# Fit-for-Purpose Multiplex Panels and Their Utility in Biomarker Screening

## **1** Abstract

Purpose: Exploratory studies that are conducted for the identification of biomarkers important to drug discovery and clinical diagnostics may include screening for 100+ biomarkers. Identification of potential leads can be misleading due to interference from large multiplex panels. In this study, smaller multiplex panels were evaluated for their utility in screening without a priori knowledge.

Methods: A biomarker screening panel was developed based on MSD's MULTI-ARRAY<sup>®</sup> technology, requiring less than 1 mL of sample to measure 122 analytes. The assays were grouped into 15 different multiplex panels following a fit-for-purpose approach. The dilution factors, diluent components, and specificity of reagents were optimized for each panel. Panels included MSD's analytically validated V-PLEX<sup>®</sup> Human Biomarker 40-Plex, which consists of biomarkers relevant to inflammation, immunology, angiogenesis, and vascular injury. The remaining assays were combined into multiplex panels of up to ten assays.

Results: Multiplex panels were developed in a 10-plex format to facilitate optimization of assay protocols and performance. Assays typically exhibited less than 1.0% non-specific binding. The dynamic range of each assay was 3 to 4 orders of magnitude, enabling quantification of samples from both normal and diseased states. Patient sample sets including serum, EDTA-plasma, cerebrospinal fluid, and urine were measured. Individual assays had good reproducibility across plates. For the majority of the assays, the median intra-plate coefficient of variation (CV) was <10% across samples that were within the quantitative range of the assay.

Conclusion: Biomarker screening by an unbiased approach allowed rapid identification of targets of potential clinical significance. Measurements across multiplex panels aided in stratification of patient populations and could be used to monitor disease activity. Use of multiplexes was ideal for screening a large number of analytes using minimal sample volume.

## **2** Methods

Samples were screened on a biomarker screening panel based on MSD's MULTI-ARRAY technology. Utilizing a fit-for-purpose methodology, 122 assays were grouped into 15 different multiplex panels. Dilution factors, diluent components, and specificity of reagents were optimized for each panel. Five of the panels were comprised of assays from MSD's analytically validated V-PLEX Human Biomarker 40-Plex Kit. The remaining assays were combined into multiplex panels of up to 10 assays. Less than one mL of sample was required to measure all 122 analytes.



### Electrochemiluminescence Technology

- Minimal non-specific background and strong responses to analyte yield high signal-tobackground 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** Specificity

To determine detection antibody specificity, blended calibrators were tested with individual detection antibodies. Testing was conducted for each of the 15 panels. We found that non-specific interactions were below 1.0% for most analytes. Representative data is shown below.

### non – specific signal %Non – specificity = specific signal

	Calbindin	Eotaxin-2	MIP-5	MMP-1	MMP-3	MMP - 9	Osteoactivin	P-Cadherin	TNF-RI	TNF-RII
Calibrator Conc. Tested (pg/mL)	6250	500	2500	25000	25000	125000	10000	25000	2500	625

	Blended Calibrator with Individual Detectors									
Spot	Calbindin	Eotaxin-2	MIP-5	MMP-1	MMP-3	MMP - 9	Osteoactivin	P-Cadherin	TNF-RI	TNF-RII
Calbindin	100%	< 1.0%	< 1.0%	< 1.0%	< 1.0%	< 1.0%	< 1.0%	< 1.0%	< 1.0%	< 1.0%
Eotaxin-2	< 1.0%	100%	< 1.0%	< 1.0%	< 1.0%	< 1.0%	< 1.0%	< 1.0%	< 1.0%	< 1.0%
MIP-5	< 1.0%	< 1.0%	100%	< 1.0%	< 1.0%	< 1.0%	< 1.0%	< 1.0%	< 1.0%	< 1.0%
MMP-1	< 1.0%	< 1.0%	< 1.0%	100%	< 1.0%	< 1.0%	< 1.0%	< 1.0%	< 1.0%	< 1.0%
MMP-3	< 1.0%	< 1.0%	< 1.0%	< 1.0%	100%	< 1.0%	< 1.0%	< 1.0%	< 1.0%	< 1.0%
MMP-9	< 1.0%	< 1.0%	< 1.0%	< 1.0%	< 1.0%	100%	< 1.0%	< 1.0%	< 1.0%	< 1.0%
Osteoactivin	< 1.0%	< 1.0%	1.1%	< 1.0%	< 1.0%	< 1.0%	100%	< 1.0%	< 1.0%	< 1.0%
P-Cadherin	< 1.0%	< 1.0%	< 1.0%	< 1.0%	< 1.0%	< 1.0%	< 1.0%	100%	< 1.0%	< 1.0%
TNF-RI	< 1.0%	< 1.0%	< 1.0%	< 1.0%	< 1.0%	< 1.0%	< 1.0%	< 1.0%	100%	< 1.0%
TNF-RII	< 1.0%	< 1.0%	< 1.0%	< 1.0%	< 1.0%	< 1.0%	< 1.0%	< 1.0%	< 1.0%	100%

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# **4** Sensitivity

The lower limit of detection (LLOD) is a calculated concentration based on a signal that is 2.5 standard deviations over the blank. At least 6 runs were used to calculate the median LLOD. The upper limit of detection (ULOD) is the highest calibrator concentration. Detection limits are reported at their dilution-adjusted concentrations in the table below.

Most assays tested used the same dilution factor for all matrices. CRP, ICAM-1, SAA, and VCAM-1 are tested at a 1000-fold dilution for serum and plasma, and at a 5-fold dilution for CSF and urine.

$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	Assay	Dilution	Median LLOD	Median ULOD	Units	Assay	Dilution	Median LLOD	Median ULOD	Units
Acthe GLP1 2 0.20 1000 pgml   Angionedin M00 6.7 80000 ngml 14.6 2 0.19 1600 pgml   Angionedin M 2 0.099 2.00 ngml 14.6 2 0.61 500 pgml   Angionedin M 2 0.010 500 pgml 14.7 2 0.77 1400 pgml   CA153 20 0.33 20000 mUmin 18.8 2 0.13 1000 pgml   CA163 20 0.73 20000 ngml 14.76 4 0.28 1000 pgml   CKM 4 88 2000 ngml MCP-1 4 0.28 2000 pgml   CLVsront 4 88 2000 ngml MCP-1 4 0.48 200 pgml   CLVsront 4 28 0.000 9.000 ngml MCP-1 4 0.4 11 10000 pgml </td <td>A2M</td> <td>4000</td> <td>0.26</td> <td>2700</td> <td>µg/mL</td> <td>IL-4</td> <td>2</td> <td>0.056</td> <td>390</td> <td>pg/mL</td>	A2M	4000	0.26	2700	µg/mL	IL-4	2	0.056	390	pg/mL
Arigonochin 0.000 6.7 BR000 ngimt   Angopopelin 1 2 0.099 200 ngimt   B2M 4000 1.6 6500 ngimt   L-7 2 0.27 1400 pgimt   CA153 20 0.33 2000 ngimt L-8 2 0.13 1000 pgimt   CA163 20 0.33 2000 mtlimt L-8 2 0.13 1000 pgimt   CA164 20 0.19 2000 ngimt L-8 2 0.053 100 pgimt   CA40 20 4.1 3.000 ngimt L-7 4 0.28 2000 pgimt   CA41 4.000 0.056 B30 pagmt MCP-3 4 4.23 2000 pgimt   CCMA8 4 8.200 ngimt MCP-3 4 4.23 2000 pgimt   CLMA8 4 0.007 1000 ngimt <td>Active GLP-1</td> <td>2</td> <td>0.20</td> <td>1000</td> <td>pg/mL</td> <td>IL-5</td> <td>2</td> <td>0.19</td> <td>1600</td> <td>pg/mL</td>	Active GLP-1	2	0.20	1000	pg/mL	IL-5	2	0.19	1600	pg/mL
Angiopolin 1 2 0.099 200 nprm   Bagopolin 7 7 5.0 70000 pprm   CA125 20 0.033 20000 mUmit   CA155 20 0.033 20000 mUmit   CA155 20 0.033 20000 mUmit   CA4 20 0.19 20000 multimit   CA4 20 0.19 20000 mgmm   CA4 20 0.19 20000 mgmm   CA4 20 0.19 20000 mgmm   CA44 20 40 3000 mgmm   CA44 20 40 3000 pgmm   CA44 20 40 404 2000 pgmm   CA44 20 20 20000 pgmm MCP4 4 50 20000 pgmm   CA44 4000 0.007 2000 pgmm MCP4 11 410000 pgmm   CA44<	Adiponectin	4000	6.7	80000	ng/mL	IL-6	2	0.66	1500	pg/mL
	Angiopoietin 1	2	0.099	200	ng/mL	IL-6R	50	0.010	500	ng/mL
	Angiopoietin 2	2	5.0	20000	pg/mL	IL-7	2	0.27	1400	pg/mL
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	B2M	4000	1.6	6500	na/ml	II -8	2	0.13	1000	pg/ml
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	CA 125	20	0.0034	100	kIU/mI	Insulin	2	36	50000	pg/mL
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	CA 15 3	20	0.33	20000	mll l/ml	IP-10	<u> </u>	0.36	11000	ng/mL
Cabindin 10 0.23 250 ng/ml 1 1 2 0.053 100 ng/ml   CEA 20 0.19 2000 ng/ml MCP-1 4 0.28 2000 pg/ml   CKR 4 88 2200 ng/ml MCP-3 4 0.48 2500 pg/ml   CKR 400 0.059 800 µg/ml MCP-3 4 0.48 2500 pg/ml   CRP 1000 0.0026 290 µg/ml MCP-4 4 5.0 2000 pg/ml   CrInt 4 0.0076 100 ng/ml MCP-4 4.0 0.098 40000 pg/ml   CrInt 4 0.077 4000 ng/ml MIP-1a 4 1.4 10000 pg/ml   Edatin 4 6.4 510 ng/ml MIP-1a 4 1.4 10000 pg/ml   Edatin 4 6.4 200 ng/ml <t< td=""><td>CA 50</td><td>20</td><td>79</td><td>80000</td><td>mll l/ml</td><td>I-TAC</td><td><u> </u></td><td>23</td><td>10000</td><td>ng/mL</td></t<>	CA 50	20	79	80000	mll l/ml	I-TAC	<u> </u>	23	10000	ng/mL
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	Calhindin	10	0.23	250	na/ml	Lentin	2	0.053	10000	ng/mL
C-Kit 20 4.0 3000 ng/mit MCP-2 2 2.1 10000 ng/mit   CKMB 4 88 2700 ng/mit MCP-3 4 0.49 2500 pg/mit   CKPE 1000 0.0076 790 µg/mit MCP-4 4 5.0 2600 pg/mit   CKP 1000 0.0076 790 µg/mit MCP-4 4 5.0 2600 pg/mit   CFM 4 0.0076 700 pg/mit MCP-4 4 5.0 2600 pg/mit   CTI 4 0.0076 100 ng/mit MBC 2 27 66000 pg/mit   CTIT 4 0.95 200 ng/mit MIF 4 1.4 4000 pg/mit   Exaderian 2 0.44 2000 ng/mit MIF 4 1.5 44000 pg/mit   Exaderian 30 0.033 500 ng/mit MIF </td <td></td> <td>20</td> <td>0.23</td> <td>2000</td> <td>ng/mL</td> <td>MCP_1</td> <td>Z</td> <td>0.000</td> <td>2000</td> <td>ng/mL</td>		20	0.23	2000	ng/mL	MCP_1	Z	0.000	2000	ng/mL
CK08 70 80 3000 pg/mL   CK08 2 0.0 pg/mL MCP3 4 0.48 2500 pg/mL   CAstenia 4000 0.059 B00 µg/mL MCP3 2 0.072 2500 pg/mL   CRP 1000 0.0026 290 µg/mL MCC 4 1.4 10000 pg/mL   CTACK 4 2.8 602000 pg/mL MCC 4 1.1 41000 pg/mL   CTACK 4 2.8 602000 pg/mL Mesoffelin 500 0.068 1200 ng/mL   Cytokeratin-8 2 0.44 2000 ng/mL MIF 4 3.4 4000 pg/mL   Edosin 500 0.038 500 pg/mL MIP-36 4 1.1 40000 pg/mL   Edosin 4 8.2 10000 pg/mL MMP-36 0.066 1000 ng/mL   Edosin	c-Kit	20	4.0	3000	ng/mL	MCP-2		2 1	10000	ng/mL
Chasterin 4000 0.059 8000 pg/mL   Celeptite 2 285 50000 pg/mL   MCCFA 4 5.0 2600 pg/mL   CRP 1000 0.0226 290 pg/mL   CTACK 4 28 60000 pg/mL   MCCFA 10 0.0026 290 pg/mL   CTACK 4 28 60000 pg/mL   MCTA 4 0.0074 1000 ng/mL   Cytoteraln-3 2 0.44 2000 ng/mL   Cytoteraln-3 2 0.44 2000 ng/mL   Ecalmerin 20 0.077 40000 ng/mL   MIP-3 4 1.5 44000 pg/mL   Endosin 4 6.4 6100 pg/mL   MIP-3 10 0.039 1000 pg/mL   Edasin-3 4 8.2 10000 pg/mL   Edasin 4 0.5 <t< td=""><td></td><td></td><td>90</td><td>2200</td><td>ng/mL</td><td>MCD 3</td><td>Z</td><td>0.48</td><td>2500</td><td>ng/mL</td></t<>			90	2200	ng/mL	MCD 3	Z	0.48	2500	ng/mL
	Clustorin	4	00	2200	ug/mL		4	5.0	2500	pg/mL
	C Dontido	4000 ว	0.037	5000	ng/mL		<u>4</u> ົ	0.072	2000	pg/mL
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $		 1000	20	20000	py/mL	MDC	Z	11	2500	pg/mL
C1ACK 4 C0006 pyrrll.   CTn1 4 0.0066 1200 ng/mL   CTn1 4 0.0076 100 ng/mL   Contact 4 0.0076 100 ng/mL   Cytokeratin 8 2 0.44 2000 ng/mL   Exacherin 20 0.017 4000 ng/mL   Endogin 50 0.038 5000 ng/mL   Entacyin 4 6.4 6100 pg/mL   MIP-3a 4 1.1 40000 pg/mL   Entacyin 3 4 8.2 19000 pg/mL   MIP-3a 4 0.19 2500 pg/mL   Fabra VIL 4000 0.053 1000 ng/mL   Fabra VIL 4000 7.0 6600 ng/mL   Fabra VIL 4000 7.0 6600 ng/mL   Fabra VIL 4000 7.1 60000 pg/mL   Fabra VIL 4000 10000		1000	0.0020	290	µg/mL	Negathalin	4 FO		41000	pg/mL
		4	28	00000	pg/mL	Met	00	0.008	1200	ng/mL
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $		4		100	ng/mL	NIEL NIE	20	0.098	4000	ng/mL
$\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$		4	0.95	200	ng/mL		<u> </u>		60000	pg/mL
E-t-Canetin 20 0.077 4000 ng/mL MIP-1 G 4 3.4 4200 pg/mL   Endogin 50 0.038 500 ng/mL MIP-16 4 1.5 4400 pg/mL   Edtakin-2 10 3.2 20000 pg/mL MIP-36 4 0.19 2500 pg/mL   Eotakin-3 4 8.2 19000 pg/mL MIP-10 0.039 1000 ng/mL   EPO 2 1.7 10000 mg/mL MIP-3 10 0.016 5000 ng/mL   Factor VII 4000 7.0 6600 ng/mL MMP-3 10 0.016 5000 ng/mL   Fast 2 0.45 5000 pg/mL MTpr0RNP 4 8.6 20000 pg/mL   Fits Ligand 20 0.71 60000 pg/mL Osteonectin 2 0.69 2000 ng/mL   GR0-a 4 1.4 10000 pg/mL		2	0.44	2000	ng/mL	MIG	4	1.4	10000	pg/mL
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	E-Cadherin	20	0.0//	4000	ng/mL	MIP-1a	4	3.4	4200	pg/mL
	ENA-78	2	0.91	2500	pg/mL	MIP-1β	4	1.5	4400	pg/mL
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	Endoglin	50	0.038	500	ng/mL	MIP-3a	4	0.19	2500	pg/mL
	Eotaxin	4	6.4	6100	pg/mL	MIP-3β	4	1.1	40000	pg/mL
	Eotaxin-2	10	3.2	20000	pg/mL	MIP-5	10	0.0050	100	ng/mL
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	Eotaxin-3	4	8.2	19000	pg/mL	MMP-1	10	0.039	1000	ng/mL
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	EPO	2	1.7	10000	mIU/mL	MMP-3	10	0.076	1000	ng/mL
	E-Selectin	2	0.053	400	ng/mL	MMP-9	10	0.16	5000	ng/mL
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	FABP3	4	0.25	400	ng/mL	Myl3	4	0.17	220	ng/mL
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	Factor VII	4000	7.0	6800	ng/mL	Myoglobin	4	17	40000	ng/mL
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	Fas	50	0.062	250	ng/mL	Nectin-4	2	0.52	20000	pg/mL
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	FasL	2	0.85	5000	pg/mL	NT-proBNP	4	8.6	20000	pg/mL
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	FGF (basic)	2	0.17	4100	pg/mL	Osteoactivin	10	0.16	400	ng/mL
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	Flt-1	2	1.3	16000	pg/mL	Osteocalcin	50	2.1	10000	ng/mL
$\begin{array}{ c c c c c c c c c c c c c c c c c c c$	Flt-3 Ligand	20	0.71	60000	pg/mL	Osteonectin	2	0.69	2000	ng/mL
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	Fractalkine	4	0.10	400	ng/mL	Osteopontin	20	14	4000	ng/mL
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	GIP	2	4.9	2500	pg/mL	Osteoprotegerin	2	0.010	200	ng/mL
$\begin{array}{ c c c c c c c c c c c c c c c c c c c$	Glucagon	2	29	10000	pg/mL	P-Cadherin	10	0.099	1000	ng/mL
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	GM-CSF	2	0.27	1900	pg/mL	PIGF	2	0.53	7100	pg/mL
$\begin{array}{ c c c c c c c c c c c c c c c c c c c$	GRO-α	4	14	10000	pg/mL	P-Selectin	2	0.13	400	ng/mL
$\begin{array}{ c c c c c c c c c c c c c c c c c c c$	I-309	4	0.28	1000	pg/mL	PYY (total)	2	9.3	3000	pg/mL
$\begin{array}{ c c c c c c c c c c c c c c c c c c c$	ICAM-1	1000	1.4	69000	ng/mL	RANTES	50	0.012	500	ng/mL
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	ICAM-3	2	0.0040	400	ng/mL	Resistin	50	0.016	130	ng/mL
$\begin{array}{ c c c c c c c c c c c c c c c c c c c$	IFN-α	2	2.1	10000	pg/mL	SAA	1000	0.018	240	µg/mL
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	IFN-γ	2	0.67	2100	pg/mL	SCF	2	0.36	10000	pg/mL
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	IL-10	2	0.060	630	pg/mL	SDF-1a	2	870	40000	pg/mL
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	IL-12/IL-23p40	2	0.54	5800	pg/mL	TARC	4	1.1	6300	pg/mL
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	IL-12p70	2	0.27	810	pg/mL	Tenascin C	4000	2.8	690	ng/mL
$\begin{array}{ c c c c c c c c c c c c c c c c c c c$						Thrombomoduli				
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	IL-13	2	1.7	990	pg/mL	n	2	0.0041	400	ng/mL
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	IL-15	2	0.30	1400	pg/mL	Tie-2	2	0.038	160	ng/mL
IL-1720.939500pg/mLTNF-RII101.425000pg/mLIL-17B29.65000pg/mLTNF-α20.12640pg/mLIL-17D24.75000pg/mLTNF-β20.0791200pg/mLIL-1824.12500pg/mLTPO44.440000pg/mLIL-1Ra500.046250ng/mLTRAIL20.4120000pg/mLIL-1β20.13670pg/mLVCAM-110006.370000ng/mLIL-220.172800pg/mLVEGF-A20.932000pg/mLIL-2122.110000pg/mLVEGF-D25.347000pg/mLIL-3322.71500pg/mLYKL-40500.0382500ng/mL	IL-16	2	5.1	4900	pg/mL	TNF-RI	10	0.032	100	ng/mL
IL-17B29.65000pg/mLIL-17D24.75000pg/mLIL-1824.12500pg/mLIL-18a500.046250ng/mLIL-1α20.13670pg/mLIL-1β20.161000pg/mLIL-2120.172800pg/mLIL-3322.71500pg/mL	IL-17	2	0.93	9500	pg/mL	TNF-RII	10	1.4	25000	pg/mL
IL-17D24.75000pg/mLIL-1824.12500pg/mLIL-1Ra500.046250ng/mLIL-1α20.13670pg/mLIL-1β20.161000pg/mLIL-220.172800pg/mLIL-2122.110000pg/mLIL-3322.71500pg/mL	IL-17B	2	9.6	5000	pg/mL	TNF-α	2	0.12	640	pg/mL
IL-1824.12500pg/mLIL-1Ra500.046250ng/mLIL-1α20.13670pg/mLIL-1β20.161000pg/mLIL-220.172800pg/mLIL-2122.110000pg/mLIL-3322.71500pg/mL	IL-17D	2	4.7	5000	pg/mL	TNF-β	2	0.079	1200	pg/mL
IL-1Ra500.046250ng/mLTRAIL20.4120000pg/mLIL-1α20.13670pg/mLVCAM-110006.370000ng/mLIL-1β20.161000pg/mLVEGF-A20.932000pg/mLIL-220.172800pg/mLVEGF-C22744000pg/mLIL-2122.110000pg/mLVEGF-D25.347000pg/mLIL-3322.71500pg/mLYKL-40500.0382500ng/mL	IL-18	2	4.1	2500	pg/mL	TPO	4	4.4	40000	pg/mL
IL-1α20.13670pg/mLVCAM-110006.370000ng/mLIL-1β20.161000pg/mLVCAM-110006.370000ng/mLIL-220.172800pg/mLVEGF-A20.932000pg/mLIL-2122.110000pg/mLVEGF-C22744000pg/mLIL-3322.71500pg/mLYKL-40500.0382500ng/mL	IL-1Ra	50	0.046	250	ng/mL	TRAIL	2	0.41	20000	pg/mL
IL-1β20.161000pg/mLIL-220.172800pg/mLIL-2122.110000pg/mLIL-3322.71500pg/mL	IL-1α	2	0.13	670	pg/mL	VCAM-1	1000	6.3	70000	ng/mL
IL-2 2 0.17 2800 pg/mL   IL-21 2 2.1 10000 pg/mL   IL-33 2 2.7 1500 pg/mL   VEGF-D 2 5.3 47000 pg/mL	IL-1β	2	0.16	1000	pg/mL	VEGF-A	2	0.93	2000	pg/mL
IL-21 2 2.1 10000 pg/mL VEGF-D 2 5.3 47000 pg/mL   IL-33 2 2.7 1500 pg/mL YKL-40 50 0.038 2500 ng/mL	IL-2	2	0.17	2800	pa/mL	VEGF-C	2	27	44000	pa/mL
IL-33 2 2.7 1500 pg/mL YKL-40 50 0.038 2500 ng/mL	IL-21	2	2.1	10000	pa/mL	VEGF-D	2	5.3	47000	pa/mL
	IL-33	2	2.7	1500	pg/mL	YKL-40	50	0.038	2500	ng/mL



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# **5** Sample Testing

Twenty human serum, 20 EDTA plasma, 8 urine, and 8 CSF samples were tested across the 15 panels. For the majority of assays, samples were detectable. IL-17B, IL-17D, and IL-21 were not detectable in normal samples.

Concentration units are listed on the left in the table with the limits of detection.

# 6 Reproducibility

The median CV was calculated for samples within the limits of quantification. For assays included in the V-PLEX Human Biomarker 40-Plex, the lower and upper limits of quantification (LLOQ and ULOQ, respectively) were obtained from the certificate of analysis for the kit. For the additional assays, the limits of quantification were estimated. The LLOQ was estimated as 5 times the median LLOD. The ULOQ was estimated as 80% of the ULOD.

For serum, EDTA plasma, and CSF, at least 90% of the assays had a median CV of less than 10%. For urine, 70% of the assays had a



Elevated CVs often correlated with low endogenous levels (see Section 5). The following assays had a median CV of greater than 20%: • Serum – IL-2, IL-13, IL-17, PYY (total), and Osteopontin.

CSF – IL-2, IL-4, IL-13, and RANTES.

Urine – IL-1α, VEGF-A, IL-16, C-Peptide, E-Selectin, NT-proBNP, cTnT, Myl3, CKMB, Myoglobin, Osteoprotegerin, MCP-2, and MET.

The median CV was calculated for the standard curve. The median signal CV was less than 10% for 98% of the assays at standard five concentration (Cal-5).

The CKMB assay had a median CV of greater than 20%. This assay was further optimized to expand the dynamic range of the assay (data not shown).

Reproducibility (precision) was assessed with matrix-based controls tested across 6 plates on a single day of testing. Representative data is shown below.

Assay	Sample	Runs	Avg Conc.	Units	Avg Intra-plate %CV	Inter-plate %CV
A2M	Sample 1	6	1170	µg/mL	3.3	5.0
	Sample 2	6	1219	µg/mL	7.6	9.3
	Sample 3	6	2391	µg/mL	9.1	11.1
	Sample 4	6	1149	µg/mL	2.4	8.5
Adinonactin	Sample 1	6	54704	ng/mL	5.1	5.5
	Sample 2	6	50878	ng/mL	8.5	7.1
Adiponectin	Sample 3	6	64417	ng/mL	4.5	5.0
	Sample 4	6	21521	ng/mL	4.2	3.9
	Sample 1	6	26.1	µg/mL	5.0	6.5
Clustorin	Sample 2	6	20.4	µg/mL	8.4	9.8
Clusielli	Sample 3	6	9.82	µg/mL	5.1	5.6
	Sample 4	6	27.0	µg/mL	6.1	7.4
	Sample 1	6	400	ng/mL	4.2	4.5
Factor VII	Sample 2	6	440	ng/mL	4.8	5.1
FACION VII	Sample 3	6	361	ng/mL	3.1	2.9
	Sample 4	6	888	ng/mL	3.9	4.6
	Sample 1	6	1885	pg/mL	2.8	4.2
FCF (basic)	Sample 2	6	194	pg/mL	5.6	6.0
FOF (Dasic)	Sample 3	6	21.2	pg/mL	5.9	6.4
	Sample 4	6	1.88	pg/mL	5.1	6.2
	Sample 1	6	6560	pg/mL	1.8	2.9
Elt 1	Sample 2	6	698	pg/mL	1.8	2.3
1 11-1	Sample 3	6	68.1	pg/mL	4.1	5.5
	Sample 4	6	79.3	pg/mL	4.9	6.4
	Sample 1	6	2961	pg/mL	6.1	7.4
PIGE	Sample 2	6	324	pg/mL	5.7	6.7
PIGF	Sample 3	6	36	pg/mL	6.9	9.7
	Sample 4	6	26.7	pg/mL	2.7	7.5
Tenascin C	Sample 1	6	37.6	ng/mL	4.8	11.1
	Sample 2	6	34.4	ng/mL	9.6	11.6
	Sample 3	6	29.4	ng/mL	13.3	13.7
	Sample 4	6	35.6	ng/mL	3.6	13.8
Tio 2	Sample 1	6	67.7	ng/mL	3.7	4.9
	Sample 2	6	8.82	ng/mL	3.5	10.7
HC-Z	Sample 3	6	2.05	ng/mL	4.0	3.4
	Sample 4	6	11.8	ng/mL	5.4	12.3

MSD's Biomarker Screening Panel includes a robust menu of assays that quantitatively measure relevant biomarkers The assays have a broad dynamic range that allows scientists to obtain an accurate quantification of their samples. Fe most assays, multiple sample types can be tested at a single dilution factor. The panel uses simple protocols wit measurement of up to 10 biomarkers within a plate. The 122 biomarkers were measured using less than 1 mL of sample The panel may be used to quantify the levels of biomarkers in a variety of matrices including serum, plasma, CSF, and



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