

## **EVALUATION REPORT**

**Max-Planck-Institut für Psychiatrie  
Abteilung Translationale Forschung in der Psychiatrie**

**V-PLEX Human Biomarker 54-PLEX**

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Report prepared for  
*Dr. Janine Knauer-Arloth*

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## **ABSTRACT**

Meso Scale Discovery is pleased to present this report to **Dr. Janine Knauer-Arloth**.

In this one-day evaluation we successfully measured 52 human biomarkers in 74 plasma samples from patients with psychiatric disorders like depression or fear (aliquots from internal biobank). IL-12p70 and IL-8 could not be measured (for more details please see discussion). We used the V-PLEX Human Biomarker 54-PLEX Kit that consists of six individual multiplex panels that are optimized for best assay performance in order to provide high quality data.

The kit was able to detect 37 out of the 54 biomarkers in more than 80% of plasma samples. Only IL-1 $\beta$  and IL-21 were not detectable, at all. Robustness was very good, which was demonstrated by very low intra-plate %CVs of standard curves and controls (in average below 10%). Although the kit is provided on six individual multiplex-plates, all panels could be processed and analyzed in parallel within 7 hours – including measurement on the MSD MESO QuickPlex SQ 120 Imager (read time 90 sec per plate).

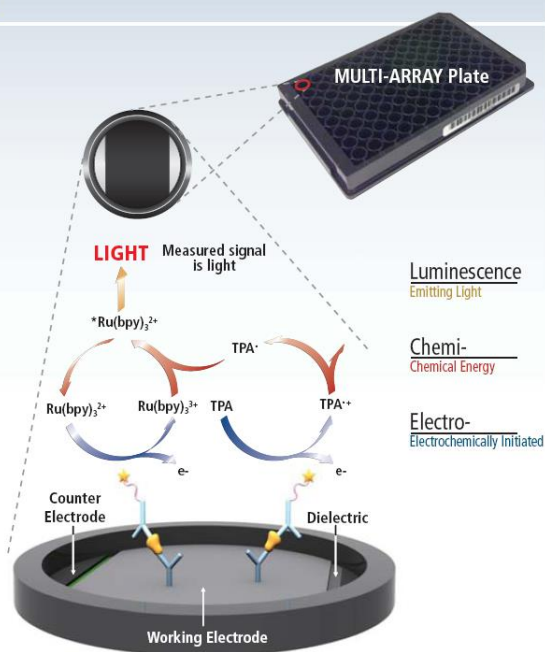
Additional instrument and software training was included in this evaluation.

## MSD TECHNOLOGY

Meso Scale Discovery's products are based on MULTI-ARRAY® technology, a proprietary combination of electrochemiluminescence detection and patterned arrays. Electrochemiluminescence detection offers a unique combination of sensitivity, dynamic range and convenience. Arrays bring speed and high density of information to discovery through miniaturization, organization and parallel processing of biological assays. Electrochemiluminescence detection uses labels that emit light when electrochemically stimulated. Background signals are minimal because the stimulation mechanism (electricity) is decoupled from the signal (light). Multiple excitation cycles of each label amplify the signal to enhance light levels and improve sensitivity. MSD's instruments use highly-efficient, custom designed optics and ultra-sensitive photodetectors to collect and quantitatively measure light emitted from the microplates. Proprietary electronics and efficient signal processing algorithms convert the measured signal into useful data quickly, keeping read time fast, even for high-density plate formats. Meso Scale Discovery offers an extensive product line of assay kits to enable the identification and quantitation of biomarkers and signaling molecules in single and multiplex formats for use in with both simple and complex matrices. A wide range of assay development materials is also available to develop new assays or transfer existing assays to improve workflow and performance as compared to traditional methods.

### Electrochemiluminescence Features:

- Minimal background signals and high signal to background ratios - the stimulation mechanism (electricity) is decoupled from the signal (light)
- Proximity - only labels bound near the electrode surface are detected, enabling non-washed assays
- Flexibility - labels are stable, non-radioactive, and are conveniently conjugated to biological molecules
- Emission at ~620 nm - eliminating problems with color quenching
- Signal amplification - multiple excitation cycles of each label enhance light levels and improve sensitivity
- Flexible surface coatings to suit most any biology
- Carbon electrode plate surface has 10X greater binding capacity than polystyrene
- Custom surface coatings and patterns



## **MATERIALS**

### V-PLEX Biomarker 54-PLEX Kit

- Kit components (including pre-coated plates, calibrators, controls, detection antibodies, diluents, wash buffer, and Read Buffer):
  - V-PLEX Chemokine Panel 1 (human) Kit
  - V-PLEX Proinflammatory Panel 1 (human) Kit
  - V-PLEX Cytokine Panel 1 (human) Kit
  - V-PLEX Cytokine Panel 2 (human) Kit
  - V-PLEX TH17 Panel 1 (human) Kit
  - V-PLEX Angiogenesis Panel 1 (human) Kit
  - V-PLEX Vascular Injury Panel 2 (human) Kit

### Other Reagents and Materials Provided by Customer

- Plate shaker
- Vortex
- Plate washer
- Reagent tubes
- Calibrated single channel pipettors that can accurately dispense 5-1000 µl.
- Calibrated multichannel pipettors that can accurately dispense 25 µl and 150 µl.
- Pipette tips
- Reagent reservoirs
- Adhesive plate seals
- Distilled water

## SAMPLES AND PROTOCOLS

In this evaluation we analyzed 74 plasma samples in singlicate analysis. V-PLEX assays require sample dilution in order to reduce matrix effects and/or to bring analyte levels into the assay range. Following sample dilutions have been done:

- V-PLEX Chemokine Panel 1: 4-fold dilution
- V-PLEX Proinflammatory Panel 1: 2-fold dilution
- V-PLEX Cytokine Panel 1: 2-fold dilution
- V-PLEX Cytokine Panel 2: 4-fold dilution
- V-PLEX TH17 Panel 1: 4-fold dilution
- V-PLEX Angiogenesis Panel 1: 2-fold dilution
- V-PLEX Vascular Injury Panel 2: 1000-fold dilution

### Brief Protocol for V-Plex Assays

**STEP 1: Add Blocker A Solution** (This step is only necessary for Angiogenesis Panel 1)

- Add 150 µL/well of Blocker A Solution to each well. Seal the plate with an adhesive plate seal. Incubate at room temperature with shaking for 1 hour.

**STEP 2: Wash and Add Sample**

- Wash plate 3 times with at least 150 µL/well of Wash Buffer.
- Add 50 µL/well of diluted sample, calibrator, or control per well (25 µL/well for Vascular Injury Panel 2). Seal the plate with an adhesive plate seal. Incubate at room temperature with shaking for 2 hours.

**STEP 3: Wash and Add Detection Antibody Solution**

- Wash plate 3 times with at least 150 µL/well of Wash Buffer.
- Add 25 µL of detection antibody solution to each well. Seal the plate with an adhesive plate seal. Incubate at room temperature with shaking for 2 hours (1 hour for Vascular Injury Panel 2).

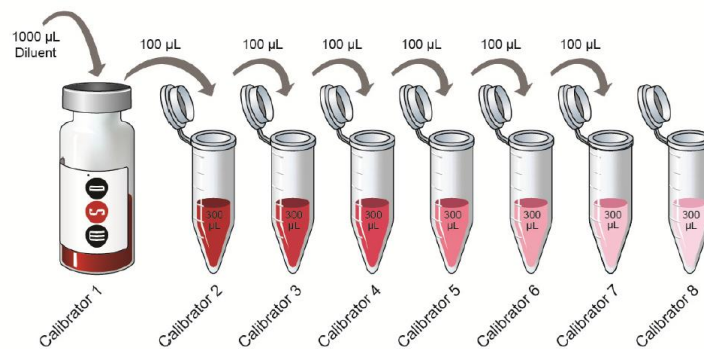
**STEP 4: Wash and Read**

- Wash the plate 3 times with at least 150 µL/well of Wash Buffer.
- Add 150 µL of 2X Read Buffer T to each well. Analyze the plate on the MSD instrument (1X Read Buffer T for Vascular Injury Panel 1).

**Note:** Chemokine Panel 1 Kit requires 10 min incubation in Read Buffer T at room temperature (no shaking).

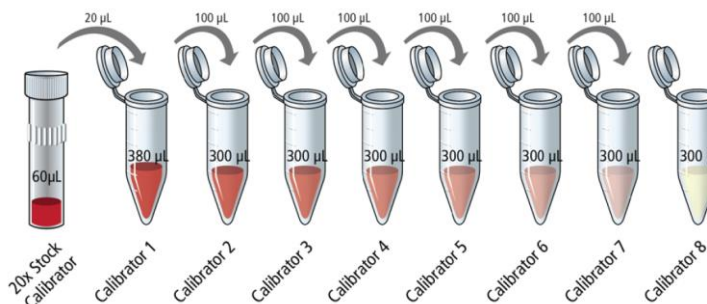
## Standards

V-PLEX Chemokine Panel 1, V-PLEX Proinflammatory Panel 1, V-PLEX Cytokine Panel 1, V-PLEX Cytokine Panel 2, V-PLEX TH17 Panel 1 kits are supplied with a multi-analyte lyophilized calibrator that yields the recommended highest calibrator concentration when reconstituted in 1000  $\mu\text{L}$  of Diluent. The concentration of each marker in the stock is listed on the lot-specific Certificate of Analysis. The graphic below demonstrates the dilution scheme for creating the standard curves.

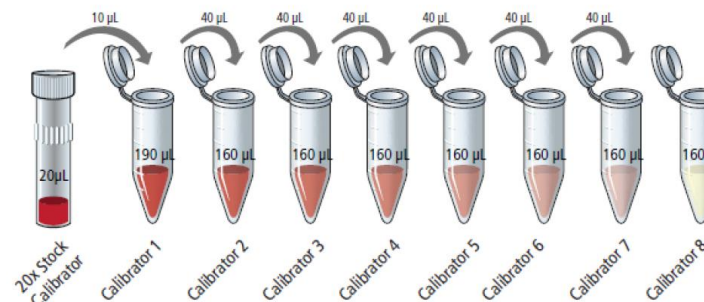


V-PLEX Angiogenesis Panel 1 and V-PLEX Vascular Injury Panel 2 kits are supplied with frozen multi-analyte calibrator stocks (20-fold concentrated). The concentration of each marker in the stock is listed on the lot-specific Certificate of Analysis. The graphics below demonstrate the dilution scheme for creating the standard curves:

### V-PLEX Angiogenesis Panel 1:



### V-PLEX Vascular Injury Panel 2:



**Plate Layout**

	1	2	3	4	5	6	7	8	9	10	11	12
A	Standard1		Sample 1	Sample 8	Sample 15	Sample 21	Sample 29	Sample 37	Sample 45	Sample 53	Sample 60	Sample 68
B	Standard2		Sample 2	Sample 9	Sample 16	Sample 22	Sample 30	Sample 38	Sample 46	Sample 54	Sample 61	Sample 69
C	Standard3		Sample 3	Sample 10	Control 3	Sample 23	Sample 31	Sample 39	Sample 47	Control 2	Sample 62	Sample 70
D	Standard4		Control 1	Sample 11	Sample 17	Sample 24	Sample 32	Sample 40	Sample 48	Sample 55	Sample 63	Control 3
E	Standard5		Sample 4	Sample 12	Control 2	Sample 25	Sample 33	Sample 41	Sample 49	Sample 56	Sample 64	Sample 71
F	Standard6		Sample 5	Sample 13	Sample 18	Sample 26	Sample 34	Sample 42	Sample 50	Sample 57	Sample 65	Sample 72
G	Standard7		Sample 6	Control 1	Sample 19	Sample 27	Sample 35	Sample 43	Sample 51	Sample 58	Sample 66	Sample 73
H	Standard8		Sample 7	Sample 14	Sample 20	Sample 28	Sample 36	Sample 44	Sample 52	Sample 59	Sample 67	Sample 74

**Note:** Only standards and controls have been analyzed in duplicates.

**DATA ANALYSIS**

The data was analyzed using MSD WORKBENCH Software 4.0. The Software uses 4 parameter logistic fit with  $1/y^2$  weighting for curve fitting.

For more information on WORKBENCH please consult the Quick Start Guides provided, and the online self-study video modules available on the MSD website. All information and a free-of-charge standalone desktop version of WORKBENCH can be found and downloaded at

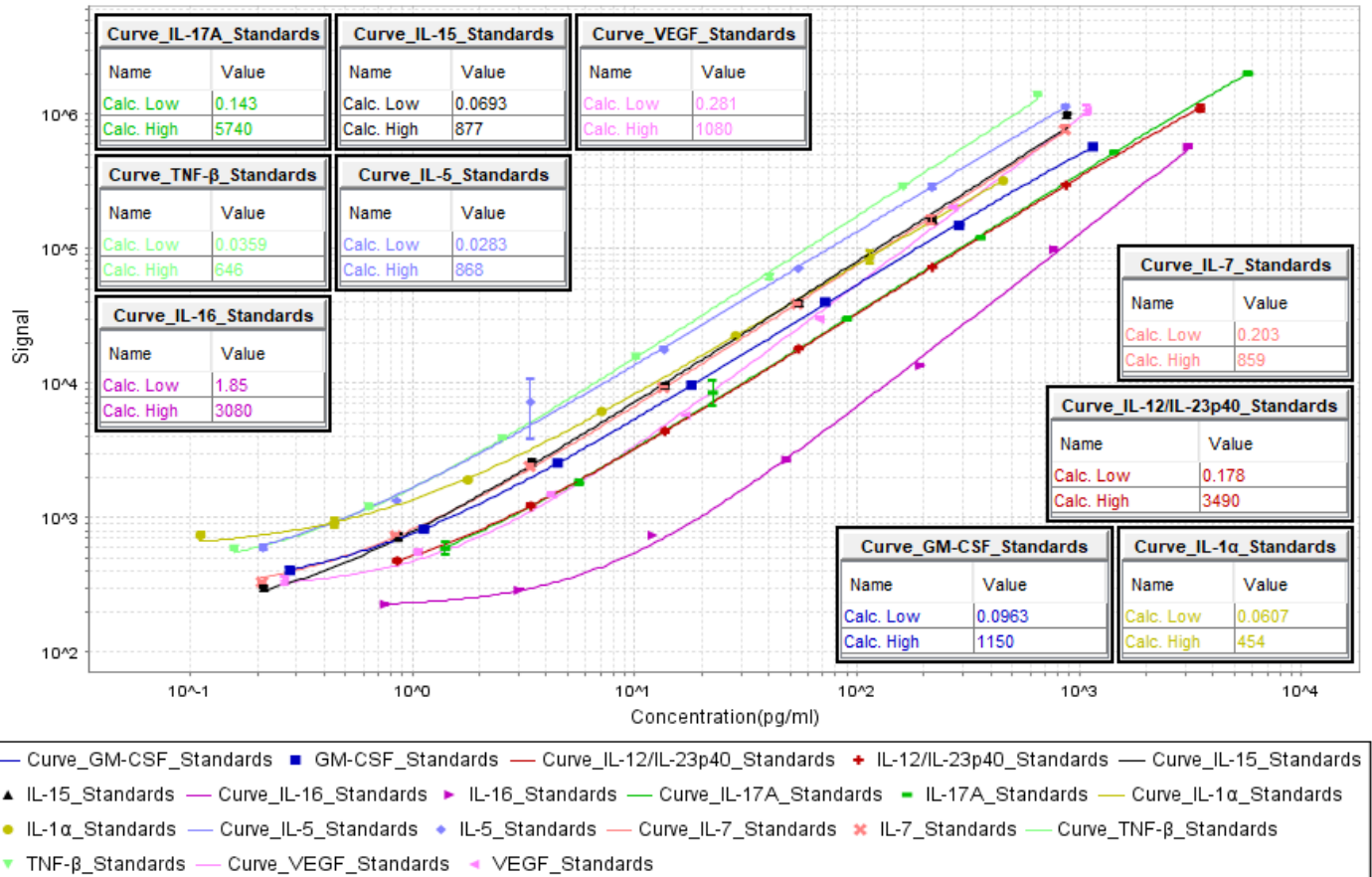
<http://www.meso-scale.com/CatalogSystemWeb/WebRoot/products/software.aspx>



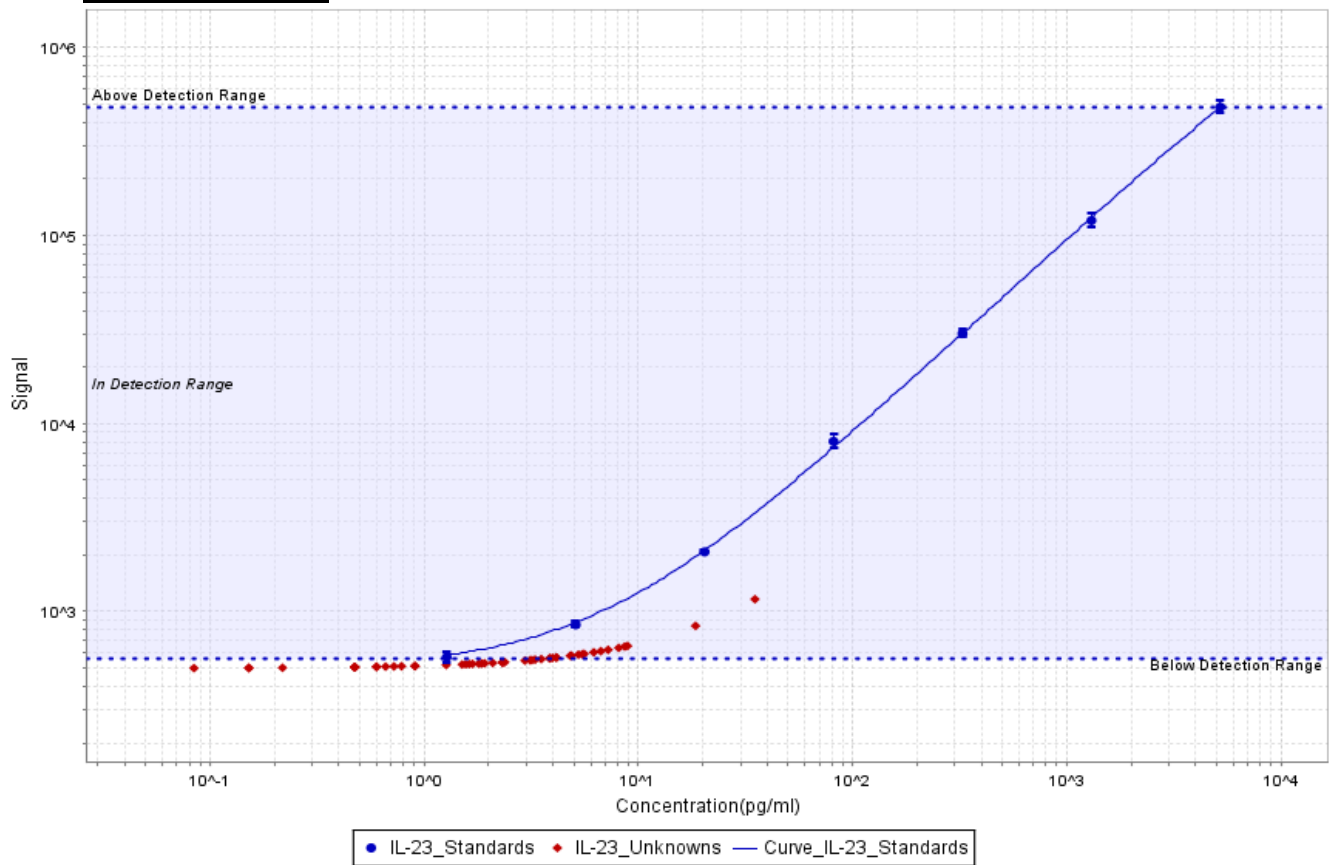
## RESULTS

### Example Plots

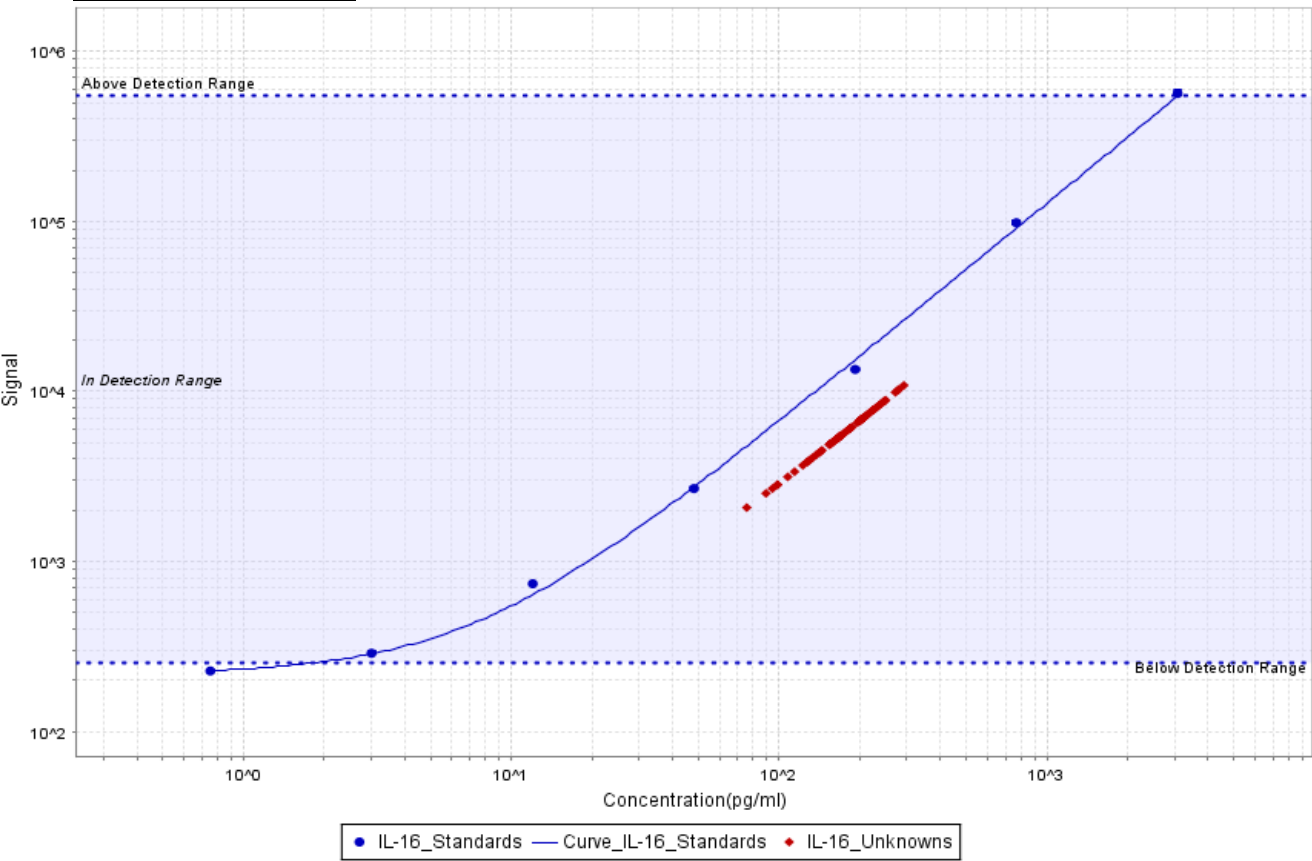
#### Cytokine Panel 1



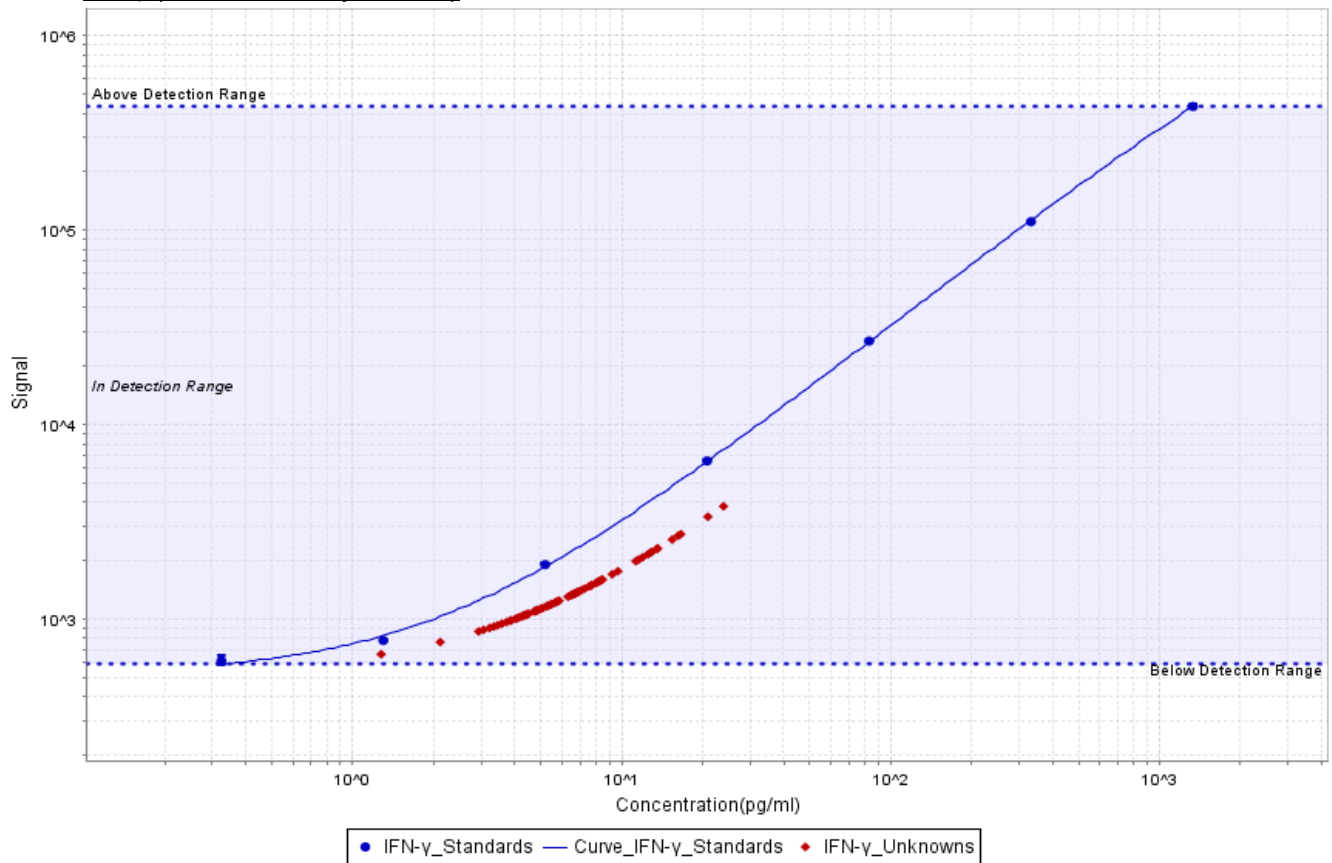
IL-23 (TH17 Panel 1)



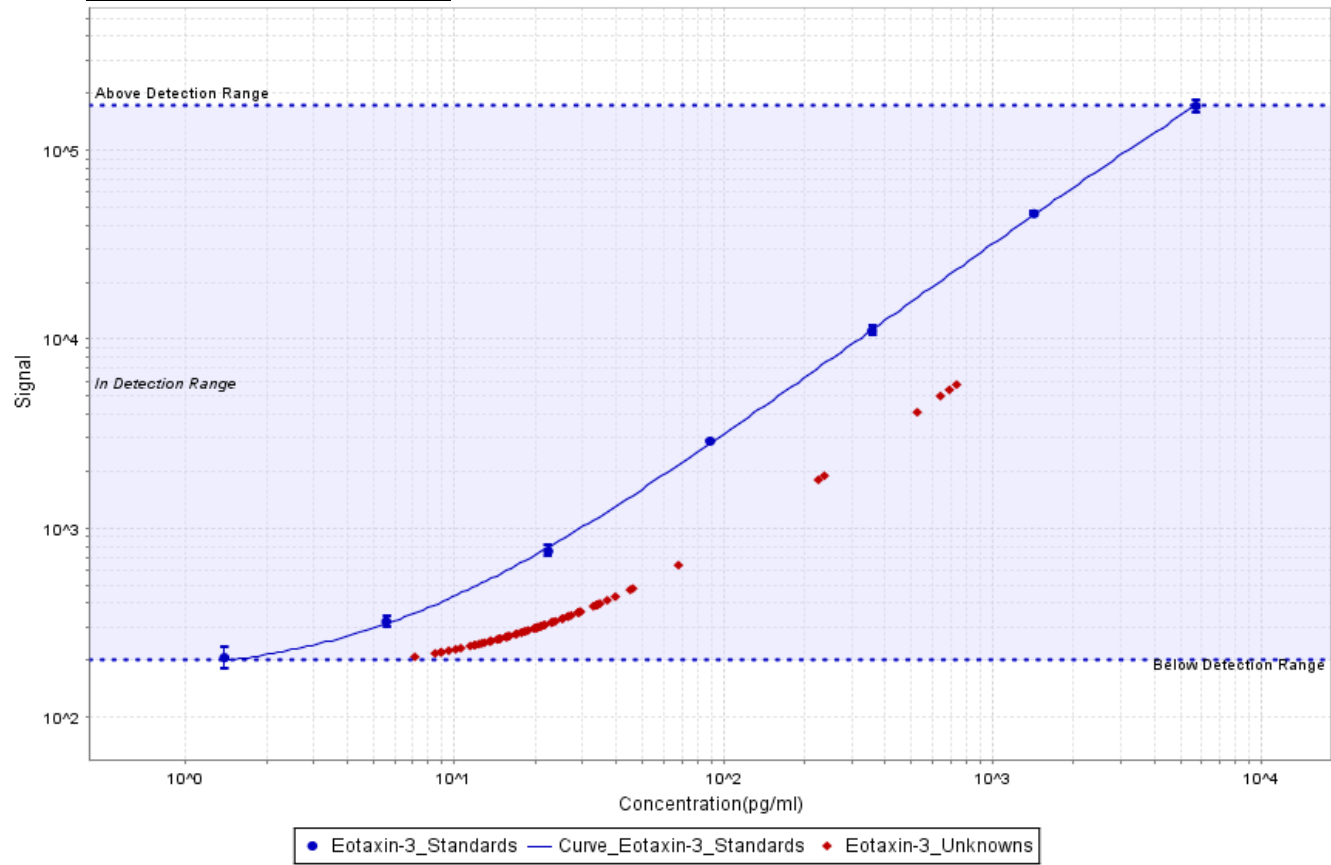
IL-16 (Cytokine Panel 1)



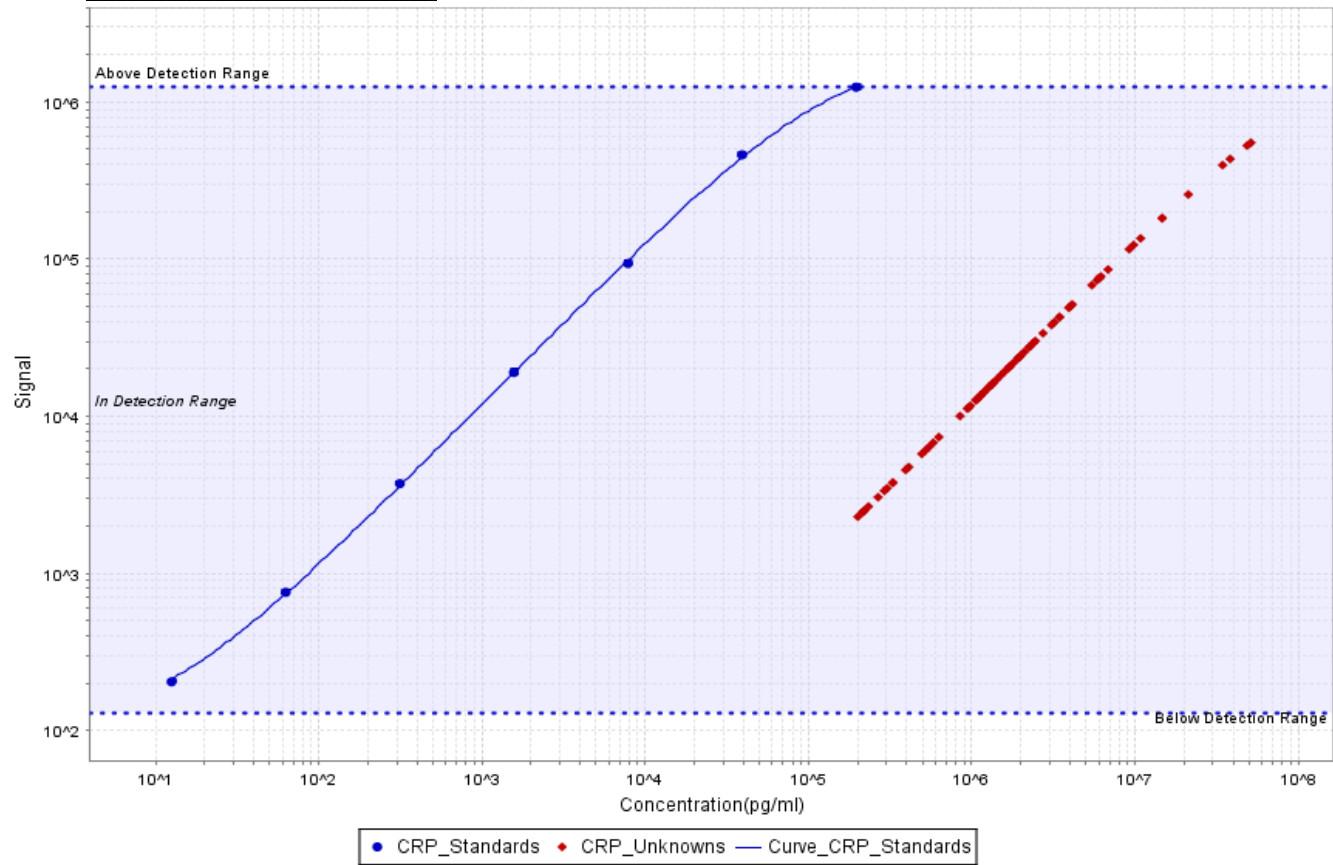
IFN- $\gamma$  (Proinflammatory Panel 1)



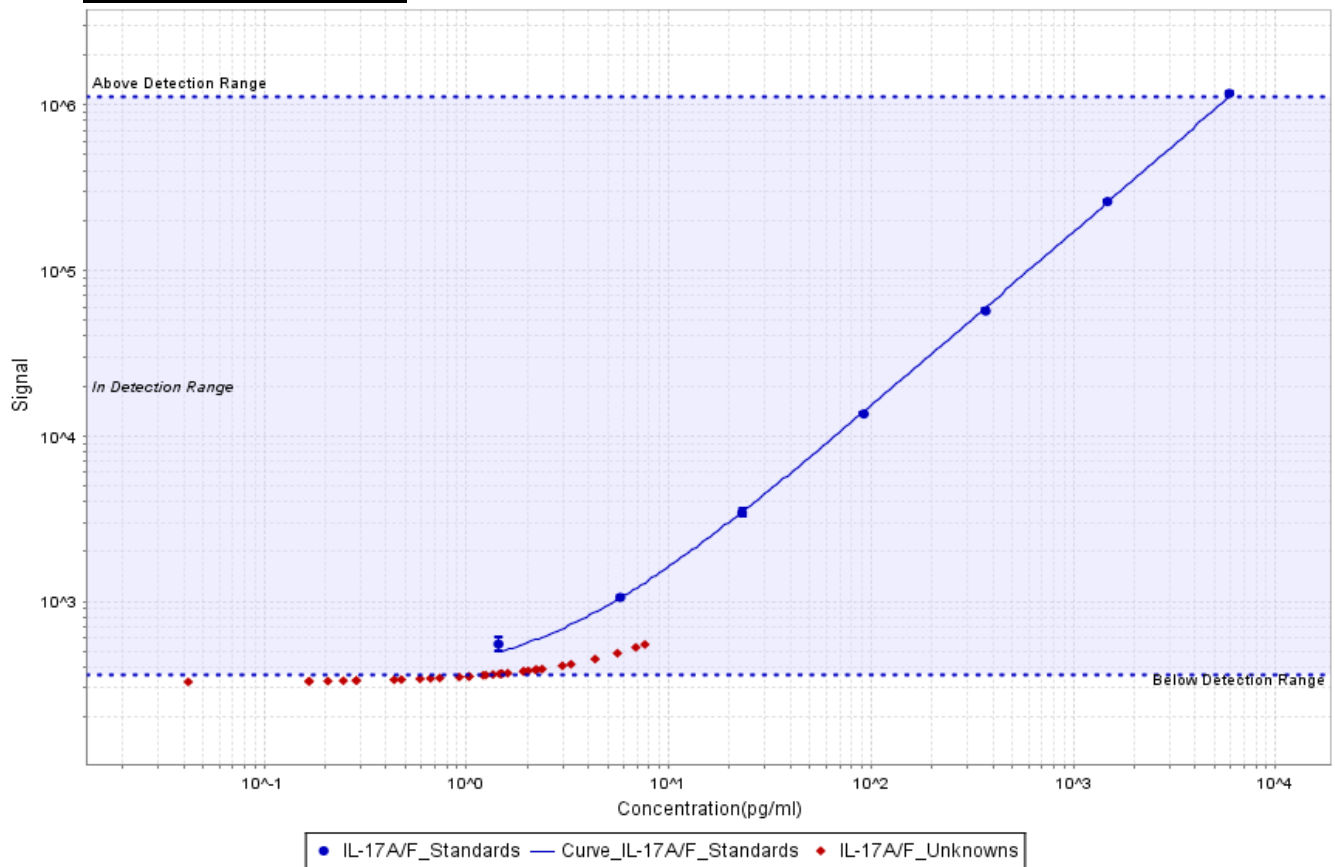
Eotaxin-3 (Chemokine Panel 1)



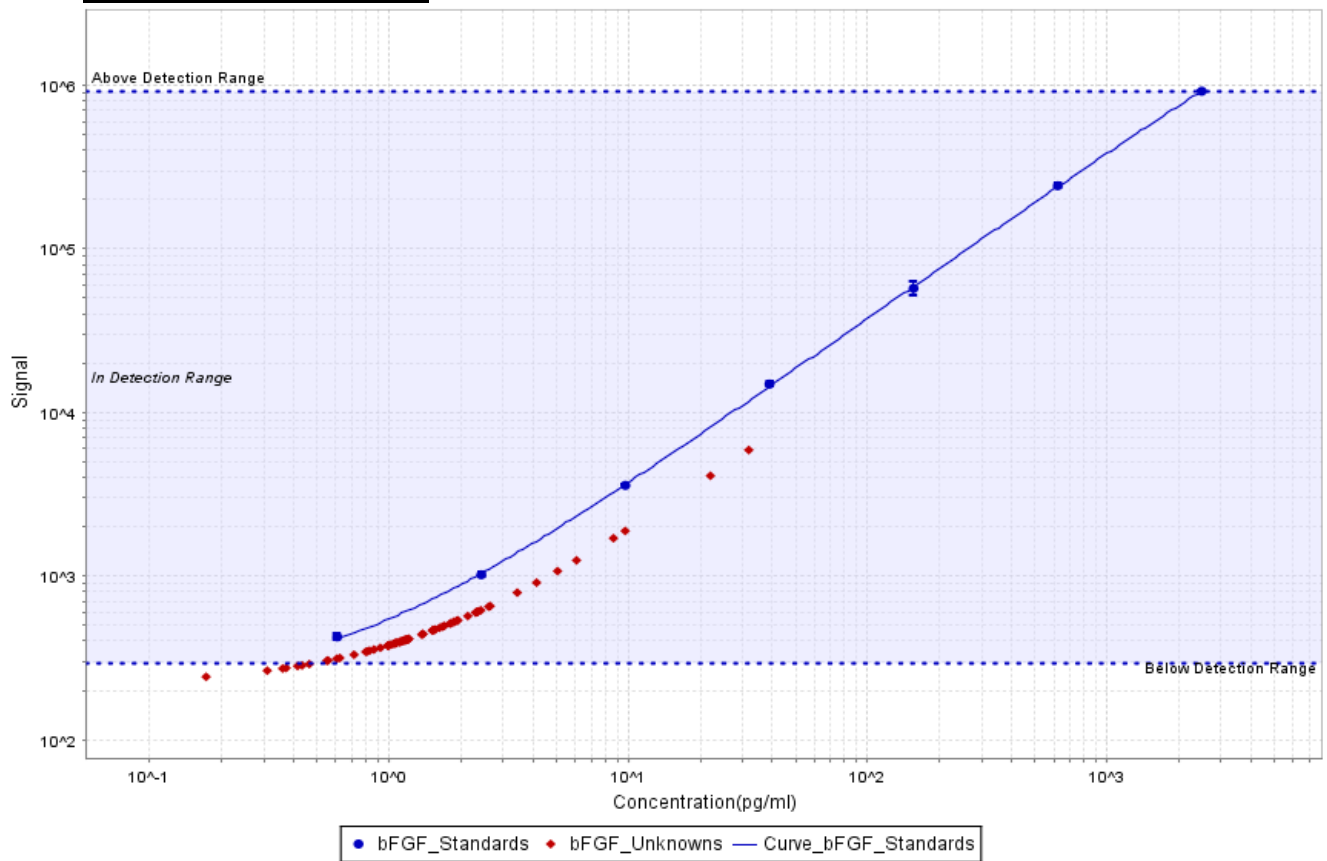
CRP (Vascular Injury Panel 2)



IL-17A/F (Cytokine Panel 2)



bFGF (Angiogenesis Panel 1)





## Assay Ranges and Sensitivities

**Angiogenesis Panel 1**

	<b>bFGF</b>	<b>Flt-1</b>	<b>PlGF</b>	<b>Tie-2</b>	<b>VEGF</b>	<b>VEGF-C</b>	<b>VEGF-D</b>
<b>LLOD (pg/ml)</b>	0.24	0.69	0.09	20.7	0.60	7.77	3.44
<b>ULOD (pg/ml)</b>	2490	9830	1210	92000	2450	24600	28400
<b>Dynamic Range</b>	4.0	4.2	4.1	3.6	3.6	3.5	3.9

**Chemokine Panel 1**

	<b>Eotaxin</b>	<b>Eotaxin-3</b>	<b>IL-8(HA)</b>	<b>IP-10</b>	<b>MCP-1</b>	<b>MCP-4</b>	<b>MDC</b>	<b>MIP-1<math>\alpha</math></b>	<b>MIP-1<math>\beta</math></b>	<b>TARC</b>
<b>LLOD (pg/ml)</b>	3.62	1.47	299	0.12	0.04	1.72	5.70	1.97	0.59	0.09
<b>ULOD (pg/ml)</b>	1790	5710	66700	2370	544	683	9350	1090	1280	1870
<b>Dynamic Range</b>	2.7	3.6	2.3	4.3	4.1	2.6	3.2	2.7	3.3	4.3

**Cytokine Panel 1**

	<b>GM-CSF</b>	<b>IL-12/IL-23p40</b>	<b>IL-15</b>	<b>IL-16</b>	<b>IL-17A</b>	<b>IL-1<math>\alpha</math></b>	<b>IL-5</b>	<b>IL-7</b>	<b>TNF-<math>\beta</math></b>	<b>VEGF</b>
<b>LLOD (pg/ml)</b>	0.10	0.18	0.07	1.68	0.14	0.06	0.03	0.20	0.04	0.28
<b>ULOD (pg/ml)</b>	1150	3490	877	3080	5740	454	868	859	646	1080
<b>Dynamic Range</b>	4.1	4.3	4.1	3.3	4.6	3.9	4.5	3.6	4.3	3.6

**Cytokine Panel 2**

	<b>IL-17A/F</b>	<b>IL-17B</b>	<b>IL-17C</b>	<b>IL-17D</b>	<b>IL-1RA</b>	<b>IL-3</b>	<b>IL-9</b>	<b>TSLP</b>
<b>LLOD (pg/ml)</b>	0.35	0.48	0.45	2.11	6.20	1.52	0.03	0.07
<b>ULOD (pg/ml)</b>	5900	1210	2790	8780	905	2590	1620	494
<b>Dynamic Range</b>	4.2	3.4	3.8	3.6	2.2	3.2	4.7	3.8

**Proinflammatory Panel 1**

	<b>IFN-<math>\gamma</math></b>	<b>IL-10</b>	<b>IL-12p70</b>	<b>IL-13</b>	<b>IL-1<math>\beta</math></b>	<b>IL-2</b>	<b>IL-4</b>	<b>IL-6</b>	<b>IL-8</b>	<b>TNF-<math>\alpha</math></b>
<b>LLOD (pg/ml)</b>	0.31	0.01		0.29	0.06	0.13	0.02	0.17		0.07
<b>ULOD (pg/ml)</b>	1330	383		500	578	1550	285	752		342
<b>Dynamic Range</b>	3.6	4.4		3.2	4.0	4.1	4.2	3.6		3.7

**TH17 Panel 1**

	<b>IL-17 Gen. B</b>	<b>IL-21</b>	<b>IL-22</b>	<b>IL-23</b>	<b>IL-27</b>	<b>IL-31</b>	<b>MIP-3<math>\alpha</math></b>
<b>LLOD (pg/ml)</b>	2.63	3.86	0.49	0.87	10.3	0.05	0.25
<b>ULOD (pg/ml)</b>	2640	1200	545	5220	19300	1060	393
<b>Dynamic Range</b>	3.0	2.5	3.0	3.8	3.3	4.3	3.2

**Vascular Injury Panel 2**

	<b>CRP</b>	<b>ICAM-1</b>	<b>SAA</b>	<b>VCAM-1</b>
<b>LLOD (pg/ml)</b>	4.15	2.29	20.5	8.67
<b>ULOD (pg/ml)</b>	196000	50000	248000	47900
<b>Dynamic Range</b>	4.7	4.3	4.1	3.7

**Tested Samples****Angiogenesis Panel 1**

Sample Type	Statistic	bFGF	Flt-1	PlGF	Tie-2	VEGF	VEGF-C	VEGF-D
Serum (N=74)	Average (pg/ml)	2.38	65.2	4.83	3484	55.9	35.4	1393
	Range (pg/ml)	0.31-31.9	35.8-121	2.77-8.46	1384-7424	13.6-746	12.1-210	118-9246
	% Detected	99	100	100	100	100	97	100

**Chemokine Panel 1**

Sample Type	Statistic	Eotaxin	Eotaxin-3	IL-8(HA)	IP-10	MCP-1	MCP-4	MDC	MIP-1 $\alpha$	MIP-1 $\beta$	TARC
Serum (N=74)	Average (pg/ml)	166	61.2	3893	416	94.2	39.3	777	11.9	60.1	39.0
	Range (pg/ml)	83.8-302	7.12-735	442-9861	212-1532	55.6-178	8.7-98.8	206-1988	2.64-105	27.5-129	5.72-114
	% Detected	100	100	8	100	100	100	100	99	100	100

**Cytokine Panel 1**

Sample Type	Statistic	GM-CSF	IL-12/IL-23p40	IL-15	IL-16	IL-17A	IL-1 $\alpha$	IL-5	IL-7	TNF- $\beta$	VEGF
Serum (N=74)	Average (pg/ml)	0.14	124	2.60	176	2.56	6.22	0.53	1.09	0.12	42.5
	Range (pg/ml)	0.10-0.23	51.4-274	1.48-4.19	72.4-286	0.69-33.8	1.63-29.5	0.11-7.51	0.22-10.3	0.04-0.26	18.0-505
	% Detected	18	100	100	100	100	100	100	97	89	100

**Cytokine Panel 2**

Sample Type	Statistic	IL-17A/F	IL-17B	IL-17C	IL-17D	IL-1RA	IL-3	IL-9	TSLP
Serum (N=73)	Average (pg/ml)	2.18	2.60	3.21	12.0	218	7.10	0.36	1.05
	Range (pg/ml)	0.44-7.62	0.52-26.4	0.55-20.0	2.34-157	18.2-3228	2.33-12.4	0.04-1.35	0.26-3.67
	% Detected	37	67	68	79	100	5	32	99

**Proinflammatory Panel 1**

Sample Type	Statistic	IFN- $\gamma$	IL-10	IL-12p70	IL-13	IL-1 $\beta$	IL-2	IL-4	IL-6	IL-8	TNF- $\alpha$
Serum (N=74)	Average (pg/ml)	7.18	0.29		1.50	ND	1.95	0.04	0.69		2.02
	Range (pg/ml)	1.28-23.9	0.02-1.52		0.33-5.76	NA	0.14-28.9	0.02-0.07	0.18-3.01		1.04-4.04
	% Detected	100	99		9	0	28	9	78		100

**TH17 Panel 1**

Sample Type	Statistic	IL-17 Gen. B	IL-21	IL-22	IL-23	IL-27	IL-31	MIP-3 $\alpha$
Serum (N=74)	Average (pg/ml)	14.0	ND	1.44	4.94	1102	0.31	8.26
	Range (pg/ml)	12.8-15.2	NA	0.58-3.10	0.91-35.0	275-2129	0.08-0.78	0.89-51.4
	% Detected	3	0	22	51	100	92	89

**Vascular Injury Panel 2**

Sample Type	Statistic	CRP	ICAM-1	SAA	VCAM-1
Serum (N=74)	Average (ng/ml)	5049	610	15961	668
	Range (ng/ml)	201-51299	275-1351	743-296823	338-1581
	% Detected	100	100	100	100

ND = Non-detectable, NA = Not Applicable

% Detected = % of samples with concentrations at or above the LLOD

# Control-Recoveries and %CVs

Angiogenesis Panel 1

		Average % Recovery	%CV
bFGF	Control 1	96	0.1
	Control 2	106	6.0
	Control 3	143	5.2
Flt-1	Control 1	76	4.4
	Control 2	80	0.7
	Control 3	128	3.0
PlGF	Control 1	99	5.1
	Control 2	88	7.5
	Control 3	114	4.0
Tie-2	Control 1	86	0.9
	Control 2	71	5.3
	Control 3	179	0.5
VEGF	Control 1	128	1.8
	Control 2	122	5.1
	Control 3	147	1.5
VEGF-C	Control 1	107	7.4
	Control 2	116	5.0
	Control 3	164	6.7
VEGF-D	Control 1	103	2.0
	Control 2	259	9.8
	Control 3	161	11.2

Chemokine Panel 1

		Average % Recovery	%CV
Eotaxin	Control 1	106	2.6
	Control 2	98	0.5
	Control 3	101	3.7
Eotaxin-3	Control 1	146	6.2
	Control 2	149	4.4
	Control 3	136	7.7
IL-8(HA)	Control 1	97	0.5
	Control 2	78	21.6
	Control 3	109	0.3
IP-10	Control 1	109	6.3
	Control 2	105	0.3
	Control 3	115	1.9
MCP-1	Control 1	109	6.8
	Control 2	87	37.1
	Control 3	87	12.6
MCP-4	Control 1	86	4.7
	Control 2	86	1.5
	Control 3	99	12.4
MDC	Control 1	97	1.9
	Control 2	87	1.7
	Control 3	96	2.9
MIP-1 $\alpha$	Control 1	100	1.4
	Control 2	82	33.3
	Control 3	101	11.1
MIP-1 $\beta$	Control 1	107	1.0
	Control 2	101	24.9
	Control 3	107	7.3
TARC	Control 1	137	6.7
	Control 2	115	15.1
	Control 3	116	20.4

Cytokine Panel 1

		Average % Recovery	%CV
GM-CSF	Control 1	101	1.5
	Control 2	105	0.3
	Control 3	107	3.3
IL-12/IL-23p40	Control 1	102	2.0
	Control 2	106	1.2
	Control 3	114	2.6
IL-15	Control 1	112	1.7
	Control 2	120	5.6
	Control 3	121	2.5
IL-16	Control 1	99	5.3
	Control 2	106	2.4
	Control 3	117	1.0
IL-17A	Control 1	99	4.4
	Control 2	103	1.2
	Control 3	102	0.0
IL-1 $\alpha$	Control 1	94	4.5
	Control 2	96	6.2
	Control 3	97	3.6
IL-5	Control 1	97	4.0
	Control 2	104	0.8
	Control 3	109	3.9
IL-7	Control 1	101	1.6
	Control 2	105	1.6
	Control 3	113	2.2
TNF- $\beta$	Control 1	100	6.0
	Control 2	100	3.1
	Control 3	113	7.0
VEGF	Control 1	100	3.9
	Control 2	103	0.6
	Control 3	111	3.5

Cytokine Panel 2

		Average % Recovery	%CV
IL-17A/F	Control 1	89	0.4
	Control 2	93	0.2
	Control 3	99	7.2
IL-17B	Control 1	111	3.5
	Control 2	116	4.5
	Control 3	116	7.4
IL-17C	Control 1	95	10.2
	Control 2	90	12.3
	Control 3	91	21.6
IL-17D	Control 1	77	9.6
	Control 2	82	0.3
	Control 3	91	4.7
IL-1RA	Control 1	92	4.8
	Control 2	90	11.2
	Control 3	95	13.4
IL-3	Control 1	86	4.8
	Control 2	94	0.9
	Control 3	98	4.4
IL-9	Control 1	106	5.1
	Control 2	121	1.3
	Control 3	102	6.7
TSLP	Control 1	85	4.9
	Control 2	94	6.1
	Control 3	117	6.3

Proinflammatory Panel 1

		Average % Recovery	%CV
IFN- $\gamma$	Control 1	107	1.9
	Control 2	115	0.0
	Control 3	123	0.3
IL-10	Control 1	104	5.1
	Control 2	107	2.9
	Control 3	114	0.3
IL-12p70	Control 1		
	Control 2		
	Control 3		
IL-13	Control 1	108	2.0
	Control 2	105	3.2
	Control 3	116	2.4
IL-1 $\beta$	Control 1	104	4.5
	Control 2	110	2.9
	Control 3	120	1.1
IL-2	Control 1	101	2.4
	Control 2	106	0.9
	Control 3	110	3.5
IL-4	Control 1	108	0.7
	Control 2	108	0.3
	Control 3	120	2.2
IL-6	Control 1	116	0.2
	Control 2	109	2.3
	Control 3	121	7.7
IL-8	Control 1		
	Control 2		
	Control 3		
TNF- $\alpha$	Control 1	107	2.9
	Control 2	112	7.9
	Control 3	120	0.5

TH17 Panel 1

		Average % Recovery	%CV
IL-17 Gen. B	Control 1	88	3.1
	Control 2	91	0.9
	Control 3	96	3.5
IL-21	Control 1	96	1.4
	Control 2	98	3.2
	Control 3	112	4.2
IL-22	Control 1	95	2.7
	Control 2	101	2.3
	Control 3	95	2.5
IL-23	Control 1	86	1.6
	Control 2	104	3.2
	Control 3	116	6.5
IL-27	Control 1	74	7.2
	Control 2	79	12.1
	Control 3	78	6.4
IL-31	Control 1	85	4.7
	Control 2	93	2.9
	Control 3	94	0.8
MIP-3 $\alpha$	Control 1	87	4.6
	Control 2	81	5.1
	Control 3	93	10.1

Vascular Injury Panel 2

		Average % Recovery	%CV
CRP	Control 1	112	0.9
	Control 2	120	0.5
	Control 3	122	1.2
ICAM-1	Control 1	116	1.1
	Control 2	122	2.4
	Control 3	133	1.7
SAA	Control 1	111	4.8
	Control 2	114	4.7
	Control 3	117	1.1
VCAM-1	Control 1	105	1.7
	Control 2	109	3.3
	Control 3	108	1.7

## DISCUSSION

Most assays performed as expected and according to the certificates of analysis. Due to missing detection antibodies in the assays IL-12p70 and IL-8 (Proinflammatory Panel 1) could not be measured. The IL-12p70 detection antibody was dried out and could not be used in the assay. IL-12p70 will be measured separately as singleplex assay. Concerning IL-8 the root cause is not clear. Most likely, we accidentally missed addition of the detection antibody. IL-1 $\beta$  and IL-21 were below limits of detection in all samples. Apparently, analyte levels were too low in these samples. 35 of 54 assays showed 100% detectability in all samples. In 5 assays at least more than 50% of samples had detectable analyte levels. 10 analytes could only be detected in less than 37% of samples. Samples have been analyzed in singlicates. Therefore, no replicate CVs are available.

%Recoveries of controls were within acceptable ranges for most analytes except for Angiogenesis Panel 1. Here, most controls showed recoveries above 120%. Further measurements will reveal, if increased control recoveries are specific to the lab equipment and site-specific nominal control values have to be established for this panel. Intra-plate CVs were very low and demonstrate excellent robustness of the V-PLEX kits and technology. The same is true for standard curves. Average %CV is below 10% for standards and controls across all panels (only few outliers were observed).

Differences in calculated concentrations of VEGF-A between Cytokine Panel 1 and Angiogenesis Panel 1 are due to different anchoring of VEGF-A calibrator to international standards (more details can be found within the according product inserts on pages 22 and 23, respectively).

Different performance of IL-17A assays (Cytokine Panel 1 and TH17 Panel 1) is due to different antibody generations. IL-17A Gen. B assay uses more specific antibodies (reduced cross-reactivity with IL-17A/F) and therefore shows lower analyte concentrations and less detectable native analyte levels in plasma samples.

Detection antibody used for IL-8(HA) assay in Chemokine Panel 1 is different from detection antibody used for IL-8 assay in Proinflammatory Panel 1, leading to different assay sensitivities.

Well C3 in Cytokine Panel 2 plate accidentally contained control in addition to sample and was not considered in analysis. The well was excluded from calculations.

## SUMMARY

- The MSD V-PLEX Human Biomarker 54-PLEX Kit was run successfully at Translationale Forschung in der Psychiatrie, MPI München.
- All assays showed sensitivity, robustness and general performance as described in the certificates of analysis and in the product inserts.
- Plasma samples could be measured at standard assay conditions without sample pre-treatment. 37 of 54 biomarkers could be detected in more than 80% of plasma samples. Only IL-1 $\beta$  and IL-21 were not detectable, at all. In addition, IL-12p70 and IL-8 were not detectable due to missing detection antibodies in the assays.
- Due to the large dynamic assay ranges (assays provide a dynamic range of 3-4 logs) all samples could be measured at the same dilution within one plate.
- The V-PLEX human Biomarker 54-PLEX Panel consists of six individual optimized panels on separate plates, which could be run in parallel within 7 hours (including measurement on the MSD MESO QuickPlex SQ 120 Imager).
- The protocols are very simple with only three to four steps throughout the whole assay.
- Measurement is rapid. Two clicks to read a plate. Read time is 90 seconds independent of size of multiplex.
- No daily maintenance or calibration is necessary for the QuickPlex instrument.
- Data can be analyzed with integrated WORKBENCH Software, which is optimized for analysis of multiplex measurements. The software can be installed and run on a desktop PC without additional costs.
- Partial plates can be run, when the number of samples to be tested is limited.
- MSD's V-PLEX assays provide high sensitivity and performance with lot-to-lot reproducibility for consistency in results over long term research studies.
- In addition to cytokine assays MSD offers over 400 kits for the quantitation of diseased focused secreted and intracellular biomarkers. MSD assays are available as singleplex assays, catalog multiplexes and custom multiplex panels.
- The new U-PLEX Platform offers high flexibility in customizing multiplex panels from a menu of 96 human assays.

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