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Accurate Measurement of Tau in Serum and Plasma Using a Novel Technology with fg/mL Sensitivity

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1 Abstract

Background: Tau has emerged as a putative therapeutic target for many neurodegenerative disorders. This protein is a major component of paired helical filaments and other large intracellular aggregates in the brains of patients with Alzheimer's disease (AD) and other neurological disorders. Clinical diagnosis of AD most often occurs several years after neurodegeneration commences. The accumulation of Tau protein in the cerebrospinal fluid (CSF) of AD patients correlates with neurodegeneration, and may also be a useful biomarker for identifying patients with mild cognitive impairment (MCI). However, collection of CSF is invasive, painful, and inconvenient for use in routine screening for early detection of the disease. Use of blood and urine is greatly preferred, but levels of Tau in blood are largely undetectable with currently available technologies. Therefore, more sensitive detection methods are required to evaluate Tau as a biomarker for AD in blood.

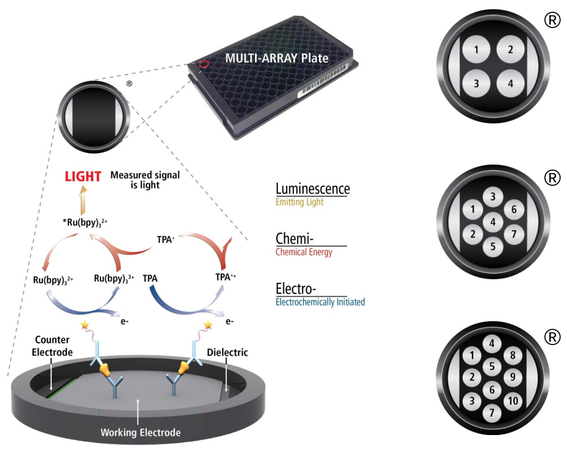
Methods: MSD® offers a Human Total Tau assay kit that has been validated for the detection of Tau protein in CSF. The MSD V-PLEX® Tau assay offers sensitivities competitive with other commercially available assays (Lower limit of quantitation [LLOQ] of 30 pg/mL). However, higher sensitivity is needed for the detection of Tau in serum or plasma. We developed a next-generation assay format, S-PLEX™, using MSD's MULTI-ARRAY® electrochemiluminescence technology. The S-PLEX technology allows quantitation of previously unmeasurable levels of biomarkers with fg/mL sensitivity and a wide dynamic range. S-PLEX assays are compatible with existing MSD instrumentation. An S-PLEX assay was developed for Tau and used to measure Tau levels in serum/plasma samples from both normal and diseased individuals. Recombinant forms of known Tau isoforms were also tested.

Results: The newly developed MSD S-PLEX Tau assay has a limit of detection (LOD) of 6 fg/mL, with lower and upper limits of quantitation of 21 fg/mL (LLOQ) and 160,000 fg/mL (ULOQ), respectively, covering a dynamic range of approximately 3 logs. The S-PLEX Tau assay detects all 6 isoforms of the protein tested. Spike recovery and dilutional linearity were between 80% and 120% for both serum and plasma samples. Levels of Tau in normal serum (n=16), normal plasma (n=16), and plasma from AD patients (n=13) were detectable in all samples, and median concentrations observed were 1.7 pg/mL (interquartile range [IQR] 1.1 - 2.5 pg/mL), 2.6 pg/mL (IQR 1.7 - 4.1 pg/mL), and 3.6 pg/mL (IQR 0.4 - 4.7 pg/mL), respectively. Elevated levels of Tau (n= 8, median concentration 13 pg/mL) were observed in plasma samples from patients with traumatic brain injury (TBI). Levels of Tau in all normal and diseased CSF samples were easily measureable using fifty-fold diluted samples.

Conclusion: MSD has developed a next-generation Tau assay that is up to 1,000x more sensitive than currently available Tau assays. This enables accurate determination of Tau concentrations in sample types, such as blood, where levels were previously unmeasurable. Only minimal amounts of CSF samples are required to measure levels of Tau, and the assay was able to recognize all isoforms of Tau tested. Importantly, Tau was measurable in all serum and plasma samples tested; from normal individuals, patients with AD, and individuals who suffered traumatic brain injuries. The MSD S-PLEX Tau assay makes it possible to reliably measure Tau protein at the low concentrations present in blood, and to evaluate its potential as a biomarker in AD and other neurological disorders.

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.
We developed the S-PLEX assay platform, a next-generation MULTI-ARRAY technology with significantly higher sensitivity.

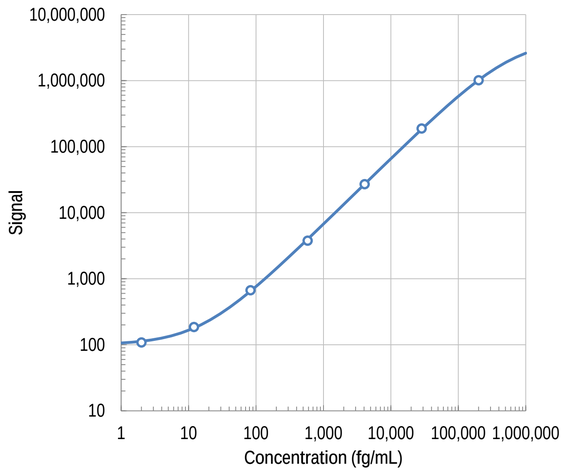


Electrochemiluminescence Technology

- 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

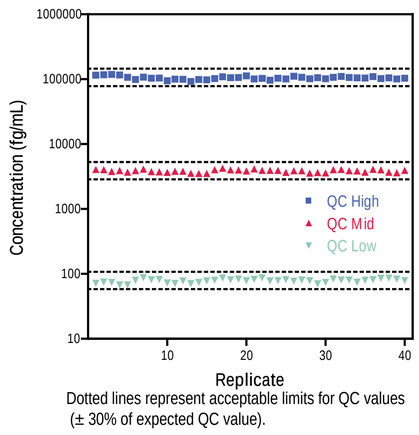
The calibration curve was generated by using serial dilutions of recombinant Tau protein. The recombinant Tau protein used for assay calibration represents the longest isoform, Tau 441. Other isoforms showed similar performance to Tau 441.
LOD is a calculated concentration corresponding to the average signal at 2.5 standard deviations above the background (zero calibrator).
LLOQ and ULOQ are established for the assay by measuring multiple levels of calibrator near the expected LLOQ and ULOQ. LLOQ and ULOQ are the lowest and highest concentrations of calibrator tested which have %CV of 20% or less, with recovered concentrations within 70-130%.



	Tau
Calibration Range	2 – 200,000 fg/mL
Hill Slope	1.00
LOD: Median (Range)	6 (4-13) fg/mL
Lot Specific LLOQ	21 fg/mL
Lot Specific ULOQ	160,000 fg/mL

Tau Isoform	Recognized by Tau Assay
Tau 441	Yes
Tau 412	Yes
Tau 410	Yes
Tau 383	Yes
Tau 381	Yes
Tau 352	Yes

4 Reproducibility



Dotted lines represent acceptable limits for QC values (± 30% of expected QC value).

	Expected fg/mL	Average Measured fg/mL	% Recovery	% CV Within Run	% CV Between Run	% CV Total
QC High	112,000	104,854	94	3.5	4.5	5.7
QC Mid	4,082	3,838	94	4.2	2.6	4.9
QC Low	83	79	95	4.7	4.8	6.8

To determine reproducibility of quality controls (QC, calibrator in diluent), 5 replicates of each sample were measured in a run for 8 runs by at least two operators over at least 4 days (n=40).
The % CV for each source of variation was determined using analysis of variance (ANOVA) with guidance from NCCLS document EP05-A2.
The % CV for QC samples ranged from 3.5% to 4.7% within run and from 2.6% to 4.8% between runs.
Average concentration recovery for all QC samples was 94-95%, with individual replicate recovery within 30% of the expected concentration.

5 Dilution Linearity, Spike Recovery

Serum, EDTA plasma, and heparin plasma samples (n=9) were diluted 2x, 4x, and 8x. Average dilution linearities for serum/plasma samples were 104%.
Serum, EDTA plasma, and heparin plasma samples (n=9) were spiked with calibrator at three concentrations. Percent recovery was calculated by taking the difference between measured concentration in spiked and native sample, and dividing by the known spike concentration [% Recovery = (Measured Spike – Measured Unspiked/Spike)]. Average spike recovery for the Tau assay was 103%.

Dilution Linearity

	Dilution Factor	Measured (fg/mL)	% Recovery
Serum 1		709	
	2x	350	99
	4x	179	101
	8x	99	111
Serum 2		1,933	
	2x	1,025	106
	4x	521	108
	8x	239	99
Serum 3		1,244	
	2x	649	104
	4x	277	89
	8x	151	97
Average % Recovery:			107

	Dilution Factor	Measured (fg/mL)	% Recovery
EDTA Plasma 1		2,838	
	2x	1,453	102
	4x	761	107
	8x	394	111
EDTA Plasma 2		4,213	
	2x	2,823	134
	4x	1,379	131
	8x	741	141
EDTA Plasma 3		2,469	
	2x	1,238	100
	4x	656	106
	8x	323	105
Average % Recovery:			103

	Dilution Factor	Measured (fg/mL)	% Recovery
Heparin Plasma 1		1,540	
	2x	699	91
	4x	341	88
	8x	184	96
Heparin Plasma 2		1,683	
	2x	873	104
	4x	440	104
	8x	233	111
Heparin Plasma 2		2,084	
	2x	754	72
	4x	495	95
	8x	252	97
Average % Recovery:			101

Overall Average % Recovery: 104

Spike Recovery

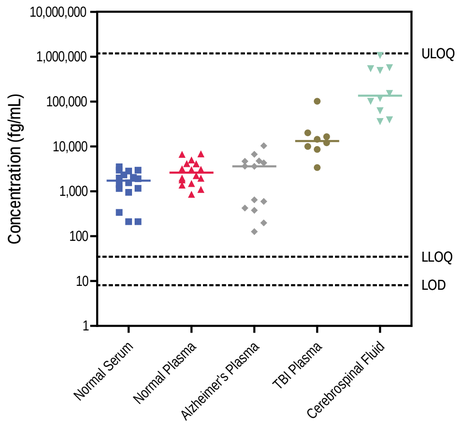
	Spike (fg/mL)	Measured (fg/mL)	% Recovery
Serum 1	Unspiked	91	
	2,000	1,831	87
	1,000	930	84
	500	520	86
Serum 2	Unspiked	210	
	2,000	1,659	72
	1,000	1,068	86
	500	704	99
Serum 3	Unspiked	134	
	2,000	1,765	82
	1,000	1,086	95
	500	677	109
Average % Recovery:			89

	Spike (fg/mL)	Measured (fg/mL)	% Recovery
EDTA Plasma 1	Unspiked	277	
	2,000	2,150	94
	1,000	1,137	86
	500	765	98
EDTA Plasma 2	Unspiked	463	
	2,000	2,303	92
	1,000	1,368	90
	500	879	83
EDTA Plasma 3	Unspiked	227	
	2,000	2,392	108
	1,000	1,186	96
	500	686	92
Average % Recovery:			93

	Spike (fg/mL)	Measured (fg/mL)	% Recovery
Heparin Plasma 1	Unspiked	184	
	2,000	2,876	135
	1,000	1,478	129
	500	855	134
Heparin Plasma 2	Unspiked	233	
	2,000	2,528	115
	1,000	1,479	125
	500	796	113
Heparin Plasma 2	Unspiked	272	
	2,000	2,681	120
	1,000	1,664	139
	500	895	125
Average % Recovery:			126

Overall Average % Recovery: 103

6 Human Samples



Sample	Median Concentration fg/mL
Normal Serum (n=16)	1,733
Normal Plasma (n=16)	2,615
Alzheimer's Plasma (n=13)	3,587
TBI Plasma (n=8)	13,229
CSF (n=10)	135,118

Thirty two matched serum and EDTA plasma samples from 16 normal donors, EDTA plasma from AD patients (n=13) and subjects with mild TBI (n=8), and 10 individual CSF samples were tested on the S-PLEX Tau assay. Some TBI and CSF samples were tested 50-fold diluted. Tau was detectable and quantifiable in 100% of the samples. CSF samples are recommended to be run 50-fold diluted.

7 Conclusions

A next-generation assay for human Tau was developed, based on MSD's ultrasensitive S-PLEX technology. This novel technology is 100 - 1,000 times more sensitive than the currently available Tau assays. This enables accurate determination of Tau concentrations in serum and plasma samples.
This new assay format can be run on a standard MSD instrument and can be performed within a normal workday using common lab equipment. The assay will initially be available through MSD's assay services.

Summary Table	Tau Assay
Calibration Range	2 – 200,000 fg/mL
Hill Slope	1.00
LOD: Median (Range)	6 (4 - 13) fg/mL
Lot Specific LLOQ	21 fg/mL
Lot Specific ULOQ	160,000 fg/mL
Median concentration in normal serum/plasma (n=32)	1,973 fg/mL
Representative CV over assay range	6%
Dilution Linearity	104%
Spike Recovery	103%
Percentage of tested serum/plasma samples above the LLOQ (n=55)	100%

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