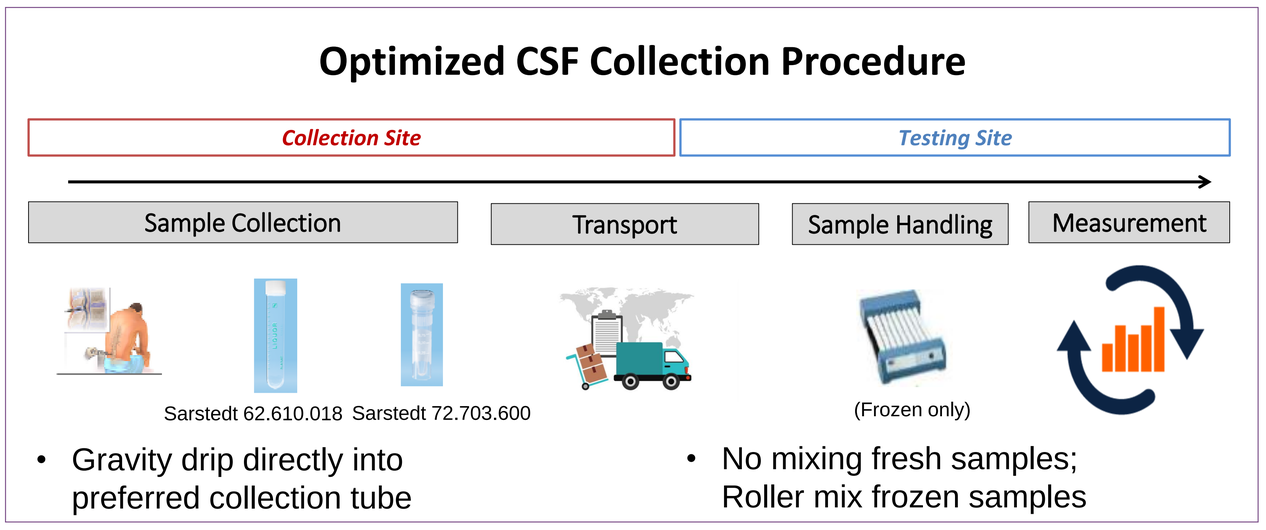
In an optimized CSF collection protocol the pTau181/Aβ1-42 ratio increases preanalytical variability over measuring Aβ1-42 alone

Esquivel, R.,[1] Ho, S.,[2] Darrow, J.,[2] Calabro, A.,[1] Thakker, P.,[2] Gannon, S.,[1] De Simone, F.,[1] Moghekar, A.[2]

[1]Fujirebio Diagnostics Inc, Malvern PA, USA [2]Johns Hopkins School of Medicine, Baltimore MD, USA

Background

Core cerebrospinal fluid (CSF) biomarker concentrations for β-amyloid1-42 (Aβ1-42), β-amyloid1-40 (Aβ1-40), and pTau181 are valuable in the diagnosis of Alzheimer's Disease (AD). Specifically, when used in a ratio Aβ1-42/Aβ1-40 and pTau181/Aβ1-42 have shown high concordance with amyloid PET. However, questions remain on the robustness of these ratios when used in clinical routine due to the tendency of amyloid to adsorb to surfaces causing amyloid loss that may result in misdiagnosis. Stringent handling procedures of CSF have been proposed to reduce amyloid loss including the use of a single polypropylene tube type. The proposal of a single tube complicates CSF collection and creates doubt in results obtained from alternative tube types. In this study the effect of varying polypropylene tube type within clinical routine on amyloid concentration using Aβ1-42/Aβ1-40 and ptau181/Aβ1-42 ratios was evaluated in freshly collected CSF. The utility of the ratios to correct for pre-analytical variability and bring concentrations within +/- 5% of baseline as indicated by recently published Alzheimer's Association International Guidelines for CSF handling[2] was examined.



Methods

- **CSF collection** CSF was obtained from 22 patients examined in the Johns Hopkins Center for CSF disorders who provided consent for biospecimen banking for research.
- **Sample Testing** Testing and handling was performed using the Lumipulse Aβ1-42, Aβ1-40, and pTau 181 assay parameters and IUO package inserts.
- **Sample Treatment** CSF was collected directly into tubes A (Sarstedt #72.703.600), B (Sarstedt #62.610.018) or C (Sarstedt #63.614.699). Collected CSF was transported securely in the upright position at ambient temperature. Tubes A and B were filled to 80% fill volume and CSF was analyzed post centrifugation (2000 xg, 10 minutes, 5 ± 3°C). Tube C was filled at both 80% or 100% fill volume. All aliquots were directly tested on the LUMIPULSE G1200 in triplicate. After baseline testing aliquots were frozen at -80°C ± 10°C for a minimum of 8 hours. Frozen samples were thawed at room temperature for a minimum of 1 hour and then roller mixed for 20 minutes before direct testing. Extended sample cap contact was evaluated by storing samples upright or upside down at 4°C for 1 week or upright or upside down at -80°C for 1 month.

Results

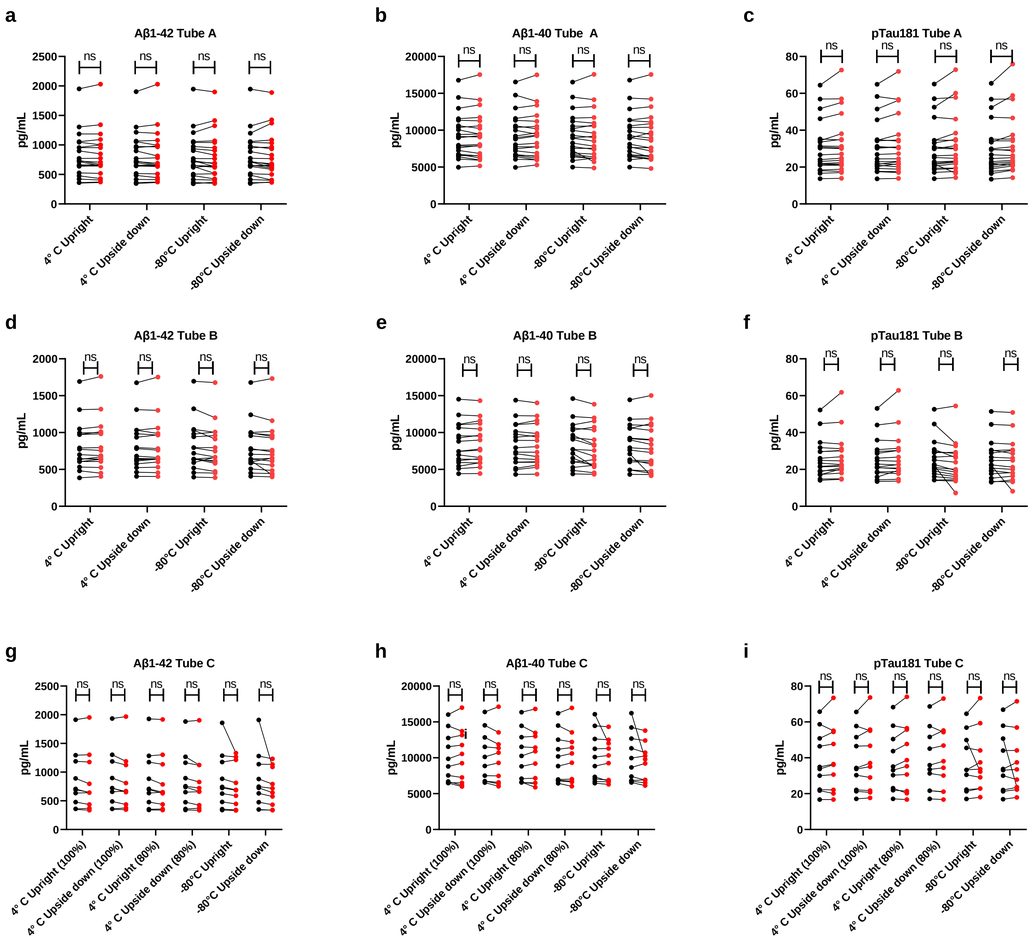


Figure 1. Fill volume and storage conditions did not significantly affect concentrations of individual biomarkers independent of polypropylene tube type. Concentrations of Aβ1-42 (a,d,g), Aβ1-40 (b,e,h), and pTau181 (c,f,i) in Tube A (a,b,c), Tube B (d,e,f), and Tube C (g,h,i).

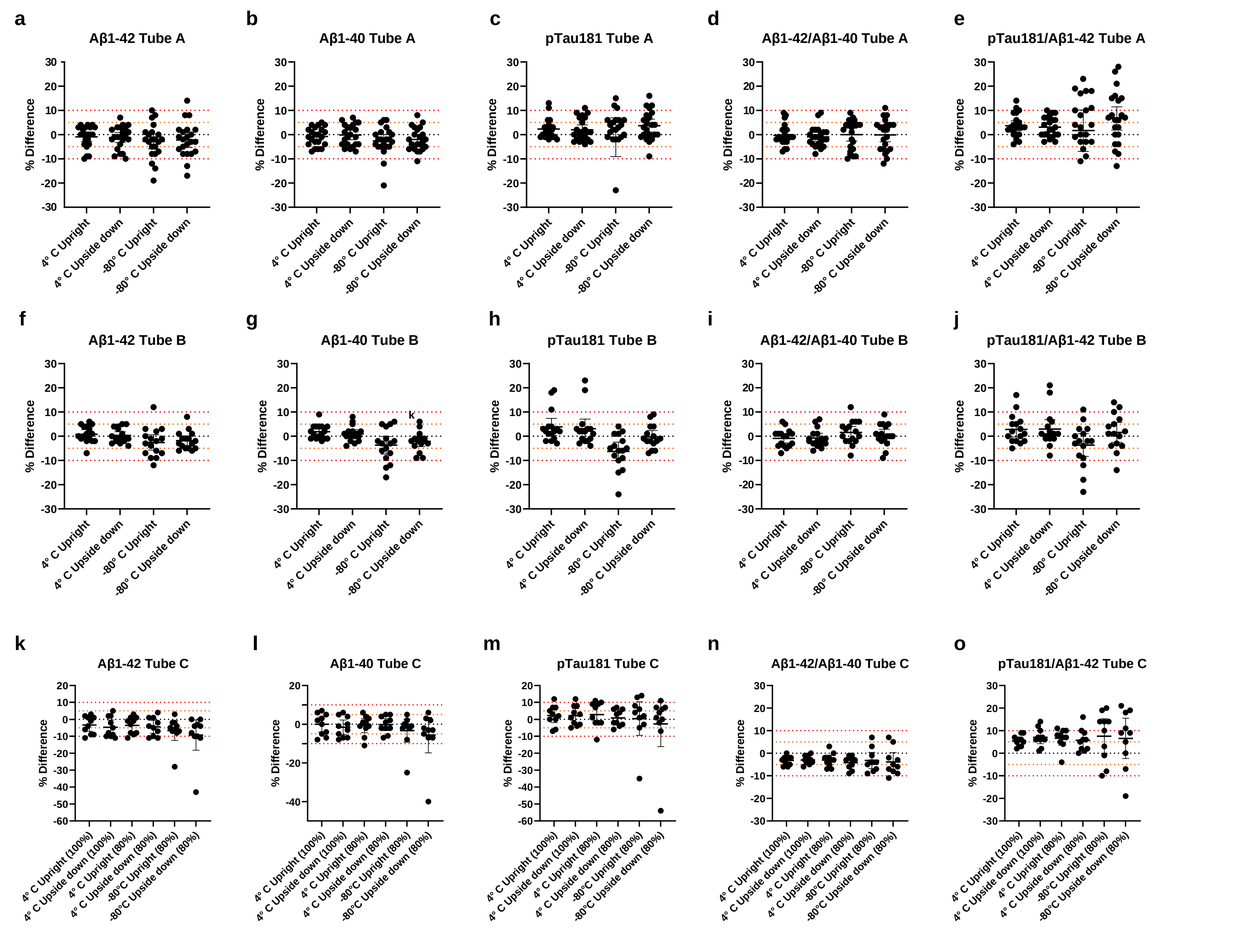


Figure 2. pTau181/Aβ1-42 ratio values were more variable when compared to the individual biomarkers or the Aβ1-42/Aβ1-40 ratio. Percent difference of values from baseline for Aβ1-42 (a,f,k), Aβ1-40 (b,g,l), pTau181 (c,h,m), Aβ1-42/Aβ1-40 (d,i,n) and pTau181/Aβ1-42 (e,j,o) under fresh and frozen storage conditions.

Conclusions

- Individual amyloid proteins and the pTau181/Aβ1-42 ratio had significant variability dependent on tube type and handling. Variability of pTau181/Aβ1-42 was higher in all three tubes after storage at -80°C.
- The pTau181/Aβ1-42 ratio may amplify preanalytical variability even when amyloid loss is minimal. Measuring the Aβ1-42/Aβ1-40 ratio alongside pTau181 as an individual analyte may provide most consistent results in studies relying on biobanked samples.
- Tube A, Sarstedt Tube #72.703.600, had the least variability when measuring individual analytes and is recommended for -80°C storage.
- In clinical routine the Aβ1-42/Aβ1-40 ratio is preferable to minimize the impact of deviations from an optimized collection and handling protocol on patient diagnosis.

References

1. Hansson O, Rutz S, Zetterberg H, et al. Pre-analytical protocol for measuring Alzheimer's disease biomarkers in fresh CSF. Alzheimers Dement (Amst). 2020 Dec 18;12(1):e12137.

2. Hansson O, et al. The Alzheimer's Association international guidelines for handling of cerebrospinal fluid for routine clinical measurements of amyloid β and tau. Alzheimers Dement. 2021 Sep;17(9):1575-1582.

Conflicts of Interest: This study was funded by Fujirebio Diagnostics Inc. FD, AC, SG, and RE are employed by Fujirebio Diagnostics Inc.