Development and Implementation of the S-PLEX® Platform for Converting Standard Immunoassays to High-Sensitivity Assays

Anahit Aghvanyan, Jen Gillies, Leonid Dzantiev, Yeming Wang, Banita Tamot, Chun-Jiang Zhang, Lumu Manandhar, Lalitha Janaki, Jon Buhrman, John Kenten, David Stewart, Jeff Debad, and Jacob N. Wohlstadter

Meso Scale Discovery, Rockville, MD, USA

Contact Information: jbuhrman@meso-scale.com

aaps PharmSci 360®

Advancing Pharmaceutical Sciences, Careers, and Community

PURPOSE

Measurements of circulating biomarkers in biofluids are widely used as an essential aspect of pharmaceutical research and drug development applications. While many biomarkers are readily measurable in the pg/mL to µg/mL range using common immunoassay techniques, some analytes are present at much lower concentrations, making them difficult to detect. Recent advances have enabled measurements of proteins in the fg/mL range but the number of available assays is limited and they can be difficult to develop. We describe an electrochemiluminescence-based platform that enables the conversion of standard sandwich immunoassays to high-sensitivity assays using common reagents such as biotinylated antibodies and streptavidin-coated plates. The platform has been analytically validated over multiple lots of assay components and has been tested on a set of 50 standard immunoassays, with the majority of assays showing significant (10- to 1000-fold) enhancement in sensitivity.

OBJECTIVES

MSD's next generation S-PLEX platform was developed with fg/mL sensitivity to enable measurement of low abundant analytes in normal donor samples that previously are not measurable by standard immunoassay methods.

METHODS

MSD's electrochemiluminescence detection technology uses SULFO-TAG™ labels that emit light upon electrochemical stimulation initiated at the electrode surfaces of 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.

S-PLEX assay components such as plates, diluents, blockers, labels, and other reagents were specifically designed to enable conversion of standard immunoassays to high sensitivity assays. Multiple lots of the components were produced and characterized, and the protocol for the new S-PLEX format was optimized for robustness.

Fifty MSD® immunoassays were converted to the S-PLEX format and evaluated. A group of 19 of these assays were selected for further characterization including measuring the limit of detection (LOD), lower limit of quantitation (LLOQ), upper limit of quantitation (ULOQ), dilution linearity, spike recovery and measurement of analyte concentrations in multiple sample matrices including serum, EDTA/heparin/citrate plasma, CSF, and stimulated cell supernatants (up to 72 samples for each assay). Concordance of the new S-PLEX assays with standard immunoassay formats was measured to demonstrate similar quantitation.

SENSITIVITY IMPROVEMENTS USING S-PLEX

Of the 50 assays converted to the S-PLEX format, 36 demonstrated sensitivities in the fg/mL range, and greater than 50% showed 10- to 1000-fold improvement over standard immunoassay formats. Representative assays demonstrating greater than 100-fold improvement in sensitivity are shown in the table.

Assay Improvement in S-PLEX

Assay	LLOQ fg/mL (S-PLEX format)	LLOQ fg/mL (Standard format)	Fold Improvement
IL-4	4.9	900	184
IL-10	9.8	1,400	143
IL-17A	100	12,000	120
TSLP	9.0	920	102
GM-CSF	8.7	1,700	195

ASSAY PERFORMANCE

Metric	IL-2	IL-4	IL-6	IL-10	IL-17A	TSLP	TNF-α	GM-CSF	IL-12p70	IL-22	IL-5	G-CSF	IL-15	IL-16	IL-23	IFN-α2a	Eotaxin-3	IL-9	MCP-3
Avg. LLOD (fg/ml)	3.57	0.41	0.34	3.01	8.63	5.06	4.29	0.99	1.79	2.06	1.07	21.2	1.7	201	22	4.11	9.2	5.79	144
LLOQ (fg/ml)	10.3	2.86	1.29	7.55	14.6	14.8	14.9	2.16	3.37	14.8	5.9	54.2	12	428	66	7.2	34.8	38.7	719
Assay Range (logs)	4.0	4.4	4.1	3.6	4.4	4.0	4.1	4.2	4.1	4.1	4.3	4.2	4.1	3.4	3.7	4.1	4.1	4.1	3.6
Intra-plate %CV	2.0	2.9	3.0	3.4	5.3	3.6	2.7	3.5	3.6	3.6	3.2	4.6	3.5	3.5	3.8	4.6	3.3	2.1	7.4

Assay Performance Examples: IL-10 and IL-17A Assays

Top graphs compare representative standard curves for the IL-10 and IL-17A assays run in two standard immunoassay platforms (MSD U-PLEX® and V-PLEX®) and run in S-PLEX. The S-PLEX assays demonstrated more that 20X improvement in sensitivity, with LODs of 1.4 fg/ml and 38 fg/ml for IL-10 and IL-17A respectively (compared to 67 to 2,070 fg/ml for the standard assays).

Assay reproducibility was assessed by running two replicates of high (QC 01), medium (QC 02) and low (QC 03) control samples per plate on nineteen plates spread over 6 months and four operators. The lower graphs show concentration % recovery of the control samples. 100% of the IL-10 and more than 90% of IL-17A assay controls recovered within 70%-130% of the expected values, demonstrating the high robustness of the S-PLEX platform.

SAMPLE TESTING

Normal serum and plasma samples were tested on the S-PLEX assays. Data from a number of proinflammatory cytokines is shown below. These cytokines typically have very low endogenous levels that may not be detectable with standard immunoassays. The enhanced S-PLEX sensitivity allows quantitation of normal levels in nearly all of the samples tested.

Metric		IL-2	IL-4	IL-6	IL-10	IL-17A	TNF-α	IL-12p70
Serum	Median (fg/mL)	129	6.9	903	171	490	116	66.9
	Range (fg/mL)	57.2 - 563	2.1 - 36.5	397 - 6763	73 - 871	133 - 1872	78.8 - 962	33.0 – 259
	% Detected	100	100	100	100	100	100	100
EDTA Plasma	Median (fg/mL)	148	4.4	759	153	531	78.2	60.6
	Range (fg/mL)	74.2 - 291	0.4 - 9.4	315 - 1299	76 - 381	166 - 1883	54.8 - 118	30.1 – 87.6
	% Detected	100	90	100	100	100	100	100
Heparin Plasma	Median (fg/mL)	150	6.5	990	158	499	110	40.9
	Range (fg/mL)	78.1 - 349	1.6 - 19.8	340 - 1360	76 - 381	154 - 2003	79.8 - 210	35.2 – 96.5
	% Detected	100	100	100	100	100	100	100
Citrate Plasma	Median (fg/mL)	122	4.06	748	160	435	83.5	61
	Range (fg/mL)	59.0 - 267	0.3 - 11.5	327 - 1341	77 - 871	127 - 1360	57.0 - 143	27.8 – 82.1
	% Detected	100	90	100	100	100	100	100

A comparison of normal sample levels of IL-10 (n=32) and IL-17A (n=16) in healthy donors measured on S-PLEX and two standard MSD immunoassay formats is shown below. Most samples with low endogenous levels were either undetectable or near the LOD of the standard assays, but readily detectable with the S-PLEX assays.

SAMPLE CONCORDANCE

Sample concentrations measured on S-PLEX and standard immunoassay formats for IL-10 and IL-17A assays showed good correlation (r² > 0.95, slopes 0.8-1.1). Representative concordance data from a single run of stimulated samples spiked into healthy donor sera are shown below.

MATRIX PERFORMANCE

Dilution linearity was evaluated in serum and plasma from four donors and is shown below as average percent recovery in the matrices tested. Recovery ranged from 84% to 119% across all assays and dilutions tested.

Metric	Fold Dilution	IL-2	IL-4	IL-6	IL-10	IL-17A	TNF-α	IL-12p70
Serum	2	104	105	108	102	108	104	93
	4	107	102	108	100	128	103	93
	8	109	104	108	101	115	102	96
EDTA Plasma	2	97	101	104	102	95	105	100
	4	106	107	108	102	96	105	109
	8	119	112	108	108	102	105	107
Heparin Plasma	2	106	106	102	99	99	102	105
	4	111	110	104	101	92	105	110
	8	119	116	105	103	110	103	112
Citrate Plasma	2	102	107	104	93	100	109	92
	4	104	108	101	98	100	109	93
	8	106	117	107	102	103	110	99

Spike recovery was also evaluated in different sample matrices for four donors. Average percent recovery is shown below, and ranged from 75% to 106%.

Metric	Spike Level	IL-2	IL-4	IL-6	IL-10	IL-17A	TNF-α	IL-12p70
Serum	High	94	92	94	92	85	93	92
	Med	97	94	94	93	85	82	89
	Low	96	90	96	97	85	89	89
EDTA Plasma	High	88	87	92	94	75	98	92
	Med	93	89	91	95	77	90	89
	Low	95	85	91	97	79	92	91
Heparin Plasma	High	94	106	96	95	83	95	96
	Med	94	106	92	95	83	89	89
	Low	87	98	99	95	84	91	95
Citrate Plasma	High	100	96	99	98	88	96	93
	Med	99	104	95	86	88	106	90
	Low	96	99	98	92	88	98	91

CONCLUSIONS

S-PLEX is a highly sensitive immunoassay format that can provide detection limits in the fg/ml range. Standard immunoassays can easily be converted to the S-PLEX format; improvements in sensitivity up to 1000-fold have been demonstrated.

The increased sensitivity of S-PLEX assays can allow quantitation of endogenous analytes which are not detectable using standard immunoassay formats.

The S-PLEX high-sensitivity format can be applied to bioanalytical assay formats other than sandwich immunoassays, with applications in various fields including pharmacodynamics, anti-drug antibody (ADA) and other anti-antibody detection assays.

Stimulated Samples
V-PLEX LLoD
S-PLEX LLoD
V-PLEX LLoQ
S-PLEX LLoQ

Meso Scale Discovery
A division of Meso Scale Diagnostics, LLC.
www.mesoscale.com®