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:**	HLH
DOB:	1-Jan-2000	**Relevant medications:**	-
Specimen type:	Plasma	**Specimen source:**	MitogenDX
Date/time collected:	1-Jan-2023 / 0:00	**Report date:**	2-Oct-2023

Cytokine, Chemokine & Growth Factor Panel

Laboratory Developed Test (LDT)

Analyte	Results (pg/mL)		Reference Interval†	Analyte	Results (pg/mL)		Reference Interval†
6CKine	363		41.0 - 892	IL-17F*	25.9		0 - 48.1
BCA-1	1910	HIGH	12.0 - 252	IL-18	1090	HIGH	3.0 - 270
CTACK	1101		323 - 1526	IL-20*	287		0 - 546
EGF	24.3		0 - 94.8	IL-21*	<2.4		0 - 10.0
ENA-78	110		19.0 - 1142	IL-22*	115		0 - 133
Eotaxin	62.2	HIGH	5.4 - 53.8	IL-23*	144		0 - 1558
Eotaxin-2	82.8		34.0 - 1249	IL-27	8948	HIGH	265 - 4087
Eotaxin-3	13.6		0 - 25.7	IL-28A*	6.9		0 - 377
FGF-2*	96.8		16.0 - 145	IL-33*	2.5		0 - 55.1
FLT-3L	51.1	HIGH	0.6 - 30.0	IP-10	>2500	HIGH	19.0 - 336
Fractalkine	615	HIGH	41.0 - 218	LIF*	11.4		0 - 17.1
G-CSF*	64.8		0 - 69.1	M-CSF	498	HIGH	3.0 - 147
GM-CSF*	51.6		0 - 51.7	MCP-1	1787	HIGH	33.0 - 298
GROα	16.0		0 - 41.2	MCP-2	70.1	HIGH	9.7 - 49.6
I-309	4.6		0.4 - 4.7	MCP-3	61.7	HIGH	3.7 - 27.3
IFN-α2	89.0		11.0 - 117	MCP-4*	133	HIGH	0 - 82.6
IFNγ	27.0	HIGH	0 - 8.9	MDC	2143	HIGH	90.0 - 1160
IL-1α	94.6	HIGH	0 - 58.6	MIG	9267	HIGH	346 - 5598
IL-1ß*	15.2		0 - 39.0	MIP-1α	157	HIGH	11.5 - 76.5
IL-1RA	99.8	HIGH	0.8 - 34.0	MIP-1ß*	28.8		9.0 - 57.6
IL-2*	5.9	HIGH	0 - 3.3	MIP-1δ*	11489	HIGH	576 - 5356
IL-3	1.0		0 - 4.2	PDGF-AA	260		20.0 - 1347
IL-4*	2.2		0 - 3.0	PDGF-AB/BB	7630		2045 - 18756
IL-5	9.9		0.5 - 19.8	RANTES	1492		167 - 1844
IL-6	180	HIGH	0.2 - 14.4	sCD40L	1776	HIGH	20.0 - 1199
IL-7	6.3		0 - 13.1	SCF*	46.3		0 - 55.4
IL-8	1.0		0 - 13.5	SDF-1α+ß	8089	HIGH	495 - 7690
IL-9*	6.3		0 - 14.2	TARC	3.9		3.0 - 135
IL-10	>5000	HIGH	0 - 18.8	TGFα	12.1		0 - 17.5
IL-12p40	350	HIGH	7.0 - 228	TNFα	353	HIGH	12.0 - 122
IL-12p70*	18.8	HIGH	0 - 15.4	TNFß	28.0		0 - 33.1
IL-13*	337	HIGH	5.0 - 153	TPO*	140		0 - 623
IL-15	35.4	HIGH	1.2 - 29.6	TRAIL	71.3		12.0 - 115
IL-16*	983	HIGH	0 - 385	TSLP	<0.3		0 - 10.6
IL-17A*	<0.8		0 - 14.5	VEGF-A	41.3		0 - 180
IL-17E/IL-25*	588		54.0 - 1315				

Report comment:

-

Reviewed by: DP

Eve Technologies Corporation 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:**	HLH
DOB:	1-Jan-2000	**Relevant medications:**	-
Specimen type:	Plasma	**Specimen source:**	MitogenDX
Date/time collected:	1-Jan-2023 / 0:00	**Report date:**	2-Oct-2023

Analyte	Results (pg/mL)		Reference Interval†
GROUP A - INNATE / AUTOIMMUNE INFLAMMATION			
FGF-2*	96.8		16.0 - 145
IFN-α2	89.0		11.0 - 117
IL-1α	94.6	**HIGH**	0 - 58.6
IL-1ß*	15.2		0 - 39.0
IL-2*	5.9	**HIGH**	0 - 3.3
IL-17A*	<0.8		0 - 14.5
IL-17E/IL-25*	588		54.0 - 1315
MIP-1α	157	**HIGH**	11.5 - 76.5
GROUP B1 - PRO-INFLAMMATORY CYTOKINES			
Fractalkine	615	**HIGH**	41.0 - 218
GM-CSF*	51.6		0 - 51.7
IFNγ	27.0	**HIGH**	0 - 8.9
TNFα	353	**HIGH**	12.0 - 122
GROUP B2 - T HELPER CELL-MEDIATED INFLAMMATION			
IL-4*	2.2		0 - 3.0
IL-5	9.9		0.5 - 19.8
IL-9*	6.3		0 - 14.2
IL-12p40	350	**HIGH**	7.0 - 228
IL-12p70*	18.8	**HIGH**	0 - 15.4
IL-13*	337	**HIGH**	5.0 - 153
IL-22*	115		0 - 133
MCP-3	61.7	**HIGH**	3.7 - 27.3
TNFß	28.0		0 - 33.1
GROUP B3 - INNATE INFLAMMATION / CYTOKINE 'STORM'			
BCA-1	1910	**HIGH**	12.0 - 252
FLT-3L	51.1	**HIGH**	0.6 - 30.0
I-309	4.6		0.4 - 4.7
IL-1RA	99.8	**HIGH**	0.8 - 34.0
IL-6	180	**HIGH**	0.2 - 14.4
IL-8	1.0		0 - 13.5
IL-10	>5000	**HIGH**	0 - 18.8
IL-18	1090	**HIGH**	3.0 - 270
IL-27	8948	**HIGH**	265 - 4087
IP-10	>2500	**HIGH**	19.0 - 336
M-CSF	498	**HIGH**	3.0 - 147
MCP-1	1787	**HIGH**	33.0 - 298
MCP-2	70.1	**HIGH**	9.7 - 49.6
MIG	9267	**HIGH**	346 - 5598
MIP-1ß*	28.8		9.0 - 57.6

Analyte	Results (pg/mL)		Reference Interval†
GROUP C - UNCLASSIFIED ANALYTES			
RANTES	1492		167 - 1844
TRAIL	71.3		12.0 - 115
GROUP D - TYPE 2 / TYPE 3 / MUCOSAL IMMUNE RESPONSE			
Eotaxin-3	13.6		0 - 25.7
IL-16*	983	**HIGH**	0 - 385
IL-17F*	25.9		0 - 48.1
IL-20*	287		0 - 546
IL-21*	<2.4		0 - 10.0
IL-23*	144		0 - 1558
IL-28A*	6.9		0 - 377
IL-33*	2.5		0 - 55.1
LIF*	11.4		0 - 17.1
MCP-4*	133	**HIGH**	0 - 82.6
SCF*	46.3		0 - 55.4
TGFα	12.1		0 - 17.5
TPO*	140		0 - 623
TSLP	<0.3		0 - 10.6
GROUP E - EOSINOPHILIC INFLAMMATION			
Eotaxin	62.2	**HIGH**	5.4 - 53.8
Eotaxin-2	82.8		34.0 - 1249
GROUP F - HEMATOPOIETIC GROWTH FACTORS			
G-CSF*	64.8		0 - 69.1
IL-3	1.0		0 - 4.2
IL-7	6.3		0 - 13.1
IL-15	35.4	**HIGH**	1.2 - 29.6
GROUP G - HOMEOSTATIC CHEMOKINES			
6CKine	363		41.0 - 892
CTACK	1101		323 - 1526
MDC	2143	**HIGH**	90.0 - 1160
MIP-1δ*	11489	**HIGH**	576 - 5356
SDF-1α+ß	8089	**HIGH**	495 - 7690
GROUP H - PLATELET ACTIVATION / WOUND HEALING			
EGF	24.3		0 - 94.8
ENA-78	110		19.0 - 1142
GROα	16.0		0 - 41.2
PDGF-AA	260		20.0 - 1347
PDGF-AB/BB	7630		2045 - 18756
sCD40L	1776	**HIGH**	20.0 - 1199
TARC	3.9		3.0 - 135
VEGF-A	41.3		0 - 180

Report comment:

High or trending-high results were observed across groups B1, B2, and B3 - refer to page 3 for grouping descriptions. The profile of high or trending high values for specific analytes could suggest a strong type 1 (Th1-type; IFNγ, TNFß, IL-2, IL-12p70, IL-18, IP-10, MIG, TNFα, GM-CSF, fractalkine, MIP-1α, IL-27) adaptive immune response.

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 - 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) immunity, 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 - 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 - GROUP B2 - T HELPER CELL-MEDIATED INFLAMMATION
The analytes in this group are largely associated with adaptive 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 - 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 - GROUP C - UNCLASSIFIED ANALYTES
Neither of these analytes were found to correlate strongly with any other in the panel.
GROUP D - 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 - GROUP E - EOSINOPHILIC INFLAMMATION
Elevated levels of biomarkers in this group could indicate a type 2 inflammatory response with eosinophil involvement.
GROUP F - 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 - GROUP G - HOMEOSTATIC CHEMOKINES
The analytes in this group are homeostatic (constitutively expressed) chemokines that can be upregulated in inflammatory contexts.
GROUP H - 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 and/or take part in angiogenic, tissue remodeling or inflammatory processes during wound healing. High results in this group are often observed in conditions typically associated with vascular injury, angiogenesis and/or thrombocytosis, such as AOSD, Kawasaki disease, juvenile arthritis, FMF, COVID-19 and Crohn’s disease, whereas lower results have been observed in conditions associated with thrombocytopenia such as HLH, lymphocytic leukemia, and hematopoietic stem cell transplantation. 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 Diagnostics.

Clusters of co-expressing cytokines were determined with unsupervised clustering analysis of >250 plasma-EDTA specimens as described 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 reflect functional similarities of certain 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.