Abstract #36726

Ultrasensitive Assays for the Detection of Total and Phosphorylated Tau in Serum and Plasma

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1 Abstract

Objectives: Increased Tau phosphorylation followed by the accumulation of Tau in cerebrospinal fluid (CSF) correlate with neurological disorders, including Alzheimer's Disease (AD). However, collection of CSF is invasive and inconvenient for use in screening of the disease. Serum and plasma are less invasive alternatives to CSF, but Tau levels in blood cannot be detected by standard immunoassay techniques. To address this, we have developed ultrasensitive assays for measuring total Tau and phosphorylated versions of Tau.

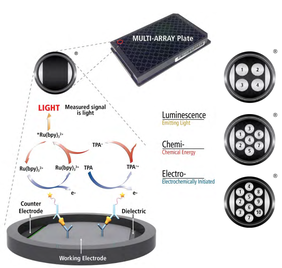
Methods: MSD's ultrasensitive assay format, S-PLEX®, uses MSD's MULTI-ARRAY® electrochemiluminescence technology and allows for quantitation of biomarkers at fg/mL levels, up to 1000-fold lower than with standard immunoassay formats. Three Tau S-PLEX assays were developed: a total Tau (tTau) assay to quantitate all forms of Tau, and assays to quantitate Tau phosphorylated at two different sites (threonine 181 [pTau181] and threonine 231 [pTau231]). The assays were qualified to measure the three markers in serum and plasma samples.

Results: The new MSD® S-PLEX tTau and pTau(181) assays have a limit of detection (LOD) below 200 fg/mL and below 3000 fg/ml for pTau(231) based on full length Tau concentration. All assays have a dynamic range of at least 3 logs. The assays were used to measure levels of the biomarkers in samples from normal individuals and individuals with AD. Levels of total Tau were detectible in all normal samples and were elevated in disease samples. Phosphorylated Tau was detectable in a subset of normal samples and was elevated in a number of the AD samples.

Conclusions: Ultrasensitive assays for total Tau and phosphorylated versions of Tau have been developed using MSD's S-PLEX format, with improved sensitivity over standard immunoassay formats. The assays' improved sensitivity allows for quantitation of tTau and pTau in serum and plasma, and their utility has been demonstrated by measuring elevated levels in disease samples with AD.

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 and MULTI-SPOT® microplates.

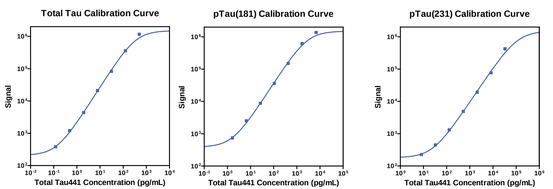


Electrochemiluminescence Technology

- Minimal non-specific background and strong responses to analyte yield high signal-to-background ratios.
- The stimulation mechanism (electricity) is decoupled from the response (light signal), minimizing matrix interference.
- Only labels bound near the electrode surface are excited, enabling non-washed assays.
- Labels are stable, non-radioactive, and directly conjugated to biological molecules.
- Emission at ~620 nm eliminates problems with color quenching.
- Multiple rounds of label excitation and emission enhance light levels and improve sensitivity.
- Carbon electrode surface has 10X greater binding capacity than polystyrene wells.
- Surface coatings can be customized.

3 Calibration Curve, Assay Range

Calibration curves for tTau, pTau(181) and pTau(231) are shown below, along with a table that includes Hill slopes, limits of detection (LOD), lower limits of quantitation (LLOQ) and upper limits of quantitation (ULOQ).
The LLOD corresponds to a signal at 2.5 standard deviations above the background. LLOQ was assigned as the lowest concentration measured with a CV of 20% or less and recovery within 80%-120%. Similarly, ULOQ was assigned as the highest concentration with a CV of 20% or less and recovery within 80%-120%.
The calibrator is recombinant full-length Tau protein (Tau441); anti-Tau antibodies used in the assays recognize all 6 isoforms of Tau. A non-phosphorylated version of the calibrator was expressed in *E. coli* and used for the tTau assay, while a phosphorylated version was expressed in a mammalian system and used for the tTau assays. 100% phosphorylation was assumed for both the 181 and 231 sites for assigning calibrator concentrations (mass spectrometry indicated at least 60% phosphorylation at each site).
The tTau detection limit is 10 fg/ml, which is at least 1000-fold more sensitive than standard immunoassays.
The detection limits for the pTau assays are 166 fg/ml for pTau(181) and 2,663 fg/ml for pTau(231). The assays are likely more sensitive given that the calibrator may not be 100% phosphorylated (further characterization of the calibrator is in process). Compared to pTau assays on the market, the S-PLEX pTau(181) assay is approximately 1000-fold more sensitive and the S-PLEX pTau(231) assay is approximately 35-fold more sensitive (after correcting for calibrator size/weight).



	tTau	pTau(181)	pTau(231)
Hill Slope	0.98	1.05	1.05
LOD, pg/mL: Median (Range)	0.010 (0.080 – 0.012)	0.166 (0.155 – 0.171)	2.66 (2.40 – 2.84)
Lot Specific LLOQ, pg/mL	0.12	0.26	10.0
Lot Specific ULOQ, pg/mL	200	1,733	25,000

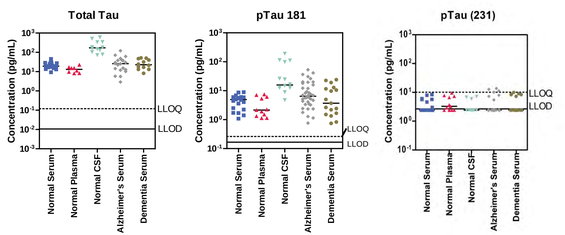
4 Phospho-Tau Assay Specificity

The specificity of the pTau assays was assessed by measuring their cross reactivity to non-phosphorylated and phosphorylated Tau protein. Dilution curves of both forms of calibrator were measured on all three assays.
The data confirms that the pTau assays do not recognize non-phosphorylated Tau, while the tTau assay recognizes phosphorylated and non-phosphorylated tau equivalently.

Sample	Total Tau441 Concen. (pg/mL)	Total Tau				pTau (181)				pTau (231)			
		Phosphorylated Tau Calibrator		Non-phosphorylated Tau Calibrator		Phosphorylated Tau Calibrator		Non-phosphorylated Tau Calibrator		Phosphorylated Tau Calibrator		Non-phosphorylated Tau Calibrator	
		ECL	%CV	ECL	%CV	ECL	%CV	ECL	%CV	ECL	%CV	ECL	%CV
Cal-1	2,000	1,248,209	1.1	1,231,394	2.1	260,648	0.6	251	4.4	42,406	3.2	243	2.7
Cal-2	500	675,095	0.8	651,122	3.2	57,830	2.6	182	1.1	9,335	2.4	175	2.2
Cal-3	125	177,774	2.2	175,592	4.1	13,856	2.7	172	3.5	2,559	5.2	166	0.3
Cal-4	31.2	40,073	1.7	41,003	1.0	3,542	1.3	171	1.2	750	0.9	159	4.9
Cal-5	7.81	8,793	5.6	9,013	3.0	946	5.2	163	7.1	299	6.8	162	3.1
Cal-6	1.95	2,294	8.1	2,502	3.2	368	3.7	153	5.2	191	5.9	154	5.5
Cal-7	0.49	697	4.5	961	5.5	212	5.4	161	5.4	160	2.6	149	2.5
Cal-8	0	272	6.6	272	6.6	161	4.8	161	4.8	154	5.2	154	5.2

5 Human Samples

Serum (n=27) and plasma (n=8-11) from healthy donors, serum from individuals with Alzheimer's (n=20-38) and dementia (n=12-17) and cerebral spinal fluid (CSF, n=12-13) were measured on the three tau assays.
Samples were tested neat for the pTau assays and diluted 2-fold for the tTau assay.
Total Tau and pTau(181) were quantifiable in 100% of the samples with some higher samples noted in the diseased groups; tTau is more concentrated in CSF, as expected.
pTau(231) was below the LLOD in the majority of normal samples (undetectable samples are assigned the LLOD concentration in the graphs below).



Subject	Sample Type	Statistic	Total Tau	pTau181	pTau231
Normals	Serum	Number Tested	20	20	20
		Median (pg/mL)	19.2	4.95	5.72
		Range (pg/mL)	18.3 – 45.5	1.09 – 8.86	ND – 8.42
		% Detected	100	100	35
	Plasma	Number Tested	8	11	11
		Median (pg/mL)	13.1	2.14	7.57
		Range (pg/mL)	8.08 – 22.5	1.12 – 7.45	ND – 9.57
		% Detected	100	100	36
	CSF	Number Tested	12	13	13
		Median (pg/mL)	170	16.7	6.68
		Range (pg/mL)	71.8 - 606	4.77 -191	ND – 7.08
		% Detected	100	100	23
Alzheimer's	Pooled Serum	Number Tested	20	38	38
		Median (pg/mL)	25.7	12.1	4.70
		Range (pg/mL)	2.90 - 120	0.776 – 53.2	ND – 4.88
		% Detected	100	100	18
Dementia	Pooled Serum	Number Tested	12	17	17
		Median (pg/mL)	22.9	3.70	8.49
		Range (pg/mL)	8.19 – 51.7	744 – 24.1	ND – 9.99
		% Detected	100	100	35

6 Spike Recovery

Serum, EDTA plasma and heparin plasma samples (n=4) were spiked with recombinant calibrator at three different concentrations spanning the dynamic range of each assay.
Percent recovery was calculated by dividing the difference between the measured concentration in the spiked and non-spiked samples by the expected spike concentration. (% Recovery= (Measured Spiked - Measured non-Spiked) / Spike).
Average spike recovery for the tTau assay was within 70%-130% in serum and EDTA plasma.
The pTau assays recovered within 70%-130% in serum and heparin plasma.

Matrix	Spike Level	Total Tau		pTau (181)		pTau (231)	
		Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range
Serum	High Spike	96	86-104	107	87-116	89	62-113
	Mid Spike	74	60-86	105	86-113	91	69-113
	Low Spike	83	75-90	105	85-116	92	65-117
EDTA Plasma	High Spike	113	102-118	149	140-158	137	122-160
	Mid Spike	96	74-111	144	136-151	138	126-148
	Low Spike	102	87-113	138	135-142	143	131-155
Heparin Plasma	High Spike	55	36-71	112	79-129	89	58-108
	Mid Spike	62	40-80	118	80-151	88	58-100
	Low Spike	69	46-88	115	82-134	93	59-109

7 Dilution Linearity

Serum, EDTA and heparin plasma samples (n=2) spiked with recombinant calibrator were diluted 2x, 4x, and 8x and measured on all three assays.
The recovery was normalized to the recommended sample dilution level: 2x for tTau and 1x (neat) for pTau assays.
Average dilution linearity for serum/plasma samples was within 70%-130% for the tTau and pTau(181) assays.
The pTau231 assay recovered ~90% on average in EDTA plasma but showed over-recovery in serum and heparin plasma.

Sample Matrix	Dilution Factor	Total Tau			pTau181			pTau231		
		ECL Signal	Concen. (pg/mL)	% Recovery	ECL Signal	Concen. (pg/mL)	% Recovery	ECL Signal	Concen. (pg/mL)	% Recovery
Serum (N=2)	None	507,707	208	81	33,321	310		19,750	1,736	
	2x	313,226	257		16,680	319	103	12,619	2,232	129
	4x	157,630	260	101	7,486	293	94	7,174	2,551	147
	8x	76,526	253	98	3,796	298	96	3,529	2,503	144
EDTA Plasma (N=2)	None	760,475	311	100	45,357	418		32,616	2,845	
	2x	380,541	312		19,042	361	87	15,618	2,756	97
	4x	182,474	301	96	7,870	307	74	7,314	2,600	91
	8x	81,398	269	86	3,665	288	69	3,452	2,4478	86
Heparin Plasma (N=2)	None	410,470	168	68	41,374	382		17,783	1,566	
	2x	303,106	249		19,373	368	96	14,466	2,555	163
	4x	157,795	260	104	7,803	305	80	7,402	2,631	168
	8x	96,752	320	128	5,833	456	119	3,576	2,537	162

8 Conclusion

Ultrasensitive assays for total Tau and phosphorylated versions of Tau have been developed using MSD's S-PLEX format, with improved sensitivity over standard immunoassay formats.
These assays allow quantitation of tTau and pTau181 in serum and plasma, providing a new tool for researching these important biomarkers of neurodegeneration.

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