An Ultrasensitive Assay for the Detection of Phosphorylated Tau in Human Samples

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1 Abstract

Objectives: The accumulation of Tau protein in the cerebrospinal fluid (CSF) of Alzheimer's disease (AD) patients correlates with neurodegeneration. Tau phosphorylation is connected to Tau aggregation, a pathological hallmark of multiple neurodegenerative disorders including AD. It would be advantageous to measure total and phosphorylated Tau in serum and plasma as an alternative to CSF; however, this requires assays with higher sensitivity than those currently available. Previously, we reported an ultrasensitive S-PLEX℠ assay detecting total Tau (Nikolenko *et al*., 2015). In this study, we developed an ultrasensitive assay that specifically measures Tau phosphorylated at threonine 181 (T181).

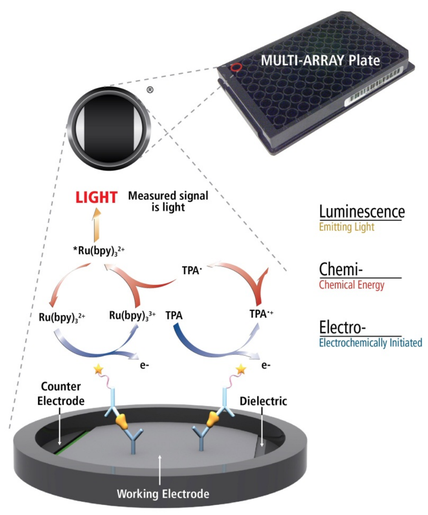
Methods: An S-PLEX immunoassay was developed using antibodies that detect Tau phosphorylated at T181. This assay was used to measure phosphorylated Tau in 200 biological samples including human serum and plasma.

Results: The S-PLEX phospho-Tau T181 assay has a dynamic range of 4 logs and a lower limit of detection of 40 fg/mL. The assay specifically measures Tau phosphorylated at T181, with less than 0.01% cross-reactivity with un-phosphorylated Tau. Phosphorylated Tau was detectable in 95% of human samples tested (CSF, plasma, serum, and urine) and in cell lysates from lung, kidney, breast, and bone marrow cell lines, with concentrations ranging from 40 to 100,000 fg/mL.

Conclusions: The MSD® S-PLEX phospho-Tau T181 assay enables accurate measurement of phosphorylated Tau at low concentrations in serum and plasma and may be used to detect phosphorylated Tau in biological samples.

2 Methods

MSD's electrochemiluminescence detection technology uses SULFO-TAG™ labels that emit light upon electrochemical stimulation initiated at the electrode surfaces of MULTI-ARRAY® microplates. We developed the S-PLEX assay platform, a next-generation MULTI-ARRAY technology with significantly higher sensitivity.



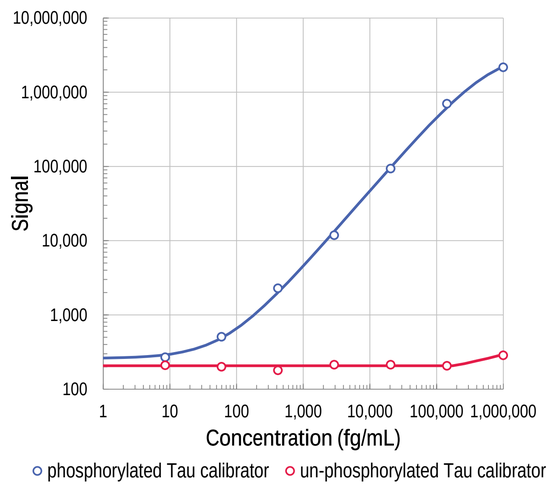
3 Calibration Curve and Assay Range

A calibration curve was generated using serial dilutions of recombinant phosphorylated Tau protein. Recombinant Tau protein used for assay calibration represents the longest isoform, Tau 441, which is phosphorylated at the T181 site.

Limit of detection (LOD) is a calculated concentration corresponding to the average signal at 2.5 standard deviations above the background (zero calibrator).

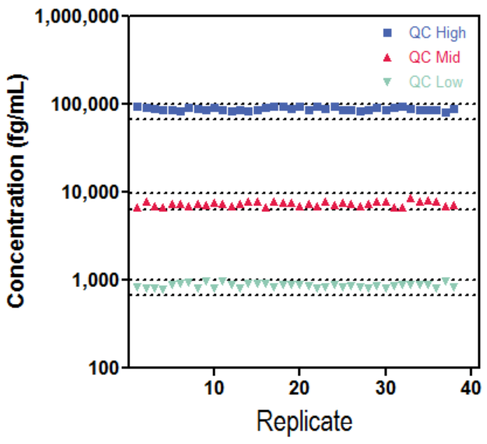
Lower limit of quantitation (LLOQ) and upper limit of quantitation (ULOQ) are established for the plate lot by measuring multiple levels of calibrator near the expected LLOQ and ULOQ. LLOQ and ULOQ are the lowest and highest (respectively) concentrations of calibrator tested which have %CVs of 20% or less, with recovered concentrations within 70-130%.

The assay specifically measures Tau phosphorylated at T181, with less than 0.01% cross-reactivity with un-phosphorylated Tau.



	pTau T181 assay
Calibration Range	40 – 800,000 fg/mL
Hill Slope	1.04
LOD: Median (Range)	40 (20-50) fg/mL
LLOQ (Lot Specific)	60 fg/mL
ULOQ (Lot Specific)	600,000 fg/mL

4 Reproducibility



Dotted lines represent acceptable limits for QC values (± 20% of expected QC value)

	Expected concentration (fg/mL)	Measured concentration (fg/mL)	% Recovery	Interplate %CV
QC High	85,000	88,628	104	4.1
QC Mid	8,000	7,399	92	5.9
QC Low	850	865	102	5.8

To determine reproducibility of quality controls, two replicates of a high, medium, and low QC sample were measured in each run for 19 runs across 30 days.

5 Dilution Linearity

Serum, EDTA plasma, and heparin plasma samples (n=9), spiked with recombinant calibrator, and neat, unspiked CSF samples (n=2) were diluted 2x, 4x, and 8x. Average dilution linearity was 85% for serum/plasma samples and 76% for CSF samples.

	Dilution Factor	Measured (fg/mL)	% Recovery
Serum 1	0	10,892	
	2x	6,555	120
	4x	2,193	81
	8x	1,046	77
Serum 2	0	7,481	
	2x	3,430	92
	4x	1,399	75
	8x	673	72
Serum 3	0	8,767	
	2x	3,797	87
	4x	1,613	74
	8x	809	74
Average % Recovery:			83

	Dilution Factor	Measured (fg/mL)	% Recovery
EDTA Plasma 1	0	9,635	
	2x	4,423	92
	4x	1,897	79
	8x	777	64
EDTA Plasma 2	0	6,060	
	2x	2,632	87
	4x	1,270	84
	8x	597	79
EDTA Plasma 3	0	11,146	
	2x	6,143	110
	4x	2,680	96
	8x	1,042	75
Average % Recovery:			85

	Dilution Factor	Measured (fg/mL)	% Recovery
Heparin Plasma 1	0	7,509	
	2x	3,891	104
	4x	1,574	84
	8x	782	83
Heparin Plasma 2	0	7,815	
	2x	4,753	122
	4x	1,780	91
	8x	763	78
Heparin Plasma 3	0	9,781	
	2x	3,769	96
	4x	1,618	83
	8x	703	72
Average % Recovery:			90

	Dilution Factor	Measured (fg/mL)	% Recovery
CSF 1	0	5,037	
	2x	2,158	86
	4x	785	62
	8x	444	71
CSF 2	0	11,338	
	2x	5,700	101
	4x	2,229	79
	8x	858	61
Average % Recovery:			76

6 Spike Recovery

Serum, EDTA plasma, and heparin plasma (n=9) and CSF samples (n=3) were spiked with calibrator at three concentrations. Percent recovery was calculated by dividing the difference between measured concentration in spiked sample and unspiked sample to expected spike concentration. [% Recovery = (Measured Spiked- Measured Unspiked) / Spike)].

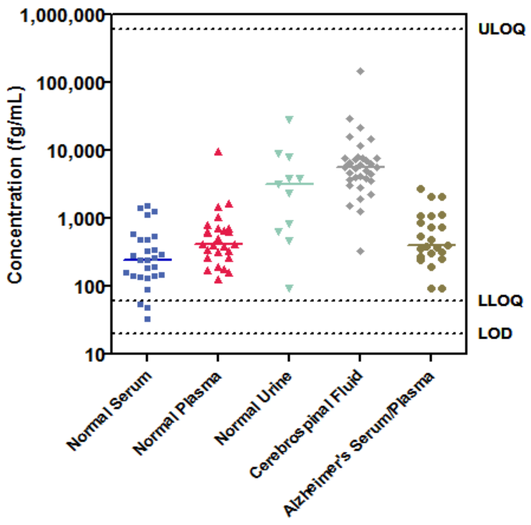
	Spike (fg/mL)	Measured (fg/mL)	% Recovery
Serum 1	Unspiked	140	
	85,000	109,652	129
	5,500	5,735	102
	850	1,048	107
Serum 2	Unspiked	138	
	85,000	70,636	83
	5,500	4,901	87
	850	824	81
Serum 3	Unspiked	111	
	85,000	104,619	123
	5,500	7,871	141
	850	962	100
Average % Recovery:			106

	Spike (fg/mL)	Measured (fg/mL)	% Recovery
EDTA Plasma 1	Unspiked	149	
	85,000	86,196	101
	5,500	6,429	114
	850	1,138	116
EDTA Plasma 2	Unspiked	112	
	85,000	62,921	74
	5,500	5,032	89
	850	930	96
EDTA Plasma 3	Unspiked	97	
	85,000	82,389	97
	5,500	7,423	133
	850	1,066	114
Average % Recovery:			104

	Spike (fg/mL)	Measured (fg/mL)	% Recovery
Heparin Plasma 1	Unspiked	360	
	85,000	129,013	151
	5,500	7,115	123
	850	1,145	92
Heparin Plasma 2	Unspiked	176	
	85,000	100,495	118
	5,500	7,690	137
	850	1,092	108
Heparin Plasma 3	Unspiked	318	
	85,000	114,629	134
	5,500	7,604	132
	850	1,208	105
Average % Recovery:			122

	Spike (fg/mL)	Measured (fg/mL)	% Recovery
CSF 1	Unspiked	153	
	85,153	80,735	95
	5,653	6,692	116
	1003	1,286	113
CSF 2	Unspiked	136	
	85,136	88,744	104
	5,636	6,769	118
	986	866	74
CSF 3	Unspiked	141	
	85,141	84,588	99
	5,641	5,765	100
	991	866	73
Average % Recovery:			99

7 Human Samples



Sample	Median Concentration (fg/mL)
Normal Serum (n=26)	243
Normal Plasma (n=26)	417
Normal Urine (n=11)	3,125
CSF (n=34)	5,629
Alzheimer's Serum/Plasma (n=23)	403

Twenty-six normal serum, 26 normal EDTA plasma, 11 normal urine, 34 cerebrospinal fluid (non-Alzheimer's disease samples), and 23 Alzheimer's disease samples (serum and plasma) were tested neat on the S-PLEX phospho-Tau T181 assay. Phospho-Tau T181 was quantifiable in 95% of all samples tested.

8 Conclusions

A next-generation assay for human phosphorylated Tau at T181 site was developed, based on MSD's ultrasensitive S-PLEX technology. This novel technology is at least 1,000 times more sensitive than the currently available phospho-Tau T181 assays. The MSD assay enables accurate determination of phospho-Tau T181 at low concentrations in serum and plasma and can be used to detect phosphorylated Tau in biological samples.

Summary Table	
	pTau T181 assay
Calibration Range	40 – 800,000 fg/mL
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LOD: Median (Range)	40 (20-50) fg/mL
LLOQ (Lot Specific)	60 fg/mL
ULOQ (Lot Specific)	600,000 fg/mL
Median concentration in normal serum/plasma (n=52)	335 fg/mL
Percentage of tested samples above the LLOQ (n=120)	95%

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