Eve Technologies Corporation

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Patient initials:	XX	**Date/time collected:**	12-Mar-22 / 7:30
Specimen ID:	1234	**Doctor:**	Dr. Smith
SSN:	22-071-012345	**Reason for testing:**	HLH/MAS
Age:	38	**Relevant medications:**	-
Gender:	M	**Location:**	MitogenDx
Sample type:	Plasma-EDTA	**Report date:**	15-Mar-22

Cytokine, Chemokine & Growth Factor 71-Plex Clinical RUO Discovery Assay

For Research Purposes Only

Analyte	Results (pg/mL)		Reference Interval†
6CKine	926		39 - 1176
BCA-1	1578	HIGH	11 - 149
CTACK	2176	HIGH	348 - 2083
EGF	5.8		0 - 72.7
ENA-78	48.2		19 - 1061
Eotaxin	13.5		6.0 - 52.8
Eotaxin-2*	323		17 - 838
Eotaxin-3*	< 3		0 - 19.5
FGF-2*	118		19 - 148
FLT-3L	54.9	HIGH	1.4 - 28.9
Fractalkine	590	HIGH	14 - 193
G-CSF*	51.5	HIGH	0 - 44.9
GM-CSF*	93.0	HIGH	0 - 54.4
GROα	21.6		0 - 32.0
I-309	83.9	HIGH	0.2 - 3.4
IFNα2	68.2		12 - 127
IFNγ	12.2	HIGH	0 - 7.9
IL-1α	50.4	HIGH	0 - 48.5
IL-1β	24.0		0 - 34.1
IL-1RA	59.2	HIGH	0.5 - 34.2
IL-2*	1.8		0 - 3.4
IL-3	4.3	HIGH	0 - 2.5
IL-4*	0.2		0 - 2.2
IL-5	20.1	HIGH	0.1 - 9.9
IL-6	27.3	HIGH	0.1 - 11.6
IL-7	4.3		0 - 9.0
IL-8	18.2	HIGH	0 - 13.3
IL-9*	13.3	HIGH	0 - 12.3
IL-10*	82.5	HIGH	0 - 18.3
IL-12p40	73.0		4 - 176
IL-12p70*	2.3		0 - 6.6
IL-13	< 4		0 - 163
IL-15*	120	HIGH	1.5 - 25.5
IL-16*	248	HIGH	0 - 247
IL-17A*	3.4		0 - 15.1
IL-17E/IL-25*	300		0 - 735
IL-17F	5.7		0 - 28.2
IL-18	3616	HIGH	1 - 115
IL-20*	< 195.3		0 - 349
IL-21*	4.1		0 - 8.1
IL-22*	< 16		0 - 85.9
IL-23*	< 24.4		0 - 926
IL-27	3579		179 - 4103
IL-28A*	< 4.88		0 - 169
IL-33*	< 2.44		0 - 36.2
IP-10	> 2500	HIGH	13 - 374
LIF*	6.4		0 - 15.8
MCP-1	322		34 - 356
MCP-2	50.1		9.7 - 52.0
MCP-3	15.3		3.0 - 31.8
MCP-4*	87.0	HIGH	0 - 80.0
M-CSF*	615	HIGH	7 - 190
MDC*	105	LOW	115 - 941
MIG	22241	HIGH	318 - 5193
MIP-1α	72.5	HIGH	8.0 - 63.9
MIP-1β	98.2	HIGH	5.0 - 38.8
MIP-1δ	4912		624 - 5302
PDGF-AA*	117		51 - 1272
PDGF-AB/BB*	2972		1129 - 16513
RANTES*	1423		362 - 2029
sCD40L	206		12 - 905
SCF*	70.2	HIGH	0 - 48.9
SDF-1α+β	7800	HIGH	402 - 7655
TARC	14.1		2.4 - 61.6
TGFα	4.4		0 - 13.1
TNFα	351	HIGH	4 - 113
TNFβ	1.7		0 - 21.9
TPO*	746	HIGH	0 - 610
TRAIL	44.8		3 - 142
TSLP*	< 0.31		0 - 9.0
VEGF-A	14.8		0 - 74.7

Report comment:

Reviewed by: DP

† Reference intervals estimated by data-mining ≥3500 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

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Sample type:	Plasma-EDTA	**Report date:**	15-Mar-22

Analyte	Results (pg/mL)		Reference Interval†
GROUP A – INNATE / AUTOIMMUNE INFLAMMATION			
FGF-2*	118		19 - 148
IFNα2	68.2		12 - 127
IL-1α	50.4	HIGH	0 - 48.5
IL-1β	24.0		0 - 34.1
IL-2*	1.8		0 - 3.4
IL-17A*	3.4		0 - 15.1
IL-17E/IL-25*	300		0 - 735
MIP-1α	72.5	HIGH	8.0 - 63.9
GROUP B1 – PRO-INFLAMMATORY CYTOKINES			
Fractalkine	590	HIGH	14 - 193
GM-CSF*	93.0	HIGH	0 - 54.4
IFNγ	12.2	HIGH	0 - 7.9
TNFα	351	HIGH	4 - 113
GROUP B2 – T-HELPER CELL-MEDIATED INFLAMMATION			
IL-12p70*	2.3		0 - 6.6
TNFβ	1.7		0 - 21.9
MCP-3	15.3		3.0 - 31.8
IL-22*	< 16		0 - 85.9
IL-13	< 4		0 - 163
IL-4*	0.2		0 - 2.2
IL-9*	13.3	HIGH	0 - 12.3
GROUP B3 – INNATE INFLAMMATION / CYTOKINE 'STORM'			
BCA-1	1578	HIGH	11 - 149
FLT-3L	54.9	HIGH	1.4 - 28.9
I-309	83.9	HIGH	0.2 - 3.4
IL-1RA	59.2	HIGH	0.5 - 34.2
IL-6	27.3	HIGH	0.1 - 11.6
IL-8	18.2	HIGH	0 - 13.3
IL-10*	82.5	HIGH	0 - 18.3
IL-18	3616	HIGH	1 - 115
IL-27	3579		179 - 4103
IP-10	> 2500	HIGH	13 - 374
MCP-1	322		34 - 356
MCP-2	50.1		9.7 - 52.0
M-CSF*	615	HIGH	7 - 190
MIP-1β	98.2	HIGH	5.0 - 38.8
MIG	22241	HIGH	318 - 5193
GROUP C – UNCLASSIFIED BIOMARKERS			
IL-12p40	73.0		4 - 176
TRAIL	44.8		3 - 142

Analyte	Results (pg/mL)		Reference Interval†
GROUP D – TYPE 2 / TYPE 3 IMMUNE RESPONSE			
IL-17F	5.7		0 - 28.2
IL-20*	< 195.3		0 - 349
IL-21*	4.1		0 - 8.1
IL-23*	< 24.4		0 - 926
IL-33*	< 2.44		0 - 36.2
LIF*	6.4		0 - 15.8
MCP-4*	87.0	HIGH	0 - 80.0
TGFα	4.4		0 - 13.1
TPO*	746	HIGH	0 - 610
TSLP	< 0.31		0 - 9.0
IL-16*	248	HIGH	0 - 247
IL-28A*	< 4.88		0 - 169
SCF*	70.2	HIGH	0 - 48.9
GROUP E – EOSINOPHILIC INFLAMMATION			
Eotaxin	13.5		6.0 - 52.8
Eotaxin-2*	323		17 - 838
Eotaxin-3*	< 3		0 - 19.5
IL-5	20.1	HIGH	0.1 - 9.9
GROUP F – HEMATOPOIETIC GROWTH FACTORS			
G-CSF*	51.5	HIGH	0 - 44.9
IL-3	4.3	HIGH	0 - 2.5
IL-7	4.3		0 - 9.0
IL-15*	120	HIGH	1.5 - 25.5
GROUP G – HOMEOSTATIC CHEMOKINES			
6CKine	926		39 - 1176
CTACK	2176	HIGH	348 - 2083
MDC*	105	LOW	115 - 941
MIP-1δ	4912		624 - 5302
SDF-1α+β	7800	HIGH	402 - 7655
GROUP H – PLATELET ACTIVATION / WOUND HEALING			
EGF	5.8		0 - 72.7
ENA-78	48.2		19 - 1061
GROα	21.6		0 - 32.0
PDGF-AA*	117		51 - 1272
PDGF-AB/BB*	2972		1129 - 16513
RANTES*	1423		362 - 2029
sCD40L	206		12 - 905
TARC	14.1		2.4 - 61.6
VEGF-A	14.8		0 - 74.7

Report comment: High values across the biomarkers in groups B1 and B3 is a signature observed in conditions associated with cytokine 'storm', such as MAS, HLH, Kawasaki, and AOSD, as well as lymphoproliferative diseases.

† Reference intervals estimated by data-mining ≥3500 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 biomarkers in this group are mainly associated with type 1 (Th1-mediated; IFNα2, IL-2, MIP-1α) or type 3 (Th17-mediated; IL-17A, IL-1) immune responses, so high levels of these markers could indicate infection with intracellular or extracellular pathogens, or autoimmune inflammation. IL-1, type I interferons, IL-17, and FGF-2 are known contributors to autoimmune disorders, and elevated IL-2 promotes active inflammation. 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 (a key driver of autoimmunity), while IFNα2, IL-2 and IL-17E/IL-25 may be negative regulators of Th17 activity. IL-1 is also one of the major drivers of innate inflammatory responses.
GROUP B1 – PRO-INFLAMMATORY CYTOKINES
The biomarkers in this group are potent pro-inflammatory cytokines that can both initiate and propagate innate inflammation and contribute to adaptive immune responses. While IFNγ is associated with type 1 immune responses, TNFα, GM-CSF and fractalkine all contribute to type 1, type 2, and type 3 immune responses. Elevated levels of biomarkers in this group are most often associated with high levels of the markers in either group B2 (T-cell mediated inflammation) or B3 (innate inflammation/cytokine storm).
GROUP B2 – T-HELPER CELL-MEDIATED INFLAMMATION
The biomarkers in this group are largely associated with CD4+ T-helper cell mediated responses. Markers may indicate responses orchestrated by Th1 (IL-12p70, IFNγ, TNFβ; intracellular pathogen infection/autoimmunity), Th2 (IL-4, IL-13; helminth infection/allergy), and/or Th17 cells (IL-22; extracellular pathogen infection/autoimmunity) along with Th9 (IL-9) and Th22 cells (IL-22, IL-13), both potentially involved in allergy and autoimmunity. The pattern of biomarkers could also point to mixed T-cell responses (e.g., mixed Th1/Th2 response in severe asthma, Th1/Th17 in Crohn's disease, etc.), or plasticity in T-helper cell function (e.g., IL-13-expressing Th1 and Th17 cells).
GROUP B3 – INNATE INFLAMMATION / CYTOKINE 'STORM'
Elevated levels of biomarkers in this group, often with the biomarkers in group B1, may be associated with innate immune responses, including 'cytokine storm' (Cytokine Release Syndrome). TNFα, IL-6 and IL-18 have been identified as important regulators to CRS, and high levels of IL-10 are observed in severe COVID-19. The chemokines in this group are mostly inducible and pro-inflammatory, and elevated levels of IL-8, IP-10, and MCP-1 specifically have been identified in the COVID-19-induced CRS, and BCA-1 has been identified as a marker of lethal COVID-19 cases. Elevated values across the biomarkers in this group are observed in other conditions associated with CRS, such as MAS, HLH, Kawasaki, and AOSD.
GROUP C – UNCLASSIFIED BIOMARKERS
Neither of these biomarkers correlate strongly with any other, and both can have anti-inflammatory properties.
GROUP D – TYPE 2 / TYPE 3 IMMUNE RESPONSE
Elevated biomarkers in this group could indicate type 2 (IL-33, TSLP) and/or type 3 (IL-17F, IL-23, IL-21) immune responses, along with the differentiation, proliferation and activation of many lymphocytes and leukocytes (TPO, SCF, IL-16). IL-28A (IFNλ2) is associated with type 1 responses, and may be a negative regulator of type 2 and type 3 responses. This group has been associated with severe COVID-19 cases corresponding with increased coagulopathy and mortality.
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 biomarkers in this group are hematopoietic growth factors and elevated levels could indicate inflammation characterized by the expansion and activation of lymphocytes (IL-7, IL-15) and/or leukocytes (IL-3, G-CSF).
GROUP G – HOMEOSTATIC CHEMOKINES
The biomarkers in this group represent homeostatic (constitutively expressed) chemokines that can also be upregulated in inflammatory conditions.
GROUP H – PLATELET ACTIVATION / WOUND HEALING
Elevated levels of most or all of the biomarkers in this group could indicate a platelet activation/wound healing response, as all of these factors are stored in and released by platelets. Levels of the biomarkers in this group are significantly higher in serum samples compared to plasma samples drawn from the same subjects.

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

Cytokine groupings were determined with unsupervised clustering analysis using different methods (k-means, hierarchical clustering using Pearson and Spearman correlation coefficients) and identifying consensus groupings between the clustering methods on >250 plasma-EDTA specimens provided to us for diagnostic testing from patients with a variety of inflammatory / autoimmune conditions. The analytes in groups A, B, D, and H were observed to cluster together consistently across the different clustering methods and so those groupings likely represent common inflammatory signatures. The placement of the analytes in groups C, E, F, and G were more variable in the clustering analysis, so those groups are informed by both the unbiased clustering results and by the physiological functions of the analytes. 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.