

Simoa® Planar Array Human Biomarker Panel 1

The Simoa Planar Array Human Biomarker Panel 1 is a multiplex immunoassay designed for the Quanterix SP-X™ Imaging and Analysis system, which simultaneously measures six important inflammation-associated biomarkers in blood. The six soluble proteins measured by the assay include Myeloperoxidase (MPO), Intercellular adhesion molecule-1 (ICAM-1; also known as CD54), Neutrophil gelatinase-associated Lipocalin (NGAL), also called Lipocalin-2, RANTES (Regulation on Activation Normal T-cell Expressed and Secreted), TIMP-1 (Tissue Inhibitor of metalloproteinases-1), and VCAM-1 (Vascular Cellular Adhesion Molecule 1, also known as CD106). Each Biomarker detected by this assay is found in abundance in human blood; the high sensitivity of the assay allows for 1:1000 sample dilution, enabling low sample volume requirements and elimination of potential matrix effects.

Description – MPO Test

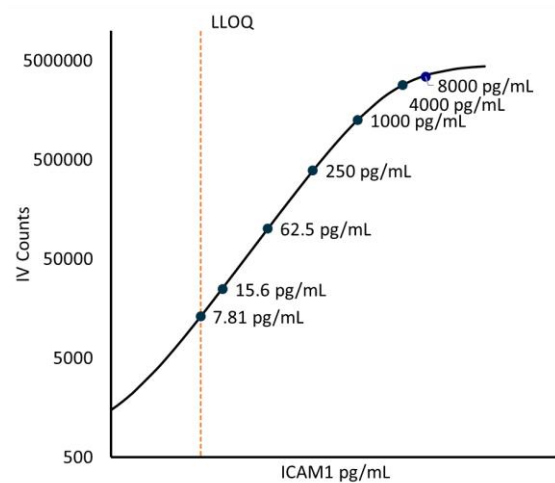
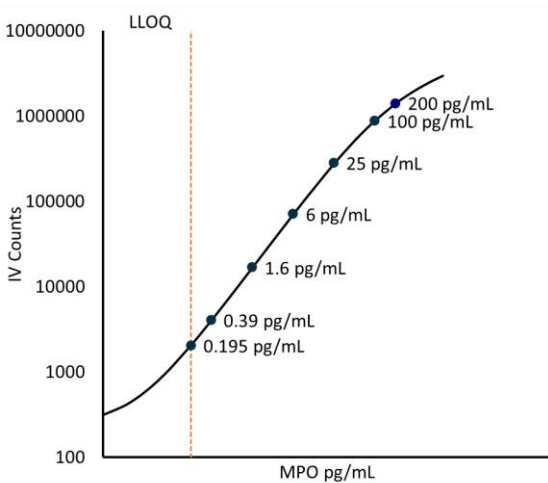
Myeloperoxidase (MPO) is a peroxidase enzyme that in humans is most abundantly expressed in neutrophil granulocytes. In the presence of hydrogen peroxide and halides, MPO catalyzes the formation of reactive oxygen intermediates. MPO has been demonstrated to be a local mediator of tissue damage and the resulting inflammation in various inflammatory diseases, and may be an important therapeutic target for the treatment of inflammatory conditions. Measurement of MPO in plasma may contribute to cardiovascular disease (CVD) risk stratification.

Description – ICAM-1 Test

Intercellular adhesion molecule-1 (ICAM-1; also known as CD54) is a 90kDa member of the immunoglobulin (Ig) superfamily and plays a central role in leukocyte trafficking, activation of lymphocytes, and numerous additional immune functions. ICAM-1 is constitutively expressed on many immune, endothelial and epithelial cell types, and its expression is upregulated in inflammatory conditions, specifically by TNF- α , IL-1 β , IFN- γ and other cytokines. By interacting with lymphocyte function associated molecule (LFA)-1 and others, ICAM-1 acts as a leukocyte adhesion molecule. ICAM-1 is highly expressed in inflammatory conditions, chronic diseases and a number of malignancies.

MPO Curve: Calibrator concentrations, and Lower Limit of Quantification depicted.

ICAM-1 Curve: Calibrator concentrations, and Lower Limit of Quantification depicted.



Description – NGAL Test

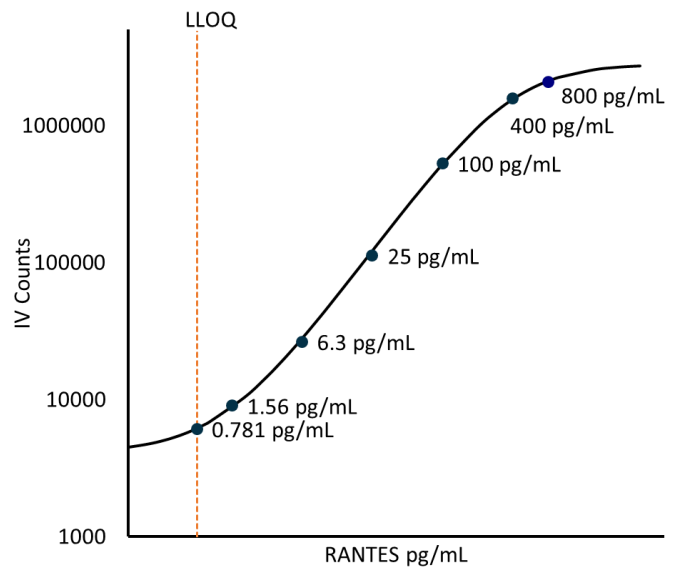
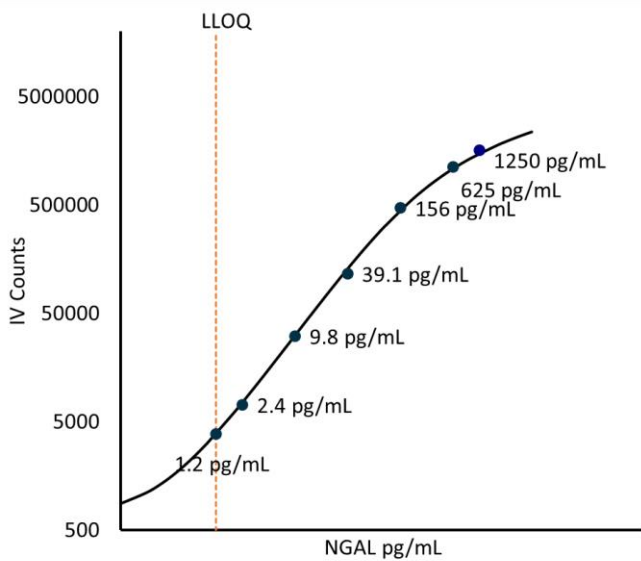
Neutrophil gelatinase-associated Lipocalin (NGAL), also called Lipocalin-2, is expressed in neutrophils and normally produced at low levels by epithelial cells, kidney tubular cells and in case of injury or inflammation by hepatocytes. Increased NGAL expression is seen in cases of damage of epithelial cells of the kidneys, large intestine, liver and lungs. NGAL is one of the most widely studied biomarkers for Acute Kidney Injury, and NGAL formation is induced in many human tumors and is a predictor of poor prognosis.

Description – RANTES Test

RANTES (Regulation on Activation Normal T-cell Expressed and Secreted) is a chemotactic cytokine (also known as CCL5), produced by macrophages, epithelial cells, platelets, megakaryoblasts, T lymphocytes and eosinophils. RANTES signals through CCR1, CCR3 and CCR5 receptors and recruits T cells, macrophages, eosinophils and basophils to sites of inflammation. In addition, it stimulates histamine secretion by mast cells, IgE and IgG production by lymphocytes, increases CD80 expression on antigen-presenting cells, and induces activation and proliferation of NK cells.

NGAL Curve: Calibrator concentrations, and Lower Limit of Quantification depicted.

RANTES Curve: Calibrator concentrations, and Lower Limit of Quantification depicted.



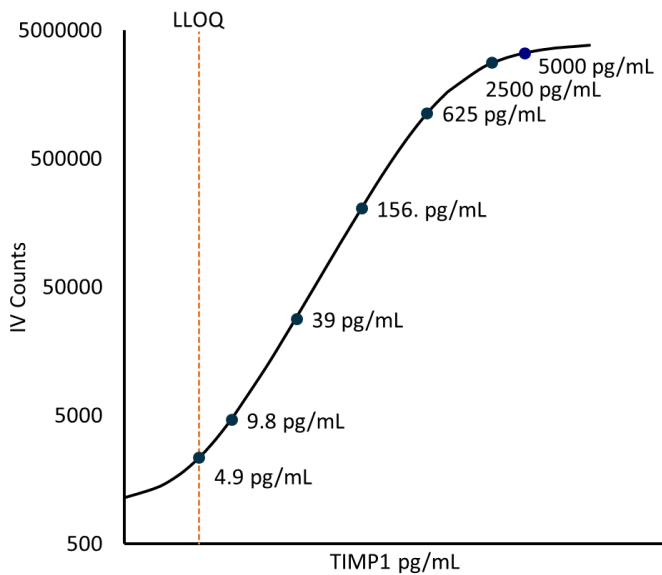
Description – TIMP-1 TEST

TIMP-1 (Tissue Inhibitor of metalloproteinases -1) is a widely expressed glycoprotein which regulates matrix metalloproteinases (MMPs), and disintegrin-metalloproteinases (ADAMs and ADAMTSs). In addition to its inhibitory role against most of the known MMPs, the encoded protein is able to promote cell proliferation in a wide range of cell types, and may also have an anti-apoptotic function. TIMP-1 is thought to play a significant role in cancer development; increased expression of TIMP-1 has been found to be associated with poor prognosis for laryngeal carcinoma and melanoma.

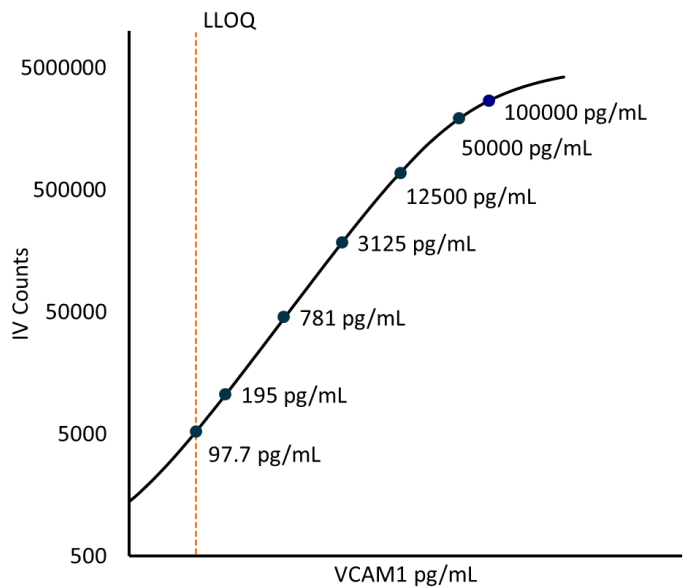
Description – VCAM-1 Test

VCAM-1 (Vascular Cellular Adhesion Molecule 1 also known as CD106) is a 110 kDa transmembrane glycoprotein expressed exclusively on cytokine-activated vascular endothelium. VCAM-1 protein mediates the adhesion of lymphocytes, monocytes, eosinophils, and basophils to vascular endothelium, and also functions in leukocyte-endothelial cell signal transduction. VCAM-1 has been shown to play an important role in the development of atherosclerosis and rheumatoid arthritis, and soluble VCAM-1 is a useful biomarker for many inflammatory diseases.

TIMP-1 Curve: Calibrator concentrations, and Lower Limit of Quantification depicted.



VCAM-1 Curve: Calibrator concentrations, and Lower Limit of Quantification depicted.



Minimum Required Dilution (MRD) and Tests per Kit

Diluted Sample volume (1:1000 Dilution) *	50 µL per measurement
Tests per kit	96

*See Kit Instruction for details

Lower Limit of Quantification (LLOQ): Triplicate measurements of serially diluted calibrator were read back on the calibration curve (13 runs total). Analytical Lower Limit of Quantification (LLOQ) is the lowest calibration standard with back-calculated concentration pooled CV <20% and relative error <25%.

	Analytical LLOQ	Functional LLOQ (x MRD)
MPO	0.195 pg/mL pooled CV 2.9% mean recovery 98.8%	195 pg/mL
ICAM-1	7.813 pg/mL pooled CV 3.3% mean recovery 98.6%	7813 pg/mL
NGAL	1.221 pg/mL pooled CV 3.5% mean recovery 98.5%	1221 pg/mL
RANTES	0.781 pg/mL pooled CV 4.0 % mean recovery 100.5%	781 pg/mL
TIMP-1	4.883 pg/mL pooled CV 5.0 % mean recovery 102.7%	4883 pg/mL
VCAM-1	97.656 pg/mL pooled CV 4.5 % mean recovery 98.0%	97,656 pg/mL

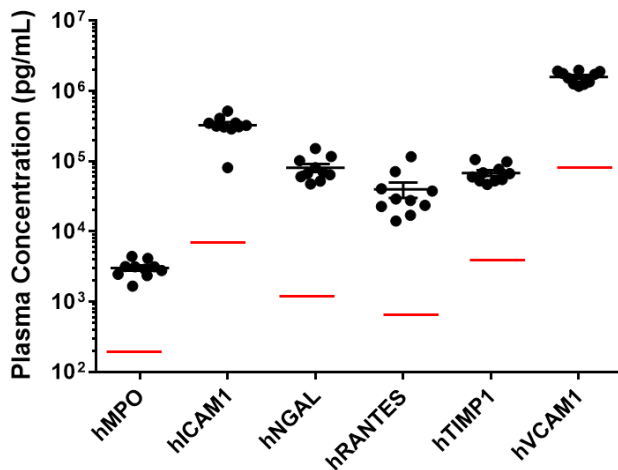
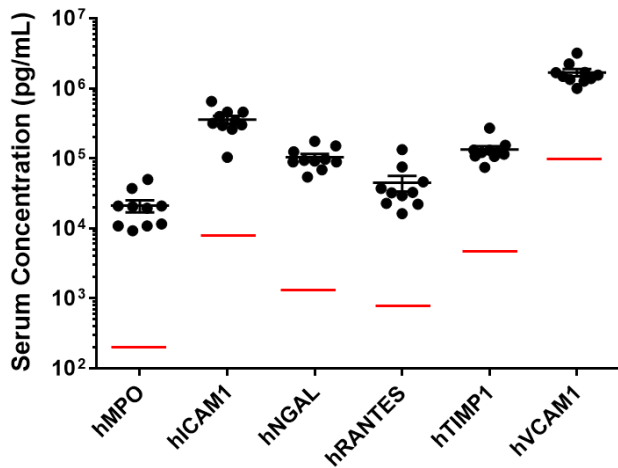
Limit of Detection (LOD): Calculated as 2.5 standard deviations from the mean of background signal read back on each calibration curve across (13 runs total).

	LOD
MPO	0.046 pg/mL range 0.031 – 0.061
ICAM-1	0.229 pg/mL range 0.009 – 0.517
NGAL	1.794 pg/mL range 0.030 – 5.796
RANTES	0.274 pg/mL range 0.039 – 0.609
TIMP-1	0.192 pg/mL range 0.124 – 0.261
VCAM-1	4.262 pg/mL range 0.180 – 8.149

Assay Ranges: The upper end of the dynamic range is equal to the top calibrator concentration multiplied by MRD.

	Assay Range
MPO	0 – 200 ng/mL
ICAM-1	0 – 8000 ng/mL
NGAL	0 – 1250 ng/mL
RANTES	0 – 800 ng/mL
TIMP-1	0 – 5,000 ng/mL
VCAM-1	0 – 100,000 ng/mL

Endogenous Serum and Plasma Readings: Healthy donor matched EDTA plasma (n=10) and serum (n=10) samples were measured. Bars depict median with interquartile range. Red lines represent functional LLOQ.



	Sample Type	Median Conc ng/mL	% Above LOD	% Above LLOQ
MPO	Serum	21.1	100%	100%
	EDTA	3.01	100%	100%
ICAM-1	Serum	358.7	100%	100%
	EDTA	323.3	100%	100%
NGAL	Serum	103.5	100%	100%
	EDTA	80.7	100%	100%
RANTES	Serum	44.9	100%	100%
	EDTA	39.7	100%	100%
TIMP-1	Serum	133.5	100%	100%
	EDTA	67.9	100%	100%
VCAM-1	Serum	1680.5	100%	100%
	EDTA	1574.7	100%	100%

Dilution Linearity: 3 serum and 3 plasma samples were diluted 1000x according to the MRD, and then serially diluted 2x with Sample Diluent eight times, for final dilution of 1:512,000.

MPO	Mean 99% range 94%–101%
ICAM-1	Mean 100% range 96%–104%
NGAL	Mean 95% range 93%–100%
RANTES	Mean 96% range 92%–101%
TIMP-1	Mean 82% range 76%–90%
VCAM-1	Mean 99% range 96%–103%

Single-plex Correlation: Sample concentrations derived from single-plex standard curves were compared to the same samples calculated from the 6-plex standard curve. The average correlation between multi-plex and single-plex assays over the entire dynamic range is shown in the table below.

	Bias
MPO	1.0%
ICAM-1	5.0%
NGAL	2.2%
RANTES	8.5%
TIMP-1	8.9%
VCAM-1	6.8%

Cross-reactivity: During assay validation, cross-reactivity was assessed by testing single antigen at the concentration of the third highest calibrator in the presence of all detection antibodies (maximum 0.6% of cross reactivity), and single detection antibodies in the presence of all antigens (maximum 0.9% of cross reactivity) in assay buffer. In addition, cross-reactivity of single detection antibodies was assessed in sample matrix (maximum 0.7% of cross reactivity).

Precision: Measurements of 2 serum or plasma-based panels and 2 calibrator-based controls. Triplicate measurements were made across 3 reagent lots (12 runs total).

Mean (ng/mL)	MPO	ICAM-1	NGAL	RANTES	TIMP-1	VCAM-1
Control 1	20.4	662.8	204.5	42.6	668.3	6,976.7
Control 2	1.5	56.5	10.7	5.5	37.0	543.3
Panel 1	3.4	407.2	6.8	2.7	82.3	858.9
Panel 2	3.1	272.9	85.3	18.1	79.9	1,491.7

Inter-run CV	MPO	ICAM-1	NGAL	RANTES	TIMP-1	VCAM-1
Control 1	11.4%	17.2%	11.5%	16.3%	14.0%	15.3%
Control 2	14.7%	24.8%	13.7%	19.1%	23.1%	17.3%
Panel 1	4.9%	12.0%	9.5%	13.2%	13.6%	8.1%
Panel 2	10.3%	12.9%	14.6%	16.4%	14.8%	12.1%

Intra-run CV	MPO	ICAM-1	NGAL	RANTES	TIMP-1	VCAM-1
Control 1	2.9%	3.7%	4.0%	2.3%	2.9%	5.4%
Control 2	4.6%	4.5%	10.9%	3.1%	4.5%	4.2%
Panel 1	4.4%	3.1%	5.4%	6.9%	3.4%	2.1%
Panel 2	4.0%	5.4%	8.0%	3.3%	6.8%	1.8%