Quantitation of Alzheimer's disease biomarkers in plasma using SMC® high sensitivity immunoassays

Joseph Hwang, Matthew Lippold, Raad Gitan, Deborah Droll, Elizabeth Adkisson, and Rick Wiese

Introduction

Background: Alzheimer's Disease (AD) affect millions worldwide and is becoming more prevalent as the population ages. Current understanding of AD pathology centers around monitoring neurodegenerative biomarkers in blood. In recent years there specifically has been a growing interest in measuring Aβ42, Aβ40, NF-L, and Tau in blood, but these low-abundant biomarkers require higher sensitivity immunoassays for detection in blood samples.

Method: We developed and verified novel SMC® NF-L/Tau and Aβ42/Aβ40 2-plex high sensitivity immunoassay kits that can accurately quantitate these biomarkers simultaneously in human plasma and serum samples using the FemtoQuest™ platform, the next generation SMC® instrument. Here we report the results from testing plasma samples from apparently healthy and AD samples using the new immunoassays and instrument.

Result: The NF-L/Tau and Aβ42/Aβ40 2-plex high sensitivity immunoassays detected NF-L, Tau, Aβ42, and Aβ40 in plasma, serum, and CSF above each assay's respective lower limit of quantitation (LLOQ) of 0.87, 2.31, 1.95, and 1.95 pg/mL. These kits also identified a clear differentiation between apparently healthy and AD plasma samples with p-values of 0.0002 for NF-L, 0.0001 for Tau, and 0.0001 for Aβ40. This sample set did not show a significant difference between apparently healthy and AD plasma samples for Aβ42.

Conclusion: This study demonstrates the value of using multi-analyte high sensitivity technology to evaluate multiple biomarkers in the same sample. The SMC® NF-L/Tau and Aβ42/Aβ40 high sensitivity immunoassay kits serve as powerful non-invasive biomarker tools for researching the progression of neurodegenerative diseases such as AD while saving time and sample. Further studies into other combinations of AD biomarkers will determine if their ratios would also serve as valuable measures of AD-related neurodegeneration.

Methods

SMC® Technology

SMC® immunoassays achieve high sensitivity assay performance while following a workflow like that of a traditional ELISA, as shown in **Figure 1A**. By combining a unique assay elution step and robust digital counting, researchers achieve improved signal-to-noise ratios over traditional immunoassay methods, as shown in **Figure 1C**. The FemtoQuest™ instrument (**Figure 1B**) provides enhanced quantification at both low and high levels of expression on one complete system.

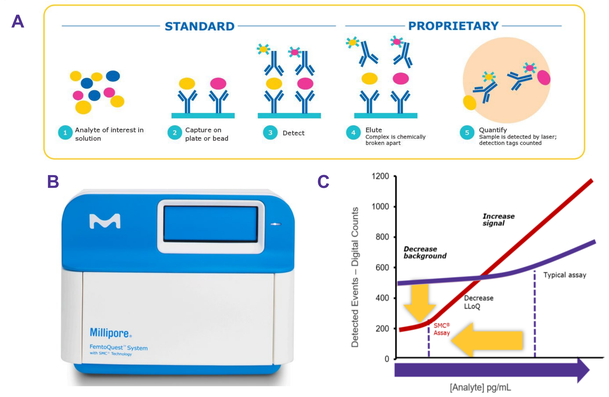

Figure 1: Ultrasensitive SMC® technology for the FemtoQuest™ platform. **(A)** SMC® immunoassays follow a simple assay protocol. Magnetic beads conjugated to a capture antibody bind to the analyte. A fluorescently-labeled detection antibody then forms a sandwich complex with the analyte and capture beads. Using a proprietary elution step, individual detection antibodies are counted to allow for ultrasensitive measurement. **(B)** SMC® immunoassays are available for the FemtoQuest™ instrument, which employs a scanning confocal laser to perform digital molecular counting. **(C)** SMC® immunoassays shows broader dynamic range and greater sensitivity than standard ELISAs.

Results

We assessed the concentrations of four neurodegenerative biomarkers in apparently healthy and AD plasma, serum, and CSF samples on the high sensitivity FemtoQuest™ platform using SMC® technology. All analytes were detectable in blood samples indicating the utility of these assays for less invasive sample testing.

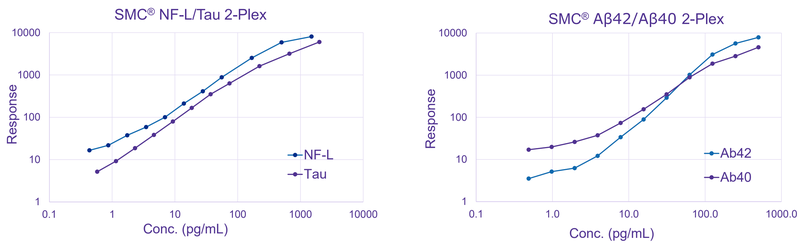

Figure 2. SMC® 2-Plex Standard Curves. The SMC® NF-L/Tau and Aβ42/Aβ40 2-plex immunoassays were run according to kit protocols. Standard curves were generated using the Belysa® Immunoassay Curve Fitting Software (Cat. No. 40-122). Response represents arbitrary fluorescent units.

Kit	Analyte	Standard Curve Range (pg/mL)	Sensitivity	Precision		Accuracy		
			LLOQ (pg/mL)	Avg. Intra-Assay (% CV)	Avg. Inter-Assay (% CV)	Mean % Recovery (Serum)	Mean % Recovery (Plasma)	Mean % Recovery (CSF)
SMC® NF-L/ Tau 2-Plex	NF-L	0.43 – 1,500	0.87	<10	<15	84	86	99
	Tau	0.58 – 2,000	2.31	<10	<15	105	77	88
SMC® Aβ42/ Aβ40 2-Plex	Aβ42	0.49 - 500	1.95	<10	<15	89	113	90
	Aβ40	0.49 - 500	1.95	<10	<15	70	126	116

Table 1. SMC® NF-L/Tau and Aβ42/Aβ40 2-plex immunoassay kit performance characteristics. Data was generated during kit development.

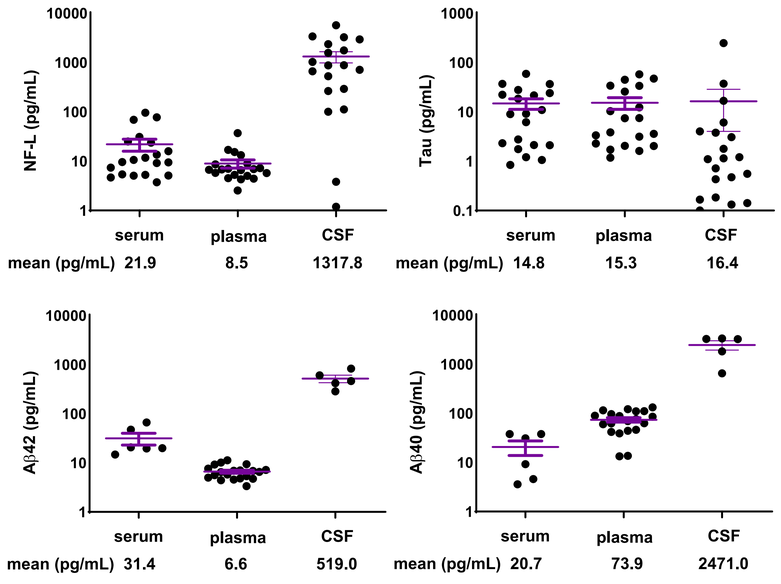

Figure 3. Biomarker concentrations in control samples by sample type. Dotplots representing distribution of interpolated, dilution-corrected concentrations for control sample cohorts corresponding to human serum, EDTA plasma, and CSF. Purple bars correspond to mean and standard deviation values.

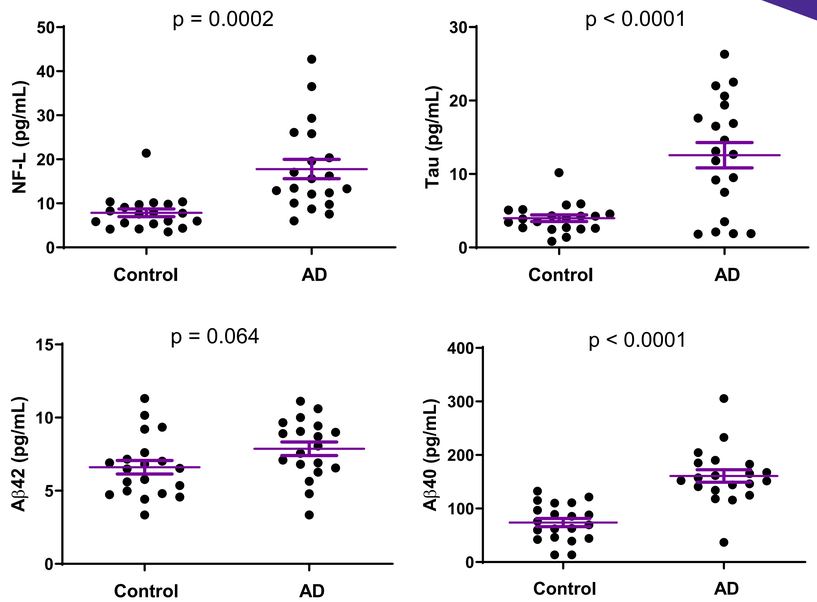

Figure 4: Control vs. AD plasma measurement. SMC® NF-L/Tau and Aβ42/Aβ40 2-plex immunoassay kits were run according to vendor protocol. Twenty apparently healthy and 20 AD plasma samples were tested. NF-L, Tau, and Aβ40 showed statistically significant differences between apparently healthy and AD samples. P-values indicate unpaired t-testing p-values comparing the two groups.

Single Analyte SMC® Biomarker Assays for Neuroscience Research		
NF-L (03-0202-00)	Tau (03-0185-00)	NPTX2 (03-0199-00)
Aβ 1-40 (03-0145-00)	p-Tau T181 (03-0184-00)	TDP-43 (03-0205-00)
Aβ 1-42 (03-0146-00)	p-Tau T217 (03-0210-00)	UCHL1 (03-0183-00)
α-synuclein (03-0196-00)	p-Tau T231 (03-0211-00)	SNAP-25 (03-0206-00)
p-α-synuclein (S129) (03-0188-00)	GFAP (03-0203-00)	BDNF (03-0171-00)

Table 2. Verified neuroscience biomarker kits for the SMC® platform. Assays for the listed analytes (kit catalog numbers noted in parentheses) are available for use with the FemtoQuest™ ultrasensitive immunoassay platform. All kits are for research use only, not for use in diagnostic procedures.

Conclusion

The FemtoQuest™ platform is an ultrasensitive, high-performance immunoassay platform that enables measurement of previously undetectable proteins in blood-based samples.

SMC® 2-Plex NF-L/Tau and Aβ42/Aβ40 immunoassay kits demonstrated the feasibility of using these analytes as blood biomarkers for AD research.

Fit-for-purpose immunoassays give researchers the flexibility to investigate a broad selection of proteins related to AD and other neurological disorders.

Explore more at
SigmaAldrich.com/smc-neuro

MilliporeSigma is the U.S. and Canada Life Science business of Merck KGaA, Darmstadt, Germany.