

pTau 217 Analyte Stability In Plasma Using Lumipulse® G

Caroline Dobbels, Jelle D’hont, Roger Moonen, Jessie Theuns, Nathalie Le Bastard, Manu Vandijck, Ina Vandenbroucke

Contact: manu.vandijck@fujirebio.com; caroline.dobbels@fujirebio.com

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

Introduction

Cerebrospinal fluid (CSF) and amyloid Positron Emission Tomography (PET) biomarkers are well-established tools to assess neurodegenerative pathology. In recent years, however, increasing attention has shifted toward blood-based biomarkers, driven by their practicality and suitability for routine clinical and research applications. Among these, tau phosphorylated at threonine 217 (pTau 217) has emerged as a sensitive marker for amyloid pathology associated with Alzheimer’s disease. As levels of pTau 217 in K₂EDTA plasma are typically low, especially in healthy individuals, results may be influenced by pre-analytical factors such as sample handling, storage conditions, and freeze/thaw cycles. A clear understanding of analyte/sample stability under realistic laboratory conditions is therefore essential to ensure reliable and reproducible pTau 217 measurements.

Lumipulse G pTau 217 Plasma 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 format is given in [Table 1](#).

Study Objective

The aim of this study was to systematically evaluate the pre-analytical stability of pTau 217 in K₂EDTA plasma, measured using the Lumipulse® G pTau 217 Plasma assay (Fujirebio Europe N.V.). Analyte stability was assessed under commonly encountered handling and storage conditions, including room temperature, refrigerated and frozen storage, repeated freeze/thaw cycles, and comparisons of fresh versus frozen plasma samples in order to support robust specimen management recommendations for clinical research and routine laboratory use.

Table 1: Lumipulse G pTau 217 Plasma assay format.

Assay	Configuration	Storage	Content	Volume
Immunoreaction Cartridge	3 x 14 Tests	2°C to 8°C	Capturing mAb on magnetic particles / Assay specific solution / Detection conjugate (mAb-ALP)	Sample volume 100 µL
Calibrators	1 x 5 CAL	-25°C to -15°C	Synthetic peptide pTau 217; Conc. range 0 to 10 pg/mL	1.5 mL / vial
Controls	2 x 2 CTRL	-25°C to -5°C	Synthetic peptide pTau 217; L1 and L2	1.5 mL / vial

Method

K₂EDTA plasma samples were collected from apparently healthy adult donors (n = 10) under an ethics committee–approved protocol. Following centrifugation, plasma was pooled per donor (40 mL), aliquoted (600 µL), and stored under defined pre-analytical conditions prior to analysis. Concentrations of pTau 217 were measured using the Lumipulse G pTau 217 Plasma assay on the LUMIPULSE G1200 platform, with samples analyzed in duplicate for each condition and time point. Analyte stability was evaluated under fresh sample handling conditions, storage at room temperature (25°C) and refrigerated conditions (2-10°C) for up to 7 days, frozen storage at -20°C and -80°C for up to 6 months, multiple freeze/thaw cycles (1x to 5x), and by comparing fresh and frozen samples. Stability was assessed in comparison to fresh sample results using predefined acceptance criteria of 100 ± 20% recovery at individual sample level and a mean change within ±10% across all samples. For samples with pTau 217 <0.150 pg/mL, an additional criterion of ±3 Standard Deviation (SD) (based on assay precision at the relevant concentration range) was applied, as percentage-based evaluation may overestimate pre-analytical variability at low concentrations.

Results

pTau 217 concentration range and general considerations

Across all samples, pTau 217 concentrations were low, ranging from approximately 0.044 pg/mL to 0.287 pg/mL in K₂EDTA plasma. At these concentrations, small absolute differences can result in relatively large percentage changes, potentially overestimating variability. Therefore, in a limited number of cases, the acceptance criterion of ±3 SD was applied to assess stability.

Fresh versus frozen samples

Concentrations of pTau 217 measured in frozen K₂EDTA plasma samples were comparable to their results obtained from the fresh samples, with no systematic differences observed across storage conditions. The mean change between fresh and frozen samples was 4%. These results support the equivalence of fresh and frozen specimens when analyzed using the Lumipulse® G pTau 217 Plasma assay (light blue and yellow bars in [Figure 1](#)).

Freeze/thaw stability

The analyte pTau 217 demonstrated stability in K₂EDTA plasma across multiple freeze/thaw cycles. Although one isolated sample in one condition exceeded the predefined acceptance criterion, when evaluated using percentage variation over the five freeze/thaw cycles, all samples remained within the acceptance limits based on absolute measurement differences relative to fresh samples (±3 SD). The mean change across all samples ranged from -2% to 5% over five freeze/thaw cycles. These results support the stability of pTau 217 in K₂EDTA plasma up to five freeze/thaw cycles when analyzed with the Lumipulse G pTau 217 Plasma assay (purple bars in [Figure 1](#)).

Short-term stability at room temperature and refrigerated conditions

Concentrations of pTau 217 remained stable in K₂EDTA plasma during short-term storage at both room temperature (25°C) and refrigerated conditions (2-10°C). While two samples exceeded the predefined acceptance criterion when evaluated using percentage variation, all samples remained within the acceptance limits defined by absolute measurement differences relative to fresh samples (±3 SD). The mean change across all samples ranged from -7% to 2% after 7 days of storage at 25°C, and from -2% to 6% after 7 days of storage at 2-10°C. Based on this assessment, pTau 217 is considered stable for up to 7 days in K₂EDTA plasma at both 25°C and 2-10°C (green and brown bars in [Figure 1](#)).

Long-term frozen storage stability at –20°C and –80°C

For frozen storage at -20°C and -80°C, pTau 217 concentrations remained stable in K₂EDTA plasma during long-term storage. Although two samples exceeded the predefined acceptance criterion based on percentage variation at one of the measurement points, all samples remained within the acceptance limits based on absolute measurement differences relative to fresh samples (±3 SD). A mean change of 3% across all samples was obtained after 6 months of storage at -20°C, and a mean change of -1% after 6 months of storage at -80°C. Based on these results, pTau 217 was considered stable in K₂EDTA for up to 6 months at -20°C and at -80°C (red and pink bars in [Figure 1](#)).

Summary of stability outcomes

Overall, pTau 217 analyte stability was confirmed across a broad range of pre-analytical conditions in the obtained K₂EDTA plasma sample set ([Figure 1](#)). Although the predefined acceptance criteria based on percentage variation were not met in a limited number of cases, all samples remained within the acceptance limits when evaluated using absolute measurement differences relative to fresh samples (±3 SD). This approach provided a more robust and concentration-appropriate assessment of stability than percentage variation alone, supporting the stability of pTau 217 under the evaluated conditions ([Table 2](#)).

Table 2: Proven stability of pTau 217 in K₂EDTA plasma under common pre-analytical conditions.

Condition	Results
Fresh vs Frozen	Equivalent
Freeze/thaw	Up to 5 F/T
Room temperature (25°C)	Up to 7 days
Refrigerated (2-10°C)	Up to 7 days
Frozen -20°C	Up to 6 months
Frozen -80°C	Up to 6 months

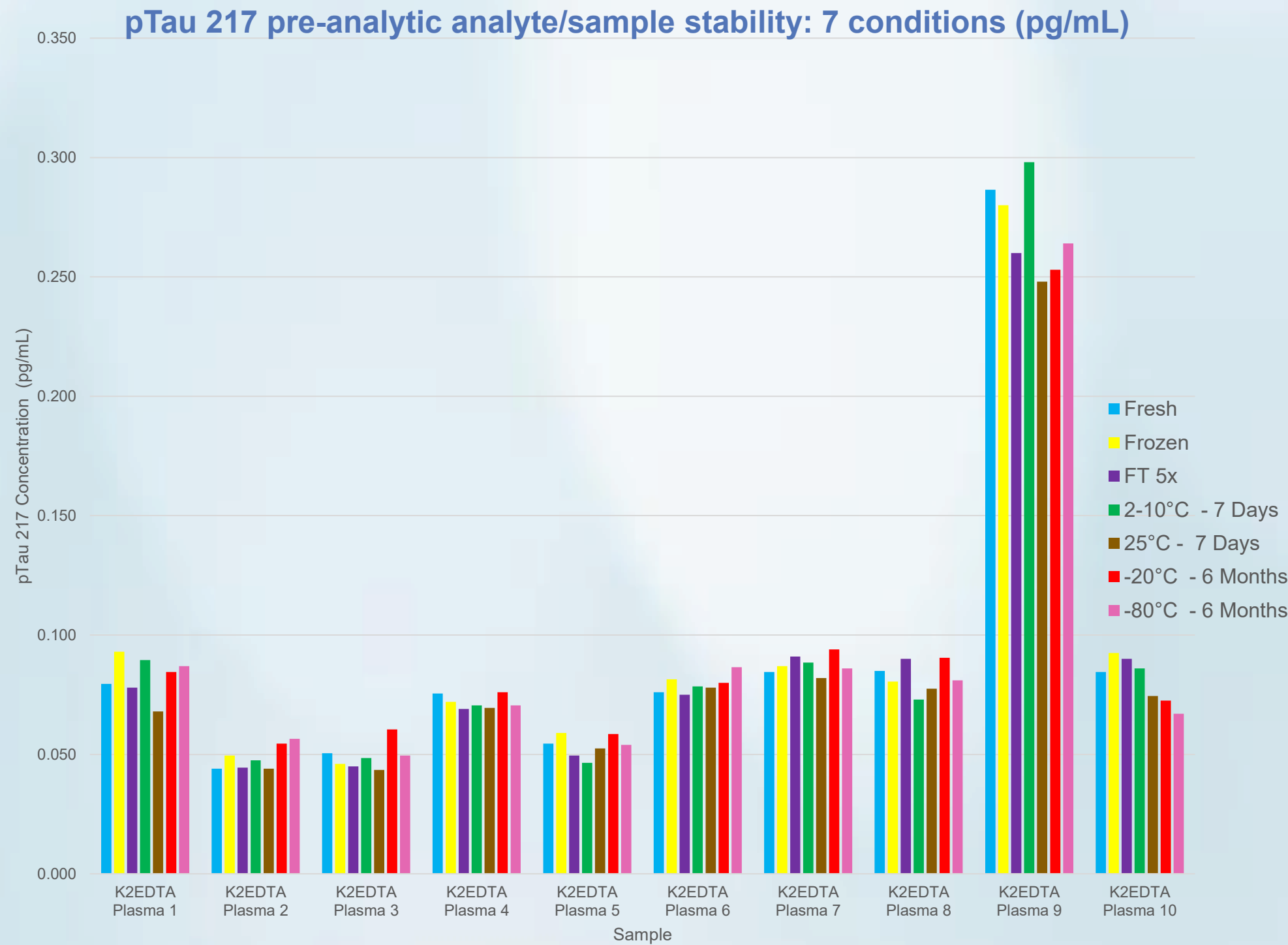


Figure 1: pTau 217 concentrations (pg/mL) per sample under different pre-analytical conditions, including fresh samples, room temperature (RT, 25°C) and 2-10°C for 7 days, frozen storage at -20°C and -80°C for 6 months, and up to 5 freeze/thaw (FT) cycles. Bars represent mean concentration per condition. RT, room temperature; FT, freeze/thaw cycles.

Conclusion

This study evaluates the analyte pTau 217 in 10 K₂EDTA plasma of apparently healthy donors under commonly encountered pre-analytical handling and storage conditions when measured using the Lumipulse G pTau 217 plasma assay. Under the conditions assessed, concentrations of pTau 217 were observed to remain stable for up to 7 days of storage at room temperature and at 2-10°C, and for up to 6 months of storage at -20°C, as well as at -80°C. In addition, pTau 217 was observed to be robust to multiple freeze/thaw cycles, supporting practical laboratory workflows. No meaningful differences were observed between fresh and frozen samples within the scope of this study. Overall, these findings suggest the potential for flexible and reliable specimen management for pTau 217 testing on the LUMIPULSE G platform, which may facilitate its application in clinical research and routine laboratory settings.

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