Quantitation of neurodegenerative biomarkers in plasma using Single Molecule Counting (SMC®) high sensitivity immunoassays

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Introduction

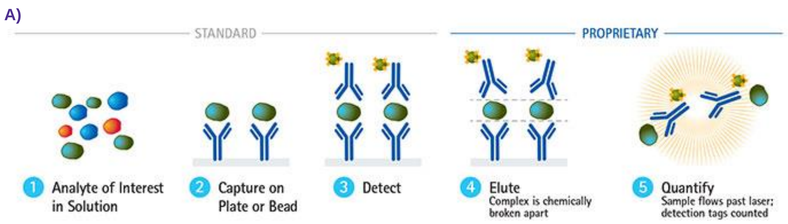
Quantification of protein biomarkers in patients with Alzheimer's Disease (AD) and Parkinson's disease (PD) is important for monitoring neurodegeneration. Current understanding of AD and PD centers around monitoring neurodegenerative biomarkers in cerebrospinal fluid (CSF). However, due to the invasive nature of collecting CSF samples, there has been great interest in analyzing biomarkers in blood. Many neurodegenerative disease biomarkers, however, are not detectable in some blood samples due to low abundance and thus require higher sensitivity immunoassays. To this end, we have developed high sensitivity immunoassays for Aβ40, Aβ42, Tau, phosphorylated Tau (T181), TDP-43, SNAP-25, GFAP, NPTX2, UCHL1, α-synuclein, and phosphorylated α-synuclein (S129) for analysis of human blood samples. Based on results from screening commercially-available CSF and plasma samples using Single Molecule Counting (SMC®) high sensitivity immunoassays, Aβ42, Tau, phosphorylated Tau (T181), TDP-43 and SNAP-25 show significantly-different concentrations in CSF between normal and AD groups. However, no significant difference was observed for Tau and SNAP-25. The most significant difference in normal versus AD plasma samples was observed for Aβ40 ($p<0.005$), Aβ42 ($p<0.05$), TDP-43 ($p<0.02$), p-α-synuclein S129 ($p<0.0007$) and total α-synuclein ($p<0.0004$). In summary, SMC® high sensitivity immunoassay kits can provide a powerful non-invasive biomarker tool for research on monitoring the progression of neurodegenerative diseases such as Alzheimer's and Parkinson's disease.

Methods

Single Molecule Counting (SMC®) Technology

SMC® immunoassays achieve high sensitivity assay performance while following a workflow like that of a traditional ELISA, as shown below. By combining a unique assay elution step and robust digital counting, researchers can achieve improved signal-to-noise ratios over traditional immunoassay methods. The SMCxPRO® instrument thus provides enhanced quantification at both low and high levels of expression on one complete system.

A)



B)

Figure 1: Ultrasensitive SMC® technology for the SMCxPRO® platform.
(A) SMC® immunoassays are available for the SMCxPRO® instrument, which employs a scanning confocal laser to perform digital molecular counting.
(B) SMC® immunoassays follow a simple assay protocol. Magnetic beads conjugated to a capture antibody bind to the analyte. A biotinylated 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.

Analyte	Cat. No.	Standard Curve Range (pg/mL)	Sensitivity	Precision		Accuracy	
			LLOQ (pg/mL)	Intra-Assay (% CV)	Inter-Assay (% CV)	% Recovery (CSF)	% Recovery (Plasma)
Aβ40	03-0145-00	2.93 - 3,000	5.86	< 10	< 10	102	97
Aβ42	03-0146-00	0.24 - 250	0.98	< 15	< 20	94	93
pTau T181	03-0184-00	0.23 - 240	0.94	< 10	< 15	108	97
tTau	03-0185-00	1.17 - 1,200	2.34	< 10	< 10	91	82
TDP-43	03-0205-00	2.6 - 6,000	5.21	< 10	< 15	89	100
SNAP-25	03-0206-00	0.13 - 300	0.26	< 10	< 10	89	91
α-synuclein	03-0196-00	0.49 - 500	1.95	< 10	< 15	96	96
p-α-synuclein S129	03-0188-00	0.05 - 50	0.10	< 10	< 10	100	87
NPTX2	03-0199-00	0.29 - 1,000	0.29	< 10	< 10	88	104
UCHL1	03-0183-00	1.30 - 2,000	2.60	< 10	< 10	92	93
GFAP	03-0203-00	0.06 - 300	0.12	< 10	< 10	101	115
NF-L (NEW!)	03-0202-00	0.43 - 1,500	0.87	< 15	< 10	123	94

Table 1: SMC® Immunoassay kits used in this study, available as research use only kits for analysis of human cerebrospinal fluid and blood samples.

Results

SMC® kits are high sensitivity immunoassays used for measuring low-abundant biomarkers, such as biomarkers of neurodegenerative disease. We examined biomarker concentrations in the CSF of normal and AD patients (traditionally the matrix of choice for such studies), before examining whether these kits could measure selected biomarkers in plasma. While several analytes demonstrated potential as blood-based biomarkers of AD, the low abundance of some proteins necessitated the development of assays on a high-sensitivity platform using SMC® technology.

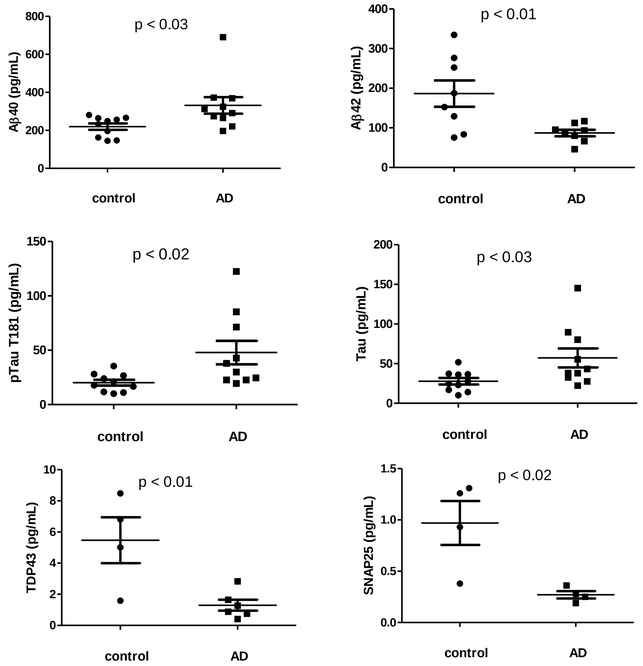


Figure 2: CSF measurement of AD biomarkers.
SMC® immunoassay kits were run according to vendor protocols. Analysis of control and AD CSF samples with each of these kits revealed expected statistically significant difference in Aβ40 ($p<0.03$), Aβ42 ($p<0.01$), phosphorylated Tau T181 ($p<0.02$), total Tau ($p<0.03$), TDP-43 ($p<0.01$) and SNAP-25 ($p<0.02$). Control and AD CSF samples were purchased from various vendors.

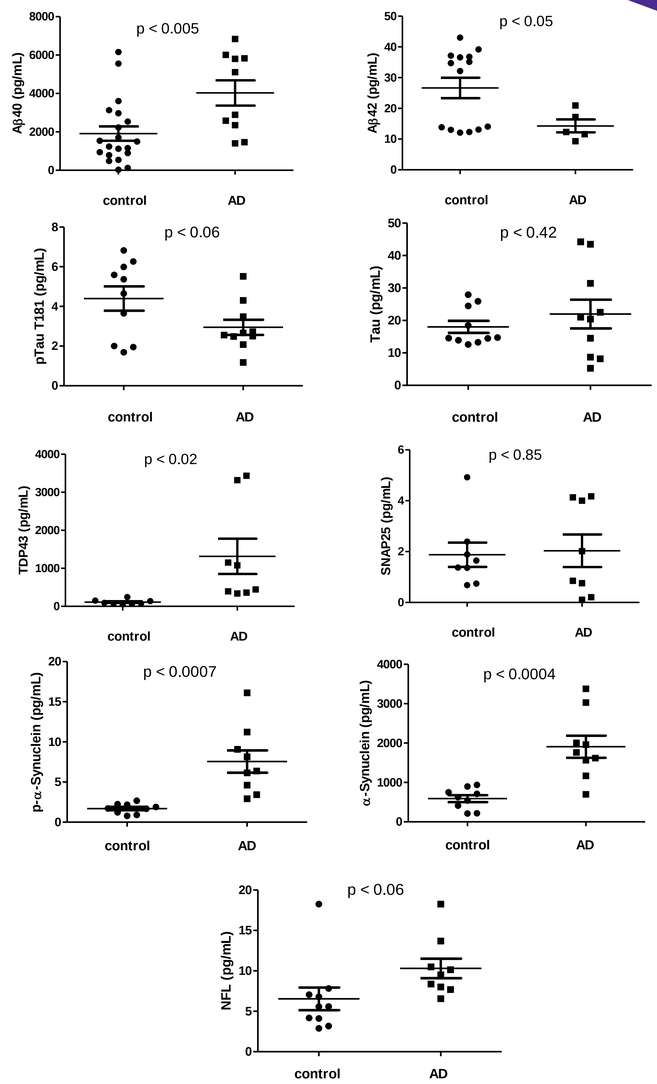


Figure 3: Plasma measurement of AD biomarkers.
SMC® immunoassay kits were run according to vendor protocols. Analyte concentrations from control and AD plasma samples are reported. Aβ40 ($p<0.005$), Aβ42 ($p<0.05$), TDP-43 ($p<0.02$), p-α-synuclein S129 ($p<0.0007$) and total α-synuclein ($p<0.0004$) showed statistically significant difference. Control and AD plasma samples were purchased from BioIVT.

Summary

The SMCxPRO® platform is an ultrasensitive, high-performance immunoassay platform that enables measurement of previously undetectable proteins.

SMC® immunoassay kits were used to measure both established and novel AD biomarkers in human CSF and plasma samples.

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

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