Blood sample matrix validation, impact of sample freezing and method comparison analysis using the Lumipulse *G* NfL blood prototype assay

Aurélie Ladang[1], Guy Mersch[2], Martine Dauwe[2], Koen De Geyter[2], Paul Vandeponseele[2], Stephanie Kovacs[1], Els Huyck[2], Nathalie Le Bastard[2], Ina Vandenbroucke[2], Etienne Cavalier[1]

[1]CHU Liège, Clinical Chemistry, Liège, Belgium, [2]Fujirebio Europe, Product Development, Ghent, Belgium

Introduction

Neurofilament light (NfL) is expressed in myelinated axons and secreted into CSF and subsequently into blood. Blood NfL levels are elevated in multiple neurological disorders. A limited number of fully automated assays are currently available for determination of blood NfL levels.
The current study investigated the agreement between matched serum and plasma samples on the Lumipulse ***G*** NfL Blood prototype assay, the impact of one freeze-thaw cycle of EDTA plasma samples on NfL concentrations, and a method comparison between the Lumipulse assay and the Quanterix Simoa NF-light Advantage kit.

Study Objective

The current study was designed to:

i. Investigate of the analytical impact of the sample matrix on the agreement between paired serum, K3EDTA plasma, and LiHeparin plasma samples on the Lumipulse ***G*** NfL Blood prototype assay.
ii. Investigate the impact of freezing plasma samples and the agreement between those fresh and frozen plasma samples on the Lumipulse ***G*** NfL Blood prototype assay.
iii. Explore the comparison between the novel Lumipulse ***G*** NfL Blood prototype assay and the commercially available Quanterix Simoa NF-light Advantage (V1) kit.

LUMIPULSE *G* platform and NfL Blood assay presentation

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. Time to result in these test cartridges takes about 30 minutes. Sequential immunoreaction steps are carried out while the single use cartridge is transported through the system. The principle of the Lumipulse ***G*** NfL Blood assay is a two-step specific assay that includes an automated pre-mixing of the sample with an Assay Specific Diluent before being mixed with the capturing beads. After washing, capturing beads with mAb are combined with a detection antibody, directly labelled with alkaline phosphatase (ALP). An overview of the assay set-up is given in Fig. 1.

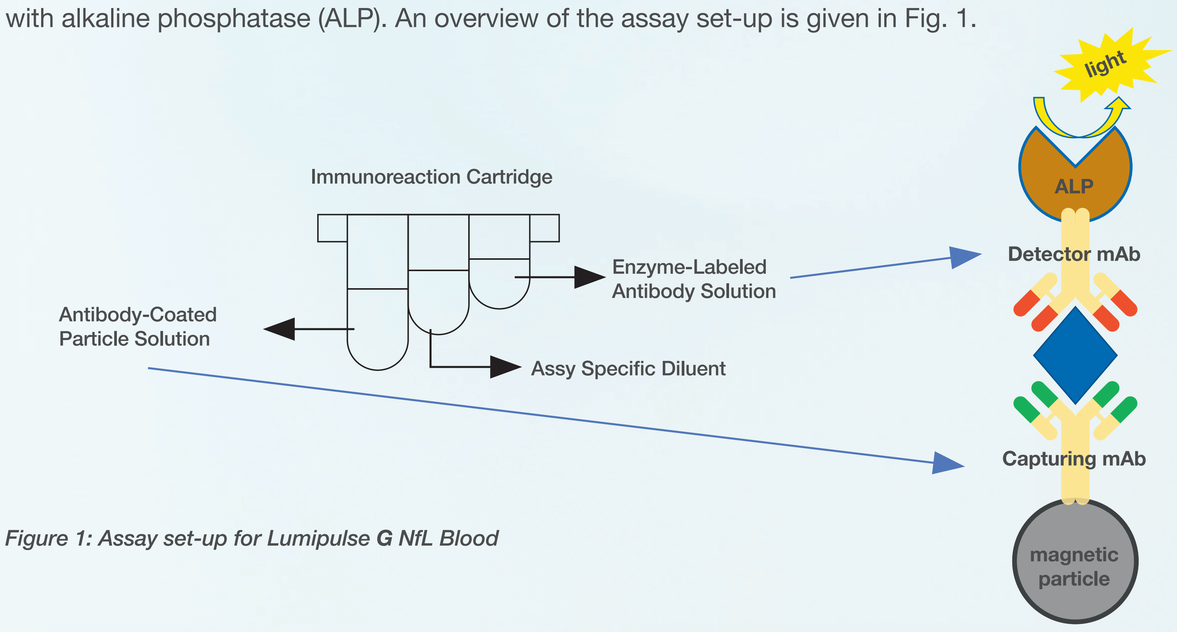

*Figure 1: Assay set-up for Lumipulse **G** NfL Blood*

Paired samples

A set of 20 paired serum and (two types of) plasma samples have been collected in serum gel (Sarstedt ref 04.1935.001), K3EDTA (Sarstedt ref 02.1066.001), and LiHeparin gel (Sarstedt ref 04.1940.100) collection tubes, processed and stored in a polypropylene microtube (Sarstedt ref 72.703). Samples were transferred to Hitachi cups prior to analysis on the LUMIPULSE G1200 instrument. Data were analyzed using Passing-Bablok regression fit (Fig. 2). A strong positive correlation on NfL concentration can be observed between all tested matrices (Table 2), suggesting that all tested matrices are accurate to measure blood NfL levels with the Lumipulse ***G*** NfL Blood assay.

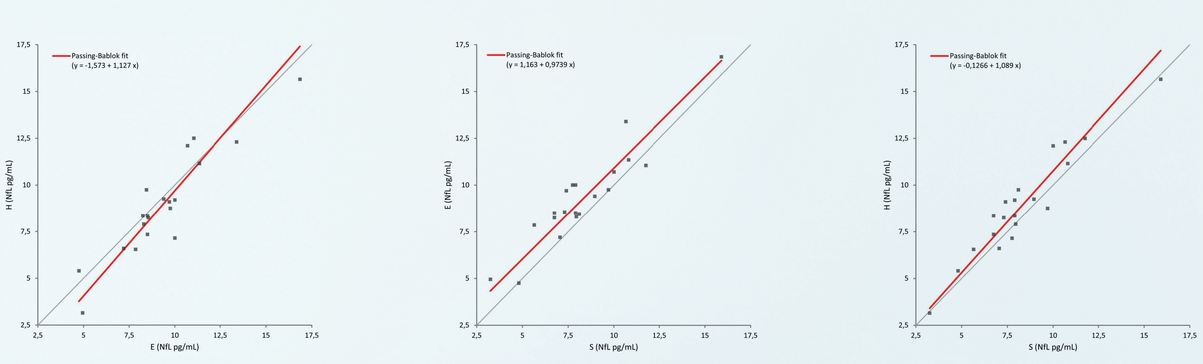

*Figure 2: Passing-Bablok regression fit of paired K3EDTA plasma samples (E), LiHeparin plasma samples (H), and serum samples (S) for the measurement of NfL levels with the Lumipulse **G** NfL Blood assay.*

Fresh/Frozen

Forty fresh plasma samples have been aliquoted in 2 aliquots, of which 1 aliquot was tested the same day and 1 aliquot was tested after 1 week (±1 day) of storage at -20°C. Plasma samples were collected in K2EDTA tubes (BD ref 368856) and stored in the same polypropylene microtube as mentioned above. The Passing-Bablok regression analysis showed no significant difference in NfL concentration between fresh and frozen plasma when measured with the Lumipulse ***G*** NfL Blood assay (slope = 0.99; Fig. 3).
Both fresh and frozen plasma are providing reliable and accurate measurements of NfL levels.

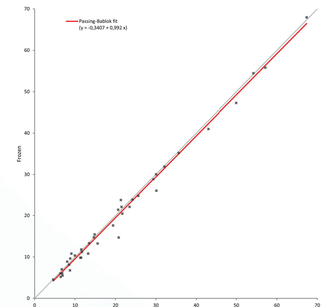

*Figure 3: Passing-Bablok regression fit of the measurement of NfL levels between fresh and frozen K2EDTA plasma samples measured with the Lumipulse **G** NfL Blood assay.*

Method comparison

In total 100 non-matched clinical leftover serum and plasma EDTA samples (n=41 each) and LiHeparin plasma samples (n=18) have been selected for the method comparison analysis. Serum samples were collected in serum separator tubes (BD ref 367957), while plasma was collected in K2EDTA tubes (BD ref 368856) and LiHeparin gel tubes (BD ref 367526), processed and finally stored in the same polypropylene microtube as mentioned above. All samples have been tested using the Lumipulse ***G*** NfL Blood assay and the Quanterix Simoa NF-light Advantage kit (V1). Passing-Bablok analysis was performed (Fig. 4), and the analysis showed a correlation coefficient of >0.9 and a slope close to 1.0 for all sample types (Table 2) indicating that the results obtained by the two methods are highly correlated and show a high level of agreement.

For the data visualization, samples with a higher measurement value (>200 pg/mL) have not been included in the analysis. The omitted data for both methods is listed in Table 1.

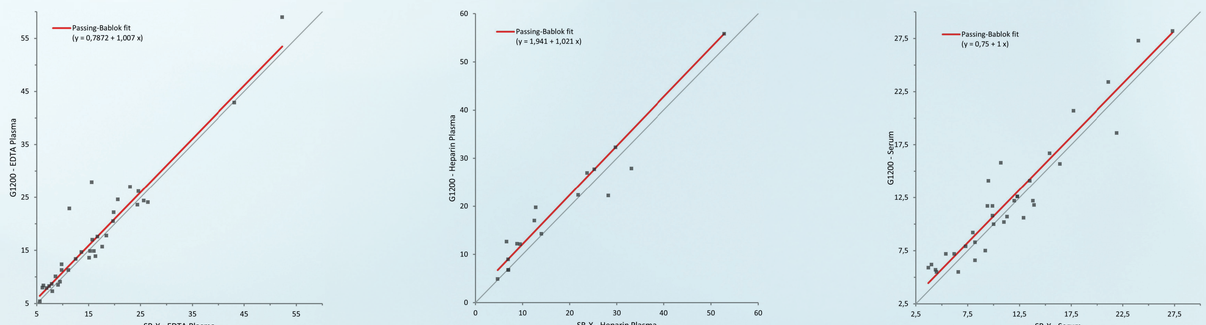

*Figure 4: Representation of a method comparison with Passing-Bablok analysis between Lumipulse **G** NfL Blood assay (on G1200) and the Quanterix Simoa NF-light Advantage kit (V1) (measured on SR-X) for the quantification of NfL levels in K2EDTA plasma, LiHeparin plasma, and serum samples.*

Sample Matrix	Lumipulse [pg/mL]	Simoa [pg/mL]
Serum	226.19	190.93
Serum	478.10	391.51
Serum	940.81	846.55
Serum	345.68	296.74
Serum	372.46	313.31
Serum	747.59	741.34
EDTA plasma	292.30	242.93
EDTA plasma	477.47	364.38
EDTA plasma	1070.42	591.12
EDTA plasma	343.96	277.67
EDTA plasma	376.22	355.38
EDTA plasma	829.20	777.27
Heparin plasma	903.49	1170.54

*Table 1: Measurement values obtained by Lumipulse **G** NfL Blood assay (Lumipulse) and Quanterix Simoa NF-light Advantage kit (V1) (Simoa). Measurement values > 200 pg/mL, not included in the method comparison by the Passing-Bablok regression analysis.*

Conclusion

Paired samples: There is a strong positive correlation between the concentration of NfL in serum, K3EDTA plasma and LiHeparin plasma, this suggesting that either matrix is a good representative of the blood NfL levels when measured with the Lumipulse ***G*** NfL Blood assay. This allows flexibility regarding blood sample type as well as anticoagulant for this assay.

Pre-analytics (Freezing of plasma samples): There is no significant difference between fresh and frozen plasma samples for determining the concentration of NfL when measured with the Lumipulse ***G*** NfL Blood assay, allowing flexibility in study or trial set-up.

Method comparison: A correlation coefficient of >0.95 and a slope close to 1.0 indicate that the results obtained by the two methods are highly correlated and show a high level of agreement. The measurement of NfL levels by the Lumipulse ***G*** NfL Blood assay and the Quanterix Simoa NF-light Advantage kit (V1) are interchangeable for all sample types tested (K2EDTA plasma, LiHeparin plasma, and serum samples).

	Intercept (95% CI)	Slope (95% CI)	Correlation - r	n
Paired				
EDTA plasma - Heparin plasma	-1.57 (-4.732 to 0.873)	1.13 (0.865 to 1.500)	0.92	20
Serum - EDTA plasma	1.16 (-0.664 to 3.463)	0.97 (0.704 to 1.164)	0.94	20
Serum - Heparin plasma	-0.13 (-2.092 to 1.384)	1.09 (0.910 to 1.353)	0.95	20
Fresh Frozen				
Fresh - Frozen	-0.34 (-1.075 to 0.356)	0.99 (0.964 to 1.020)	0.99	40
Method Comparison: SR-X vs G1200				
Serum	0.75 (-1.314 to 2.287)	1.00 (0.800 to 1.148)	0.91	34
Heparin plasma	1.94 (-0.604 to 5.839)	1.02 (0.808 to 1.147)	0.97	35
EDTA plasma - Heparin plasma	0.79 (-1.063 to 1.957)	1.01 (0.913 to 1.132)	0.95	17

Table 2: Overview of the regression data obtained by Passing-Bablok analysis for all analyses described in this poster.

*Disclaimer: Lumipulse **G** NfL CSF is for Research Use Only (RUO). Not for use in diagnostic procedures.*

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