ΑΝΟΣΟΦΑΙΝΟΤΥΠΟΣ ΛΕΜΦΟΚΥΤΤΑΡΩΝ ΔΕΙΓΜΑΤΩΝ COVID-19



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KIT ΜΕΤΡΗΣΗΣ ΤΩΝ Τ & Β ΛΕΜΦΟΚΥΤΤΑΡΩΝ ΤΗΣ ΕΤΑΙΡΕΙΑΣ EXBIO ΤΣΕΧΙΑΣ. DRYFLOWEX ASC SCREEN KIT DRYFLOWEX ACT T SCREEN KIT

- ΤΑ ΚΙΤ ΕΊΝΑΙ ΤΩΝ 50 ΤΕΣΤ, ΣΕ ΜΟΝΗΡΗ ΣΩΛΗΝΑΡΙΑ ΣΕ ΞΗΡΑ ΜΟΡΦΗ, ΠΑΚΕΤΑΡΙΣΜΕΝΑ ΑΝΑ 5
 ΣΩΛΗΝΑΡΙΑ ΣΕ ΑΛΟΥΜΙΝΕΝΙΑ ΣΥΣΚΕΥΑΣΙΑ, ΜΕ 7 (B CELLS) Ή 8 (T CELLS) ΑΝΤΙΣΩΜΑΤ/
- ΠΕΡΙΛΑΜΒΑΝΟΥΝ 7 Ή 8 ΣΩΛΗΝΑΡΙΑ , ΑΝΑ ΚΙΤ, 1ΣΕΤ, ΓΙΑ COMPENSATION.
- ΑΠΟΘΗΚΕΥΣΗ ΣΕ ΘΕΡΜΟΚΡΑΣΙΑ ΔΩΜΑΤΙΟΥ.
- ΠΡΟΣΤΑΤΕΥΜΕΝΑ ΑΠΌ ΤΗΝ ΥΓΡΑΣΙΑ.
- ΛΥΤΙΚΟ ΜΕ ΦΟΡΜΑΛΔΕΥΔΗ







DryFlowEx ACT T Screen Kit

		PD-1			CXCR5			
Specificity	CD4	(CD279)	HLA-DR	CD3	(CD185)	CD38	CD45	CD8
Clone	MEM-241	EH12.2H7	L243	SK7	J252D4	HIT2	2D1	MEM-31
lsotype								
(murine)	lgG1	lgG1	lgG2a	lgG1	lgG1	lgG1	lgG1	lgG2a
			PerCP-				Pacific	Pacific
Fluorochrome	FITC	PE	Cy5.5	PE-Cy7	APC	APC-Cy7	Blue	Orange
λ excitation	488 nm	488 nm	488 nm	488 nm	633 nm	633 nm	405 nm	405 nm
Emission								
maxima	525 nm	575 nm	692 nm	780 nm	660 nm	780 nm	455 nm	551 nm







Target population(s) percentage range acquired from normal healthy human whole blood specimens

n=15	Avg	Min. (Avg - 2 SD)	Max. (Avg + 2 SD)
Circulating Folicular Helper T- cells	2,24%	0,90%	3,59%
Activated CD3 ⁺ CD4 ⁺ T-cells	0,66%	0,29%	1,03%
Activated CD3 ⁺ CD8 ⁺ T-cells	0,67%	0,00%	1,60%

95% Range





Target population(s) percentage change in time acquired from whole blood specimens of hospitalized patients diagnosed with COVID-19





DryFlowEx ASC Screen Kit

Specificity	lgD	CD27	CD24	CD19	CD21	CD38	CD45
Clone	IA6-2	LT27	SN3	LT19	LT21	HIT2	2D1
Isotype							
(murine)	lgG2a	lgG2a	lgG1	lgG1	lgG1	lgG1	lgG1
			PerCP-				Pacific
Fluorochrome	FITC	PE	Cy5.5	PE-Cy7	APC	APC-Cy7	Blue
λ excitation	488 nm	488 nm	488 nm	488 nm	633 nm	633 nm	405 nm
Emission							
maxima	525 nm	575 nm	692 nm	780 nm	660 nm	780 nm	455 nm







Singlet events
 CD45+ singlets - leukocytes



3 CD45+ SSClow mononuclear cells
4 CD45+CD19+ lymphocytes (B lymphocytes)

- 5 CD45+CD19+CD27+ B lymphocytes (memory B cells)
 6 CD45+CD19+CD27+CD38+ Plasmablasts
 7 ASC target populations:
 - IgD+ Non-switched Plasmablasts
 - IgD- Class-switched Plasmablasts



Table 1 – Target population(s) percentage range acquired from normal healthy human whole blood specimens

95% Range

n=15	Avg	Min. (Avg - 2 SD)	Max. (Avg + 2 SD)
Class-switched Plasmablasts	1,18%	0,00%	2,50%
Non-switched Plasmablasts	0,03%	0,00%	0,08%





Fig. 1 Target population(s) percentage change in time acquired from whole blood specimens of hospitalized patients diagnosed with COVID-19











More B lymphocytes subsets to be evaluated during viral infection:

Transitional B cells

- CD45+ CD19+ CD27- CD24++ CD38+ (CD21+ IgD+)
- Do not secrete antibodies

Marginal zone-like B cells

- CD45+ CD19+ CD27+ IgD+ (CD21+/++ CD24+ CD38dim)
- T cell independent immune response

CD21Iow B cells

- CD45+ CD19+ CD21- CD38-
- Auto-immunity connected cells

Näive B cells

• CD45+ CD19+ CD27- CD24+ CD38- (IgD+)





Βρέθηκαν ότι οι απόλυτοι αριθμοί του περιφερικού CD4 και CD8 T κύτταρων μειώθηκαν ουσιαστικά, ενώ ενεργοποιήθηκαν, όπως αποδεικνύεται από το υψηλές αναλογίες HLA-DR (CD4 3,47%) και CD38 (CD8 39,4%) διπλά θετικά κλάσματα. Επιπλέον, υπήρξε αυξημένη συγκέντρωση εξαιρετικά προφλεγμονώδους CCR6 + Th17 σε CD4 T κύτταρα. Επιπλέον, βρέθηκαν CD8 T κύτταρα να έχουν υψηλές συγκεντρώσεις κυτταροτοξικών κοκκίων, στα οποία 31,6% κύτταρα ήταν θετικά στην περφορίνη, 64,2% κύτταρα ήταν θετικά στην γκρανουλυσίνη, και 30,5% κύτταρα ήταν granulysin και perforin διπλά θετικά. Τα αποτελέσματά μας υπονοούν ότι η υπερβολική ενεργοποίηση των Τ κυττάρων, εκδηλώνεται με αύξηση του Th17 και υψηλή κυτταροτοξικότητα του CD8 T κυττάρων και αντιπροσωπεύουν, εν μέρει, το σοβαρό ανοσοποιητικό τραυματισμό σε αυτούς τους ασθενείς.





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Table 3. Immunological features of patients with COVID-19

	All patients (<i>n</i> = 21)	Severe cases $(n = 11)$	Moderate cases (<i>n</i> = 10)	P value	Normal range
Total T lymphocytes, %	60.5 (54.4–70.3)	55.1 (52.2-60.5)	68.8 (64.7-75.2)	0.020	50-84
Total T lymphocyte count, ×10 ⁶ /L	486.5 (267.0-664.8)	294.0 (169.3–415.3)	640.5 (588.3–789.5)	0.011	955-2860
Decreased, n/N (%)	13/14 (92.9%)	8/8 (100.0%)	5/6 (83.3%)	0.43	
<400, n/N (%)	6/14 (42.9%)	6/8 (75.0%)	0/6 (0.0%)	0.010	
Total B lymphocytes (%)	16.9 (10.8-22.4)	20.2 (17.6–39.5)	10.8 (10.3–12.4)	0.025	5–18
Increased, n/N (%)	7/14 (50.0%)	6/8 (75.0%)	1/6 (16.7%)	0.10	
Total B lymphocyte count, ×10 ⁶ /L	115.5 (57.8–249.3)	184.0 (42.8–273.3)	115.5 (102.8–133.5)	0.35	90-560
Decreased, n/N (%)	4/14 (28.6%)	3/8 (37.5%)	1/6 (16.7%)	0.58	
CD4⁺ T cells, %	36.7 (32.0-40.0)	36.7 (30.7–37.3)	36.4 (32.0-40.6)	0.56	27-51
CD4⁺T cell count, ×10º/L	241.5 (135.0–363.8)	177.5 (104.0–249.8)	381.5 (255.0–451.0)	0.018	550-1440
Decreased, n/N (%)	14/14 (100.0%)	8/8 (100.0%)	6/6 (100.0%)	NA	
CD8⁺T cells, %	22.2 (15.7–26.9)	17.4 (14.7–23.4)	25.2 (22.8-34.2)	0.093	15-44
CD8⁺T cell count, ×10º/L	169.5 (86.0–281.5)	89.0 (61.5–130.3)	254.0 (183.3-312.8)	0.035	320-1250
Decreased, n/N (%)	12/14 (85.7%)	7/8 (87.5%)	5/6 (83.3%)	1.00	
<150, <i>n/N</i> (%)	6/14 (42.9%)	6/8 (75.0%)	0/6 (0.0%)	0.010	
NK cells, %	14.8 (10.3–21.9)	14.7 (7.5–21.0)	15.1 (11.6–22.8)	0.62	7–40
NK cell count, ×10 ⁶ /L	89.0 (58.8–207.0)	60.5 (27.5–109.0)	180.5 (115.0–228.0)	0.27	150-1100
Decreased, n/N (%)	8/14 (57.1%)	6/8 (75.0%)	2/6 (33.3%)	0.28	
<77, n/N (%)	6/14 (42.9%)	6/8 (75.0%)	0/6 (0.0%)	0.010	
CD28+CD4+ T cells/CD4+ T, %	98.3 (96.8–98.8)	97.5 (96.8–98.7)	98.6 (97.2–99.0)	1.00	84.11-100.00
CD28+CD8+ T cells/CD8+ T, %	64.8 (44.6-75.9)	44.6 (37.5-73.1)	70.3 (63.3–76.9)	0.20	48.04-77.14
HLA-DR⁺CD8⁺T cells/CD8⁺T, %	42.3 (30.9–48.2)	46.2 (42.3-48.2)	28.6 (25.4–37.9)	0.19	20.73-60.23
CD45RA+CD4+ T cells/CD4+ T, %	32.8 (31.7-40.3)	32.8 (31.8–36.4)	36.0 (29.3-40.5)	0.54	29.41-55.41
CD45RO*CD4* T cells/CD4* T, %	67.2 (59.7–68.3)	67.2 (63.6–68.2)	64.0 (59.5–70.7)	0.54	44.44-68.94
Treg, %	4.1 (3.5-4.9)	4.7 (2.6-5.4)	3.9 (3.6-4.3)	0.92	5.36-6.30
CD45RA⁺ Treg, %	0.8 (0.5–1.1)	0.5 (0.3–0.7)	1.1 (1.0–1.3)	0.020	2.07-4.55
CD45RO⁺ Treg, %	3.3 (2.4-3.8)	3.8 (1.9–4.9)	2.9 (2.5–3.4)	0.59	1.44-2.76
IFN-γ−expressing CD4⁺ T cells, %	19.1 (13.0–22.8)	14.1 (9.4–18.8)	22.8 (18.8–25.4)	0.063	14.54-36.96
IFN-γ–expressing CD8+T cells, %	50.1 (44.2-53.6)	47.2 (39.2–52.7)	51.2 (47.3–54.1)	0.49	34.93-87.95
IFN- γ -expressing NK cells, %	73.3 (65.7–79.7)	71.2 (63.8-72.9)	79.7 (71.9-81.5)	0.25	61.2-92.65

Data are the median (IQR) or n/N (%), where N is the total number of patients with available data. P values comparing severe cases and moderate cases are from χ^2 , Fisher's exact test, or unpaired 2-sided Student's t test. COVID-19, coronavirus disease 2019; IQR, interquartile range; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.





The innate immune response and adaptive immune responses of Coronaviruses (CoV) infection during an infection. A, CoV infects macrophages, and then macrophages present CoV antigens to T cells. This process leads to T cell activation and differentiation, including the production of cytokines associated with the different T cell subsets (ie, Th17), followed by a massive release of cytokines for immune response amplification. The continued production of these mediators due to viral persistence has a negative effect on NK, and CD8 T cell activation. However, CD8 T cells produce very effective mediators to clear CoV. B, Attachment of CoV to DPP4R on the host cell through S protein leads to the appearance of genomic RNA in the cytoplasm. An immune response to dsRNA can be partially generated during CoV replication. TLR-3sensitized by dsRNA and cascades of signaling pathways (IRFs and NF-κB activation, respectively) are activated to produce type I IFNs and proinflammatory cytokines. The production of type I IFNs is important to enhance the release of antiviral proteins for the protection of uninfected cells. Sometimes, accessory proteins of CoV can interfere with TLR-3 signaling and bind the dsRNA of CoV during replication to prevent TLR-3 activation and evade the immune response. TLR-4 might recognize S protein and lead to the activation of proinflammatory cytokines through the MyD88-dependent signaling pathway. Virus-cell interactions lead to the strong production of immune mediators. The secretion of large quantities of chemokines and cytokines (IL-1, IL-6, IL-8, IL-21, TNF-β,and MCP-1) is promoted in infected cells in response to CoV infection. These chemokines and cytokines, in turn, recruit lymphocytes to the site of infection. Red lines refer to inhibitory effects. Green lines refer to activating effects



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Number of immune cell subsets and proportion of **IFN-y expression in patients with COVID-19.** (A) Flow cytometric analysis of NK cells, CD4+ T cells, CD8+ T cells, and Tregs as well as production of IFN- γ by CD4+ T cells, CD8+ T cells, and NK cells from a representative patient. (B) A series of compari-sons of absolute number of total T and B lymphocytes, CD4+ T cells, CD8+ T cells, and NK cells between severe cases (n = 8) and moderate cases (n = 6). (C) A series of comparisons of production of IFN- γ by CD4+T cells, CD8+ T cells, and NK cells between severe cases (n = 6) and moderate cases (n = 7). All data presented as the mean \pm SEM. Differences were tested using unpaired 2-sided Student's t test.





ASE



normal Sample	GeoMean	severe Infection COVID-19	GeoMean	moderate Infection COVID-19	GeoMean
IFNg		IFNg		IFNg	
Geometric Mean : FL2-H	1,49	Geometric Mean : FL2-H	22,9	Geometric Mean : FL2-H	8,84
IL-1b		IL-1b		IL-1b	
Geometric Mean : FL2-H	1,41	Geometric Mean : FL2-H	32,8	Geometric Mean : FL2-H	12,3
IL-2		IL-2		IL-2	
Geometric Mean : FL2-H	2,78	Geometric Mean : FL2-H	20,6	Geometric Mean : FL2-H	6,38
IL-6		IL-6		IL-6	
Geometric Mean : FL2-H	1,49	Geometric Mean : FL2-H	23,1	Geometric Mean : FL2-H	19,1
IL-10		IL-10		IL-10	
Geometric Mean : FL2-H	1,23	Geometric Mean : FL2-H	6,34	Geometric Mean : FL2-H	2,35
IL-12		IL-12		IL-12	
Geometric Mean : FL2-H	16,1	Geometric Mean : FL2-H	47,8	Geometric Mean : FL2-H	21,6
TNFa		TNFα		TNFa	
Geometric Mean : FL2-H	1,93	Geometric Mean : FL2-H	42	Geometric Mean : FL2-H	14
IL-4		IL-4		IL-4	
Geometric Mean : FL2-H	1,31	Geometric Mean : FL2-H	24,5	Geometric Mean : FL2-H	2,86
IL-5		IL-5		IL-5	
Geometric Mean : FL2-H	1,4	Geometric Mean : FL2-H	19,3	Geometric Mean : FL2-H	3,63
IL-8		IL-8		IL-8	
Geometric Mean : FL2-H	1,32	Geometric Mean : FL2-H	9,12	Geometric Mean : FL2-H	8,4

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