Plasma oAβ in ADNI

Ting Yang,1 YiRan Xu1,Shirley Cai,1 and Dennis Selkoe1

1. Ann Romney Center for Neurologic Diseases, Brigham and Women’s Hospital, 60 Fenwood Road, Boston, MA 02115

|  |  |
| --- | --- |
| **Contents** | |
| Page 1 | Introduction |
| Page 1  Page 2  Page 3  Page 3 | Summary  Methodology and Quality Control  References  About the Authors |

# Introduction

# Our team has recently revised and enhanced our ultra-sensitive immunoassay for quantifying soluble Aβ oligomers in human plasma and CSF using EMD Millipore’s SMCxPRO platform.1-6 This assay offers a long-awaited approach to detect and monitor the species believed to be the crucial bioactive form of Aβ in Alzheimer's disease (AD), thus making it a target for disease-modifying therapeutics. This assay has been moved from beads-based to plate-based, upgrading its stability and condensing its workflow in a cost-effective manner, that requires significantly less biological sample to perform than the original assay.

# Summary (or Abstract)

# We sought a set of plasma samples from participants of the Alzheimer’s Disease Neuroimaging Initiative (ADNI) spanning the full range of AD pathology development. We requested 200 baseline plasma samples from participants from each category of healthy control, mild cognitive impairment, and Alzheimer disease, who all had received at least one amyloid PET scan. The following sample size was identified in the ADNI repository: 275 healthy control, 379 mild cognitive impairment, and 131 Alzheimer disease.

In total, we received 829 de-identified plasma samples. The oAβ assays were conducted between 7/12/2023 and 7/27/2023. While oAβ measurements were obtained for all 829 plasma samples, some were derived from fewer than the standard three replicates per sample (details below). Notably, when normalized by the internal control, no significant day-to-day variation was observed. Among the 829 plasma samples, all values exceeded the lower limit of quantitation (LLoQ). All samples were assayed in triplicate, and we identified and excluded outliers from 40 samples (4.8%), where the outlier value was more than three standard deviations away from the mean of the other two measurements Additionally, 21 samples (2.5%) exhibited a coefficient of variation (CV) equal to or greater than 20%.

# Methodology and Quality Control

**Generation of Aβ oligomer specific antibodies 71A1**

Monoclonal antibodies 71A1 (a subclone of the parent clone 7A1a) was developed using a synthetic conformational peptide immunogen designed to simulate the three-dimensional structure formed upon monomeric Aβ dimerization.7

**Calibrators**

Amyloid-β derived diffusible ligands (ADDLs) [[1](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8938295/#R1)] and S26C-dimer were prepared as per previous reports.8

**SMCxPRO Immunoassay**

The SMCxPRO (platform EMD Millipore) is based on single-molecule-counting technology that typically allows a 20- to 100-fold increase in sensitivity compared with traditional ELISA detection systems. Biotinylated capture mAbs (71A1) were conjugated to streptavidin-coated 96-well microplates (Pierce Thermo Fisher Scientific) at 0.15 ug per well overnight. A three-step assay protocol was executed to quantify oligomeric Aβ. In step 1, 50 μl of standard, blank, or biofluid were added to plates conjugated with capture antibody and incubated at 600 rpm on a shaker for 30 min at RT. The plates were washed three times with TBS-T (10 mM sodium phosphate, 0.15 M NaCl, 0.05% Tween, pH 7.5) using a Hydroflex plate washer (Tecan Group AG). In step 2, fluorescently labeled (Alexa-647 dye) detection antibody 3D6 (50 μL, 1:50k) was added and incubated for 60 min at 600 rpm using a Jitterbug shaker (Boekel) in dark, and the plates were washed three times. In step 3, fluorescently labeled 3D6 detection antibody was released by shaking in EMD Millipore’s Elution Buffer B (25 μL/well) for 15 min at 25°C. 25 μL of the eluates were then transferred to a clean 96-well microplate containing the Millipore Neutralization Buffer D (25 μL/well). The neutralized sample (25 μL/well) was then transferred to a black 384-well-read plate (Aurora) and read onthe SMCxPRO plate reader. When fluorescently labeled antibodies are excited by a 642 nm laser and passes through the interrogation space, the emitted fluorescence is measured by a confocal microscope lens and a photon detector. The output from the detector is a train of pulses, with each pulse representing one photon that was detected. The lower limit of reliable quantification (LLoQ) was defined as the lowest back-interpolated standard that provides a signal two-fold the background with a percentage of recovery calculated between 80% and 100% and coefficient of variance (CV) ≤20%.

The samples were assayed over ten runs spanning fifteen days, with pooled-plasma internal controls assayed concurrently with ADNI samples on each plate to ensure reproducibility across runs. All patient plasma samples were run in triplicate at a 8x dilution. The average CV for all samples was 9% (range 0-46%; with 21/829 above the ideal 20%). The average CV for the internal control was 8%. There are 40 samples with only duplicates measurements, where the outlier value was more than three standard deviations away from the mean of the other two measurements and was removed from analysis.

# References­

1. Liu, L. , Kwak, H. , Lawton, T. L. , Jin, S.‐X. , Meunier, A. L. , Dang, Y. , Ostaszewski, B. , Pietras, A. C. , Stern, A. M. , & Selkoe, D. J. (2021). An ultra‐sensitive immunoassay detects and quantifies soluble Aβ oligomers in human plasma. Alzheimer's & Dementia, 18(6), 1186–1202. 10.1002/alz.12457

2. Liu L, Stern A, Dang Y, Ostaszewski B, Chhatwal JP, Selkoe DJ. Predicting development of AD clinical symptoms and their progression through a collection of novel plasma Aβ immunoassays: Biomarkers (non-neuroimaging): Blood based biomarkers. Alzheimer’s & Dementia 2020; 16: e043670.

3. Yang T, Dang Y, Ostaszewski B, Mengel D, Steffen V, Rabe C et al. Target engagement in an Alzheimer trial: Crenezumab lowers amyloid β oligomers in cerebrospinal fluid. Annals of neurology 2019; 86: 215–224.

4. Yang T, Li S, Xu H, Walsh DM, Selkoe DJ. [Large Soluble Oligomers of Amyloid β-Protein from Alzheimer Brain Are Far Less Neuroactive Than the Smaller Oligomers to Which They Dissociate.](https://pubmed.ncbi.nlm.nih.gov/28053038/) J Neurosci. 2017 Jan 4;37(1):152-163. doi: 10.1523/JNEUROSCI.1698-16.2016.

5. Yang T, O’Malley TT, Kanmert D, Jerecic J, Zieske LR, Zetterberg H, Hyman BT, Walsh DM, Selkoe DJ (2015) A highly sensitive novel immunoassay specifically detects low levels of soluble Abeta oligomers in human cerebrospinal fluid. Alzheimers Res Ther 7:14.

6. Yang T, Hong S, O’Malley T, Sperling R, Walsh DM, Selkoe DJ. New ELISAs with high specificity for soluble oligomers of amyloid β-protein detect natural Aβ oligomers in human brain but not CSF. Alzheimers Dement. 2013;9:99–112. doi: 10.1016/j.jalz.2012.11.005.

7. Van Helmond Z, Heesom K, Love S. Characterisation of two antibodies to oligomeric Abeta and their use in ELISAs on human brain tissue homogenates. J Neurosci Methods 2009; 176: 206–212.

8. O’Nuallain B, Freir DB, Nicoll AJ, Risse E, Ferguson N, Herron CE et al. Amyloid beta-protein dimers rapidly form stable synaptotoxic protofibrils. J Neurosci 2010; 30: 14411–14419.

# About the Authors

This document was prepared by Ting Yang and YiRan Xu. For more information, please contact Ting at tyang@bwh.harvard.edu.

*Notice: This document is presented by the author(s) as a service to ADNI data users.  However, users should be aware that no formal review process has vetted this document and that ADNI cannot guarantee the accuracy or utility of this document.*