

September 26th, 2022

Monocyte SFC panel #3

Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly	Ly+Mon	Total	3E+6	2E+6
ND025	ad NY		2.5E6	09/24/07		0.5ml					→
ND032	ad NY		2.5E6	01/25/08	Broken - LN2 checked	0.5ml					→

8:11 AM
250 ml RPMI (5 batches) 8:36 reagents prepped

45 ml RAI = 225 ml
#5 FBS = 25 ml
0.5 Light = 2.5 ml
0.150 gentamycin 0.250 μ l

250 ml 10% FBS

25 + 225 ml PBS

50 μ l DNase

500 μ l RAI + 2.5 μ l PPD
@ 9:14 AM

Reagents for experiment
& cell mixes prepped @
10:08 AM

↑ 1 ml (~1.5 ml) → 300 μ l (1/5th)
10:14 cell sort unstained for each specimen
1 hr ~ PPD

10:17 cells first spin

LyD @ 10:29 → 10:44 am
= 3 μ l block

2 ml 1400 6 min @ 10:48 pm

37°C @ 10:59 - 11:29 AM
unstained + br stain buffer for background

3rd @ 11:30 1400 6 min

11:40 am → 12:10 pm

Rbc lysis @ 12:10 (11) → 12:14

Date		Time		Location		Remarks	
10/10/10	10:00	10:15	10:30	10:45	11:00	11:15	11:30
10/10/10	11:45	12:00	12:15	12:30	12:45	13:00	13:15
10/10/10	13:30	13:45	14:00	14:15	14:30	14:45	15:00
10/10/10	15:15	15:30	15:45	16:00	16:15	16:30	16:45
10/10/10	17:00	17:15	17:30	17:45	18:00	18:15	18:30
10/10/10	18:45	19:00	19:15	19:30	19:45	20:00	20:15
10/10/10	20:30	20:45	21:00	21:15	21:30	21:45	22:00
10/10/10	22:15	22:30	22:45	23:00	23:15	23:30	23:45
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10/10/10	239:15	239:30	239:45	240:00	240:15	240:30	240:45
10/10/10	241:00	241:15	241:30	241:45	242:00	242:15	242:30
10/10/10	242:45	243:00	243:1				

Monocyte SFC panel

Antibody Name	Marker	Clone	Vial lot #	I/D + FC blocker 3uL/rxn	37°C (RT) for 30min	2	4°C for 30min	2
Fluorochrome								
BUV395	CD18	(6.7)		0.8	0.8			
APC350								
BUV496								
BUV563	CCR2	(S132.109)		0.8	0.8			0
BUV615								
BUV661	CD3CR1	(2A9-1)		1	1			
BUV737								
BUV805	CD14	(M5E2)					1.0	1
BU421	FCER1a						1.0	1
Super Bright 438							1.5	1.5
APC670								
Pacific Blue	CD5						1.5	1.5
BV480	CD11a	(H1111)					0.6	0.6
BV510 (dim)				0	0			
BV570	CD16	(3G8)					1.5	1.5
APC670								
BU605	CD56	(5.4H11)					1.0	1
BU650	CD11c	(B4-6)					1.0	1
BU711								
BU750	CCR5	(2D7)		1.5	1.5			
BU785	CD141			1.5	1.5			
BB515 / VioBright515							1.5	1.5
FITC	CD11b							
Spark blue 550							0.8	0.8
PE	CD88	(SS-1)		0.6	0.6			
PE-Dazzle594	CD3						0.6	0.6
PE-Cy5	CD86	(IT2.2)		0.5	0.5			
BB-700 better								
PerCP-Cy5.5	CD123	(7G3)		2	2			
PE-Vio770	H1A-DR	(REA805)					0.5	0.5
APC								
Alexa Fluor 647	CD163	(GH1/61)					1.0	1
Spark NIR 685								
APC-R700	CD40	(5C3)		1.0	1			
Zombie NIR	L/D			<1.2500>				
APC/Fire 750	CD64	(10.1)		1	1			
APC/Fire 810	CD38	(HIT2)		1.0	1			
True Stain FCx				0.5	0.5		0.5	0.5
Antibody Total								
PBS				12.2	12.2		13.0	13
Pipette draw volume/sample				50	50		50	50
				61.2			62.0	

Simplified Protocol

Aliquot cells 1.2×10^6 cells/tube

Wash with 2ml PBS, 1300rpm, 8min

2500x diluted Zombie NIR, 1E+6/1ml + 3ul Fc blocker at RT,

Wash with 2ml 2%FBS-PBS-2mM EDTA

Spin at 1300rpm for 8min

x ul of 370C Ab mix, at 370C for 30min

Remove RBC with 1ml lysing solution for 3min

Wash with 2ml 2%FBS-PBS-2mM EDTA

Spin at 1300rpm for 8min

100 μ l of 40C Ab mix, at 40C for 30min

Treat with 600ul of 1x Lysing solution at RT for 3min

Wash with 2ml 2%FBS-PBS-2mM EDTA

Spin at 1300rpm for 8min

Resuspend in 0.4%PFA-PBS

Spideen ps

LD 201 201 113

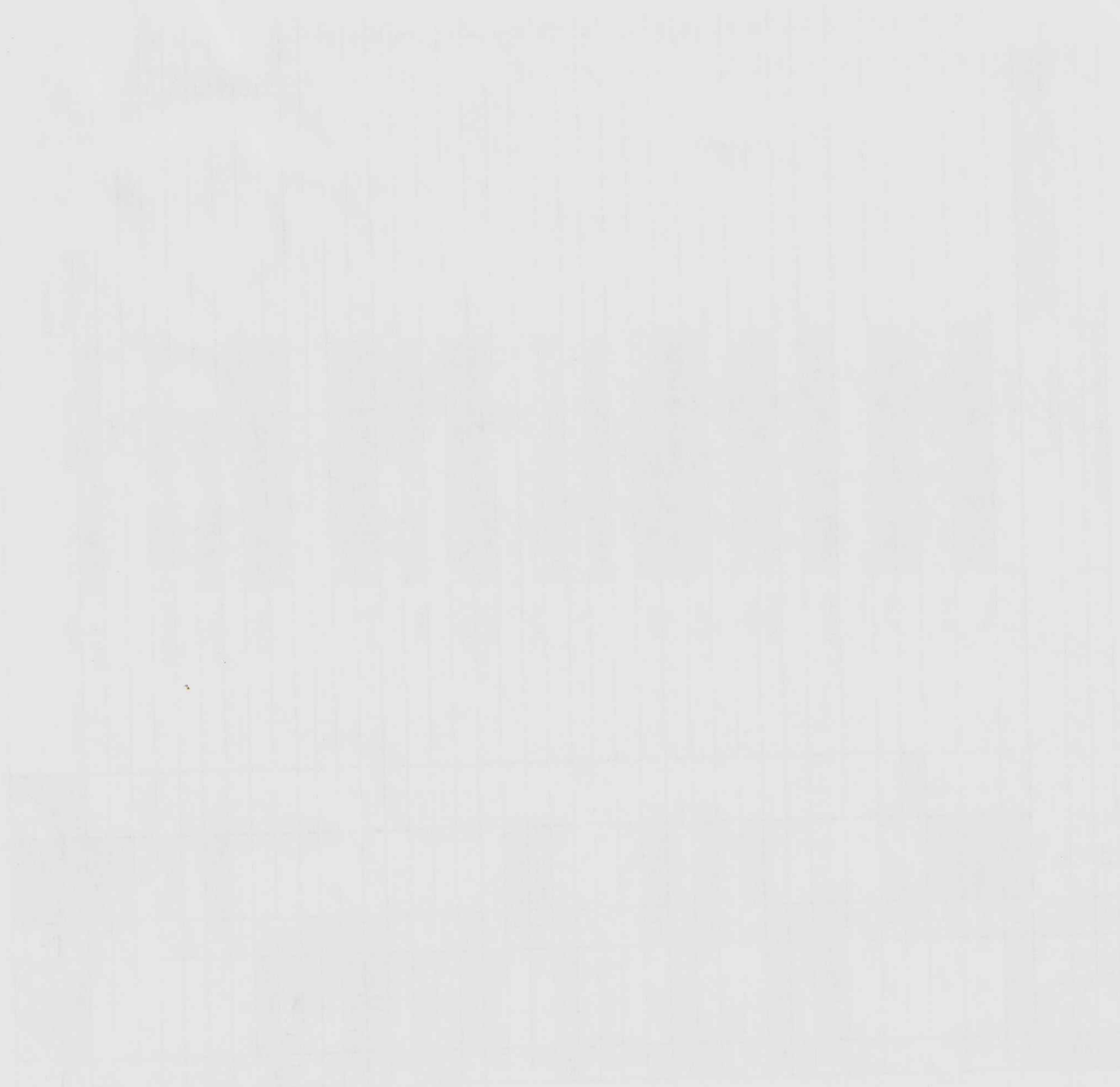
10

2 1/2 x 1 1/2

2nd 2nd

6m1 2 12m1

$10^2 - 2 \times 10^1 + 10^0$
 $100 - 20 + 1$
 81



9/26/2022

[illegible]

CD40L

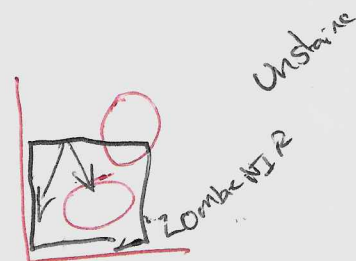
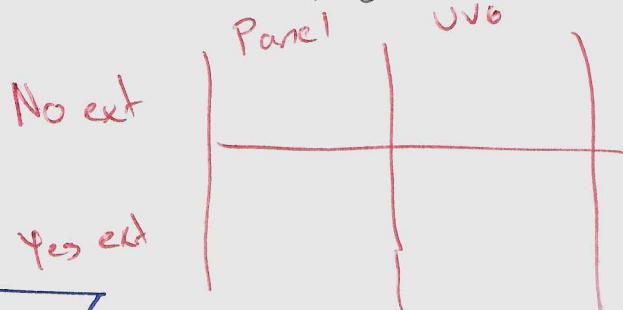
September 26th, 2022

Monocyte SFC panel

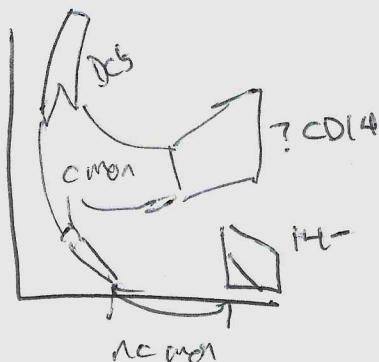
NY025 → 62.8 Leukocytes
 NY032 → 21.9 Leukocytes (rest dead, shrinking cells)

Unst NY025 → 2 AFs ~ Lymphocytes → UV7, V7 (V3)
 Unst NY032 → 1 AFs ~ Monocytes →
 2 AFs ~ Monocytes → UV7, V7 (V3), (one makes ↑ B1, B2, B3)
 1 AFs ~ Lymphocytes →

common shared monocyte signature



CD16 dot?

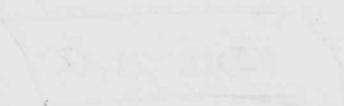


CD18 lower dc's & oddball monocytes
 CCR2 absent nc monocytes, lower Dcs oddballs
 CX3CR1 edge nc & cMon
 CD14 primarily classical feature
 FcER1a ~ classical boundary, not Dcs
 CD5 ~ primarily cDC1/2 boundary
 CD11a lowest oddballs
 CD16 nc mon feature
 CD56 ~ mainly Dc feature
 CD11c ~ dc ~ nc & some classicals
 CCR5 mainly absent
 HLA pan cDC1s, some elsewhere
 CD11b ~ mainly absent Dcs, nc, one oddball cluster
 CD88 absent not monocytes, ↓ oddball
 CD3 absent
 CD86 ~ Monocytes & nc especially, not oddballs
 CD123 ~ cDC1
 HLA-DR high Dcs, class, less oddballs/nc
 CD103 low level dc's. 40 low levels cMon
 CD64 high cMon & oddball internal
 CD38 absent oddballs, nc, highest cDC1s, 2, cMon

Subject: Mathematics



Pythagoras' Theorem states that in a right-angled triangle, the square of the hypotenuse (the side opposite the right angle) is equal to the sum of the squares of the other two sides. This can be written as:

$$a^2 + b^2 = c^2$$


Example: Find the length of the hypotenuse of a right-angled triangle with a base of 3 units and a height of 4 units.

Solution: Using Pythagoras' Theorem, we have:

$$3^2 + 4^2 = c^2$$
$$9 + 16 = c^2$$
$$25 = c^2$$
$$c = \sqrt{25}$$
$$c = 5$$

Therefore, the length of the hypotenuse is 5 units.

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