# Critical evaluation and comparison of biomarker values in commercial CSF with Lumipulse® to support assay development for clinical trials

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## Introduction

Cerebrospinal fluid (CSF) from human donors is an important matrix used in multiple applications in the study of central nervous system drug delivery and biomarker analysis. CSF requires a thorough analysis of multiple factors contributing to its quality of use. The ccharacterization and use of CSF is highly dependent on protocols, reagents, and methods used to collect, store, handle, and analyze the CSF, as well as underlying diagnostic criteria for the selected subjects.

The complexity increases when CSF is obtained from commercial suppliers, because only limited information is typically available on sample quality, collection procedures, and levels of the accepted Alzheimer's disease (AD) protein biomarkers.

Pre-analytical variables (e.g., time, temperature, freezing, blood contamination) can directly impact biomarker concentrations and various assay parameters. The lack of such information can affect timelines for assay development and outcomes of clinical sample analysis.

In this study, human CSF samples obtained from PrecisionMed LLC were used to evaluate and compare biomarker levels and material loss in selected reagent tubes.

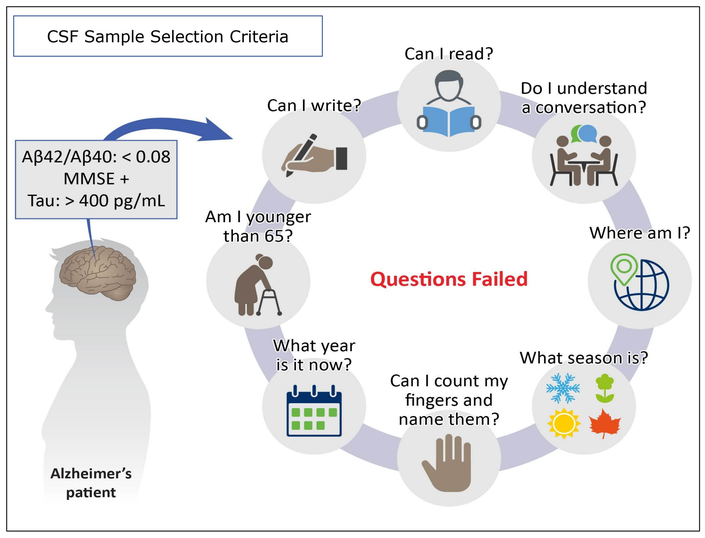


**Figure 1. CSF Selection criteria**
CSF sample selection was initially based on biomarker cut-off levels (top left). Information provided in patient CRFs includes the Mini-Mental State Examination (MMSE) and satisfies NINCDS-ADRDA criteria. The circle exemplifies questions included in the patient CRFs which evaluate different cognitive domains such as memory, language, perceptual skills, attention, constructive abilities, orientation, problem solving and functional abilities.

### STUDY OBJECTIVES

- Define selection criteria for CSF samples purchased from PrecisionMed LLC (Carlsbad, CA, USA).
- Perform a comparative quantitation of CSF biomarkers with the recently FDA-cleared Lumipulse® (β-Amyloid 1-42/1-40) assays and Lumipulse® assays for total tau and pTau181.
- Qualify reagent tube vendor for use in the INTERCEPT-AD trial (Code: ACU001).
- Select CSF samples based on biomarker levels and Case Report Form(CRF) diagnostic criteria.

## Methods

The CSF research sample inventories at PrecisionMed LLC (PM) provide a comprehensive list of patient information [diagnosis at baseline, demographics, number of visits, clinical information (e.g., MMSE, ADAS-cog), protein biomarkers (Aß**N**-38, Aß**N**-40, Aß**N**-42, tTau), aliquots and volumes]. Each sample is provided with a unique Case Report Form (CRF) that includes measures of the eight cognitive domains specified in the NINCDS-ADRDA criteria for AD diagnosis.
For this study, we selected five healthy control (HC) and seven AD CSF samples that met our pre-defined biomarker criteria for classification of AD and HC subjects and reviewed the diagnostic information provided in the patient CRFs (**Figure 1**).

To evaluate protein biomarker recovery and potential loss via passive adsorption to the reagent tube walls, we first aliquoted each CSF sample into two different low protein binding reagent tubes: Tube A. used by Fujirebio (Sarstedt cat 63.614.625, 2.5ml, false bottom); Tube B. Sarstedt (cat 72.694.600, 2ml) and frozen at -80°C. (**Figure 2**). An additional serial transfer of CSF into Tube B was done as described in B1, whereby CSF samples were first incubated for 30 minutes at room temperature, transferred to a new tube and subsequently frozen at -80°C. Another set of Tube B vials was pre-washed with sample diluent before addition of the CSF sample (B2) and freezing at -80°C .

All samples were stored at -80°C and subsequently processed for biomarker concentration analysis (batch-mode) in the facilities of Fujirebio (Philadelphia, USA). CSF biomarker levels were quantified with Lumipulse® (β-Amyloid 1-40/1-42) assays and Lumipulse® assays for total tau and pTau181.

Statistics: For each of the analytes and ratios of proteins, a linear mixed model was applied to the log-transformed data with sample code as random effect to correct for the within sample clustering. Differences are expressed as percentage difference between geometric means. P-values were adjusted for multiple testing (3 pairwise comparisons per analyte or ratio) using Bonferroni correction.

### REAGENT FLOWCHART

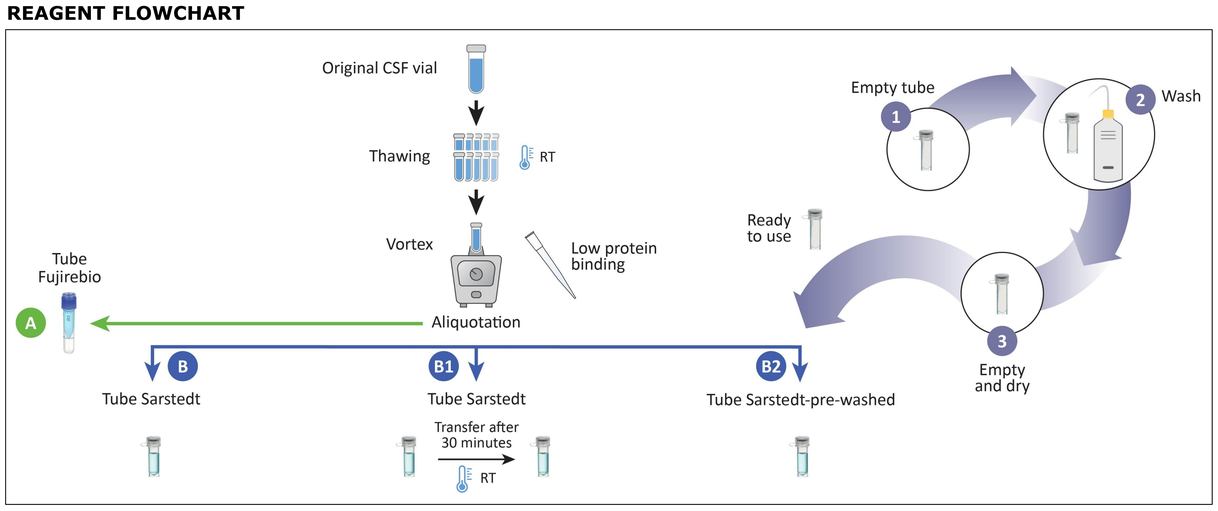


**Figure 2. Experimental protocol for CSF aliquotation**

## Results

False bottom 2.5ml vs standard 2ml reagent tube | Frozen vs 30min at RT | Prewash vs no prewash

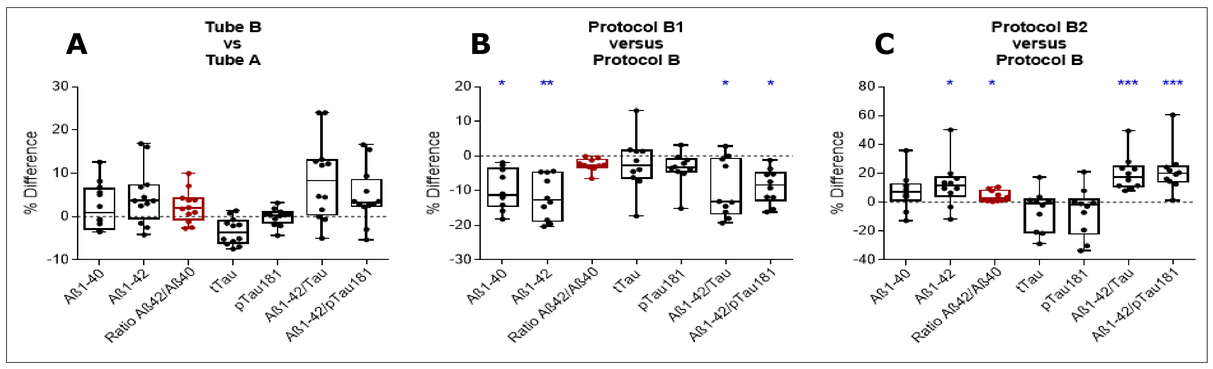


**Figure 3. Differences in CSF analyte concentrations comparing different aliquotation procedures**
The % difference for each sample (n=12) between CSF sample treatment protocols is presented as box-plots. Box-plots for the ratio Aß42/Aß40 are plotted in red.
* p<0.05; ** p<0.01; *** p<0.001.

CSF analytes were measured with the Lumipulse® immunoassays in all aliquots (12 subjects, 4 protocols). Results were compared to the biomarker levels reported by PrecisionMed LLC using the Meso Scale discovery platform (MSD).

**Part 1. Analyte concentrations**
Data presented in **Figure 3A** did not show any significant difference (p> 0.05) in analyte concentration or ratio of analytes (Aß1-42/Aß1-40, Aß1-42/tTau, Aß1-42/P-Tau181) between CSF added to Tubes A (Fujirebio) or Tubes B (p>0.05).

**Part 2. Passive adsorption**
Serial transfer of CSF from Tube B into Tube B1 (one additional transfer) resulted in minor, but statistically significant reductions in Aß1-40 and Aß1-42 values, amounting to -9.5% (LL, UL; -16.1%, - 2.5%; p=0.02) and -11.8% (-18.9, -3.9; p=0.007), respectively. This difference was not significant for the ratio Aß1-42/Aß1-40 (p=0.33) (**Figure 3B**).
In contrast, slightly higher values were quantified after a pre-wash of Tubes B (B2 versus B) for Aß1-42 (+11.9%) (+2.7, +21.9;p=0.02), for the ratio Aß1-42/Aß1-40 (+4.1%) (+1.0, +7.2; p=0.02), ratio Aß1-42/tTau (+20.4%) (+11.4,+30.1; p=<0.001) and ratio Aß1-42/P-Tau181 (+21.3%) (+12.7, +30.5) (p=<0.001). The difference for Aß1-40 (+7.2%) (-0.3, +15.8) was not significant (p=0.2). Also, no statistically significant differences were noted for tTau (p=0.6) or P-Tau181 (p=0.6)(**Figure 3C**).

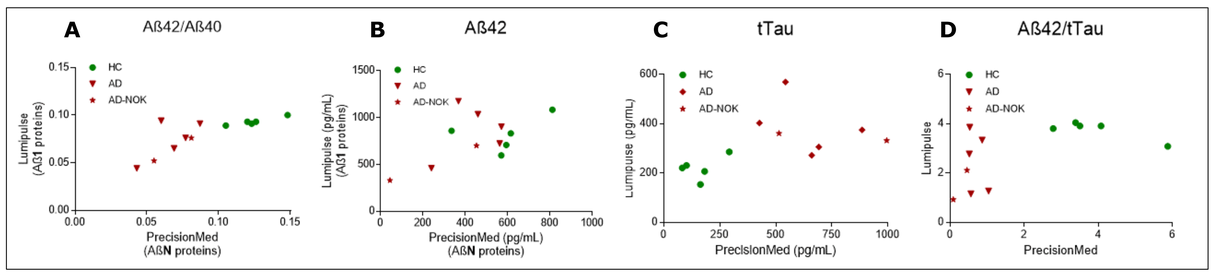


**Figure 4. Comparison of Lumipulse® and MSD results**
Correlation of PrecisionMed (MSD) and Lumipulse® data for Aß42/Aß40 (A), Aß42 (B), tTau (C), and Aß42/tTau (D). HC (green) and AD (Red) were initially identified based on the biomarker data provided by PrecisionMed LLC and subsequent analysis provided by Fuijirebio. AD-NOK (*) indicates samples with factors such as normal MRI imaging data, brain surgery, and lack of proficiency in English that may have affected biomarker levels or AD diagnosis based on the analysis of CRF questionnaires provided by PrecisionMed LLC.

**Part 3. Critical review of case reports and diagnosis for individual CSF samples**
The selection criteria applied to the PrecisionMed CSF biomarker results allowed us to differentiate AD from HC samples and subsequently compare the reported biomarker results with data obtained with the Lumipulse® immunoassays for total tau, pTau181, Aß1-42, and Aß1-40. The best separation of AD and non-AD samples was initially obtained with the PrecisionMed LLC samples using a cut-off value of 0.08 for the ratio of Aß1-42/Aß 1-40 (**Figure 4**). This cut off value is different from the Lumipulse® assay cut off value of 0.072. The difference is likely due to the assay format where 6E10 is used as a detector antibody in the MSD assay (reported by PrecisionMed) and 3D6 is used as detector antibody in the Lumipulse® assay. In addition, no harmonization of results with International Federation of Clinical Chemistry (IFCC) certified reference materials was performed for Aß1-42 in the MSD assay. Inclusion of the cognitive test criteria provided in the CRF further reduced the number of AD positive CSF samples. Two AD subjects were identified as non-AD (AD-NOK *) after review of the subject questionnaires.

### RESEARCH HIGHLIGHTS

- Both tested Sarstedt tubes are suitable for CSF biomarker analysis. The number of tube transfers and inclusion of tube pre-wash had a small, but statistically significant, impact on biomarker recovery.
- A cut-off value of 0.08 for the Aß1-42/Aß 1-40 ratio differentiates AD from HC samples from PrecisionMed LLC using the MSD assay.
- Of the biomarkers tested, the Aß1-42/Aß1-40 ratio values best correlate between the MSD and Lumipulse® assays.
- Inclusion of patient CRF information reduced AD sample selection due to MRI and diagnostic observations.

**Data presented at CTAD, San Franciso, CA; November 2022.**
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