P1098

Measuring fg/mL Concentrations of Cytokines in Cerebrospinal Fluid (CSF)

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1 Abstract

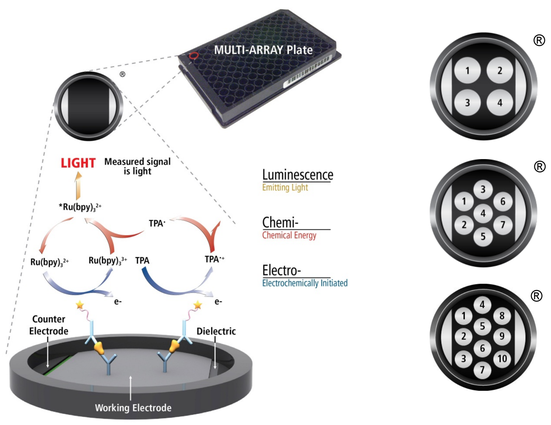
Measuring low-abundance cytokines in CSF may be useful when studying brain and nervous system disorders. We recently developed novel immunoassays with fg/mL and sub-fg/mL detection limits that enable quantitation of low-abundance biomarkers in CSF. S-PLEX℠ assays, utilizing MSD's MULTI-ARRAY® electrochemiluminescence technology, were developed and analytically characterized and the concentrations of cytokines in CSF were measured using these assays.

Assays for the following cytokines were used: IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-17A, IL-21, IFN-γ, GM-CSF, TSLP, and TNF-α. The lower limits of detection achieved for S-PLEX assays for these analytes ranged from ~0.2 fg/mL to ~50 fg/mL. Spike recovery and dilution linearity were between 80% and 120%. Depletion experiments demonstrated specificity for each analyte. Cytokine levels in the majority of apparently healthy CSF samples were measurable for 7 out of the 12 analytes tested.

We developed a next-generation assay format that is 100 to 1,000 times more sensitive than the current limits of standard ELISA technology. This increased sensitivity enabled accurate quantitation of cytokine levels in apparently healthy CSF samples. These assays will allow for a better understanding of the role these cytokines play in brain and nervous system disorders.

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.

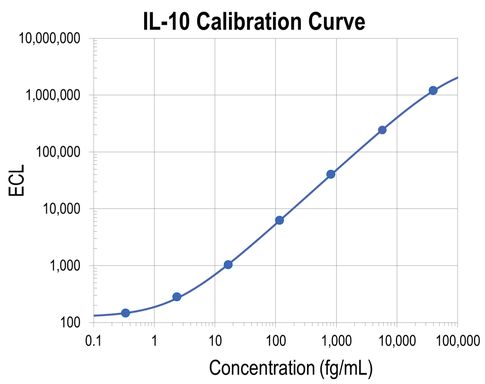


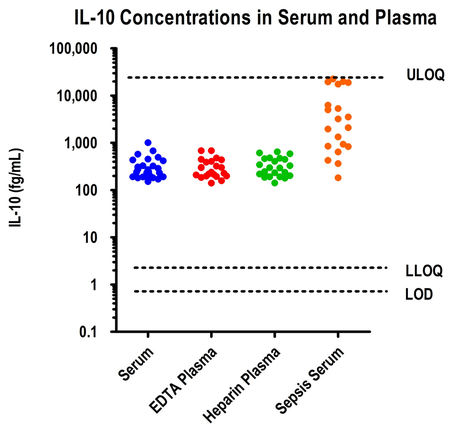
Electrochemiluminescence Technology

- 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 Assay Performance (IL-10)

Example data for the S-PLEX IL-10 assay are shown below. Similar assay performance verification experiments were conducted for the other assays evaluated in this study. The table in section 6 shows limits of detection (LOD) for all assays.





The graph to the upper left shows a representative calibration curve for the S-PLEX IL-10 assay. The LOD was 0.7 fg/mL. The graph on the upper right shows IL-10 concentrations in apparently healthy serum and plasma samples and in sepsis serum samples. The lower limit of quantitation (LLOQ) and the upper limit of quantitation (ULOQ) were 2.3 fg/mL and 25,000 fg/mL, respectively.

4 Assay Specificity (IL-10)

Assay specificity was determined by spike recovery, dilution linearity, and depletion experiments. Spike recovery data are shown for the S-PLEX IL-10 assay. All analytes described achieved spike recovery and dilution linearity between 80% and 120%. Specificity of all assays was also demonstrated by depleting samples with antibodies that were not included in the assay (data not shown).

Spike Recovery

	Spike (fg/mL)	Measured (fg/mL)	% Recovery
Serum 1	unspiked	170	
	5,600	5,696	99
	2,800	3,251	110
	800	1,041	109
Serum 2	unspiked	176	
	5,600	6,161	107
	2,800	3,220	109
	800	1,130	119
Serum 3	unspiked	128	
	5,600	5,634	98
	2,800	2,807	96
	800	961	104
Average % Recovery:			106

	Spike (fg/mL)	Measured (fg/mL)	% Recovery
EDTA Plasma 1	unspiked	166	
	5,600	5,679	98
	2,800	3,023	102
	800	1,110	118
EDTA Plasma 2	unspiked	158	
	5,600	5,840	101
	2,800	3,074	104
	800	1,077	115
Average % Recovery:			106

	Spike (fg/mL)	Measured (fg/mL)	% Recovery
Heparin Plasma 1	unspiked	227	
	5,600	5,412	93
	2,800	2,944	97
	800	1,151	115
Heparin Plasma 2	unspiked	196	
	5,600	6,095	105
	2,800	3,020	101
	800	1,127	116
Average % Recovery:			105

Dilution Linearity

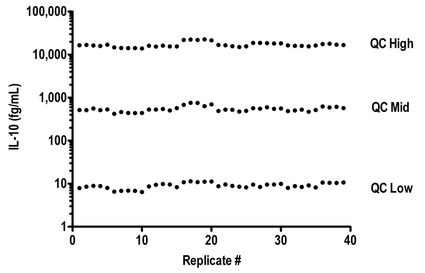
	Dilution Factor	Measured (fg/mL)	% Recovery
Serum 1	undiluted	578	
	2x	323	112
	4x	153	106
	8x	82	113
Serum 2	undiluted	492	
	2x	253	103
	4x	138	112
	8x	65	106
Serum 3	undiluted	738	
	2x	385	104
	4x	191	104
	8x	105	114
Average % Recovery:			108

	Dilution Factor	Measured (fg/mL)	% Recovery
EDTA Plasma 1	undiluted	563	
	2x	293	104
	4x	141	100
	8x	74	105
EDTA Plasma 2	undiluted	724	
	2x	391	108
	4x	190	105
	8x	101	111
EDTA Plasma 3	undiluted	556	
	2x	286	103
	4x	144	103
	8x	74	106
Average % Recovery:			105

	Dilution Factor	Measured (fg/mL)	% Recovery
Heparin Plasma 1	undiluted	557	
	2x	294	106
	4x	143	103
	8x	76	109
Heparin Plasma 2	undiluted	732	
	2x	364	100
	4x	188	103
	8x	95	104
Average % Recovery:			104

5 Reproducibility (IL-10)

Assay reproducibility was assessed by running five replicates of a high, medium, and low QC sample per plate on eight plates on four days by two operators.

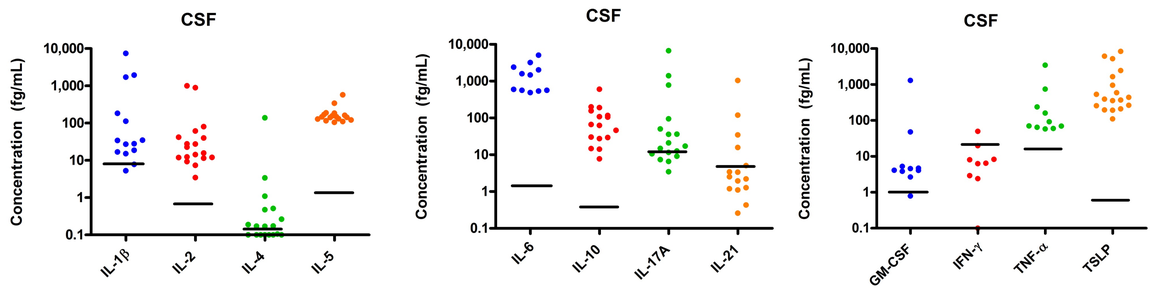


	% CV			
	Within Run	Between Run	Between Day	Total
QC High	2.6	15.1	0.0	15.3
QC Mid	4.9	14.2	2.3	15.2
QC Low	4.9	12.7	8.0	15.8

6 Measurements in CSF

The following low-abundance cytokines were selected as model analytes: IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-17A, IL-21, IFN-γ, GM-CSF, TSLP, and TNF-α. The LODs achieved for S-PLEX assays for these analytes ranged from ~0.2 to ~20 fg/mL. The native levels of these analytes were detectable in most tested serum and plasma samples.

Normal CSF samples were obtained from a sample vendor (these samples were deemed normal after culture for three days did not reveal any bacterial growth). Samples were measured neat for all analytes. The solid line indicates the LOD for each assay. Seven out of the twelve analytes tested were detected in normal CSF.



	S-PLEX Assay											
	IL-1β	IL-2	IL-4	IL-5	IL-6	IL-10	IL-17A	IL-21	GM-CSF	IFN-γ	TNF-α	TSLP
Sample Range (fg/mL)	<9 - 7,497	3 - 1,005	<0.2 - 140	105 - 59,661	489 - 21,423	8 - 25,571	<20 - 78,881	<7 - 1,045	<1 - 1,295	<20 - 61,512	58 - 3,475	111 - 8,358
Median (fg/mL)	23	19	<0.2	148	2,813	86	19	<7	4	<20	81	417
% Detected	61	100	28	100	100	100	50	22	90	20	100	100
Assay LOD (fg/mL)	9	0.8	0.2	2	2	0.4	20	7	1	20	20	0.8

7 Conclusion

We developed a next-generation assay format that is 100 to 1000 times more sensitive than the current limits of standard ELISA technology. This increased sensitivity enabled accurate quantitation of cytokine levels in the CSF of normal samples for 7 out of 12 cytokines tested. The data demonstrated that these assays are suitable for research studies that focus on understanding the role of these low-abundance cytokines in brain and nervous system disorders.

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