



Vascular Injury Biomarkers: Multiplexed Immunoassays

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Meso Scale Discovery (MSD) has developed multiplexed immunoassays for the quantitation of eight serum biomarkers related to human vascular injury and disease: Thrombomodulin, ICAM-3, E-selectin, P-selectin, serum amyloid protein A (SAA), C-reactive protein (CRP), VCAM-1 and ICAM-1.

These markers were all cited in a recent FDA expert group report suggesting that the measurement of predictive biomarkers of vascular injury may become part of the drug development process (Kerns, W. et al., Drug-Induced Vascular Injury – A Search for Biomarkers, 2001).

The assays are formatted as two 4-plexes: Panel I (ICAM-3/Thrombomodulin/E-selectin/P-selectin) and Panel II (CRP/SAA/VCAM-1/ICAM-1). The multiplexed assays are performed on the MSD® MULTI-ARRAY™ platform using MSD's MULTI-SPOT® 96-well 4-spot plates and SECTOR™ instrumentation. All assays are formatted as sandwich immunoassays: capture antibodies are pre-coated onto different spots within each well of the plates, and electrochemiluminescent-labeled detection antibodies are supplied. Assay protocols are simple: they require few wash steps, use incubation steps of either one or two hours, have a rapid read time of approximately one minute per plate, and use 10 µL or less of serum, EDTA plasma, or heparin plasma samples.

Linear ranges for the assays are 0.02 – 1000 ng/mL for Thrombomodulin, 0.07 – 1000 ng/mL for ICAM-3, 0.04 – 1000 ng/mL for E-selectin, 0.27 – 1000 ng/mL for P-selectin, 0.03 – 1000 µg/mL for SAA, 0.04 – 100 µg/mL for CRP, 0.02 – 100 µg/mL for VCAM-1 and 0.002 – 100 µg/mL for ICAM-1.

Background and Assay Format

Analyte	Clinical Ranges	Disease States
Thrombomodulin	~ 20 ng/mL	Thrombocytopenic purpura, disseminated intravascular coagulation, atherosclerosis and inflammatory connective tissue diseases
ICAM-3	~ 70 ng/mL	Initial immune response
E-Selectin	~ 50 ng/mL	Vascular infection and inflammation, allergic reactions, ocular diseases, septic shock, inflammatory bowel disease and transplantation reaction
P-Selectin	40 - 200 ng/mL	Respiratory distress syndrome, adult respiratory distress syndrome, acute lung injury, ischemiareperfusion injury, septic shock, thrombotic diseases and rheumatoid arthritis
SAA	1 - 5 µg/mL normal; elevated up to 1 mg/mL	Inflammatory disease
CRP	< 10 µg/mL normal; elevated up to 10 mg/mL	Inflammatory disease
VCAM-1	200 - 1,700 ng/mL	Vasculitis
ICAM-1	60 - 300 ng/mL normal; elevated up to 700 ng/mL	Vasculitis

- MSD vascular injury assay panels are performed in MSD MULTI-SPOT 96-well 4-spot plates, and read on MSD SECTOR Instruments.
- MSD assays use electrochemiluminescence detection that allows for very sensitive measurements (e.g. detection limit of 20 pg/mL for Thrombomodulin).
- All assays are formatted as sandwich immunoassays.
- Assay kits contain the following components:
 - Plates: MSD MULTI-SPOT 4-spot plates pre-coated with capture antibodies.
 - Detection antibody, pre-labeled with an electrochemiluminescent $\text{Ru}(\text{bpy})_3^{2+}$ compound.
 - Diluents: formatted for optimal sensitivity and precision, containing additives to eliminate HAMA and other interference.
 - Calibrators: calibration is performed using recombinant material - a high concentration of analyte is supplied for generating a dilution curve.

Note: These assays are for research use only: not for use in diagnostic procedures.

Assay Protocols

Protocol for Vascular Injury Panel I - Thrombomodulin, ICAM-3, E-Selectin and P-Selectin:

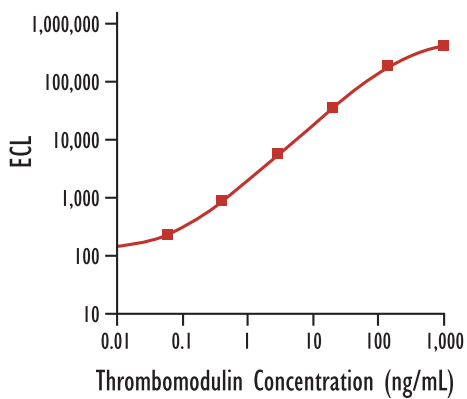
- Block MSD MULTI-SPOT plate for 1 hour, wash
- Add 40 μ L of labeled antibody solution to each well
- Add 10 μ L of calibrator or sample to each well
- Incubate with shaking for 120 minutes, wash
- Add MSD Read Buffer
- Read plate on MSD Reader

Protocol for Vascular Injury Panel II - SAA, CRP, VCAM-1 and ICAM-1:

- Block MSD MULTI-SPOT plate for 1 hour, wash
- Dilute sample 1:200 with assay diluent
- Add 40 μ L of assay diluent solution to each well
- Add 10 μ L of calibrator or diluted sample to each well
- Incubate with shaking for 60 minutes, wash
- Add 25 μ L of labeled antibody solution to each well
- Incubate with shaking for 60 minutes, wash
- Add MSD Read Buffer
- Read plate on MSD Reader

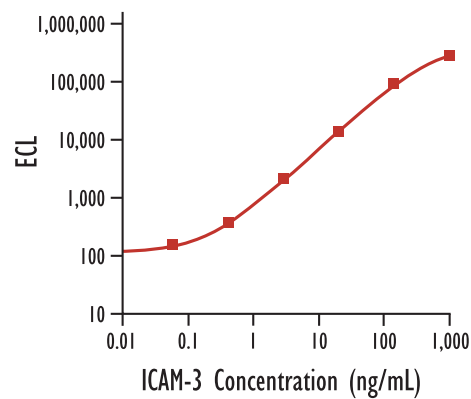
Assay Performances

Thrombomodulin Calibration Curve



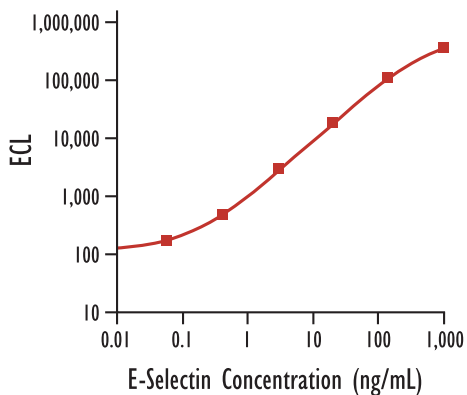
Sensitivity: 0.02 ng/mL
Dynamic Range: 0.02 to 1,000 ng/mL
Variability: less than 7% CV

ICAM-3 Calibration Curve



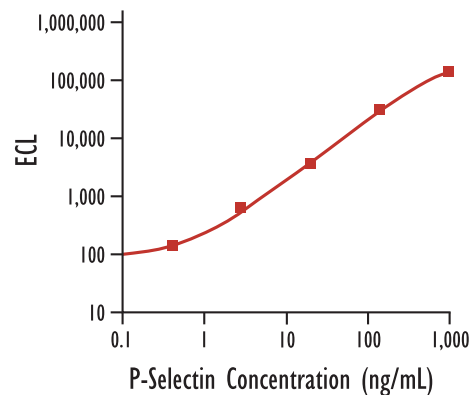
Sensitivity: 0.07 ng/mL
Dynamic Range: 0.07 to 1,000 ng/mL
Variability: less than 7% CV

E-Selectin Calibration Curve



Sensitivity: 0.04 ng/mL
Dynamic Range: 0.04 to 1,000 ng/mL
Variability: less than 7% CV

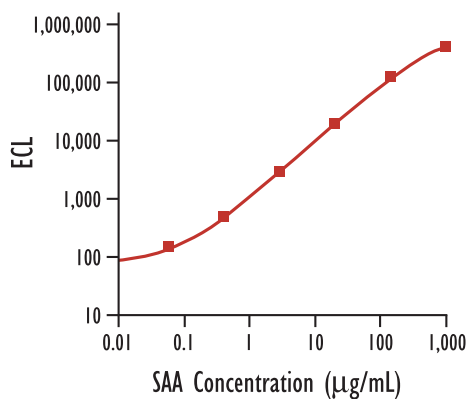
P-Selectin Calibration Curve



Sensitivity: 0.27 ng/mL
Dynamic Range: 0.27 to 1,000 ng/mL
Variability: less than 7% CV

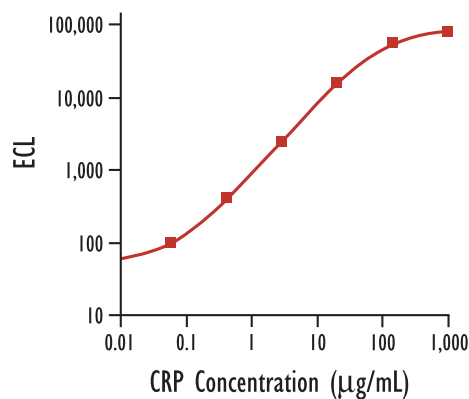
Assay Performances (continued)

SAA Calibration Curve



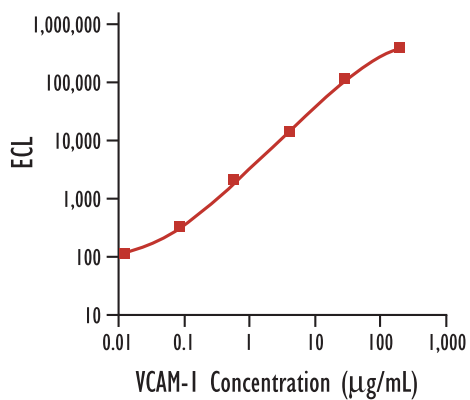
Sensitivity: 0.03 µg/mL
Dynamic Range: 0.03 to 1,000 µg/mL
Variability: less than 8% CV

CRP Calibration Curve



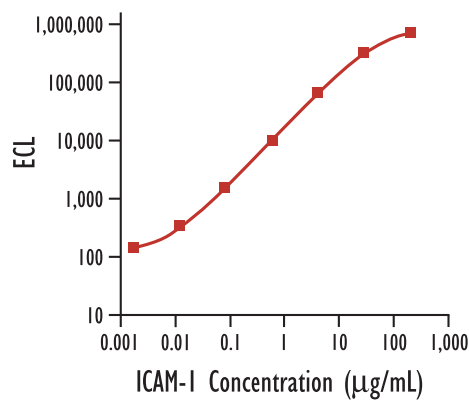
Sensitivity: 0.04 µg/mL
Dynamic Range: 0.04 to 100 µg/mL
Variability: less than 8% CV

VCAM-I Calibration Curve



Sensitivity: 0.02 µg/mL
Dynamic Range: 0.02 to 100 µg/mL
Variability: less than 8% CV

ICAM-I Calibration Curve



Sensitivity: 0.002 µg/mL
Dynamic Range: 0.002 to 100 µg/mL
Variability: less than 8% CV

Performance Summary

Analyte	MSD Vascular Assays		Clinical Analyzer and ELISA Assays		
	Analytical Sensitivity*	Linear Range	Analytical Sensitivity	Linear Range	System
Thrombomodulin	0.02 ng/mL	1,000 ng/mL	1.5 ng/mL	50 ng/mL	IMUBIND ELISA
ICAM-3	0.07 ng/mL	1,000 ng/mL	0.58 ng/mL	100 ng/mL	IBL ELISA
E-Selectin	0.04 ng/mL	1,000 ng/mL	< 0.5 ng/mL	250 ng/mL	Chemicon ELISA
P-Selectin	0.27 ng/mL	1,000 ng/mL	< 0.5 ng/mL	~50 ng/mL (no hook to 50,000 ng/mL)	R&D Systems ELISA
SAA	0.03 µg/mL	1,000 µg/mL (no hook to 5,000 µg/mL)	0.06 µg/mL	4 µg/mL	Abazyme ELISA
CRP	0.04 µg/mL	100 µg/mL (no hook to 5,000 µg/mL)	0.1 µg/mL	No hook to 4,000 µg/mL	DCP Immulite® 2,000 hsCRP
VCAM-1	0.02 µg/mL	100 µg/mL	0.01 µg/mL	4 µg/mL	R&D Systems ELISA
ICAM-1	0.002 µg/mL	100 µg/mL	< 0.007 µg/mL	1 µg/mL	R&D Systems ELISA

* For MSD assays, analytical sensitivity = analyte concentration at 2.5 StdDev above background.

Dilution Linearity

Serum samples from different individuals containing detectable amounts of Thrombomodulin, ICAM-3, E-Selectin, P-Selectin, SAA, CRP, VCAM-I or ICAM-I were diluted with the appropriate calibrator diluent from MSD vascular injury panel kits. Recoveries were measured at three dilution levels – the average recovery at each dilution level is tabulated.

Analyte	Average Recovery at 1/2 Dilution Factor	Average Recovery at 1/4 Dilution Factor	Average Recovery at 1/8 Dilution Factor
Thrombomodulin	108%	107%	116%
ICAM-3	97%	77%	76%
E-Selectin	103%	109%	102%
P-Selectin	102%	107%	91%
SAA	98%	94%	74%
CRP	97%	103%	107%
VCAM-I	104%	113%	109%
ICAM-I	98%	96%	92%

Spike Recovery

Plasma and serum samples from different individuals were spiked with known quantities of Thrombomodulin, ICAM-3, E-Selectin, P-Selectin, SAA, CRP, VCAM-I or ICAM-I at different levels and quantitated on MSD vascular injury multiplexed kits. The average recovery in each matrix is tabulated.

Analyte	Serum	Plasma EDTA	Plasma Heparin
Thrombomodulin	93%	83%	107%
ICAM-3	87%	105%	108%
E-Selectin	94%	87%	111%
P-Selectin	100%	105%	107%
SAA	89%	89%	90%
CRP	92%	86%	86%
VCAM-I	84%	81%	88%
ICAM-I	87%	83%	89%

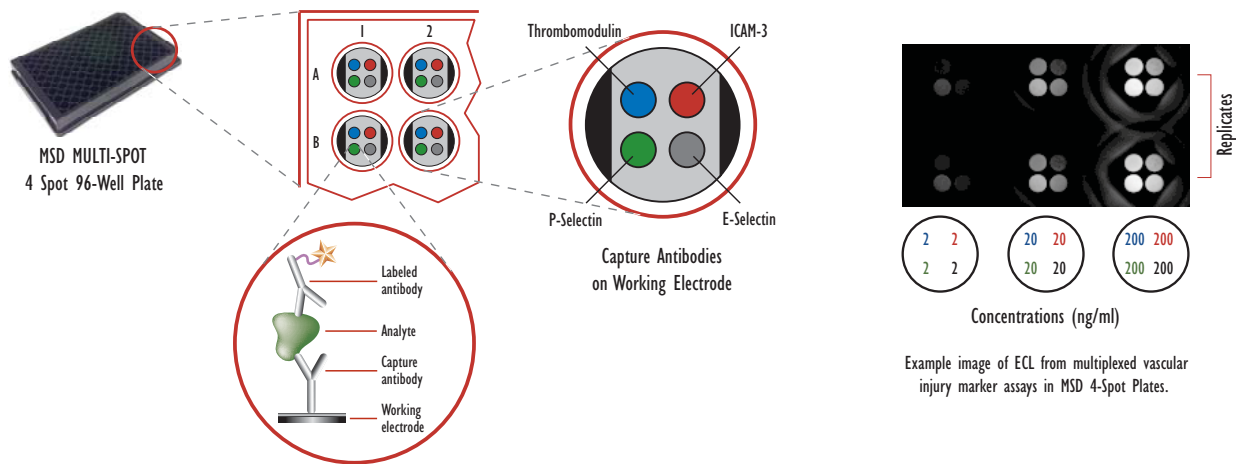
Expected Values

A reference range study was performed using matched plasma and serum samples from healthy donors, approximately half female and half male. Samples were measured on each assay to obtain the tabulated data.

Assay	N	Sample	Median	Mean	Range (95th Percentile)
Thrombomodulin	20	Serum	1.1 ng/mL	1.2 ng/mL	0.9 - 1.5 ng/mL
		Plasma EDTA	1.0 ng/mL	0.9 ng/mL	0.5 - 1.5 ng/mL
		Plasma Heparin	1.5 ng/mL	1.5 ng/mL	1.0 - 2.1 ng/mL
ICAM-3	20	Serum	23.5 ng/mL	23.2 ng/mL	16.2 - 38.8 ng/mL
		Plasma EDTA	24.4 ng/mL	24.9 ng/mL	11.0 - 41.4 ng/mL
		Plasma Heparin	25.3 ng/mL	26.6 ng/mL	16.3 - 50.1 ng/mL
E-Selectin	20	Serum	12.1 ng/mL	12.0 ng/mL	4.0 - 20.9 ng/mL
		Plasma EDTA	9.4 ng/mL	9.5 ng/mL	2.0 - 16.7 ng/mL
		Plasma Heparin	14.3 ng/mL	13.3 ng/mL	3.6 - 20.3 ng/mL
P-Selectin	20	Serum	64.9 ng/mL	60.2 ng/mL	13.9 - 95.7 ng/mL
		Plasma EDTA	42.4 ng/mL	41.7 ng/mL	13.6 - 64.8 ng/mL
		Plasma Heparin	53.9 ng/mL	51.5 ng/mL	19.5 - 85.2 ng/mL
SAA	20	Serum	3.5 µg/mL	6.6 µg/mL	0.39 - 30 µg/mL
		Plasma EDTA	3.3 µg/mL	6.6 µg/mL	0.55 - 28 µg/mL
		Plasma Heparin	3.5 µg/mL	7.0 µg/mL	0.50 - 30 µg/mL
CRP	20	Serum	1.7 µg/mL	3.8 µg/mL	0.09 - 17 µg/mL
		Plasma EDTA	1.5 µg/mL	3.7 µg/mL	1.3 - 16 µg/mL
		Plasma Heparin	1.5 µg/mL	4.0 µg/mL	0.08 - 18 µg/mL
VCAM-I	20	Serum	0.33 µg/mL	0.35 µg/mL	0.18 - 0.59 µg/mL
		Plasma EDTA	0.32 µg/mL	0.33 µg/mL	0.23 - 0.52 µg/mL
		Plasma Heparin	0.34 µg/mL	0.35 µg/mL	0.22 - 0.57 µg/mL
ICAM-I	20	Serum	0.20 µg/mL	0.20 µg/mL	0.12 - 0.29 µg/mL
		Plasma EDTA	0.19 µg/mL	0.19 µg/mL	0.12 - 0.33 µg/mL
		Plasma Heparin	0.19 µg/mL	0.20 µg/mL	0.14 - 0.32 µg/mL

Multiplexing

The MSD vascular injury assays are multiplexed in two separate 4-plexes, allowing simultaneous quantitation of four analytes in a single sample volume. The assays are formatted on MULTI-SPOT 4-spot plates containing 4 capture zones within each well, each zone containing a different capture antibody.



Conclusions

- Immunoassays for the vascular injury biomarkers VCAM-1, Thrombomodulin, P-selectin, E-selectin, ICAM-1, ICAM-3, C-reactive protein (CRP) and serum amyloid A (SAA) have been demonstrated on MSD's MULTI-ARRAY platform.
- The assays are available in two 4-plex kits, containing MULTI-SPOT plates and all reagents required to perform the assays on human serum or plasma samples.
- Assays are rapid (2 hours), simple (minimal washes), use small sample volumes (< 25 mL), are very sensitive, and have wide dynamic ranges (>3 logs).
- Assay performance is comparable or superior to clinical laboratory analyzers or ELISA kits.