

Analytical Performance of the Lumipulse® G NfL Blood Assay for Neurofilament Light Measurement

Dagmara Minczakiewicz, Martine Dauwe, Koen de Geyter, Nadia Van der Cruyssen, Peter Jagiello, Jessie Theuns, Nathalie Le Bastard, Manu Vandijck, Guy Mersch, Ina Vandenbroucke
Contact: dagmara.minczakiewicz@fujirebio.com; guy.mersch@fujirebio.com

Fujirebio Europe N.V., Technologiepark 6, 9052 Gent, Belgium

Introduction

Neurofilament light chain (NfL) is a neuron-specific intermediate filament protein released into the extracellular space following axonal degradation. Its concentration in body fluids reflects neuro-axonal damage, regardless of the underlying cause. Elevated NfL levels are associated with a wide range of neurological conditions, including multiple sclerosis, amyotrophic lateral sclerosis, frontotemporal dementia, and Alzheimer's disease, as well as acute events such as traumatic brain injury. Consequently, NfL is a promising biomarker to evaluate disease activity, progression, prognosis, and therapy monitoring. Its detectability in both cerebrospinal fluid (CSF) and blood supports its potential for routine clinical application. In this study, we report the analytical verification of the Lumipulse G NfL Blood assay performed as part of the CE-IVDR regulatory submission.

Lumipulse G NfL Blood assay

The LUMIPULSE G System (Fujirebio Inc.) is a chemiluminescent enzyme immunoassay platform that allows fully automated processing of samples using single analyte, ready-to-use immunoreaction cartridges (IRC). Sequential immunoreaction steps are carried out while the single-use cartridge is transported through the system; the analyte is captured specifically by the antibody-coated microparticles and the detection antibody fragments labeled with alkaline-phosphatase (ALP) and substrate are added, each after a thorough washing step.

The Lumipulse G NfL Blood assay (Fujirebio Europe N.V.) was developed for the quantification of NfL in serum and plasma. An overview of the assay design is given in [Table 1](#). An Assay Specific Solution is added during sample incubation to optimize analyte detection and improve the assay specificity.

Table 1: Lumipulse G NfL Blood assay design

Assay	Configuration	Storage	Content	Volume
Immunoreaction Cartridge	3 x 14 Tests	2°C to 10°C	Capturing mAb on magnetic particles / Assay Specific Solution / Detection conjugate (mAb-ALP)	Sample volume 100 µL
Calibrators	1 x 2 CAL	2°C to 10°C	Synthetic peptide NfL; Conc. range 0 and 2500 pg/mL	1.5 mL / vial
Controls	2 x 2 CTRL	2°C to 10°C	Synthetic peptide NfL; L1 and L2	1.5 mL / vial

Study objective

The aim of the study was to determine the analytical performance of the Lumipulse G NfL Blood (CE) assay, focusing on sensitivity, precision, matrix equivalence, linearity, and comparability with existing RUO assays, using plasma- and serum-based samples tested across relevant concentration ranges.

Conclusion

The Lumipulse G NfL Blood assay demonstrated robust analytical performance across all evaluated parameters. The assay showed high sensitivity, with low LoB, LoD, and LoQ values, enabling reliable quantification of low NfL concentrations. Excellent precision was observed across instruments and concentration ranges, with low total imprecision. Equivalence between serum and K₂-EDTA plasma was confirmed, supporting interchangeable use of both matrices. The assay exhibited excellent linearity over a wide measuring range, covering clinically relevant concentrations. Finally, strong agreement with the established Simoa® NF-light™ Advantage Plus assay was demonstrated. Together, these results support the suitability of the Lumipulse G NfL Blood assay for reliable and reproducible NfL measurement in blood-based samples.

"LUMIPULSE is a registered trademark of Fujirebio Inc."

Results

Sensitivity

Assay sensitivity was evaluated by determination of the Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantification (LoQ). The study was performed on the LUMIPULSE G1200 system using two reagent lots and two matrices (K₂-EDTA plasma and serum). Blank samples (n=4 per matrix) were generated by immunodepletion and tested over 3 days with 5 replicates per sample, resulting in 60 measurements per reagent lot. Low-level samples for LoD (0.10–3.00 pg/mL) and LoQ (0.30–8.00 pg/mL) were prepared by controlled depletion and/or spiking and tested over 5 days with 8 replicates per sample (40 measurements per sample and lot). Measurement order was randomized between runs. LoB was calculated non-parametrically as the 95th percentile of blank measurements. LoD and LoQ were derived using precision profile approaches, with LoD defined as $LoD = LoB + (Cp \times SD(LoD))$ and LoQ defined at 20% CV. For each parameter, the worst-case value across matrices and reagent lots was selected as the final assay specification. Final sensitivity (worst-case): LoB: 0.30 pg/mL; LoD: 0.57 pg/mL and LoQ (20%CV): 0.96 pg/mL ([Figure 1](#)).

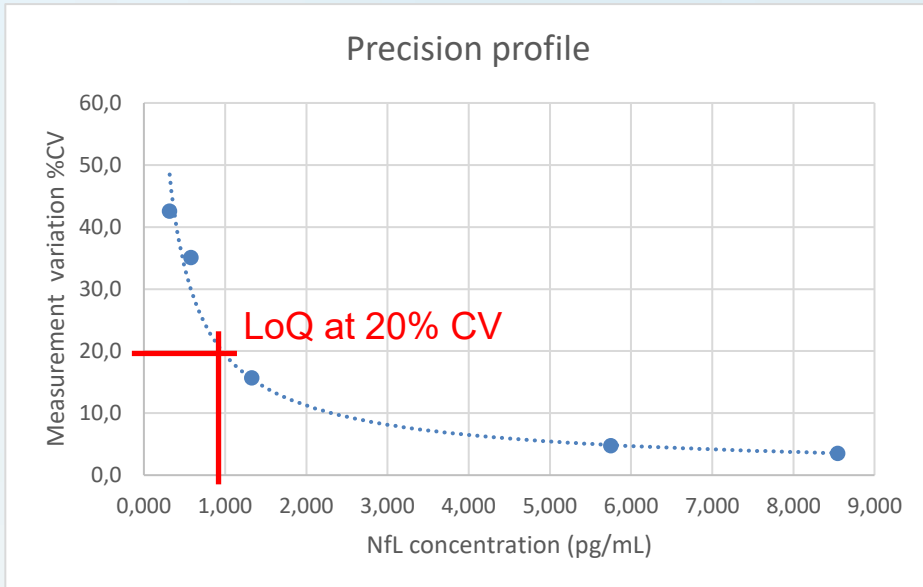


Figure 1: Sensitivity: Precision profile with indication of the LoQ at 20% CV level.

Precision

Assay precision was evaluated using a multi-day, multi-run study design on the LUMIPULSE G1200 and LUMIPULSE G600II systems. A test panel comprising six samples (serum, K₂-EDTA plasma, and buffer-based controls) covering the full measuring range was used. On the LUMIPULSE G1200, samples were tested over 20 days with 2 runs per day and 2 replicates per run (20×2×2 design). On the LUMIPULSE G600II, testing was performed over 8 days using the same 2×2 daily structure (8×2×2 design). One reagent lot was used per instrument. Sample order was randomized between runs. Precision components—including within-run (repeatability), between-run, between-day, and total within-laboratory imprecision—were estimated. Total imprecision (%CV): LUMIPULSE G1200: ≤6.4% ([Table 2](#)); LUMIPULSE G600II: ≤5.0% ([Table 3](#)).

Table 2: Precision: Outcome of within and between-assay variability study on LUMIPULSE G1200, showing SD and %CV for various sources of imprecision. S: serum; P: K₂-EDTA plasma; B: buffer based.

Sample	Mean (pg/mL)	Within Run (Repeatability)		Between Run		Within Day		Between Day		Total imprecision (Within Laboratory)	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
		0.71	5.2	0.47	3.5	0.85	6.3	0.18	1.3	0.87	6.4
S	13.59										
P1	68.01	1.79	2.6	1.15	1.7	2.13	3.1	1.32	1.9	2.51	3.7
P2	451.49	19.14	4.2	4.16	0.9	19.59	4.3	0.00	0.0	19.59	4.3
L1	16.27	0.49	3.0	0.00	0.0	0.49	3.0	0.18	1.1	0.52	3.2
L2	817.35	12.87	1.6	2.27	0.3	13.06	1.6	6.29	0.8	14.50	1.8
B	1750.77	30.36	1.7	98.14	5.6	102.73	5.9	0.00	0.0	102.73	5.9

Table 3: Precision: Outcome of within and between-assay variability study on LUMIPULSE G600II, showing SD and %CV for various sources of imprecision. S: serum; P: K₂-EDTA plasma; B: buffer based.

Sample	Mean (pg/mL)	Within Run (Repeatability)		Between Run		Within Day		Between Day		Total imprecision (Within Laboratory)	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
		0.19	1.5	0.26	2.0	0.32	2.5	0.00	0.0	0.32	2.5
S	12.77										
P1	62.44	1.13	1.8	2.92	4.7	3.13	5.0	0.00	0.0	3.13	5.0
P2	442.20	8.11	1.8	11.06	2.5	13.72	3.1	0.00	0.0	13.72	3.1
L1	13.97	0.34	2.5	0.37	2.7	0.51	3.6	0.10	0.7	0.52	3.7
L2	773.08	14.82	1.9	10.81	1.4	18.34	2.4	6.44	0.8	19.44	2.5
B	1746.90	42.78	2.4	26.12	1.5	50.12	2.9	0.00	0.0	50.12	2.9

Matrix equivalence

Sample matrix equivalence between serum and K₂-EDTA plasma was evaluated. In total, 43 paired specimens were analyzed on the LUMIPULSE G1200 system, of which 21 pairs were CSF-spiked to extend coverage across the measuring range. Each specimen pair was measured in duplicate in randomized order using a single reagent lot. Analysis based on the first replicate - reflecting routine single-measurement use - resulted in $r = 0.996$ (95% CI: 0.993–0.998) with a slope of 1.005 (95% CI: 0.9788–1.0321) ([Figure 2](#)).

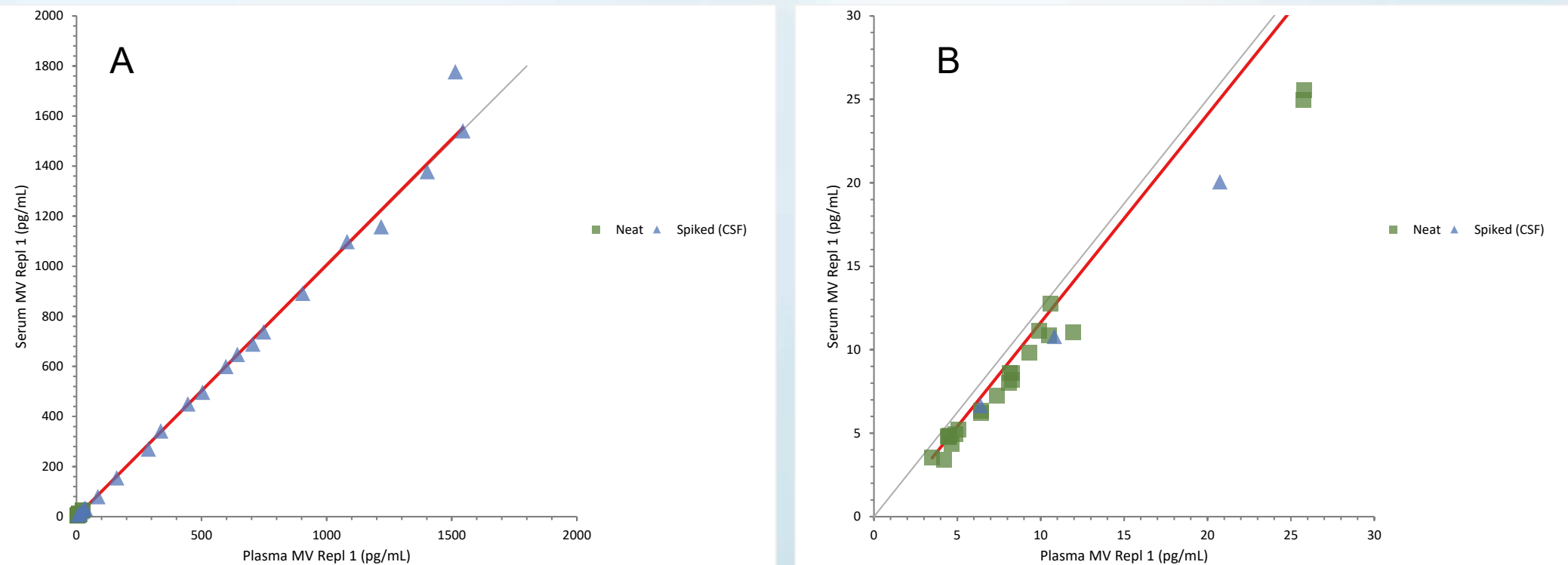


Figure 2: Matrix Equivalence: Scatterplot regression analysis using the 1st replicate of each sample in (A) the entire concentration range, and (B) zoomed into the lower concentration range.

Method Comparison

(1) Method comparison of the Lumipulse G NfL Blood assay was performed to assess agreement with the Simoa® NF-light™ Advantage Plus (RUO, For Research Use only) assay. In total, 120 patient samples (60 serum and 60 K₂-EDTA plasma) were analyzed. After exclusion of invalid results (due to high replicate variability (%CV > 20), technical errors resulting in a single valid measurement, or concentrations exceeding the assay measuring range), a final dataset of 107 (55 serum, 52 plasma) samples was retained, including 16 CSF-spiked samples to ensure coverage of higher concentrations. Samples were measured in duplicate on both platforms. In line with assay design, the first replicate of the Lumipulse G NfL Blood assay and the mean of two replicates for the Simoa assay were used for analysis. Passing-Bablok regression was applied to assess correlation. Using all valid samples, Passing-Bablok regression yielded a correlation coefficient $r = 0.992$ (95% CI: 0.988–0.994), demonstrating strong agreement between methods. The regression slope was 0.839 (95% CI: 0.816–0.863) ([Figure 4](#)). (2) The same 120 samples were also tested on the Lumipulse G NfL Blood (RUO) - v1: 81215, 81422. Passing-Bablok regression yielded a correlation coefficient $r = 1.000$ and a regression slope of 0.724 (95% CI: 0.710–0.729) ([Figure 5](#)).

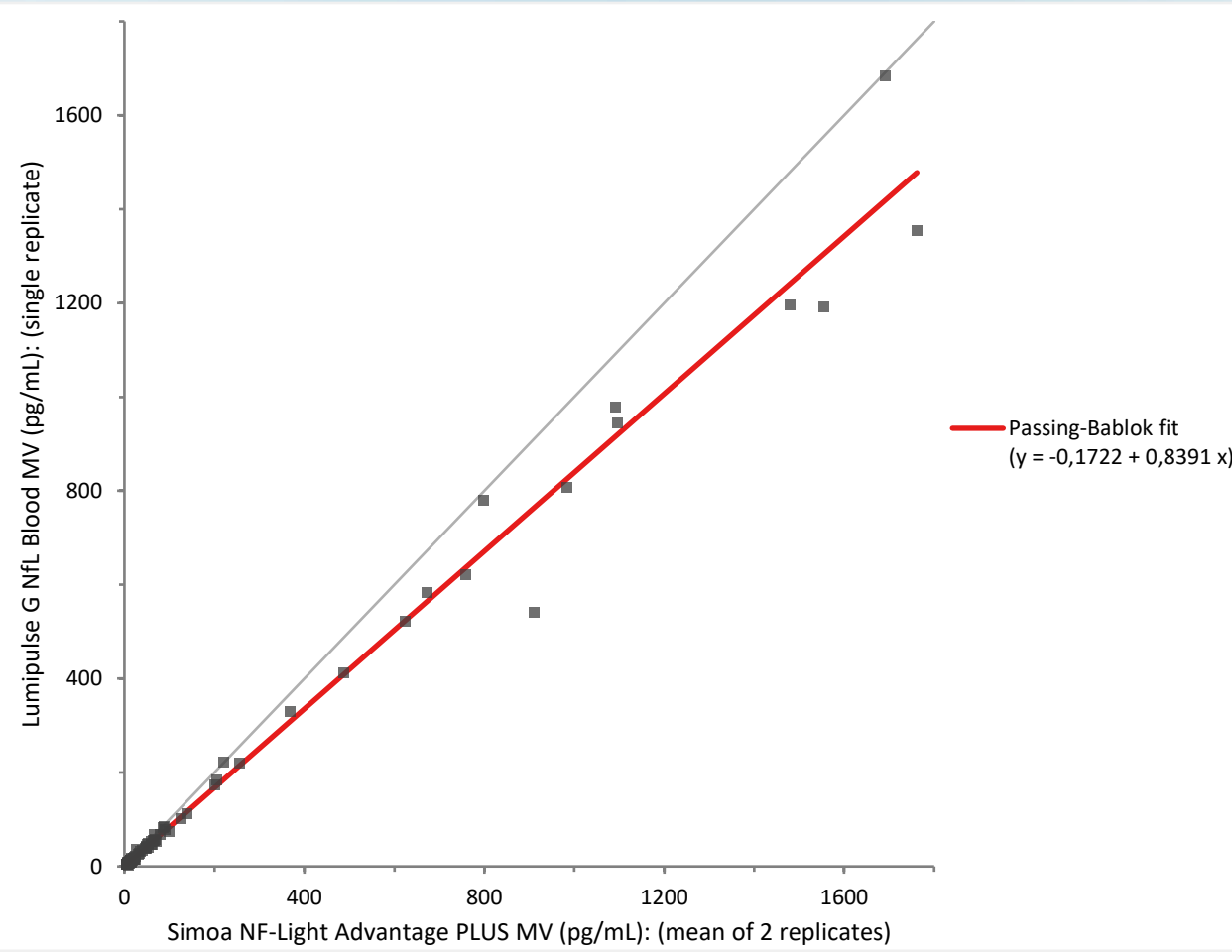


Figure 4: Method Comparison with Simoa® NF-light™ Advantage Plus (RUO) : Analysis for the complete dataset comprising 107 samples, showing scatterplot with Passing-Bablok regression fit

Linearity

The linear range of the assay was evaluated using a proportional linearity study. An equal-volume mixed panel was prepared, consisting of 11 concentration levels generated by stepwise mixing of a low-level sample (at or below the LoQ) with a high-concentration sample. All mixed panel samples were measured in four replicates on the LUMIPULSE G1200 system using a single reagent lot. Linearity was assessed by fitting a linear regression of measured versus expected concentrations. Linearity acceptance was evaluated using an allowable deviation from linearity (DL) of ±10% above the LoQ, or an absolute DL of 0.58 pg/mL at very low concentrations. Linearity was demonstrated over a concentration range of 0.89 to 2909.02 pg/mL, with a maximum deviation from linearity of 9.8%.

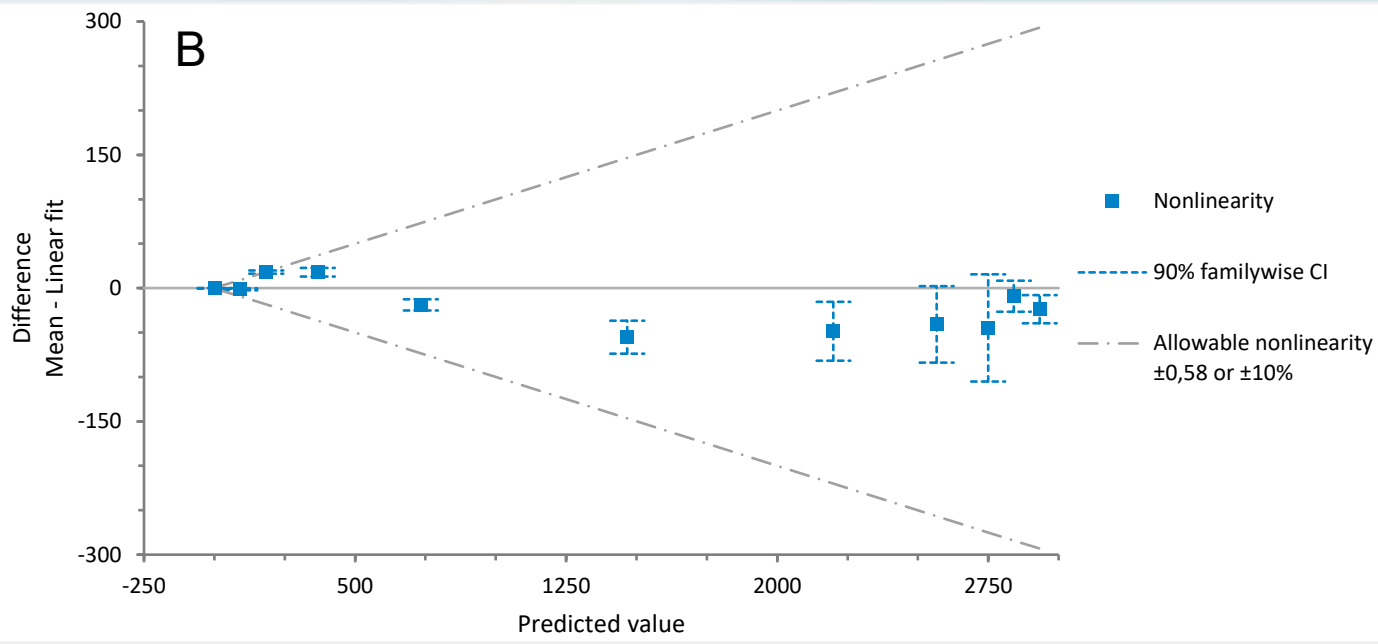
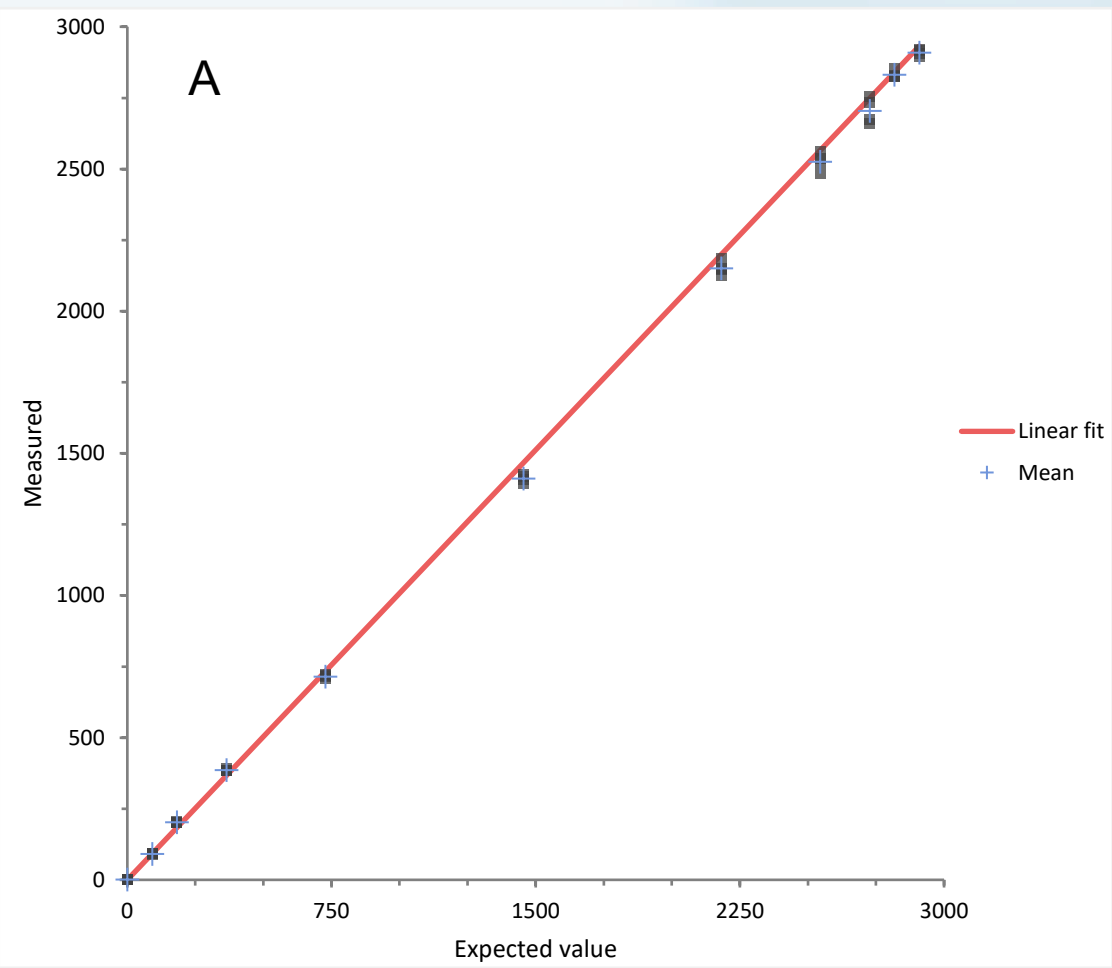


Figure 3: Proportional Linearity: (A) linear regression for mixed panel, plotting measured values against expected values. (B) Difference plot for mixed panel, plotting difference for mean towards linear fit against predicted values.

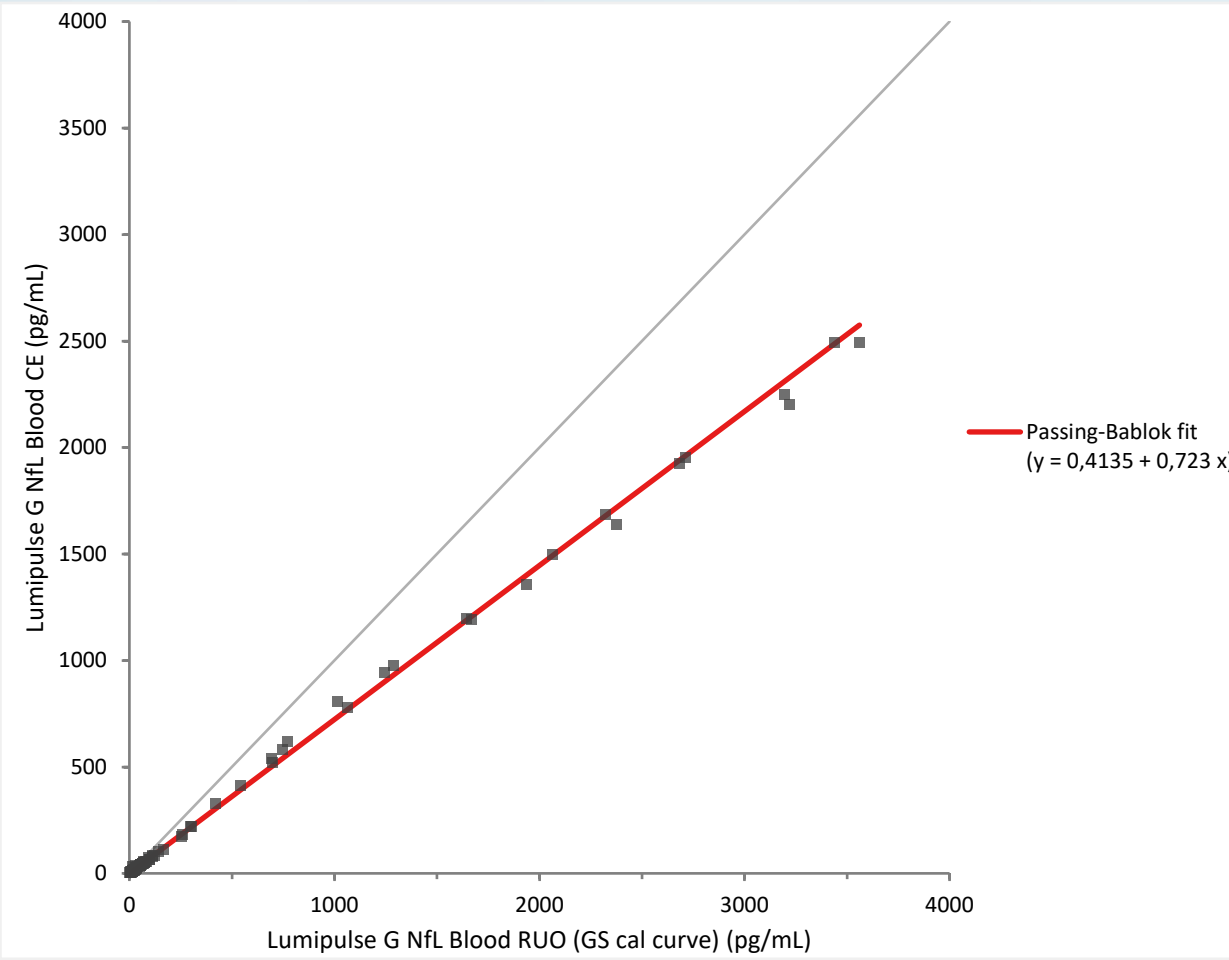


Figure 5: Method Comparison with Lumipulse G NfL Blood RUO (v1: 81215, 81422): Analysis for the complete dataset comprising 120 samples, showing scatterplot with Passing-Bablok regression fit



Fujirebio
Neuro Center
of Excellence



FUJIREBIO