Analytical performance of the Lumipulse® *G* NfL CSF <RUO>

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Introduction

Neurofilaments provide structural support to neurons. Different forms of neurofilaments exist, including Neurofilament light (NfL), which is strongly expressed in myelinated axons and secreted in the cerebrospinal fluid (CSF).

NfL is considered a biomarker of neuro-axonal injury and is increasingly used as a marker for e.g. progression, disease activity or therapy monitoring across multiple neurological conditions, including multiple sclerosis or Alzheimer's disease.

Only a few fully automated assays are currently available for determination of CSF NfL levels. Based on new monoclonal antibodies, an assay for NfL in CSF was developed on the fully automated random access LUMIPULSE platform. The objective of the current study is to demonstrate the analytical performance of the newly developed Lumipulse ***G*** NfL CSF assay.

Study Objective

The aim of the study was to determine the performance of several analytical parameters of the Lumipulse ***G*** NfL CSF assay including precision, sensitivity, proportional linearity, and impact of interfering substances. The studies included testing a series of samples at different concentration levels, and in several replicates. In addition, a method comparison with the CE certified NF-light® ELISA from Uman Diagnostics (Sweden) was performed on CSF samples (n=73).

LUMIPULSE *G* platform and NfL CSF 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 Lumipulse ***G*** NfL CSF assay contains the capturing mAb in the gel compartment and is combined with a detection antibody, directly labelled with alkaline phosphatase (ALP). An overview of the assay set-up is given in Fig. 1.

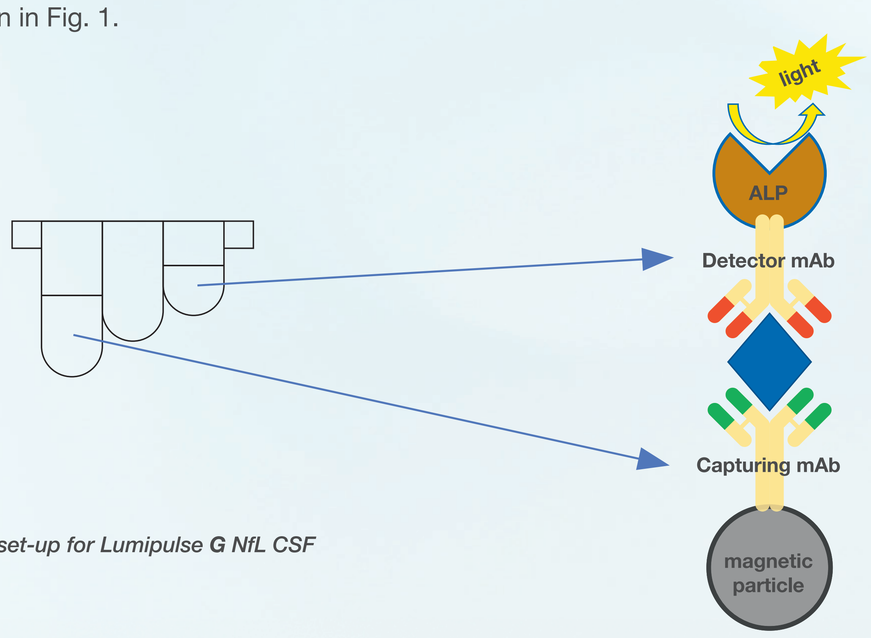

*Figure 1: Assay set-up for Lumipulse **G** NfL CSF*

Precision

A set of 3 native CSF samples and 3 calibrator material spiked buffer samples were analyzed in duplicate, in 2 runs a day, during 5 days on 3 LUMIPULSE G600II instruments (spread over 17 days). Next to total variation and repeatability, the intermediate precision for run, within day, between day, within instrument and between instrument was calculated (see Table 1).

All factors of variability were low for this assay, with %CVs between 3.3% and 7.0% for respectively the highest and lowest neat CSF sample.

Sample	Mean (pg/mL)	%CV					
		Repeatability (intra-assay)	Between run (inter-assay)	Between day	Within Instrument	Between Instrument	Reproducibility (total imprecision)
CSF L	742	1.2%	1.0%	2.2%	2.7%	6.5%	7.0%
CSF M	1799	1.2%	1.9%	1.5%	2.7%	6.4%	6.9%
CSF H	6647	1.4%	2.0%	1.1%	2.7%	1.9%	3.3%
CON L	718	1.6%	1.3%	0.5%	2.1%	5.8%	6.2%
CON M	3881	1.1%	1.8%	0.0%	2.1%	3.6%	4.2%
CON H	19387	0.9%	1.2%	1.5%	2.2%	0.0%	2.2%

*Table 1: Precision data Lumipulse **G** NfL CSF on LUMIPULSE G600II*

Analytical sensitivity

Using a first concept lot, a series (n=11) of low concentration CSF samples (range of 0.6 to 60.0 pg/mL; LoB=1.9pg/mL) was tested on 4 days in 5-fold on 2 instruments (40 replicates/sample). Based on the observed variability over the different measurements of the 5 lowest concentrated samples (from 0.6 to 5.1 pg/mL), a precision profile was built and used to determine the limit of detection (LoD) based on the calculation procedure referenced in CLSI EP17-A2. For the limit of quantification (LoQ) the observed variability of the highest concentrated samples (from 5.1 to 60.0 pg/mL), was used to build a precision profile and determine a LoQ at 20% CV (Fig. 2). The LoD and LoQ determined for this lot were found to be 3.6 pg/mL and 6.1 pg/mL respectively.

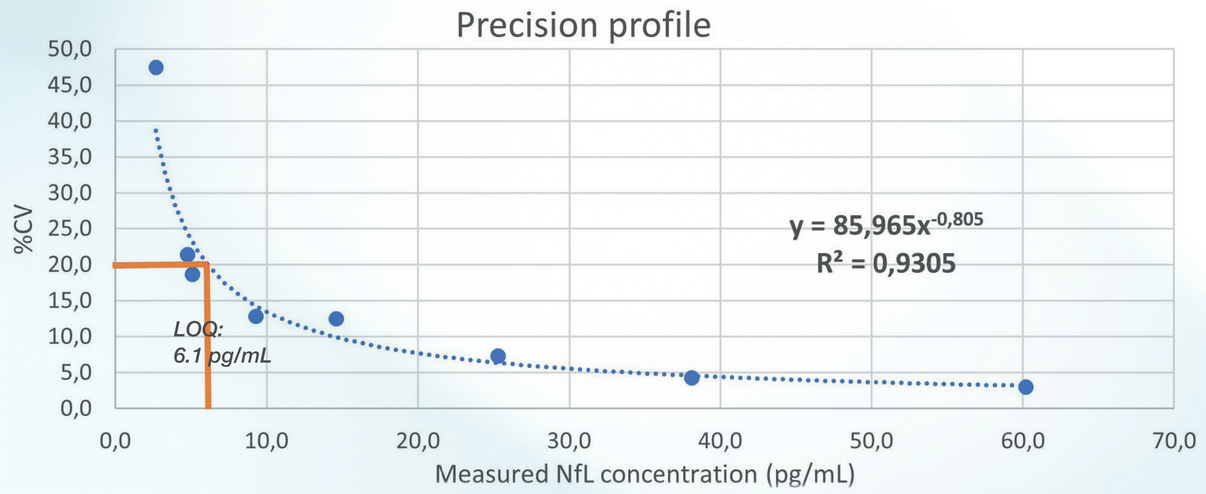

*Figure 2: Precision profile Lumipulse **G** NfL CSF with indication of LOQ as obtained on the LUMIPULSE system*

Proportional linearity

The accuracy of the assay was evaluated by means of a proportional linearity experiment. A CSF sample with an NfL concentration in the upper limit range (≈52000 pg/mL NfL) was proportionally mixed with a low concentrated NfL CSF sample (≈6 pg/mL NfL) resulting in 17 sample pools and two neat samples. All were tested in triplicate with the Lumipulse ***G*** NfL CSF assay. A linear fit was obtained when plotting the observed versus the theoretical concentration levels of the samples (Fig. 3). The maximal observed deviation from the theoretical value was 12.7%. The method can be considered as linear from 7 pg/mL up to 52218 pg/mL.

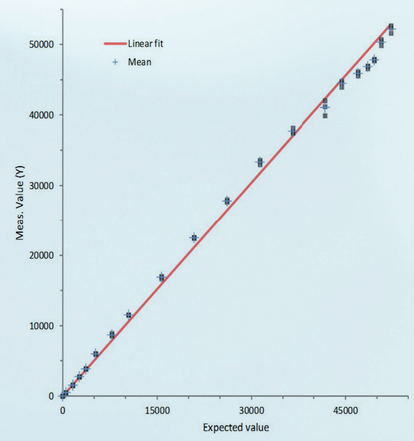

*Figure 3: Proportional linearity Lumipulse **G** NfL CSF*

Endogenous interference

The impact of some common endogenous potential interferents was assessed using a set of 3 CSF samples (CSF L: ≈15000 pg/mL; CSF M: ≈36000 pg/mL and CSF H: ≈112000 pg/mL). Each of the samples was spiked at the interferent concentration indicated in Table 2 (concentration tested) and compared to blank spiked control sample. The measurement values obtained for the test samples show a variation below 10% towards the control sample (Table 2), meaning that no significant impact of the evaluated endogenous substances in the assay was observed.

Interferent tested	Concentration tested	CSF L, M, H MV %Recovery
Intralipid	≥33 mg/dL	102 - 106%
Bilirubin F	≥0.3 mg/dL	95 - 97%
Bilirubin C	≥0.3 mg/dL	96 - 98%
Hemoglobin	≥11 mg/dL	97 - 96%
RF	≥14 IU/mL	100 - 104%
Human Serum albumin	≥0.17 g/dL	102 - 102%
Human IgG	≥0.06 g/dL	97 - 104%
Human IgM	≥0.003 g/dL	97 - 102%
Human IgA	≥0.002 g/dL	101 - 102%
Whole blood	≥0.1% v/v	102 - 104%
HAMA	≥5 ng/mL	100 - 101%
Chyle	≥14 FTU	93 - 96%

Table 2: Observed %Recovery on the measurement values (MV) for commonly tested endogenous substances

Method comparison

A set of 73 CSF samples were analyzed with the Lumipulse NfL CSF assay (on LUMIPULSE G1200) and Uman Diagnostics NF-light® ELISA CE (CSF, ref 10-7001). Eight CSF samples were excluded from analysis because the measured concentration was out of range (125 to 2500 pg/mL) based on the analysis with the Uman Diagnostics NF-light® ELISA. The outcome of Passing-Bablok regression analysis (n=65) revealed a high correlation (r=0.99) between both methods and the measurement values aligned very well (slope=0.99 [95%CI: 0.95 to 1.01; Fig. 4).

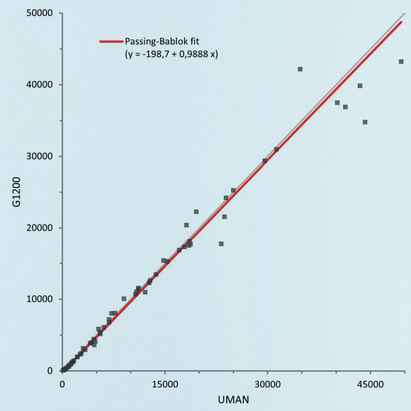

*Figure 4: Lumipulse **G** assay comparison with Uman Diagnostics NF-light® ELISA for NfL CSF*

Conclusion

The analytical performance studies of the Lumipulse ***G*** NfL CSF assay demonstrate low variability and high sensitivity enabling fast and fully automated measurement of NfL in CSF samples. The assay has a high correlation to the Uman Diagnostics NF-light® ELISA. The Lumipulse ***G*** NfL CSF assay is now ready to be explored further for clinical utility in various contexts of use.

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

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