

NfL Analyte Stability Evaluation in Blood Using Lumipulse® G

Manu Vandijck, Martine Dauwe, Koen de Geyter, Nadia Van der Cruyssen, Peter Jagiello, Jessie Theuns, Nathalie Le Bastard, Guy Mersch, Ina Vandenbroucke

Contact: manu.vandijck@fujirebio.com; guy.mersch@fujirebio.com

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

Introduction

Cerebrospinal fluid (CSF) biomarkers are well-established tools to assess neurodegenerative pathology. In recent years, however, attention has shifted toward blood-based biomarkers, driven by their practicality and suitability for routine clinical and research applications. Among these, neurofilament light chain (NfL) has emerged as a sensitive marker of neuro-axonal damage across a broad range of neurological diseases. Because blood NfL concentrations are typically low, especially in healthy individuals, results may be more easily influenced by pre-analytical factors such as sample handling, storage conditions, and freeze/thaw cycles. A clear understanding of analyte stability under realistic laboratory conditions is therefore essential to ensure reliable and reproducible NfL measurements.

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. An Assay Specific Solution is added during sample incubation to optimize analyte detection and improve the assay specificity. An overview of the assay design is given in [Table 1](#).

Study Objective

The aim of this study was to systematically evaluate the pre-analytical stability of NfL in blood, measured using the Lumipulse® G NfL Blood (CE) assay (Fujirebio Europe N.V.), in serum and K₂-EDTA plasma. Analyte stability was assessed under commonly encountered storage and handling conditions, including room temperature, refrigerated and frozen storage, multiple freeze/thaw cycles, and fresh versus frozen comparisons, in order to support robust specimen management recommendations for clinical research and routine laboratory use.

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

Method

Serum and K₂-EDTA plasma samples were collected from healthy adult donors (n = 9) under an ethics committee-approved protocol. Following centrifugation, samples were pooled per donor, aliquoted (600 µL), and stored under different pre-analytical conditions prior to analysis. NfL concentrations were measured using the Lumipulse G NfL Blood assay on the LUMIPULSE G1200 platform. For each condition and time point, samples were analyzed in four replicates, using a single reagent lot, with randomized testing order. Sample stability was evaluated under the following conditions: room temperature (25°C) for up to 7 days, refrigerated storage (2–10°C) for up to 7 days, frozen storage at –20°C for up to 6 months, frozen storage at –80°C for up to 12 months, multiple freeze/thaw cycles (1×, 2×, 3×, and 5×), and fresh versus frozen sample comparison. Stability was primarily assessed by comparing measurements obtained under each condition to the corresponding fresh sample. Results were evaluated using the predefined acceptance criterion of 100 ± 10% variation relative to fresh samples. In addition, given the low NfL concentrations observed, stability was also assessed using an absolute measurement difference criterion based on ±3× standard deviation, derived from assay precision at the respective concentration range.

Results

NfL concentration range and general considerations

Across all samples, measured NfL concentrations were consistently low, ranging from approximately 2.7 to 13.8 pg/mL in both serum and K₂-EDTA plasma. At these concentration levels, small absolute differences can translate into relatively large percentage changes, which influenced the interpretation of results based solely on percentage variation.

Fresh versus frozen samples

NfL concentrations measured in frozen samples were comparable to those obtained from fresh samples in both serum and K₂-EDTA plasma. No systematic differences were observed between fresh and frozen conditions, irrespective of storage temperature and sample matrix. These findings indicate equivalence between fresh and frozen specimens for NfL in blood.

Freeze/thaw stability

NfL demonstrated stability across multiple freeze/thaw cycles in both matrices. When evaluated using percentage variation, isolated samples exceeded the predefined acceptance limits after five freeze/thaw cycles. However, when assessed using absolute measurement differences relative to fresh samples (±3×SD), all samples remained within acceptable limits up to five freeze/thaw cycles, indicating robustness of NfL to repeated freezing and thawing.

Short-term stability at room temperature and refrigerated conditions

NfL concentrations remained stable in serum and K₂-EDTA plasma during short-term storage at room temperature (25°C) and refrigerated conditions (2–10°C). At room temperature, NfL remained stable for up to 7 days in both matrices when evaluated using absolute measurement differences. Under 2–10°C storage, isolated borderline deviations were observed at intermediate time points based on percentage variation; however, measurements at the final time point again remained within acceptable limits. Overall, absolute difference evaluation supported stability for up to 7 days under refrigerated conditions.

Frozen storage stability at –20°C and –80°C

For frozen storage at –20°C, NfL concentrations remained stable for up to 6 months in both serum and K₂-EDTA plasma based on absolute measurement differences relative to fresh samples. For long-term storage at –80°C, increased variability was observed at the 6-month time point in a limited number of samples, primarily due to replicate variability. At 12 months, NfL concentrations met acceptance criteria based on absolute measurement differences, supporting long-term stability at –80°C.

Summary of stability outcomes

Overall, NfL stability was confirmed across a broad range of pre-analytical conditions in serum and K₂-EDTA plasma. Evaluation based on absolute measurement differences (±3×SD) provided a more robust and concentration-appropriate assessment of stability than percentage variation alone ([Table 2](#), [Figure 1](#)).

Table 2: Proven stability of blood NfL under common pre-analytical conditions. Results are summarized for serum and K₂-EDTA plasma using both percentage variation and absolute difference (±3×SD) evaluation criteria.

Condition	% Variation criterion	Absolute difference (±3×SD)
Fresh vs frozen	Within specification	Within specification
Freeze/thaw	Up to 3× F/T	Up to 5× F/T
Room temperature (25°C)	Up to 3 days	Up to 7 days
Refrigerated (2–10°C)	Up to 1 day	Up to 7 days
Frozen –20°C	3 months	Up to 6 months
Frozen –80°C	6 months	Up to 12 months

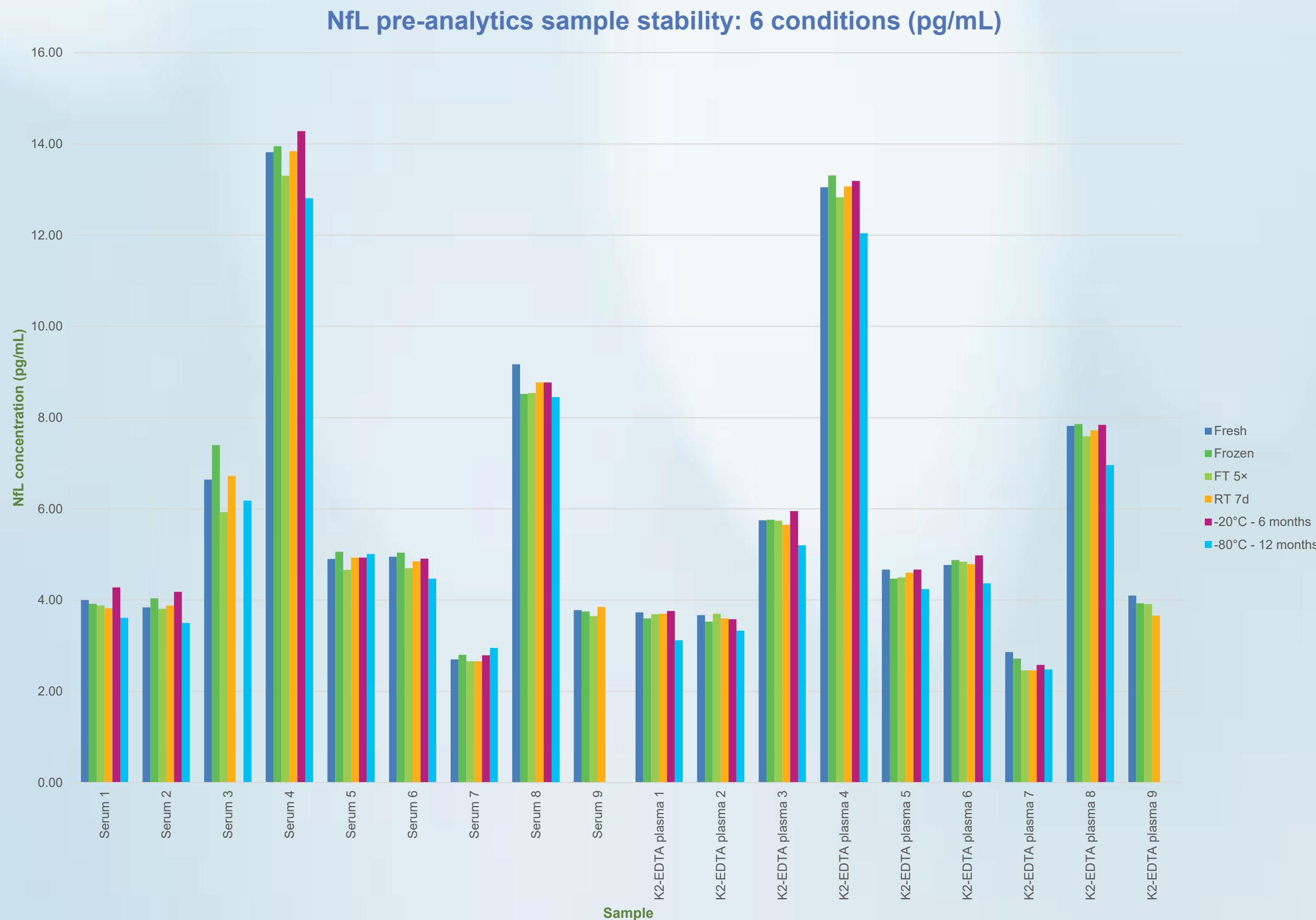


Figure 1: Concentration (pg/mL) per sample under six storage/handling conditions (Fresh, Frozen, 5× freeze–thaw, RT 7 days, –20° C for 6 months, –80° C for 12 months). Bars represent the mean value for each condition. RT = room temperature; FT = freeze–thaw cycles. Some data points are missing due to sample depletion; this applies to Serum 3 at –20° C and to Serum 9 and Plasma 9 at –80° C.

Conclusion

This study demonstrates that NfL in serum and K₂-EDTA plasma is stable under routine pre-analytical handling and storage conditions when measured using the Lumipulse G NfL Blood assay. Across both matrices, NfL concentrations remained stable for up to 7 days at room temperature and at 2–10°C, for up to 6 months at –20°C, and for long-term storage at –80°C (up to 12 months tested). In addition, NfL demonstrated robustness to multiple freeze/thaw cycles, supporting practical laboratory workflows. No meaningful differences were observed between fresh and frozen samples. Overall, these findings support flexible and reliable specimen management for blood NfL testing using the LUMIPULSE G platform, facilitating its application in both clinical research and routine laboratory settings.

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