

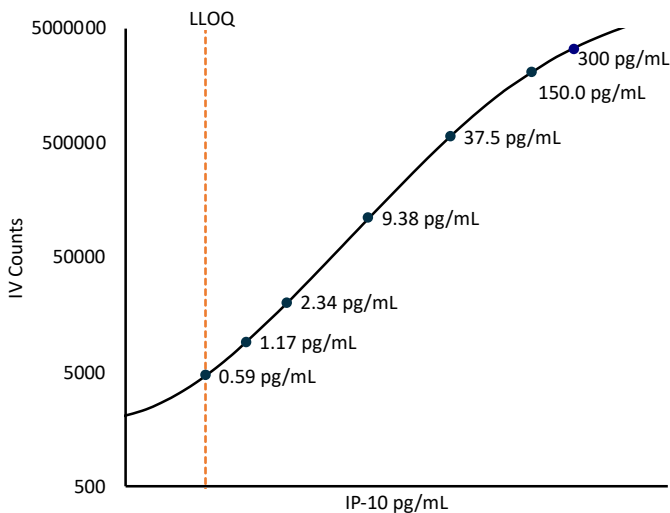
Simoa® Planar Array Human Chemokine Panel 1

The Simoa Planar Array Human Chemokine Panel 1 is a multiplex immunoassay designed for the Quanterix SP-X™ Imaging and Analysis system, which simultaneously measures four important chemokines (IP-10, ITAC, MCP-1 and MIP-3β) in human serum and plasma samples.

Description – IP-10 Test

IFN-γ-inducible protein 10 (IP-10, CXCL10) is a 10 kDa chemokine secreted from cells stimulated with type I and II interferons (IFNs) and lipopolysaccharide (LPS). IP-10 is constitutively expressed at low levels in thymic, splenic, and lymph node stroma. Expression of IP-10 is seen in many Th1-type human inflammatory diseases, including skin diseases (e.g., psoriasis), multiple sclerosis, atherosclerosis, rheumatoid arthritis, transplant rejection, and inflammatory bowel disease. Elevated levels of IP-10 protein have been found in the cerebral spinal fluid in patients with viral meningitis and multiple sclerosis. In these diseases, levels of IP-10 correlate with the tissue infiltration of T lymphocytes, suggesting that IP-10 plays an important role in the recruitment of T cells to sites of tissue inflammation.

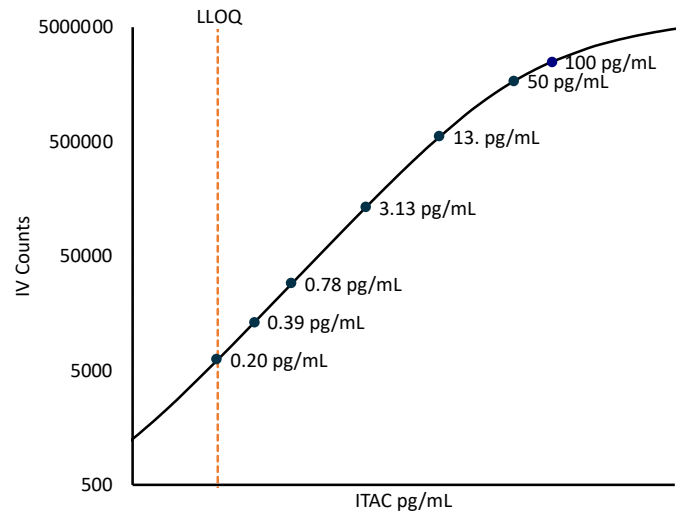
IP-10 Curve: Calibrator concentrations and Lower Limit of Quantification depicted.



Description – ITAC Test

Interferon-inducible T-cell alpha chemoattractant (ITAC), also known as CXCL11 (C-X-C motif chemokine 11), is a protein found in several organs including the pancreas, liver, thymus, spleen, and lung. ITAC is a cytokine stimulated by IFNβ and IFNγ to induce chemotaxis in activated T cells. ITAC has been linked with heart failure and central nervous system diseases.

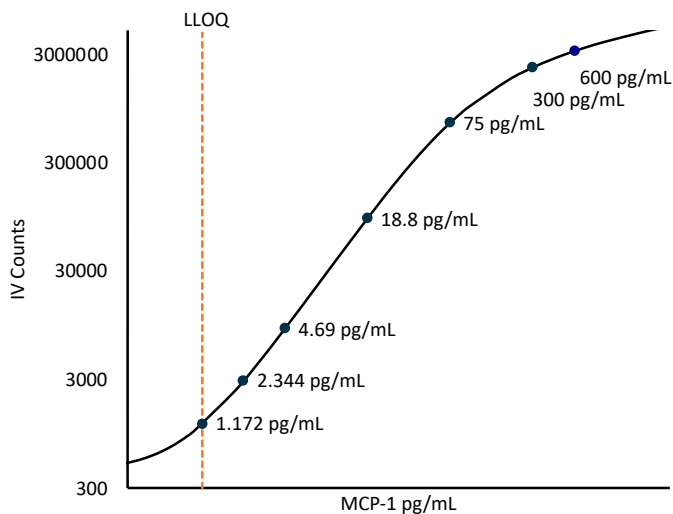
ITAC Curve: Calibrator concentrations and Lower Limit of Quantification depicted.



Description – MCP-1 Test

Monocyte chemoattractant protein-1 (MCP-1) is a 13 kDa chemokine that regulates migration and infiltration of monocytes/macrophages. The idiopathic inflammatory myopathies, including dermatomyositis (DM), polymyositis and inclusion body myositis, are a group of autoimmune diseases characterized by chronic lymphocytic and macrophagic infiltration in muscle. MCP-1 is believed to play a major role in the recruitment of these cells, especially in DM. Plasma levels of MCP-1 have also been shown to be increased in patients with high viral load compared with HIV-seropositive subjects with undetectable plasma viral RNA and healthy controls.

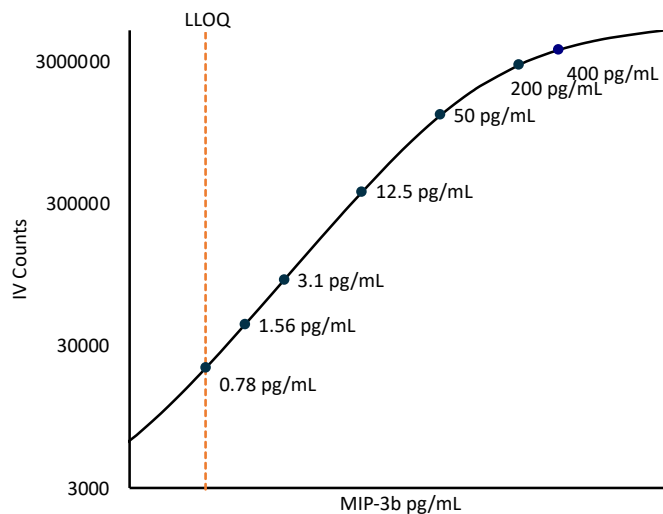
MCP-1 Curve: Calibrator concentrations and Lower Limit of Quantification depicted.



Description – MIP-3β Test

MIP-3β (macrophage inflammatory protein-3-beta), also known as (Chemokine (C-C motif) ligand 19 (CCL19) and EBI1 ligand chemokine (ELC) is a small cytokine belonging to the CC chemokine family. It is expressed at high levels in the lymph nodes, thymus and appendix, and elicits its effects on its target cells by binding to the chemokine receptor chemokine receptor CCR7. MIP-3β and plays a role not only in inflammatory and immunological responses but also in normal lymphocyte recirculation, migration and homing.

MIP-3β Curve: Calibrator concentrations and Lower Limit of Quantification depicted.



Minimum Required Dilution (MRD) and Tests per Kit

Diluted Sample volume (1:10 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 across a single reagent lot (8 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)
IP-10	0.586 pg/mL	5.859 pg/mL
ITAC	0.195 pg/mL	1.953 pg/mL
MCP-1	1.172 pg/mL	11.719 pg/mL
MIP-3β	0.781 pg/mL	7.813 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 1 reagent lot (8 runs total). LOD is not multiplied by MRD.

	LOD
IP-10	0.146 pg/mL Range 0.008 - 0.412
ITAC	0.031 pg/mL Range 0.016 - 0.042
MCP-1	0.511 pg/mL Range 0.232 - 0.712
MIP-3β	0.065 pg/mL Range 0.003 - 0.209

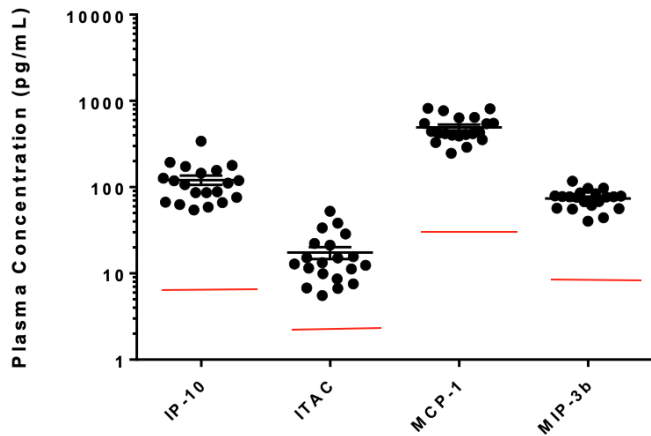
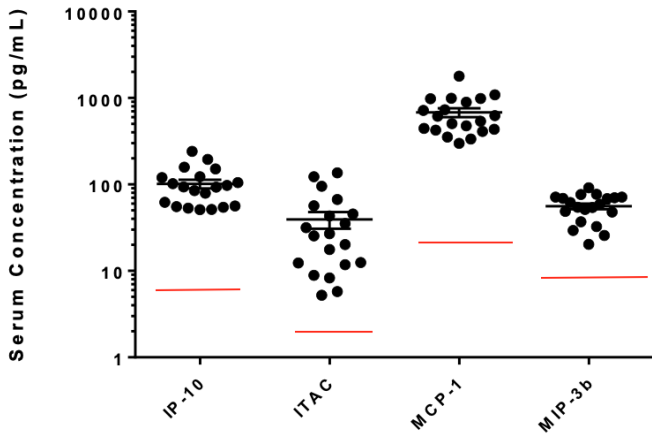
Assay Ranges: The upper end of the dynamic range is equal to the top calibrator concentration multiplied by MRD.

	Assay Range
IP-10	0 – 3000 pg/mL
ITAC	0 – 1000 pg/mL
MCP-1	0 – 6000 pg/mL
MIP-3β	0 – 4000 pg/mL

Endogenous Serum and Plasma Readings: Healthy donor matched EDTA plasma (n=20) and serum (n=20) samples were measured.

	Sample Type	Mean Conc pg/mL	% Above LOD	% Above LLOQ
IP-10	Serum	101.3	100%	100%
	EDTA	120.0	100%	100%
ITAC	Serum	39.4	100%	100%
	EDTA	17.5	100%	100%
MCP-1	Serum	681.8	100%	100%
	EDTA	492.9	100%	100%
MIP-3β	Serum	55.8	100%	100%
	EDTA	73.8	100%	100%

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.



Precision: Measurements of 2 serum or plasma-based panels and 3 calibrator-based controls. Triplicate measurements were made across a single reagent lot (8 runs total).

Mean (pg/mL)	IP-10	ITAC	MCP-1	MIP-3β
Control 1	595.0	189.4	1,131.8	674.3
Control 2	112.5	37.9	232.4	128.4
Control 3	19.9	6.6	36.8	21.6
Panel 1	535.5	14.4	628.9	75.7
Panel 2	146.6	9.9	425.5	52.2

Inter-run CV	IP-10	ITAC	MCP-1	MIP-3β
Control 1	8.8%	8.8%	2.9%	10.7%
Control 2	8.6%	9.1%	4.1%	10.4%
Control 3	9.8%	7.5%	3.7%	10.3%
Panel 1	10.5%	8.6%	6.1%	7.2%
Panel 2	9.3%	7.2%	6.6%	9.9%

Intra-run CV	IP-10	ITAC	MCP-1	MIP-3β
Control 1	2.0%	1.9%	2.0%	2.2%
Control 2	3.4%	1.6%	2.3%	3.4%
Control 3	2.0%	2.9%	2.5%	3.9%
Panel 1	2.0%	2.9%	2.5%	3.9%
Panel 2	2.4%	2.1%	1.4%	3.1%

Spike and Recovery: Three EDTA plasma samples and 4 serum samples were spiked at high, medium and low concentrations within the range of the assay.

IP-10	Mean 85% Range 81%-90%
ITAC	Mean 77% Range 76%-77%
MCP-1	Mean 84% Range 82%-87%
MIP-3β	Mean 84% Range 80%-86%

Dilution Linearity: One spiked serum and 2 spiked plasma samples were diluted 10x according to the MRD, and then serially diluted 2x with Sample Diluent eight times, for final dilution of 1:1280.

IP-10	Mean 107% Range 84%–129%
ITAC	Mean 118% Range 94%–145%
MCP-1	Mean 103% Range 92%–113%
MIP-3β	Mean 109% Range 84%–144%

Single-plex Correlation: Sample concentrations derived from single-plex standard curves were compared to the same samples calculated from the 4-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
IP-10	-1.9%
ITAC	2.3%
MCP-1	4.8%
MIP-3β	7.8%

Cross-reactivity: During assay validation, cross-reactivity was assessed by testing single antigens at the concentration of the third highest calibrator in the presence of all detection antibodies (< 0.2% average cross reactivity), and single detection antibodies in the presence of all antigens (<0.2% average cross reactivity) in assay buffer. In addition, cross-reactivity of single detection antibodies was assessed in sample matrix (<0.65% average cross reactivity).