Eve Technologies Corporation
3415A 3 Ave NW, Calgary, Alberta, T2N 0M4, Canada

Patient name:	Last, First		
Accession #:	12-345-678910	**Doctor:**	Dr. Doctor
PHN:	AB 123456789	**Reason for testing:**	-
DOB:	1-Jan-2000	**Relevant medications:**	-
Specimen type:	Plasma	**Specimen source:**	MitogenDX
Date/time collected:	1-Jan-2023 / 0:00	**Report date:**	27-Sep-2023

Cytokine, Chemokine & Growth Factor Panel

Laboratory Developed Test (LDT)

Analyte	Results (pg/mL)		Reference Interval†
6CKine	450		41.0 - 892
BCA-1	>2000	HIGH	12.0 - 252
CTACK	1206		323 - 1526
EGF	53.2		0 - 94.8
ENA-78	936		19.0 - 1142
Eotaxin	31.0		5.4 - 53.8
Eotaxin-2	299		34.0 - 1249
Eotaxin-3	36.6	HIGH	0 - 25.7
FGF-2*	328	HIGH	16.0 - 145
FLT-3L	120	HIGH	0.6 - 30.0
Fractalkine	140		41.0 - 218
G-CSF*	34.6		0 - 69.1
GM-CSF*	<3.2		0 - 51.7
GROα	39.0		0 - 41.2
I-309	5.2	HIGH	0.4 - 4.7
IFN-α2	177	HIGH	11.0 - 117
IFNγ	4.4		0 - 8.9
IL-1α	131	HIGH	0 - 58.6
IL-1ß*	96.3	HIGH	0 - 39.0
IL-1RA	49.6	HIGH	0.8 - 34.0
IL-2*	17.2	HIGH	0 - 3.3
IL-3	7.2	HIGH	0 - 4.2
IL-4*	2.1		0 - 3.0
IL-5	34.0	HIGH	0.5 - 19.8
IL-6	4.5		0.2 - 14.4
IL-7	6.7		0 - 13.1
IL-8	19.3	HIGH	0 - 13.5
IL-9	18.7	HIGH	0 - 14.2
IL-10	63.0	HIGH	0 - 18.8
IL-12p40	156		7.0 - 228
IL-12p70*	30.7	HIGH	0 - 15.4
IL-13*	88.6		5.0 - 153
IL-15	46.3	HIGH	1.2 - 29.6
IL-16*	1387	HIGH	0 - 385
IL-17A*	36.6	HIGH	0 - 14.5
IL-17E/IL-25*	401		54.0 - 1315
IL-17F*	69.2	HIGH	0 - 48.1
IL-18	234		3.0 - 270
IL-20*	412		0 - 546
IL-21*	5.3		0 - 10.0
IL-22*	46.8		0 - 133
IL-23*	4219	HIGH	0 - 1558
IL-27	5652	HIGH	265 - 4087
IL-28A*	2662	HIGH	0 - 377
IL-33*	115	HIGH	0 - 55.1
IP-10	>2500	HIGH	19.0 - 336
LIF*	25.6	HIGH	0 - 17.1
M-CSF	211	HIGH	3.0 - 147
MCP-1	219		33.0 - 298
MCP-2	58.3	HIGH	9.7 - 49.6
MCP-3	18.7		3.7 - 27.3
MCP-4*	74.0		0 - 82.6
MDC	427		90.0 - 1160
MIG	12257	HIGH	346 - 5598
MIP-1α	69.4		11.5 - 76.5
MIP-1ß*	102	HIGH	9.0 - 57.6
MIP-1δ*	7254	HIGH	576 - 5356
PDGF-AA	1089		20.0 - 1347
PDGF-AB/BB	13177		2045 - 18756
RANTES	1608		167 - 1844
sCD40L	430		20.0 - 1199
SCF*	50.0		0 - 55.4
SDF-1α+ß	8282	HIGH	495 - 7690
TARC	274	HIGH	3.0 - 135
TGFα	13.1		0 - 17.5
TNFα	147	HIGH	12.0 - 122
TNFß	<1.0		0 - 33.1
TPO*	273		0 - 623
TRAIL	55.8		12.0 - 115
TSLP	64.7	HIGH	0 - 10.6
VEGF-A	30.1		0 - 180

Report comment:

-

Reviewed by: DP

Eve Technologies is a CLIA certified High Complexity International Laboratory

† Reference intervals estimated by data-mining ≥9200 PLASMA samples drawn from both healthy and pathological subjects

* Upper reference limit defined as the 85th percentile of distributions which were incompatible with the data-mining algorithm

Cytokine Groupings - Immune Signatures

Groupings represent co-expressing cytokines identified by non-biased clustering of >250 clinical plasma-EDTA samples

Refer to Polley et al., Front. Immunol., doi: 10.3389/fimmu.2023.1223817.

Patient name:	Last, First		
Accession #:	12-345-678910	**Doctor:**	Dr. Doctor
PHN:	AB 123456789	**Reason for testing:**	-
DOB:	1-Jan-2000	**Relevant medications:**	-
Specimen type:	Plasma	**Specimen source:**	MitogenDX
Date/time collected:	1-Jan-2023 / 0:00	**Report date:**	27-Sep-2023

Analyte	Results (pg/mL)		Reference Interval†
GROUP A - INNATE / AUTOIMMUNE INFLAMMATION			
FGF-2*	328	**HIGH**	16.0 - 145
IFN-α2	177	**HIGH**	11.0 - 117
IL-1α	131	**HIGH**	0 - 58.6
IL-1ß*	96.3	**HIGH**	0 - 39.0
IL-2*	17.2	**HIGH**	0 - 3.3
IL-17A*	36.6	**HIGH**	0 - 14.5
IL-17E/IL-25*	401		54.0 - 1315
MIP-1α	69.4		11.5 - 76.5
GROUP B1 - PRO-INFLAMMATORY CYTOKINES			
Fractalkine	140		41.0 - 218
GM-CSF*	<3.2		0 - 51.7
IFNγ	4.4		0 - 8.9
TNFα	147	**HIGH**	12.0 - 122
GROUP B2 - T-HELPER CELL-MEDIATED INFLAMMATION			
IL-4*	2.1		0 - 3.0
IL-5	34.0	**HIGH**	0.5 - 19.8
IL-9	18.7	**HIGH**	0 - 14.2
IL-12p40	156		7.0 - 228
IL-12p70*	30.7	**HIGH**	0 - 15.4
IL-13*	88.6		5.0 - 153
IL-22*	46.8		0 - 133
MCP-3	18.7		3.7 - 27.3
TNFß	<1.0		0 - 33.1
GROUP B3 - INNATE INFLAMMATION / CYTOKINE 'STORM'			
BCA-1	>2000	**HIGH**	12.0 - 252
FLT-3L	120	**HIGH**	0.6 - 30.0
I-309	5.2	**HIGH**	0.4 - 4.7
IL-1RA	49.6	**HIGH**	0.8 - 34.0
IL-6	4.5		0.2 - 14.4
IL-8	19.3	**HIGH**	0 - 13.5
IL-10	63.0	**HIGH**	0 - 18.8
IL-18	234		3.0 - 270
IL-27	5652	**HIGH**	265 - 4087
IP-10	>2500	**HIGH**	19.0 - 336
M-CSF	211	**HIGH**	3.0 - 147
MCP-1	219		33.0 - 298
MCP-2	58.3	**HIGH**	9.7 - 49.6
MIG	12257	**HIGH**	346 - 5598
MIP-1ß*	102	**HIGH**	9.0 - 57.6

Analyte	Results (pg/mL)		Reference Interval†
GROUP C - UNCLASSIFIED BIOMARKERS			
RANTES	1608		167 - 1844
TRAIL	55.8		12.0 - 115
GROUP D - TYPE 2 / TYPE 3 / MUCOSAL IMMUNE RESPONSE			
Eotaxin-3	36.6	**HIGH**	0 - 25.7
IL-16*	1387	**HIGH**	0 - 385
IL-17F*	69.2	**HIGH**	0 - 48.1
IL-20*	412		0 - 546
IL-21*	5.3		0 - 10.0
IL-23*	4219	**HIGH**	0 - 1558
IL-28A*	2662	**HIGH**	0 - 377
IL-33*	115	**HIGH**	0 - 55.1
LIF*	25.6	**HIGH**	0 - 17.1
MCP-4*	74.0		0 - 82.6
SCF*	50.0		0 - 55.4
TGFα	13.1		0 - 17.5
TPO*	273		0 - 623
TSLP	64.7	**HIGH**	0 - 10.6
GROUP E - EOSINOPHILIC INFLAMMATION			
Eotaxin	31.0		5.4 - 53.8
Eotaxin-2	299		34.0 - 1249
GROUP F - HEMATOPOIETIC GROWTH FACTORS			
G-CSF*	34.6		0 - 69.1
IL-3	7.2	**HIGH**	0 - 4.2
IL-7	6.7		0 - 13.1
IL-15	46.3	**HIGH**	1.2 - 29.6
GROUP G - HOMEOSTATIC CHEMOKINES			
6CKine	450		41.0 - 892
CTACK	1206		323 - 1526
MDC	427		90.0 - 1160
MIP-1δ*	7254	**HIGH**	576 - 5356
SDF-1α+ß	8282	**HIGH**	495 - 7690
GROUP H - PLATELET ACTIVATION / WOUND HEALING			
EGF	53.2		0 - 94.8
ENA-78	936		19.0 - 1142
GROα	39.0		0 - 41.2
PDGF-AA	1089		20.0 - 1347
PDGF-AB/BB	13177		2045 - 18756
sCD40L	430		20.0 - 1199
TARC	274	**HIGH**	3.0 - 135
VEGF-A	30.1		0 - 180

Report comment:

The cytokine profile could suggest a mixed type 1 (IFN-α2, IL-2, IL-12p70, TNFα, IP-10, MIG)/type 3 (IL-17A, IL-17F, IL-23 with IL-1) immune response. High results in group A may indicate autoimmune inflammation, high results in group B3 may indicate severe innate inflammation or cytokine storm, and high results in group D could indicate a type 3/mucosal immune response. Similar profiles have been observed in conditions that are associated with severe systemic inflammation.

Reviewed by: DP

† Reference intervals estimated by data-mining ≥9200 PLASMA samples drawn from both healthy and pathological subjects
* Upper reference limit defined as the 85th percentile of distributions which were incompatible with the data-mining algorithm

Cytokine Groupings Descriptions

GROUP A - INNATE / AUTOIMMUNE INFLAMMATION
The analytes in this group are mainly associated with innate immunity (IL-1α/β, IL-17E/IL-25, IFNα2), type 1 (IFNα2, IL-2, MIP-1α) or type 3 (IL-17A, IL-1) immune responses. IL-1, type I interferons, IL-17, MIP-1α, and FGF-2 are known contributors to autoimmune diseases, and IL-2 and IL-17E/IL-25 have context-specific roles in either promoting or suppressing autoimmunity. IL-17A and FGF-2 have been shown to synergistically promote inflammation in autoimmune arthritis. IL-1, IL-17 and FGF-2 can initiate and potentiate type 3 (Th17-mediated) immune responses, which is a key driver of autoimmunity, while IL-2 and IL-17E/IL-25 have been shown to be negative regulators of Th17 activity. IFNα2 can exacerbate Th17-mediated inflammation in autoimmune diseases, such as in systemic lupus erythematosus (SLE) where IFNα2 and IL-17A contribute to a pathogenic signaling axis. IL-1α/β are also key drivers of innate inflammatory responses and are major contributors to autoinflammatory conditions.
GROUP B1 - PRO-INFLAMMATORY CYTOKINES
The analytes in this group are potent pro-inflammatory cytokines that can initiate and propagate innate inflammation and contribute to adaptive immune responses. These analytes are all associated with type 1 immune responses – IFNγ, GM-CSF and TNFα can all be released by Th1 cells and NK cells, and fractalkine can amplify polarized type 1 responses and induce the recruitment of Th1 and NK cells. TNFα, GM-CSF and fractalkine can also contribute to type 2 and type 3 immune responses in certain contexts.
GROUP B2 - T-HELPER CELL-MEDIATED INFLAMMATION
The analytes in this group are largely associated with CD4+ T-helper cell mediated responses. The cytokine profile may indicate responses orchestrated by Th1 (type 1 immunity - IFNγ, IL-12p70, TNFβ), Th2 (type 2 immunity - IL-4, IL-5, IL-13, IL-9), and/or Th17 cells (type 3 immunity - IL-22) along with Th9 (type 2 immunity - IL-9) and Th22 cells (type 3 immunity - IL-22, IL-13). IL-12p40 is a subunit of both IL-12p70 (Th1-polarizing) and IL-23 (Th17-polarizing). Patterns of mixed T cell cytokine release could reflect immune regulation, as the cytokines or each immunotype tend to suppress other immunotypes, or the heterogeneity and plasticity observed in T cells in vivo, since “hybrid” cells that co-express the signature cytokines of two different T cell subtypes (e.g., IL-4 with IFNγ - Th2/Th1; IFNγ with IL-17A, - Th17/Th1; IL-4 with IL-17A - Th2/Th17, etc.) may be common.
GROUP B3 - INNATE INFLAMMATION / CYTOKINE ‘STORM’
High levels of the analytes in this group may be associated with innate immune responses – IL-6 plays a central role in innate immunity as a key factor driving acute phase protein release, IL-18 is a pro-inflammatory alarmin released following inflammasome activation, and Flt-3L is an important factor in innate lymphoid cell development. High results across this group may indicate severe systemic inflammatory responses such as ‘cytokine storm’ (cytokine release syndrome; CRS). IL-6, IL-10, IL-18, IL-8, MIG, IP-10, MIP-1β, MCP-1, BCA-1 have been found to be important soluble mediators of cytokine storm. The anti-inflammatory factors IL-1RA and IL-10 have been found to be upregulated in cytokine storm, likely representing an insufficient regulatory response.
GROUP C - UNCLASSIFIED BIOMARKERS
Neither of these biomarkers were found to correlate strongly with any other in the panel.
GROUP D - TYPE 2 / TYPE 3 / MUCOSAL IMMUNE RESPONSE
High results of the analytes in this group could indicate a mixed type 2 (IL-33, TSLP, Eotaxin-3)/type 3 (IL-17F, IL-23, IL-21) immune response (additionally, SCF potentiates dendritic cells to induce either Th2 or Th17 differentiation, and IL-16 is a chemoattractant and activating factor for CD4+ T cells). IL-28A is associated with type 1 responses and may be a negative regulator of type 2 and type 3 responses, and LIF is released by Tregs and suppresses Th17 differentiation. Type 3 immunity is crucial in mucosal defence and epithelial integrity, and several analytes in this group are prominently derived from or act primarily upon epithelial cells, such as TSLP, IL-33, TGFα, IL-28A, and IL-20, which suggests that the group as a whole may reflect mucosal immune responses.
GROUP E - EOSINOPHILIC INFLAMMATION
Elevated levels of biomarkers in this group could indicate a type 2 inflammatory response with eosinophil involvement.
GROUP F - HEMATOPOIETIC GROWTH FACTORS
The analytes in this group are hematopoietic growth factors and could indicate the expansion and activation of lymphocytes (IL-7, IL-15) and/or leukocytes (G-CSF, IL-3).
GROUP G - HOMEOSTATIC CHEMOKINES
The analytes in this group are homeostatic (constitutively expressed) chemokines that can be upregulated in inflammatory contexts.
GROUP H - PLATELET ACTIVATION / WOUND HEALING
High levels of most or all of the analytes in this group could indicate a platelet activation/wound healing response, as all of these factors are stored in and released by platelets, or induced by platelet-released factors. High results in this group are often observed in conditions typically associated with thrombocytosis, whereas lower results have been observed in conditions associated with thrombocytopenia. Levels of the analytes in this group are significantly higher in serum samples than in plasma samples drawn from the same subjects.

Descriptions of the analytes and groupings with citations are available from Eve Technologies Corporation.

Clusters of co-expressing cytokines were determined with unsupervised clustering analysis on >250 plasma-EDTA specimens provided to us for diagnostic testing drawn from patients with a variety of inflammatory / autoimmune conditions, as detailed in our publication:

Polley DJ et al. Identification of novel clusters of co-expressing cytokines in a diagnostic cytokine multiplex test. Frontiers in Immunology. 2023-July-31 2023;14 doi:10.3389/fimmu.2023.1223817.

The groupings in this report were adapted to functional similarities of certain analytes. Specifically, the placement of the analytes in groups E, F, and G were informed by common physiological functions of the analytes, and group C is comprised of the two analytes in the panel that did not correlate strongly with any other. The designations of physiological/pathological significance assigned to each grouping are speculative, based on an analysis of the immune signatures in our database of clinical specimens and on the functional/pathological roles of the analytes in each grouping established in the scientific literature.