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Application Note

MILLIPLEX® MAP multiplex and SMC™ high-sensitivity immunoassay detection of Alzheimer's disease biomarkers in human cerebrospinal fluid, plasma, and serum

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Introduction

Monitoring protein biomarkers in cerebrospinal fluid (CSF) of patients with Alzheimer 's disease (AD) has been highly beneficial to understanding disease progression 1. Multiplex immunoassays allow researchers to measure several biomarkers simultaneously and generate an abundance of reliable data from a single experiment. While several CSF biomarkers can reproducibly distinguish normal and diseased samples, CSF is a difficult biological fluid to obtain in research studies.

The need for blood-based biomarkers of AD has driven a continuous search for novel candidates 2. However, identification of blood-based biomarkers may be limited by the sensitivity of standard immunoassay methodologies. High-sensitivity immunoassay technologies, such as Single Molecule Counting (SMC™) can transform neurodegenerative disease research by enabling the measurement of low abundant proteins in a variety of biofluids and create opportunities for the identification of novel biomarkers 3,4. To demonstrate the ultrasensitive capabilities of SMC™ technology quantifying Alzheimer's disease biomarkers in diverse sample types, commercially available MILLIPLEX® MAP Neuroscience kits were evaluated using CSF, plasma, and serum from AD patients and healthy controls.

Materials and Methods

Human CSF, plasma, and serum were obtained from AD patients and non-AD controls (normal) via commercial vendors (Discovery Life Sciences, BioIVT, and PrecisionMed). Samples were either run neat or diluted, according to the kit protocol. An unpaired t-test was used to calculate two-tail p-values for all analytes (GraphPad Prism).

MILLIPLEX® MAP multiplex assays were performed in 96-well plates according to the product instruction manuals. **Figure 1** illustrates the assay format and capability for quantitative determination of multiple protein concentrations from a single sample. Mean Fluorescence Intensity (MFI) was measured on a Luminex® 200™ System (Luminex Corporation, Austin, TX) and data was analyzed with MILLIPLEX® Analyst 5.1 Software.

MILLIPLEX® MAP Human Amyloid Beta and Tau Panel (Cat. No. HNABTMAG-68K) was used to quantitate the analytes: Amyloid β (1-40) (Aβ40), Amyloid β (1-42) (Aβ42), total Tau (tTau), and pTau T181 in 1:2 diluted CSF samples. MILLIPLEX® MAP Human Neuroscience Panel 1 (Cat. No. HNS1MAG-95K) was used to quantitate the analytes: α-Synuclein, Glial Fibrillary Acidic Protein (GFAP), Neuron-Specific Enolase (NSE), PARK5 (UCHL1), PARK7 (DJ-1), Transglutaminase 2 (TGM2) in neat CSF samples.

MILLIPLEX® MAP Human Neuroscience Panel 2 (Cat. No. HNS2MAG-95K) was used to quantitate the analytes: Angiogenin (ANG), ApoE4, FABP3, Ferritin, Neurogranin (NGRN), Prion Protein (PRNP), TREM2, in 1:10 diluted CSF, serum, and plasma samples.

SMC™Amyloid Beta 1-40 High Sensitivity Immunoassay Kit (Cat. No. 03-0145-00) and SMC™Amyloid Beta 1-42 High Sensitivity Immunoassay Kit (Cat. No. 03-0146-00) were used to quantitate amyloid beta peptides according to product instruction manuals. Two instruments were used to independently quantify SMC™ immunoassays: the ERENNA® (**Figure 2A**) and the SMCxPRO™ (**Figure 2B**). SMC™ technology utilizes a traditional sandwich immunoassay format (**Figure 2C**) that is compatible with both SMC™ instruments. Sgx Link™ and xPRO™ software were used for data collection and analysis for the ERENNA® and SMCxPRO™, respectively.

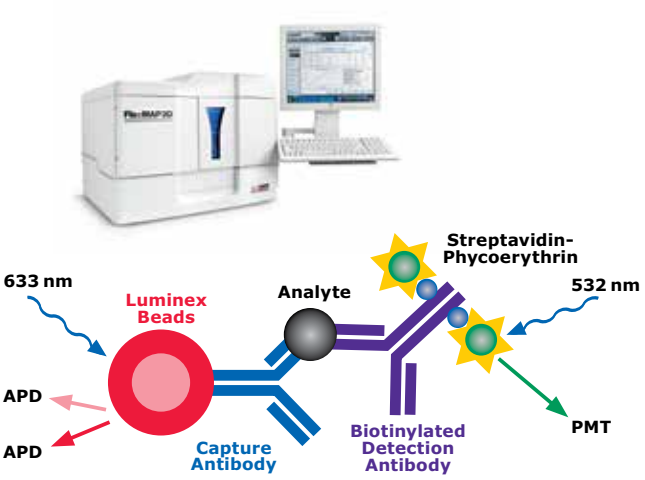

Figure 1: MILLIPLEX® MAP format

MILLIPLEX® MAP assays use magnetic microspheres (beads) conjugated to capture antibodies. Each set of beads is distinguished by different ratios of two internal dyes yielding a unique fluorescent signature to each bead set, allowing researchers to simultaneously measure the analytes targeted by the capture antibodies. Protein is analyzed by means of a "sandwich" immunoassay, pairing the capture beads with a biotinylated detection antibody. (APD: avalanche photodiode; PMT: photomultiplier tube).

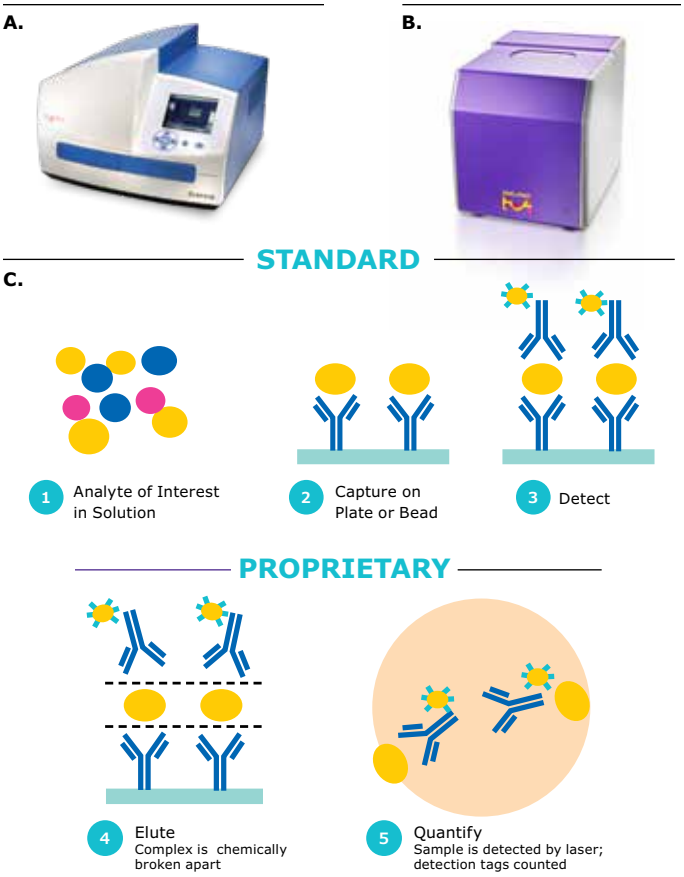

Figure 2: Ultrasensitive SMC™ technology for the ERENNA® and SMCxPRO™

Results

MILLIPLEX® MAP immunoassays are recognized as valuable tools to measure biomarkers of neurodegenerative disease. Several established and emerging neurodegenerative disease biomarkers were examined in the CSF of normal and AD patients, before assessing biomarker detection in serum and plasma.

MILLIPLEX® kits for CSF measurement of biomarkers of neurological disease include the Human Amyloid Beta and Tau panel and Human Neuroscience Panel 1. MILLIPLEX® MAP Human Neuroscience Panel 2 has been validated for both CSF and blood (serum and plasma) samples. Standard curves for each of the kits described above are shown in **Figure 3**. CSF samples from AD patients and healthy controls (normal) were evaluated with the multiplex kits, and differences in specific biomarker concentrations were determined.

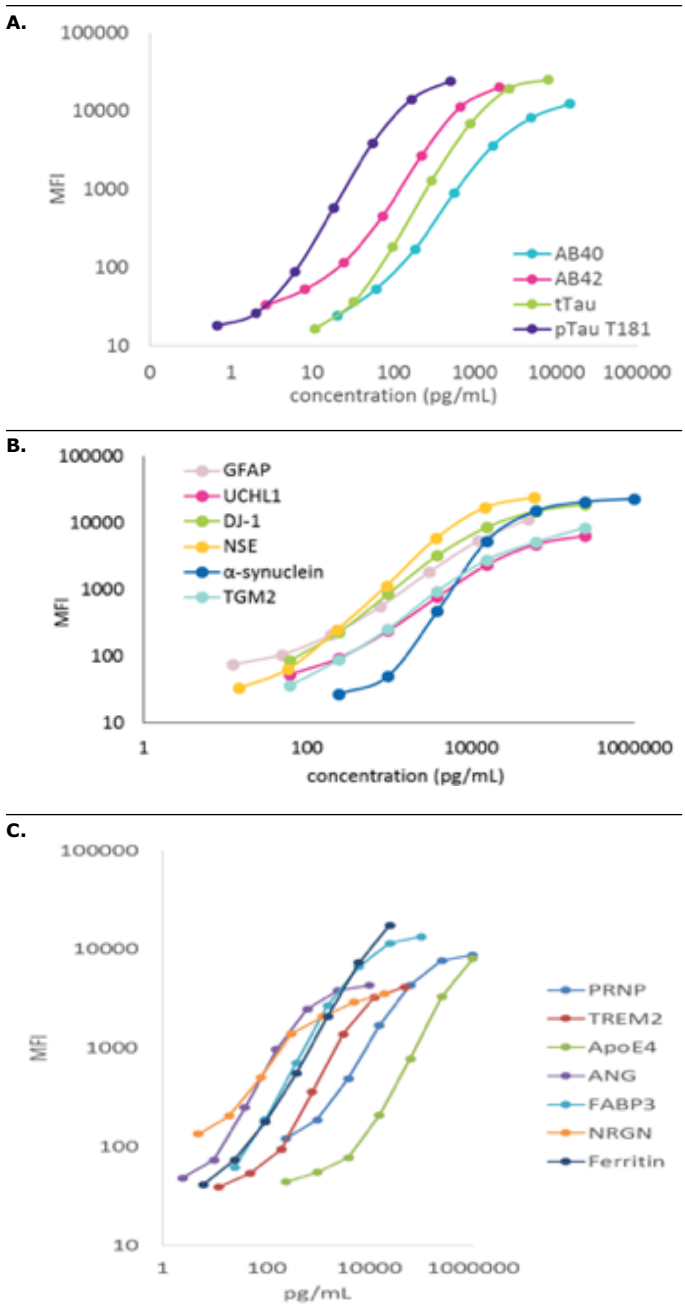

Figure 3: Multiplex immunoassays for neuroscience research

Standard curves for the MILLIPLEX® MAP (**A**) Human Amyloid Beta and Tau kit, (**B**) Human Neuroscience Panel 1, and (**C**) Human Neuroscience Panel 2 are shown above.

Each kit revealed alterations in AD CSF compared to control samples (**Figure 4**).

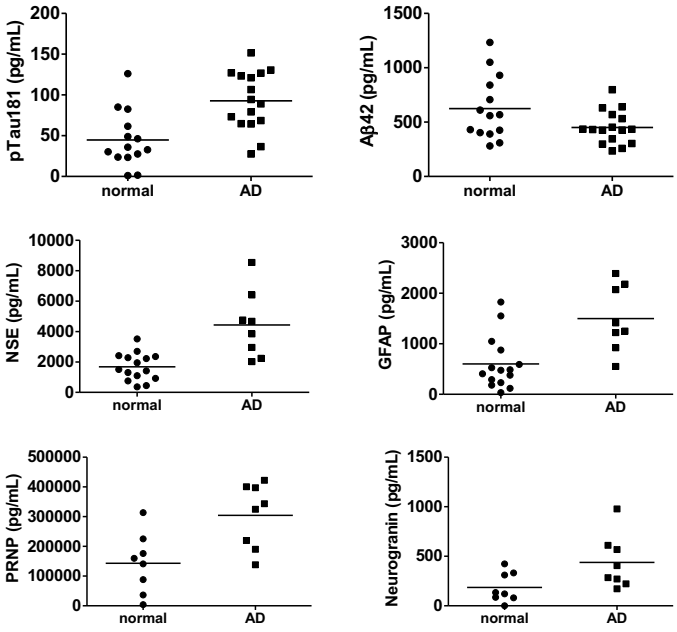

Figure 4: Multiplex biomarker analysis of AD CSF

MILLIPLEX® MAP Neuroscience kits were used to measure a total of 17 proteins in human CSF. Analysis of normal and AD CSF samples with each of these kits revealed differences in phosphorylated Tau (p=0.0009), Aβ42 (p=0.0476), NSE (p=0.008), GFAP (p=0.0016), UCHL1 (p=0.0088, data not shown), Neurogranin (p=0.0359), and prion protein (p=0.0079).

For each kit, sample sizes are as follows: Human Amyloid Beta and Tau Panel - normal CSF (n=14) and AD CSF (n=16). Human Neuroscience Panel 1 - normal CSF (n=15) and AD CSF (n=8). Human Neuroscience Panel 2 - normal CSF (n=7) and AD CSF (n=7).

In the Human Amyloid Beta and Tau Panel, Aβ42 was decreased in AD CSF, whereas phospho-Tau (Thr181) was increased, consistent with well-established observations of these canonical AD biomarkers 5. NSE and GFAP from Human Neuroscience Panel 1 were both increased in AD CSF. In Human Neuroscience Panel 2, PRNP and neurogranin NRGN exhibited higher levels in AD CSF relative to controls. As Human Neuroscience Panel 2 is also validated for serum and plasma, we next examined whether any of the analytes exhibited differential protein expression in circulation. FABP3 and PRNP were increased in both the plasma and serum of AD patients, whereas TREM2 was increased in AD serum, and NRGN was increased in AD plasma (**Figure 5**).

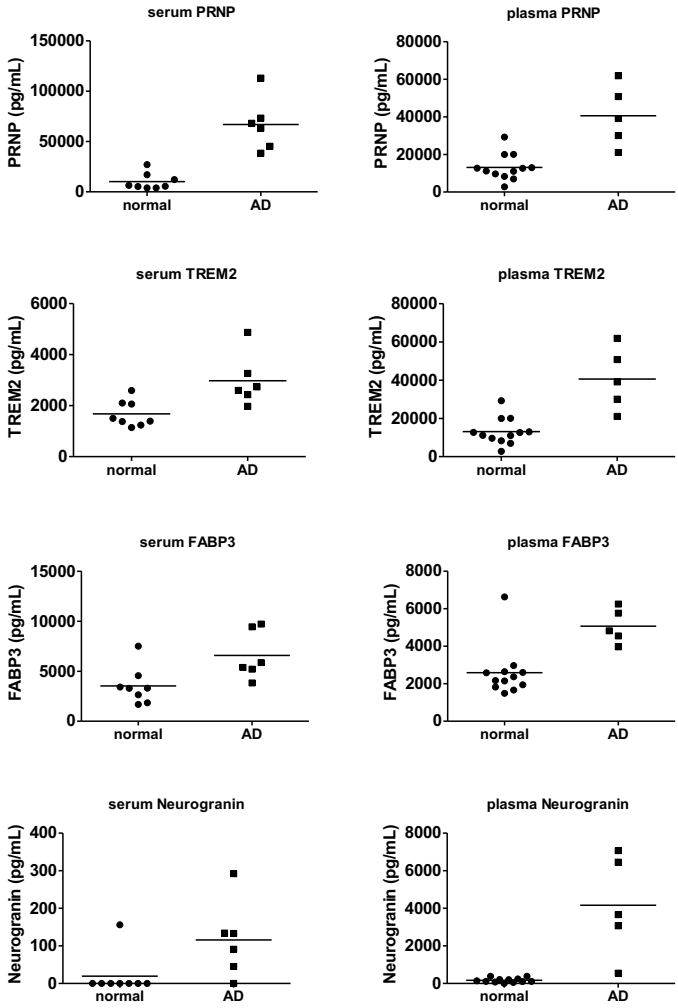

Figure 5: Biomarker measurement in plasma and serum

MILLIPLEX® MAP Human Neuroscience Panel 2 was used to measure disease-related proteins in the plasma (n=5) and serum (n=6) of AD patients versus the plasma (n=12) and serum (n=8) of healthy controls. Analysis of serum and plasma with Human Neuroscience Panel 2 identified increases of TREM2 (serum p=0.0083), FABP3 (serum p=0.0204, plasma p=0.002), prion protein (serum p=0.0002, plasma p=0.0171), and neurogranin (plasma p=0.0044) in AD samples relative to healthy controls.

While several analytes demonstrated potential as blood-based biomarkers of AD, the low abundance of other proteins necessitated the development of assays on a high-sensitivity platform using SMC™ technology.

SMC™ technology enabled measurement of the amyloid beta peptides in human plasma samples (**Figure 6**). A small increase in plasma Aβ40 (p=0.028) was associated with AD. No difference was observed in plasma Aβ42 values. Importantly, the SMC™ Aβ42 kit successfully detected the decrease in CSF Aβ42 (p=0.0116) which is characteristic of AD. We also observed a difference in Aβ40 levels in the CSF (p=0.0055). Excellent correlation between the SMC™ instrumentation platforms was demonstrated for the Aβ40 and Aβ42 kits by reading sequentially on the SMCxPRO™ followed by the ERENNA®.

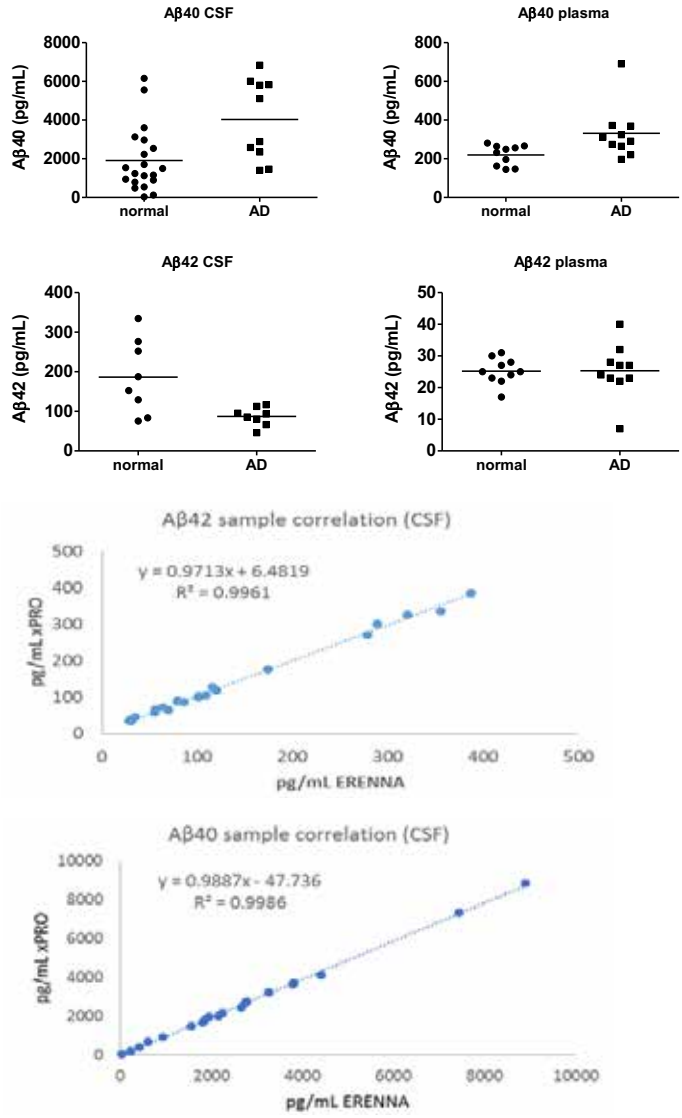

Figure 6: Aβ40 and Aβ42 SMC™ kits for ERENNA® and SMCxPRO™

Aβ40 and Aβ42 were measured in CSF and plasma from AD patients and healthy controls with their respective high-sensitivity SMC™ immunoassays. Differences in Aβ40 between AD and healthy controls were observed in both plasma (p=0.028) and CSF (p=0.0055). A decrease in Aβ42 was observed in AD CSF (p=0.0116), although no change was observed in plasma (p=0.9733). The same plates used to measure CSF on the SMCxPRO™ were subsequently analyzed on the ERENNA®, demonstrating high correlation between the two platforms.

For each kit, sample sizes are as follows: SMC™ Amyloid Beta 1-40 High Sensitivity Immunoassay Kit normal CSF (n=20), AD CSF (n=10), normal plasma (n=10), AD plasma (n=10). SMC™ Amyloid Beta 1-42 High Sensitivity Immunoassay Kit normal CSF (n=8) and AD CSF (n=8), normal plasma (n=10), and AD plasma (n=10).

Summary

MILLIPLEX® MAP and SMC™ immunoassays were used to measure a multitude of proteins linked to neurodegenerative disease in CSF, plasma, and serum. Established AD biomarkers such as Aβ42 and phospho-Tau showed the expected patterns of alteration in CSF. Additionally, proteins such as Neurogranin and GFAP demonstrated elevated concentrations in AD CSF. Potential blood-based biomarkers of AD were analyzed in plasma and serum, with several promising candidates emerging. Future studies will be integral to determining whether these observations can be corroborated with larger sample cohorts.

By adopting a fit-for-purpose approach, we were able to integrate multiple technology platforms and immunoassays to analyze AD biomarkers in CSF, plasma, and serum. These MILLIPLEX® MAP and SMC™ immunoassays provide researchers with reliable resources for studying a breadth of neurodegenerative disease biomarkers in a variety of biofluids.

Ordering Information

Product	Catalog No.
MILLIPLEX® MAP MULTIPLEX ASSAY KITS	
Human Amyloid Beta and Tau Panel	**HNABTMAG-68K**
Human Neuroscience Panel 1	**HNS1MAG-95K**
Human Neuroscience Panel 2	**HNS2MAG-95K**
SMC™ Immunoassay Kits	
Amyloid Beta 1-40 High Sensitivity	**03-0145-00**
Amyloid Beta 1-42 High Sensitivity	**03-0146-00**

References

1. Lleó, A. *et al.* Cerebrospinal fluid biomarkers in trials for Alzheimer and Parkinson diseases. *Nat Rev Neurol* **11**, 41–55 (2015).
2. Hye, A. *et al.* Plasma proteins predict conversion to dementia from prodromal disease. *Alzheimers Dement* **10**, 799-807.e2 (2014).
3. Hwang, J. *et al.* Quantitation of low abundant soluble biomarkers using high sensitivity Single Molecule Counting technology. *Methods* (2018). doi:10.1016/j.ymeth.2018.10.018
4. Understanding Biomarkers of Neurodegeneration: Ultrasensitive detection techniques pave the way for mechanistic understanding | Nature Medicine. Available at: https://www.nature.com/articles/nm.3810. (Accessed: 31st January 2019)
5. Olsson, B. et al. CSF and blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis. *Lancet Neurol* **15**, 673–684 (2016).

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