P1-205

Development of Novel Assay Panels for the Detection of Alzheimer's and Parkinson's Disease Biomarkers in Human Matrices

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1 Abstract

Objectives: Accurate and reproducible measurement of neurodegenerative disease biomarkers is required for biomarker validation and for the development of assays suitable for clinical studies. We describe the development of two novel immunoassays that measure α-Synuclein and DJ-1/PARK7 in clinically relevant human matrices. We also report the feasibility of a multiplexed immunoassay panel designed to measure both Alzheimer's disease (AD) and Parkinson's disease (PD) biomarkers, including Aβ42, Aβ40, Aβ38, total tau, sAPPα, sAPPβ, α-synuclein, and DJ-1/PARK7.

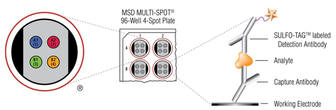
Methods: The assays were built using MSD's MULTI-ARRAY® electrochemiluminescence technology. Antibody selection was based on sensitivity, specificity, analytical characteristics, and functional performance in human matrices. Protocol format was evaluated to achieve optimal signal and assay sensitivity. Matrix tolerance was assessed using dilution linearity and spike recovery.

Results: The assays for AD and PD biomarkers demonstrated reproducible standard curves with quantitative ranges that support the accurate measurement of endogenous levels of each analyte. We present AD and PD biomarker levels measured in human cerebrospinal fluid samples (CSF) from patients with various neurological diseases.

Conclusions: The development of multiplexed assays to measure several protein biomarkers associated with AD and PD is an important goal in the neurodegeneration biomarker field. Such assays would be particularly advantageous given that some biomarkers are relevant for multiple disease types; therefore, multiplexing allows measurement of biomarkers for multiple, related diseases while conserving sample. The panel described is anticipated to aid researchers in defining the relationship between disease progression and the relative abundance of specific neurodegenerative disease biomarkers.

2 Methods

- MSD's electrochemiluminescence detection technology uses SULFO-TAG™ labels, which emit light upon electrochemical stimulation initiated at the electrode surfaces of MULTI-ARRAY® and MULTI-SPOT® microplates.
- Information regarding α-Synuclein, DJ-1/PARK7, and the other neurodegeneration biomarker assays that we offer can be found at www.mesoscale.com/assays.



Assay Protocol

1. Add 150 µL MSD® Diluent 35. Incubate for 1 hour at room temperature (RT).
2. Wash 3X with PBS-T. Add 25 µL of detection antibody to each well. Add 25 µL of standard or diluted sample. Incubate for 2 hours at RT.
3. Wash 3X with PBS-T. Add 150 µL of Read Buffer T. Read on MSD instrument.

3 Antibody Screening: Calibration Curves

Eight antibodies for α-synuclein and five antibodies for DJ-1/PARK7 were subjected to an unbiased screen to identify optimal antibody pairs for each assay. Assays that used the same antibody as both the capture and detection antibody were avoided. For the α-synuclein assay, Ab8 was selected as the capture antibody and Ab4 was selected as the detection antibody based on high sensitivity and low background signal (Figure A). Antibody screening for DJ-1/PARK7 was complicated by elevated background signals indicating that nonspecific binding interactions were obscuring the functional performance of the DJ-1/PARK7 assays (Figure B). Final antibody pairs selected for each assay are indicated by a red box.

A

α-Synuclein Capture			α-Synuclein Detection						
			Ab1 (mouse mono)	Ab2 (mouse mono)	Ab3 (mouse mono)	Ab4 (mouse mono)	Ab5 (mouse mono)	Ab6 (rabbit mono)	Ab7 (mouse mono)
Ab1	Bkgd			274		873	148	483	98
	Hill Slope	ND	1.3	ND	1.4	1.1	N/A	1.1
	STD4/Bkgd		1.17		2.15	1.37	2.29	2.43
Ab2	Bkgd	92		89	74	239	88	164
	Hill Slope	1.9	ND	1.8	1.1	2.0	N/A	0.7
	STD4/Bkgd	1.90		2.51	22.9	1.35	1.05	1.09
Ab3	Bkgd	117	91		89	179	79	99
	Hill Slope	1.9	1.6	ND	1.7	0.6	N/A	1.7
	STD4/Bkgd	1.74	2.34		28.8	1.26	1.30	3.22
Ab5	Bkgd	80	121	76	64		99	321
	Hill Slope	1.2	1.3	1.4	1.7	ND	N/A	1.2
	STD4/Bkgd	1.24	1.02	1.01	1.72		0.97	1.09
Ab6	Bkgd	2693	238	361	492	1715		379
	Hill Slope	1.4	1.1	1.0	1.5	1.3	ND	1.3
	STD4/Bkgd	0.97	0.97	0.95	1.46	1.00		1.02
Ab7	Bkgd	80	171	72	79	335	135	
	Hill Slope	N/A	N/A	N/A	1.0	N/A	N/A	ND
	STD4/Bkgd	0.89	0.91	1.08	1.33	0.98	0.79	
Ab8 (rabbit mono)	Bkgd		128	98	107			234
	Hill Slope	ND	1.3	1.6	1.3	ND	ND	1.5
	STD4/Bkgd		11.6	20.2	128			5.02

B

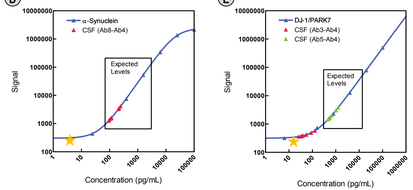
DJ-1/PARK7 Capture		DJ-1/PARK7 Detection					
		Ab1 (mouse mono)	Ab2 (rabbit poly)	Ab3 (mouse mono)	Ab4 (goat poly)	Ab5 (rat mono)
Ab1	Bkgd		283	2088	7001	375
	Hill Slope	ND	1.11	2.65	1.14	N/A
	STD4/Bkgd		2.43	1.04	1.32	0.94
Ab2	Bkgd	2706		1718	6365	186
	Hill Slope	1	ND	1.26	1.21	0.7
	STD4/Bkgd	3.57		0.97	75.0	2.56
Ab3	Bkgd	311	107		372	74
	Hill Slope	1.04	1.07	ND	1.16	1.02
	STD4/Bkgd	4.21	8.64		6.02	1.26
Ab4	Bkgd	795	455	10270		131
	Hill Slope	0.988	1.08	N/A	ND	1.1
	STD4/Bkgd	1.79	23.6	1.19		1.11
Ab5	Bkgd	8395	283	925	17557	
	Hill Slope	N/A	1.04	1.27	1.05	ND
	STD4/Bkgd	0.84	6.24	1.01	1.60	

4 Antibody Screening: Blocker Optimization and Sample Testing

In an effort to minimize nonspecific binding interactions in the DJ-1/PARK7 assay, we examined the effect of a panel of blockers on two of the monoclonal capture antibodies (Ab3 and Ab5) paired with the Ab4 detector. The presence of blockers decreased background signals and increased sensitivity for both DJ-1/PARK7 antibody pairs (Figure C compared to Figure B). Seven CSF samples from patients with various neurological diseases were tested with the selected α-synuclein pair (Ab8-Ab4) and both DJ-1/PARK7 pairs: Ab3-Ab4 and Ab5-Ab4. In-well concentrations for α-synuclein (Figure D) and DJ-1/PARK7 (Figure E) are plotted on the respective calibration curves. LLOD is indicated by a yellow star. α-Synuclein levels were detected in all samples and were similar to literature values. DJ-1/PARK7 levels measured by Ab3-Ab4 were near the LLOD while the Ab5-Ab4 pair measured DJ-1/PARK7 levels consistent with literature. For this reason, Ab5-Ab4 was selected as the final antibody pair for the DJ-1/PARK7 assay.

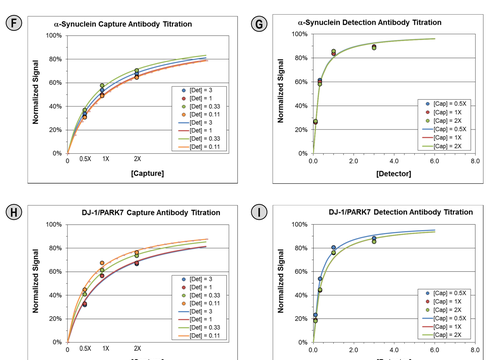
C

DJ-1/PARK7 Capture		Detection Ab4 + Blockers
Ab3	Bkgd	226
	Hill Slope	1.04
	STD4/Bkgd	9.17
Ab5	Bkgd	1736
	Hill Slope	1.33
	STD4/Bkgd	4.46



5 Antibody Pair Optimization

Antibody pair optimization was determined by evaluating the performance of three capture antibody concentrations (0.5X, 1X, and 2X) and four detection antibody concentrations (0.11 µg/mL, 0.33 µg/mL, 1 µg/mL, and 3 µg/mL). Final concentrations were selected to enhance assay robustness such that modest changes in capture or detection antibody concentrations would have minimal effect on assay performance (i.e., less than 20% change in signal). For both assays, 2X capture concentration (Figures F and H) and 1 µg/mL detection concentration (Figures G and I) were selected as the final assay conditions.

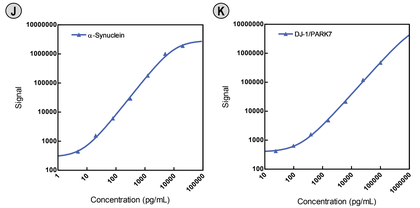


6 Calibration Curves

The following calibration curves represent the sensitivity and dynamic range of the optimized α-synuclein (Figure J) and DJ-1/PARK7 (Figure K) assays. Calibration curves were analyzed using a 4-parameter logistic model with $1/y^2$ weighting.

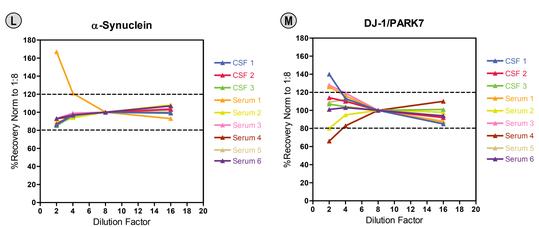
	LLOD (pg/mL)	Hill Slope
α-Synuclein	0.94	1.2
DJ-1/PARK7	12.0	1.1

The lower limit of detection (LLOD) is a calculated concentration corresponding to a signal 2.5 standard deviations above the background (zero calibrator).



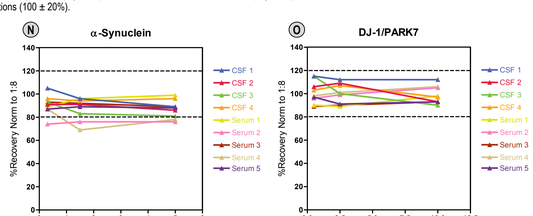
7 Matrix Tolerance: Dilution Linearity

To assess matrix tolerance, dilution linearity was measured for three normal CSF and six normal serum samples diluted 2-fold, 4-fold, 8-fold, and 16-fold in Diluent 35. Measured concentrations were corrected for dilution factor. Percent recovery at each dilution was calculated relative to the recommended sample dilution (8-fold). For α-Synuclein, 8 of 9 samples displayed dilution linearity (100 ± 20%) for all sample dilutions (Figure L). All samples measured for DJ-1/PARK7 were linear (100 ± 20 %) for dilutions between 4-fold and 16-fold (Figure M).



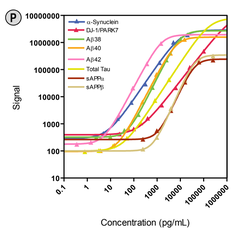
8 Matrix Tolerance: Spike Recovery

The ability of each assay to accurately measure spiked calibrator concentrations was assessed for four normal CSF and five normal serum samples. Samples were spiked with calibrator at multiple levels: 0.3 ng/mL, 1.5 ng/mL, and 5 ng/mL (α-Synuclein) or 0.5 ng/mL, 2.5 ng/mL, and 10 ng/mL (DJ-1/PARK7). Samples were diluted 8-fold and tested for % recovery (Recovery = measured/expected x 100). Seven of 9 samples for α-synuclein (Figure N) and all 9 samples for DJ-1/PARK7 (Figure O) met standard specifications for all spiked calibrator concentrations (100 ± 20%).



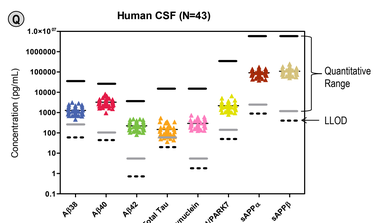
9 Sample Testing: Calibration Curves

Several MSD neurodegeneration biomarker assays, such as Aβ Peptide Panel 1 (6E10), sAPPα/sAPPβ, and Human Total Tau, were run with the α-synuclein and DJ-1/PARK7 assays described herein. Assay format was selected to enable running all of the analytes in multiplex. Calibration curves shown below were analyzed using a 4-parameter logistic model with $1/y^2$ weighting (Figure P).



10 Sample Testing: Endogenous Levels

Forty-three well-curated CSF samples from patients with various neurological diseases were tested across the MSD assay panels described in the previous section. Samples were diluted between 2-fold and 8-fold depending on the abundance of the analyte in CSF. The scatter plot shown below depicts the concentrations of Aβ38, Aβ40, Aβ42, total tau, α-synuclein, DJ-1/PARK7, sAPPα, and sAPPβ for all 43 samples (Figure Q). Quantitative range and LLOD are plotted for each analyte with adjustment for sample dilution. The dilution-adjusted mean, median, and range for each analyte are reported in Figure R.



R

	Measured Concentration (pg/mL)							
	Aβ38	Aβ40	Aβ42	Total Tau	α-Synuclein	DJ-1/PARK7	sAPPα	sAPPβ
Mean	1284	3256	222	148	295	2192	91253	109536
Median	1142	2881	190	109	237	2030	84308	102586
Minimum	463	969	82	37	128	809	39010	50979
Maximum	3146	8193	479	541	738	6584	175389	253763

11 Conclusion

We present the development of two novel sandwich immunoassays for the measurement of important PD biomarkers, α-synuclein and DJ-1/PARK7. Both assays demonstrate excellent sensitivity and a quantitative range suitable for measuring normal and disease levels of each analyte. The potential to multiplex the α-synuclein and DJ-1/PARK7 assays with other AD biomarker assays will enable researchers to advance the understanding of neurodegeneration biomarker prevalence across multiple disease types while still conserving sample. The assays described herein represent useful tools for researchers studying the relationship between neurodegeneration and the incidence of PD and AD biomarkers.

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