Billerica, USA) (UMA) was used in SBG.

2.4. Statistical analysis

All values of sNfL are expressed in pg/mL and as mean ± standard deviation (SD) in text. Data analysis was separated into two sections: 1) inter-center comparison of sNfL measured on the 6 individual Fujirebio® Lumipulse (LMP) instruments and 2) inter-technique comparison of sNfL measured on the Fujirebio® LMP (using only values of MTP) and the four others platforms. Mean, SD and coefficient of variation (CV) were determined for each pool and on each instrument, with values outside lower and upper limits of quantification systematically excluded. For inter-technique comparison, only the data of MTP were used because the CV was the closest of median CV value of the LMP for the six French centers. Classical one-way analysis of variance (ANOVA) followed by Tukey-Kramer post-hoc test were performed to compare the concentrations for the three pools obtained on LMP instrument by the six laboratories. A two-way analysis of variance was performed to estimate the combined roles of both reagent batches (Z1B3101 and Y8B4022) and LMP analyser versions (G600 and G1200) on results. For all comparisons, p-value under 0.05 was considered as statistically significant.

sNfL values of the 12 samples were expressed as Z-scores for the inter-center comparison, calculated with the following formula: Z-Score = (x-μ)/σ, with x: sNfL value, μ and σ: mean and standard deviation of sNfL. Lin's concordance correlation coefficient (ρc) was determined to evaluate precision as well as accuracy between the different methods. Correlations between sNfL values of the different analytical systems were performed using Passing-Bablok linear regression analysis and Pearson correlation coefficient (r).

3. Results

3.1. sNfL values

sNfL raw values are presented in supplementary Table 1 and are graphically summarized in Fig. 2. 4 out 296 (1.35 %) sNfL values were missing following sample shortage (2 concentrations for Pool Medium on LMP, 1 concentration for Pool Medium on SIM and 1 value for Pool Low on SIM). 7 out 296 (2.36 %) values were excluded from analysis: 6 ELL sNfL values (for sample 1 and 5 Pool Low values) were below lower limit of quantification and one UMA sNfL value (sample 12) was above upper limit of quantification.

3.2. Inter-center comparison of sNfL measurements on Fujirebio® Lumipulse (LMP)

For the three pools (low medium and high), sNfL values of LMP inter-center comparison are represented in Fig. 2A and analysis is presented in Table 2. Coefficients of variation (CV) ranged from 2.0 % (SBG – Pool High) to 16.9 % (LIL – Pool Low). The highest CV were obtained for the pool with low concentrations, decreasing with higher sNfL concentrations. Mean concentrations of sNfL were statistically different between the 6 centers for all pools ($p < 0.001$). Pairwise comparison showed specific differences in mean between centers. Of note, LYO sNfL mean values were statistically different from every other center for the three different pools. To explore these differences, we analyzed the results

Table 1
Analytical characteristics of the different immunoassays evaluated in this study.

Instrument | Kit	Company	Detection	Analytical range (pg/mL)	Specimen	Sample uptake	Measure
Lumipulse G | Nfl Blood	Fujirebio®	Chemiluminescence	2 – 5000	Blood	100 μL	Automated On-demand test
SiMOA HD-X | NF-light V2	Quanterix®	Fluorescence/Digital	1.38 – 1440	Blood | CSF	25 μL	Semi-automated 96-well plate
Ella | Human NF-L	Bio-techne®	Fluorescence	2.70 – 10,290	Blood | CSF	35 μL	Semi-automated 96-well plate
Sector 2400 | R-Plex Human Neurofilament L	MSD®	Electro-chemiluminescence	11 – 100,000	Blood | CSF	25 μL	Semi-automated 96-well plate
NA | NF-light Serum ELISA	Uman Diagnostics / Quanterix®	Absorbance	2 – 160	Blood	50 μL	Manual 96-well plate

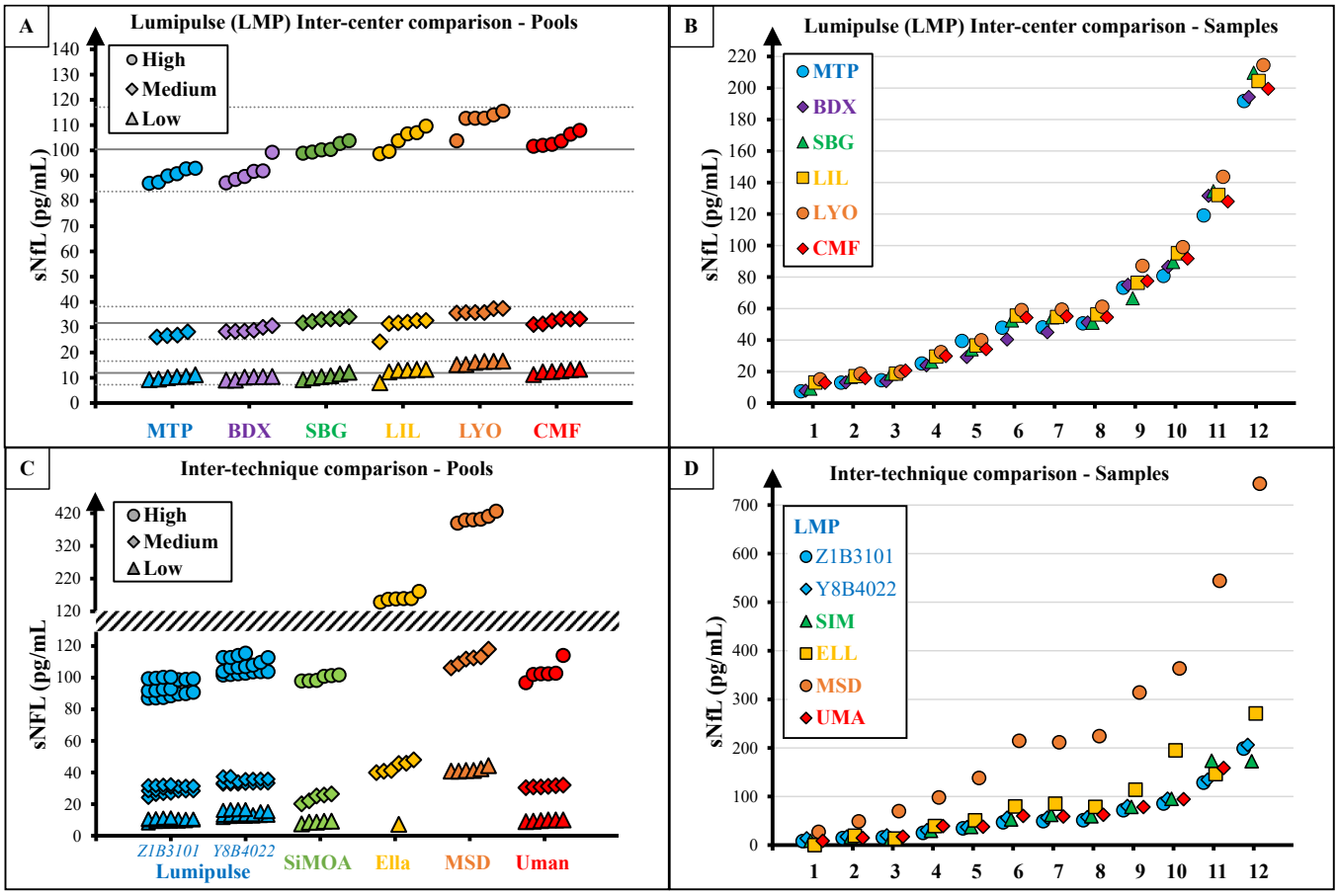

Fig. 2. Inter-center and inter-technique comparisons of pools and samples **A.** Serum neurofilament light chain (sNfL) levels on Lumipulse (LMP) for the 3 different pool levels (mean is represented by gray lines and 2*standard deviation is represented by gray dotted lines) according to each laboratory (MTP: Montpellier, BDX: Bordeaux, SBG: Strasbourg, LIL: Lille, LYO: Lyon, CMF: Clermont-Ferrand). **B.** sNfL levels on LMP for the 12 different samples according to each laboratory. **C.** sNfL levels for the 3 different pool levels according to each immunoassay; LMP G sNfL blood assay is separated into two groups according to both reagent batches. **D.** sNfL levels for the 12 different samples according to each instrument; LMP G sNfL blood assay is separated into two groups according to both reagent batches.

regarding the reagent batch and the instrument type. A significant difference was observed between sNfL mean values according to LMP reagent batches: batch Y8B4022 used by LIL, LYO and CMF yielded higher values than batch Z1B3101 used by MTP, BDX and SBG (Pool Low: 13.6 ± 2.2 vs 10.3 ± 0.8 pg/mL, Pool Medium: 33.2 ± 3 vs 30 ± 2.7 pg/mL, Pool High: 105.4 ± 5.8 vs 94.1 ± 5.7 pg/mL). No difference was observed between LMP G600 (used by BDX, LIL and LYO) and LMP G1200 (used by MTP, SBG, CMF) analyzers (Pool Low: 12.7 ± 2.9 vs 11.1 ± 1.3 pg/mL, p-value = 0.350, Pool Medium: 32.0 ± 3.7 vs 31.3 ± 2.7 pg/mL, p-value = 0.838, Pool High: 102.5 ± 9.6 vs 98.3 ± 6.5 pg/mL, p-value = 0.981).

Regarding samples values of LMP inter-center comparison (Fig. 2B and 3), Z-scores were all comprised between −2 and + 2 regardless of the sample sNfL value, from 11.0 ± 3.1 pg/mL for sample 1 to 202.4 ± 8.9 pg/mL for sample 12. It is worth noting that average Z-score was negative for all centers using reagent batch Z1B3101 (MTP: −0.85, BDX: −0.98, SBG: −0.11) and that average Z-score was positive for all centers using reagent batch Y8B4022 (LIL: +0.42, LYO: +1.32, CMF: +0.21).

3.3. Inter-technique comparison of sNfL measurements

Fig. 2C illustrates sNfL values obtained for the three pools according to the different immunoassays and the statistical analysis of the inter-technique comparison is presented in Table 3. Pool Low of sNfL values obtained with ELL were excluded from analysis as 5/6 values were below quantification limit. Coefficient of variations ranged from 1.8 % (SIM- Pool High) to 11.2 % (SIM- Pool Medium). For low concentrations, the lowest CV was obtained for MSD whereas the highest CV was obtained for SIM (3.2 % and 8.0 % respectively). For medium concentrations, the best CV was obtained for UMA whereas the highest CV was obtained for SIM (1.9 % and 11.2 % respectively). Finally, the lowest CV was obtained for SIM for high concentrations whereas the highest CV was obtained for ELL (1.8 % and 6.8 % respectively). Mean concentrations of sNfL were statistically different between the five different immunoassays for all pools tested ($p < 0.001$). Pairwise comparison showed specific differences in mean between centers. MSD and ELL concentrations were systematically different from other immunoassays, other differences were noted but remained limited to particular sNfL ranges of concentration. As examples, SIM and LMP were different for low concentrations; SIM and UMA were different for medium concentrations.

sNfL values are shown in Fig. 2D for the comparison of the 12 samples while correlation results between techniques are depicted in Fig. 4. Lin's concordance correlation coefficient revealed a strong concordance between most of the techniques (ρc = 0.735 to 0.967), except for MSD presenting lower values (ρc = 0.266 to 0.378). However, a strong correlation was obtained between the five techniques ($p < 0.0001$ in all cases), with Pearson correlation coefficient r ranging from 0.836 (between UMA and ELL) to 0.996 (between LMP and MSD). Correlation coefficients between Lumipulse and other techniques was systematically above 0.9. The immunoassay that showed the weakest correlation with the others was the ELLA technique (r = 0.836 with UMA, r = 0.885 with

Table 2
Inter-center comparison of sNfL measured on the 6 individual Fujirebio® Lumipulse (LMP) instruments (MTP: Montpellier, BDX: Bordeaux, SBG: Strasbourg, LIL: Lille, LYO: Lyon, CMF: Clermont-Ferrand). *** p-value < 0.05.

Center	MTP	BDX	SBG	LIL	LYO	CMF	MTP- BDX-SBG	LIL-LYO-CMF	BDX-LIL-LYO	MTP-SBG-CMF
Reagent batch | model	Z1B3101 | G1200	Z1B3101 | G600	Z1B3101 | G1200	Y8B4022 | G600	Y8B4022 | G600	Y8B4022 | G1200	**Z1B3101**	**Y8B4022**	**G600**	**G1200**
Serum pool Low										
sNfL (pg/mL), *mean*	10.2	9.9	10.7	12.1	16	12.5	10.3	13.6	12.7	11.1
sNfL (pg/mL), *SD*	0.7	0.7	1.1	2.0	0.6	0.7	0.8	2.2	2.9	1.3
CV (%)	7.0	6.8	10.0	16.9	4.0	5.8	8.2	16.0	22.5	11.8
p-value	**<0.001*** **						**<0.001*** **		**0.350**	
Different from	LIL-LYO-CMF	LIL-LYO-CMF	LYO	MTP-BDX-LYO	All centers	MTP-BDX-LYO				
Serum pool Medium										
sNfL (pg/mL), *mean*	27.0	29.1	33	30.8	32.4	23.9	30.0	33.2	32.0	31.3
sNfL (pg/mL), *SD*	0.9	1.0	0.9	3.2	0.9	1.0	2.7	3.0	3.7	2.7
CV (%)	3.3	3.4	2.6	10.5	2.6	4.3	8.9	9.2	11.5	8.7
p-value	**<0.001*** **						**0.005*** **		**0.838**	
Different from	SBG-LIL-LYO-CMF	SBG-LYO-CMF	MTP-BDX-LYO	MTP-LYO	All centers	MTP-BDX-LYO				
Serum pool High										
sNfL (pg/mL), *mean*	100.4	91.4	100.9	104.2	104	99.6	94.1	105.4	102.5	98.3
sNfL (pg/mL), *SD*	2.6	4.3	2.0	4.4	4.1	2.6	5.7	5.8	9.6	6.5
CV (%)	2.5	4.7	2.0	4.2	4.0	2.6	6.1	5.5	9.4	6.6
p-value	**<0.001*** **						**<0.001*** **		**0.981**	
Different from	SBG-LIL-LYO-CMF	SBG-LIL-LYO-CMF	MTP-BDX-LYO	MTP-BDX-LYO	All centers	MTP-BDX-LYO				

Table 3
Inter-technique comparison of sNfL measured on SIMOA NF-light V2 (SiMOA), Ella Human NF-L (Ella), Human Neurofilament L R-Plex (MSD), NF-light Serum ELISA (Uman) and Lumipulse G sNfL blood assay (LMP). n.a: not applicable (five values of six below lower limit of quantification). *** p-value < 0.05.

Instrument	SiMOA	Ella	MSD	Uman	LMP
Center	MTP	CMF	LYO	SBG	MTP
Serum pool Low					
sNfL (pg/mL), *mean*	8.6	n.a	41.8	9.6	10.2
sNfL (pg/mL), *SD*	0.7	n.a	1.3	0.5	0.7
CV (%)	8.0	n.a	3.2	5.0	7.0
p-value	**<0.001*** **				
Different from	MSD-LMP	n.a	All instruments	MSD	SiMOA-MSD
Serum pool Medium					
sNfL (pg/mL), *mean*	23.9	43.6	111.7	31.1	27.0
sNfL (pg/mL), *SD*	2.7	3.3	4.0	0.6	0.9
CV (%)	11.2	7.6	3.6	1.9	3.3
p-value	**<0.001*** **				
Different from	Ella-MSD-Uman	All instruments	All instruments	SiMOA-Ella-MSD	Ella-MSD
Serum pool High					
sNfL (pg/mL), *mean*	99.6	160.3	404.6	103.4	90.1
sNfL (pg/mL), *SD*	1.8	10.9	12.5	5.7	2.6
CV (%)	1.8	6.8	3.1	5.5	2.8
p-value	**<0.001*** **				
Different from	Ella-MSD	All instruments	All instruments	Ella-MSD	Ella-MSD

SIM).

4. Discussion

For the first time, we propose a multi-center study that investigates the analytical performance of Lumipulse G sNfL blood assay (Fujirebio®) technology for serum sNfL determination and compares 5 methods for assaying this biomarker.

First, within-run variability of the automated Lumipulse G sNfL blood assay (Fujirebio®) was satisfactory for all centers (Fig. 2A and Table 2). Coefficients of variation (CV) ranged from 4.0 % to 16.9 % for pool low, from 2.6 % to 10.5 % for pool medium and from 2.0 % to 4.7 % for pool high (concentrations of sNfL around 12 pg/mL, 30 pg/mL and 100 pg/mL respectively). As expected, variability was higher for the lowest values of sNfL, which were close to the limit of quantification. These results are in line with other sNfL immunoassays' variability [26–28]. When analyzing the set of 12 samples with various concentrations (Fig. 2B), the correlation of this assay was excellent between the six centers with Person's correlation coefficient r ranging from 0.995 to 0.9997 (data not shown). For these 12 samples, all the concentrations measured by the six laboratories could be considered as equivalent, as no concentration was above + 2 or below −2 z-score (see Fig. 3): 47 values (65 %) were between −1 and + 1 z-score and 25 values (35 %) were between −2 and + 2 z-score. This allows to use the cut-offs determined in research studies in a center for the other centers. Nevertheless, differences could be seen between centers, although they were not clinically relevant. A systematic difference was present between reagent batches Z1B3101 and Y8B4022 for the three pools (Table 2). On the contrary, no difference was observed comparing both Lumipulse G600 and G1200 instruments. Finally, statistical differences between centers were also observed when analyzing samples with the same instrument and the same reagent batch (as examples between MTP and SBG with Lumipulse G1200 and reagent batch Z1B3101 or between LIL and LYO with Lumipulse G600 and reagent batch Y8B4022). This heterogeneity of results could be partly due to the variability in test calibration. These results highlight the necessity for laboratories to have procedures for the quantification of this inaccuracy [29,30]. The latter should include the laboratory's own internal quality controls alongside those provided by the manufacturer and the participation to external quality control programs, making it possible to monitor longitudinal drifts and variations linked to regular calibrations.

In addition, the use of the same pools and samples in this study permitted the parallel determination of sNfL concentrations in 4 other immunoassays: SIMOA NF-light V2 (Quanterix®), Ella Human NF-L (Biotechne®), NF-light Serum ELISA (UmanDiagnostics®/Quanterix®) and Human Neurofilament L R-Plex (MesoSCaleDiscovery®). The coefficients of variation obtained with the five immunoassays were acceptable for a clinical application of these tests (Table 3). SIMOA NF-light V2 (SIM) assay exhibited the highest CV for low and medium pools (8.0 % and 11.2 % respectively) but the lowest CV for pool high (1.8 %) whereas Human Neurofilament L R-Plex (MSD) assay's variability appeared to be the most constant across the different concentrations (3.2 %, 3.6 % and 3.1 % respectively). Within-run variability observed for NF-light Serum ELISA kit (UMA), which was the sole assay performed fully in a manual way in this study, was also optimal for a clinical use of sNfL (CVs ranging from 1.9 % to 5.5 %). Differences in average concentrations were observable for the three pools, some of them appearing to be systematic (Fig. 2C). These differences were also highlighted by the values of the Lin's concordance correlation coefficient presented in Fig. 4. Indeed, concentrations were the highest when measuring sNfL with MSD assay. The concentrations obtained with ELL were lower than those obtained on MSD, but higher than those obtained with the SIM, UMA and LMP tests for the medium and high pools. SIM, UMA et LMP assays gave equivalent sNfL concentrations with a few exceptions (between SIM and LMP for pool low and between SIM and UMA for pool medium). The same differences were also observable comparing the 12 samples with increasing sNfL values (Fig. 2D). Our results are in line with previous studies. Indeed, Ulndreaj *et al.* reported that absolute sNfL concentrations acquired using MSD's R-PLEX were on average 3–4 times higher than the levels quantitated using SIMOA [31]. Some studies already reported mean sNfL concentrations measured by ELLA significantly higher than the one obtained by SIMOA [25,28,32]. Moreover, as in our study, a very high agreement was found between SIMOA and Lumipulse concentrations in the study of Vecchio *et al.* These discrepancies are mainly due to the lack of standardization of the calibrators and antibodies used by the different companies [28,30]. Despite the different concentrations obtained between tests, all the techniques showed very good correlations, with Pearson correlation coefficients (r) above 0.836 (Fig. 4).

Many factors need to be taken into account when choosing a sNfL

Center	MTP	BDX	SBG	LIL	LYO	CMF	sNfL
Instrument	G1200	G600	G1200	G600	G600	G1200	*Mean ± SD*
Reagent batch	Z1B3101	Z1B3101	Z1B3101	Y8B4022	Y8B4022	Y8B4022	(pg/mL)
Sample Z-score 1	-1.08	-0.97	-0.56	+0.69	+1.32	+0.60	11.0 ± 3.1
2	-1.20	-1.16	+0.40	+0.63	+1.28	+0.05	15.8 ± 2.3
3	-1.14	-1.34	+0.33	+0.36	+0.76	+1.04	17.8 ± 2.8
4	-0.83	-1.21	-0.49	+0.54	+1.40	+0.60	27.9 ± 3.2
5	+0.97	-1.60	-0.36	+0.25	+1.08	-0.35	35.6 ± 4.0
6	-0.56	-1.70	+0.12	+0.60	+1.13	+0.40	51.6 ± 6.6
7	-0.89	-1.48	+0.31	+0.37	+1.26	+0.43	52.8 ± 5.3
8	-0.84	-0.70	-0.76	+0.52	+1.71	+0.08	54.1 ± 4.1
9	-0.40	-0.14	-1.41	+0.06	+1.67	+0.22	76.0 ± 6.7
10	-1.51	-0.61	-0.13	+0.72	+1.33	+0.20	90.5 ± 6.4
11	-1.53	+0.01	+0.38	+0.07	+1.52	-0.44	131.5 ± 8.0
12	-1.20	-0.91	+0.83	+0.24	+1.37	-0.32	202.4 ± 8.9
Average Z-score	-0.85	-0.98	-0.11	+0.42	+1.32	+0.21	/

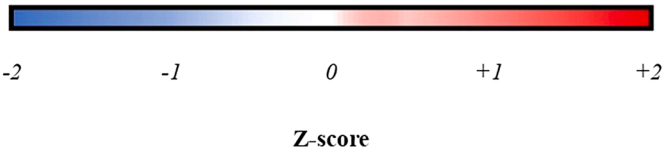

Fig. 3. Heatmap of sNfL (pg/mL) Z-scores of the 12 samples measured on the 6 individual Fujirebio® Lumipulse (LMP) analyzers (MTP: Montpellier, BDX: Bordeaux, SBG: Strasbourg, LIL: Lille, LYO: Lyon, CMF: Clermont-Ferrand). Mean and standard deviation (SD) of each sample is indicated in the right column.

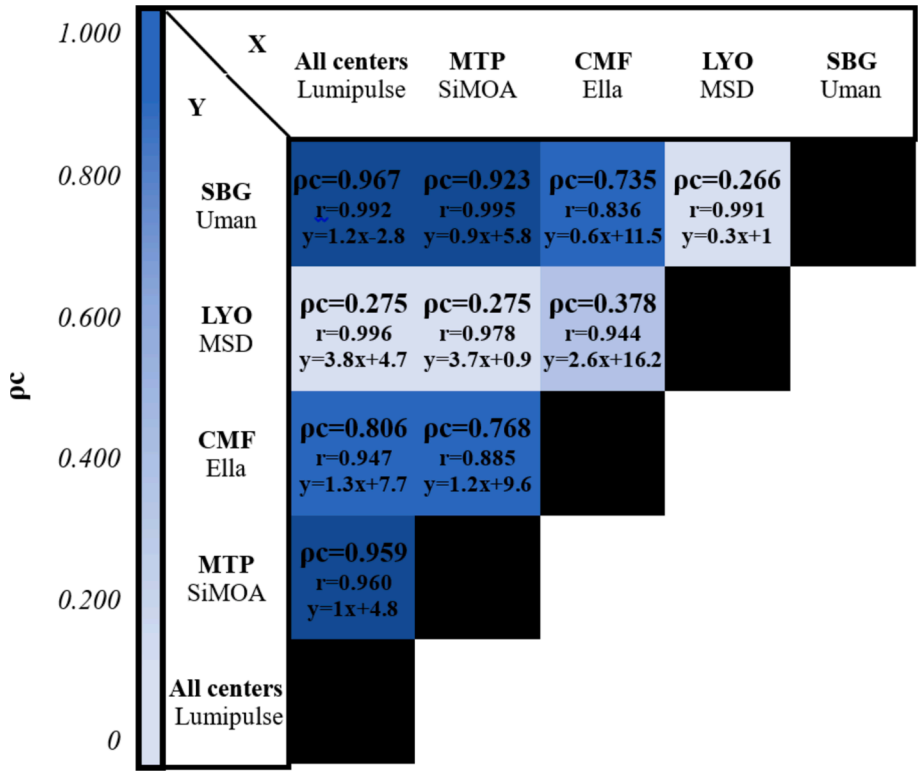

Fig. 4. Heatmap representing the Lin's concordance correlation coefficient (ρc). Pearson correlation coefficients (r) and Passing-Bablok regression equation (y = ax + b) between different techniques (MTP: Montpellier, BDX: Bordeaux, SBG: Strasbourg, LIL: Lille, LYO: Lyon, CMF: Clermont-Ferrand) of the 12 different samples. x values correspond to the first row of the table while y values correspond to the first column.

immunoassay for clinical application. The first of all depends on the accessibility of analyzers and the cost of reagents for the laboratory. So far, most studies used the SIMOA ultrasensitive method to quantify sNfL however its cost often limits its widespread availability in clinical laboratories [26,30]. Manual ELISAs are cheaper but require qualified personnel [33]. Fully automated assays with minimal pre-analytical procedures are likely to be the preferred choice of laboratories, especially when In-Vitro Diagnostic Regulation (IVDR) labels will be obtained. In this study, Lumipulse G sNfL blood assay showed good analytical performances. Other on-demand sNfL immunoassays exist such as Roche's Elecsys® sNfL or Siemens Atellica® Neurofilament Light (NFL) assay. Recently, the study from Ashrafzadeh-Kian *et al.* was the first to compare these three fully automated immunoassays to SIMOA [34]. Further real-life cohort studies should now be performed to evaluate their performances in everyday use. Other aspects to take into account include pre-analytical and analytical criteria. Sampling type (serum or plasma) will influence sNfL concentrations [27,35]. In this study, Ella immunoassay failed to accurately quantify low concentration's sample of sNfL (sample 1 and 5 on 6 measurements of pool low), which may compromise longitudinal follow-up of pre-symptomatic patients with neurodegenerative conditions [36–38]. On the contrary, the low upper limit of quantification of Uman diagnostics/Quanterix® NF-light Serum ELISA kit (160 pg/mL, Table 1) would lead to frequent dilutions for samples from patients with pathologies for which high sNfL concentrations are expected, such as amyotrophic lateral sclerosis [38]. As systematic differences concerning absolute sNfL values exist between the different immunoassays, laboratories will have to rely on or realize their own specific reference values in order to be able to interpret their data. To our knowledge, such normal values exist so far for SIMOA technology only [39,40]. Finally, once the analytical process has been adopted by the laboratory, it should be borne in mind that confounding factors such as age, body mass index and renal insufficiency might influence sNfL concentrations [12,19,41]. Further studies with inclusion of repeated samples with physiopathological, environmental or nycthemeral variations are needed to investigate intra-individual variability of sNfL.

5. Conclusion

The automated LMP yielded overall comparable inter-laboratory sNfL values and is well correlated to MSD, Uman, Simoa and Ella assays. However, further adjustments are needed to harmonize sNfL results and determine cut-offs for clinical application. Biologists and physicians must be aware of result variability between the different immunoassay suppliers.

CRediT authorship contribution statement

Etienne Mondésert: Writing – original draft, Resources, Data curation. **Susanna Schraen-Maschke:** Writing – review & editing, Resources, Data curation. **Isabelle Quadrio:** Writing – review & editing, Resources. **Olivier Bousiges:** Writing – review & editing, Resources. **Damien Bouvier:** Writing – review & editing, Resources. **Constance Delaby:** Writing – review & editing, Resources. **Aurélie Bedel:** Writing – review & editing, Supervision, Resources, Conceptualization. **Sylvain Lehmann:** Writing – review & editing, Supervision, Resources, Data curation, Conceptualization. **Anthony Fourier:** Writing – original draft, Resources, Data curation.

Declaration of Competing Interest

The specific evaluation of Lumipulse G sNfL blood assay (Fujirebio®) was carried out by the six French laboratories using kits supplied free of charge by Fujirebio® (1 calibration pack, 1 quality control pack and 1 reagent pack per laboratory). The authors declare that they have no other known competing financial interests or personal relationships that

could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2024.120007.

Data availability

Raw data are presented in supplementary Table 1.

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