Multiplex detection of four Alzheimer's Disease biomarkers in human CSF using a novel MILLIPLEX® assay panel

Introduction

Progressive neurodegenerative disorders, such as Alzheimer's Disease (AD), affect millions worldwide and are becoming more prevalent as our population ages. Two key neuropathological features that exemplify AD are extracellular Amyloid β (Aβ) plaques and intracellular neurofibrillary tangles, which are composed of the abnormally hyperphosphorylated protein Tau. Biochemical changes in Aβ and Tau reflect AD pathologic processes in the brain.

Monitoring Aβ, Tau and other protein biomarkers in cerebrospinal fluid (CSF) of patients with these neurological disorders may be highly beneficial to understanding the pathological processes involved. Previous studies in which several protein biomarkers were monitored using a multiplexed panel showed that analyzing combinations of two or more CSF biomarkers (such as phosphorylated Tau [Thr181] in combination with Aβ1-42) provided a more accurate diagnosis of AD than any single CSF biomarker[1,2].

We developed a new multiplexed assay panel, the MILLIPLEX® MAP Human Amyloid Beta and Tau Magnetic Bead Panel (Cat. No. HNABTMAG-68K), to simultaneously quantitate levels of Aβ1-40, Aβ1-42, total Tau and phosphorylated Tau (Thr181) in human CSF. Compared to other commercially available panels that do not include Aβ1-40, this 4-plex panel enables determination of the Aβ1-40:Aβ1-42 ratio, which has also been suggested as crucial for AD pathogenesis[3]. In fact, many mutations associated with familial AD increase the Aβ1-40:Aβ1-42 ratio.

Materials and Methods

Human CSF samples from undiagnosed (normal) and AD individuals were acquired from Discovery™ Life Sciences and Precision Medicine, respectively.

The multiplex assays for AD biomarkers were performed in 96-well plates according to product instructions supplied for the MILLIPLEX® MAP Human Amyloid Beta and Tau Magnetic Bead Panel (Cat. No. HNABTMAG-68K).

Results

The specificity of this kit was tested and showed no cross-reactivity nor any significant difference in analyte concentrations, regardless of whether each analyte was measured in a single-plex or multiplex (full 4-analyte kit) format (Table 1). Furthermore, sensitivity, intra- and inter-assay precision, linearity of dilution and spike recovery exhibited excellent analytical performance (Table 2).

Plex	4-plex	1-plex	4-plex	1-plex	4-plex	1-plex	4-plex	1-plex
Analyte	**Aβ40**	**Aβ40**	**Aβ42**	**Aβ42**	**tTau**	**tTau**	**pTau (T181)**	**pTau (T181)**
CSF1	1993	2060	189	172	843	805	21	20
CSF1	2136	2094	193	190	867	886	21	22
CSF2	3050	3090	400	392	915	898	28	29
CSF2	2900	3068	412	381	857	898	29	25
CSF3	1160	1178	95	93	547	508	29	28
CSF3	1240	1191	102	100	544	573	30	31
CSF4	2174	2208	260	252	919	859	27	27
CSF4	2170	2284	254	249	929	879	28	28

Table 1.

Comparison of CSF sample concentration in single-plex vs. multiplex. Measurement of human CSF sample value in single-plex bead and detection vs. 4-plex beads and detection in duplicates.

		Aβ1-40	Aβ1-42	Total Tau	Phosphorylated Tau (Thr181)
Intra-assay (%CV)		3.8	2.6	3.9	2.9
Inter-assay (%CV)		7.9	9.0	14.2	4.8
Spike recovery in buffer (%)		93	91	92	95
Spike recovery in CSF (%)		101	109	98	91
CSF dilution linearity (%)		96	101	105	112
Cross-reactivity (%)	Aβ40	100	< 2	0	< 0.8
	Aβ42	0.4	100	0	0
	Total Tau	0	0	100	0.6
	Phosphorylated Tau (Thr181)	0	0	0	100
MinDC +2 Std Dev		10.2	5.4	14.2	1.5

Table 2.

Assay performance characteristics. Inter-assay %CVs and intra-assay %CVs were calculated using positive controls from different assays. Percent spike recovery was calculated from spiked recombinant proteins in assay buffer and CSF samples. The average percent linearity was calculated from three diluted human CSF samples. Percent cross-reactivity was performed using single, purified recombinant proteins with multiplexed beads and detection cocktails. Minimum detectable concentration (MinDC) was calculated using MILLIPLEX® Analyst 5.1 Software (Cat. No. 40-087).

Given the physicochemical characteristics of Aβ peptides, we investigated whether the presence of Aβ peptides would interfere with the detection or quantitation of the other protein biomarkers when measured simultaneously. We performed an interference test (Figure 1) in which increasing concentrations of Aβ40 or Aβ42 recombinant proteins were spiked into human CSF samples. The measured concentrations of the analytes did not change, indicating that there was no detectable interference from the Aβ peptides.

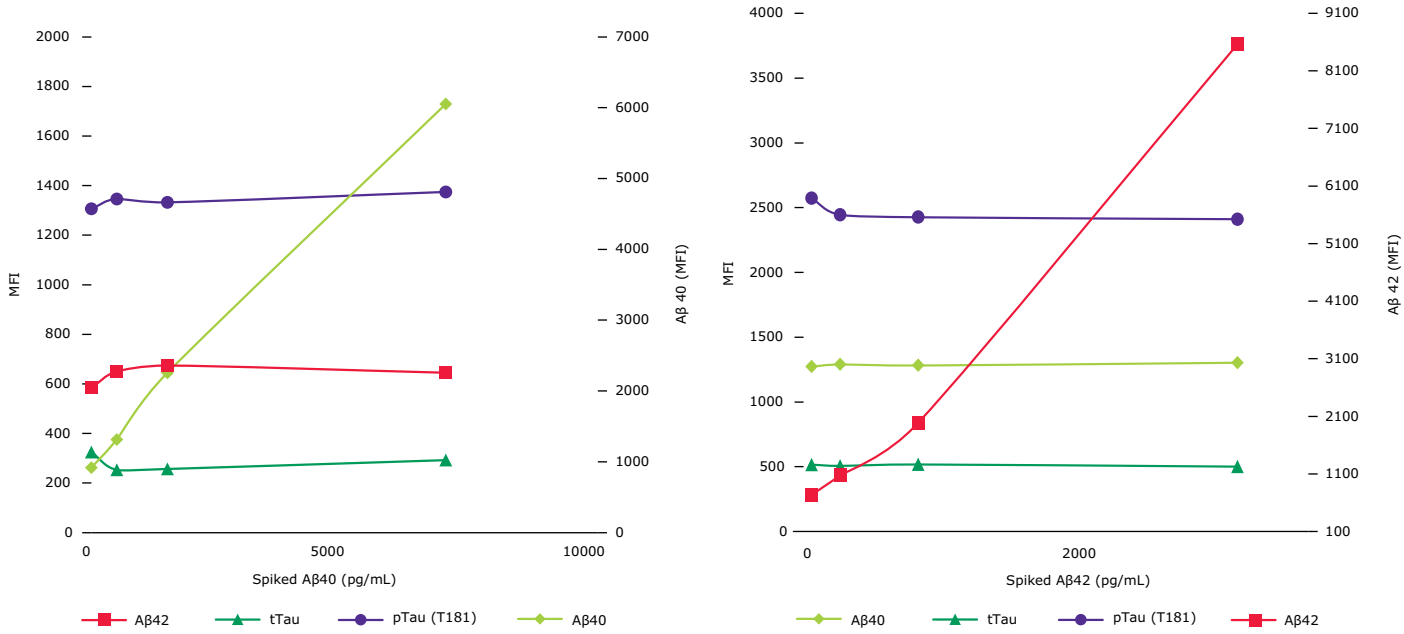

Figure 1.

MILLIPLEX® MAP Human Amyloid Beta and Tau Magnetic Bead Panel (Cat. No. HNABTMAG-68K) interference test. Increasing concentrations of Aβ40 or Aβ42 recombinant proteins were spiked into human CSF samples (n=4). No significant differences in sample concentrations were detected.

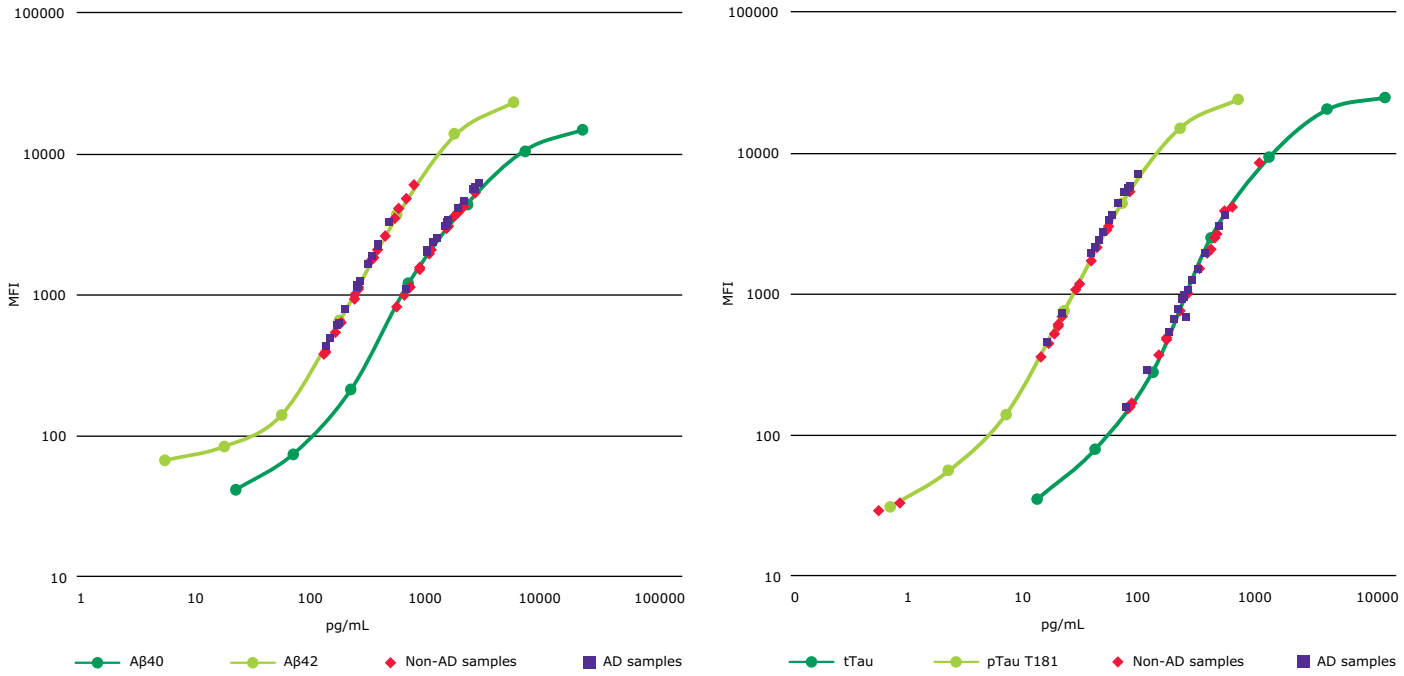

Figure 2.

CSF sample values plotted on standard curves using the MILLIPLEX® MAP Human Amyloid Beta and Tau Panel. Aβ40, Aβ42, total Tau and phosphorylated Tau (Thr181) concentrations from human CSF samples are plotted on the standard curves using the MILLIPLEX® MAP Human Amyloid Beta and Tau Magnetic Bead Panel (Cat. No. HNABTMAG-68K).

Results (continued)

Using the MILLIPLEX® MAP multiplex immunoassay kit, Aβ1-40, Aβ1-42, total Tau and phosphorylated Tau (Thr181) were detected simultaneously in age-matched AD and non-AD human CSF samples (Figure 3), minimizing the use of valuable CSF samples (12.5 µL/well). Phosphorylated Tau (Thr181) and Aβ1-42 correlated significantly with the presence of an AD diagnosis (p-value < 0.01 and p-value < 0.05, respectively). Neither total Tau nor Aβ1-40 showed a significant correlation with AD diagnosis in the CSF samples used in this study.

The ratio of phosphorylated Tau (Thr181) over Aβ1-42 improved the p-value < 0.001. This result was consistent with published data indicating that the ratio of phosphorylated Tau (Thr181) to Aβ1-42 correlates with cognitive decline[4].

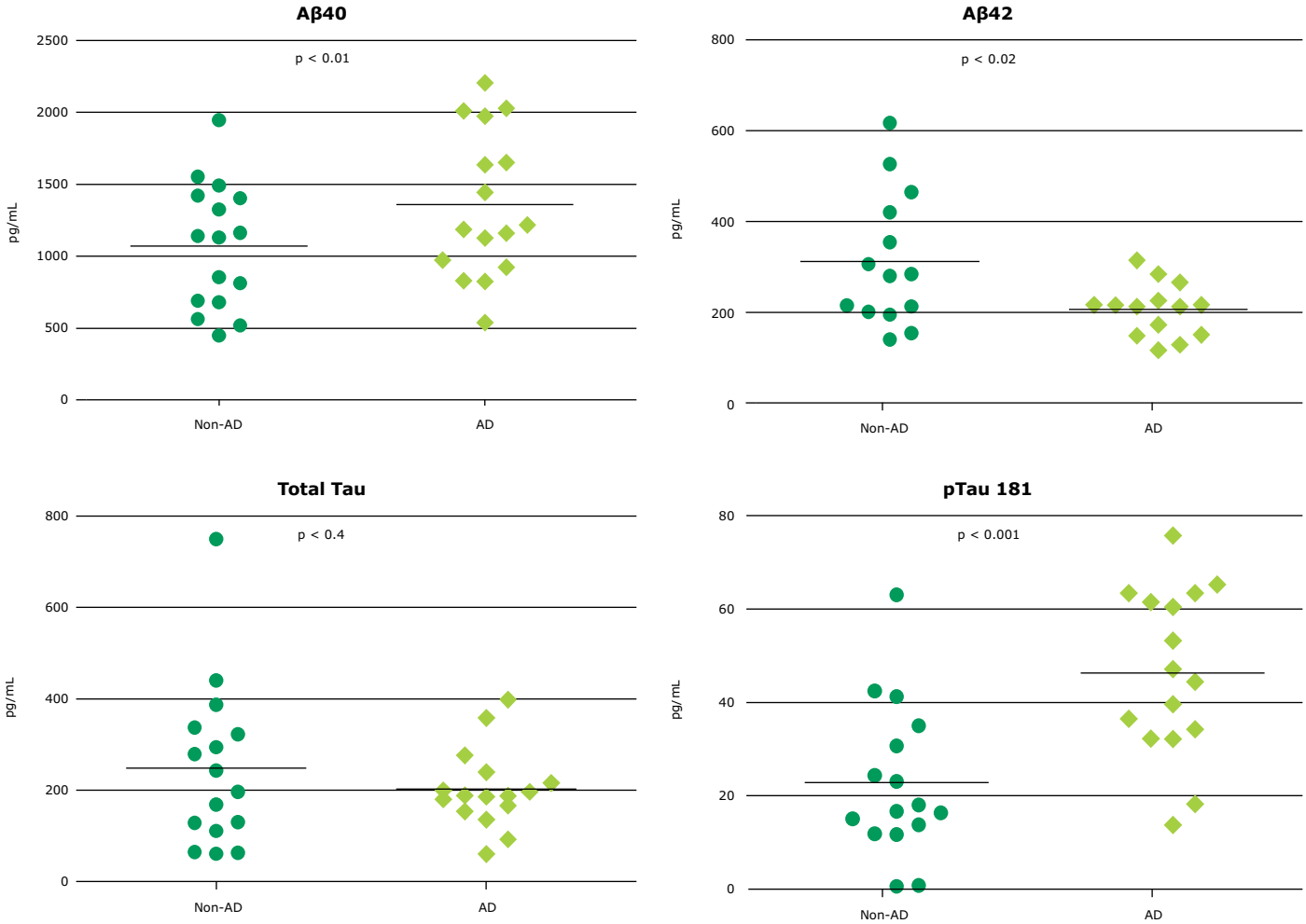

Figure 3.

Multiplex measurement of human non-AD-CSF and AD-CSF samples. Non-AD-CSF samples (n=16) and AD CSF samples (n=16) were analyzed using the MILLIPLEX® MAP Human Amyloid Beta and Tau Magnetic Bead Panel (Cat. No. HNABTMAG-68K).

Conclusion

The MILLIPLEX® MAP Human Amyloid Beta and Tau Magnetic Bead Panel (Cat. No. HNABTMAG-68K) is ideal for simultaneous measurement of Aβ1-40, Aβ1-42, total Tau and phosphorylated Tau (Thr181) for neurodegenerative disease biomarkers in human CSF. This assay is rapid, sensitive, reproducible and requires only 12.5 µL or less of CSF sample. It is important to note that the presence of either Aβ1-40 or Aβ1-42 does not interfere with the detection or quantitation of other biomarkers. The ability to determine both the Aβ1-40:Aβ1-42 ratio as well as the Aβ1-42:phosphorylated Tau ratio indicates that this assay is a unique and powerful tool for studying the complexities of the nervous system and the mechanisms of pathology in AD and other neurodegenerative disorders.

Ordering Information

MILLIPLEX® MAP Kits	Cat. No.
Human Amyloid Beta and Tau Panel	**HNABTMAG-68K**
Numan Neuroscience Panel 1	**HNS1MAG-95K**
Human Neurodegenerative Disease Panel 1	**HNDG1MAG-36K**
Human Neurodegenerative Disease Panel 2	**HNDG2MAG-36K**
Human Neurodegenerative Disease Panel 3	**HNDG3MAG-36K**
Human Neurodegenerative Disease Panel 4	**HNDG4MAG-36K**
Human Neurological Disorders Panel 3	**HND3MAG-39K**
Human Neuropeptide Panel	**HNPMAG-35K**
Human Circadian Stress	**HNCSMAG-35K**

References

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3. Dolev I, Fogel H, Milshtein H, Berdichevsky Y, Lipstein N, Brose N, Gazit N, Slutsky I. Spike bursts increase amyloid-β 40/42 ratio by inducing a presenilin-1 conformational change. Nat Neurosci. 2013 May;16(5):587-95.
4. Bombois S, Duhamel A, Salleron J, Deramecourt V, Mackowiak MA, Deken V, Sergeant N, Pasquier F, Buée L, Sablonniére B, Schraen-Maschke S. A new decision tree combining Abeta 1-42 and p-Tau levels in Alzheimer's diagnosis. Curr Alzheimer Res. 2013 May 1;10(4):357-64.

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