

September 9th, 2023

Day 17 IEG

Specimen	Status	Location	Conc	Date	Notes	Volume / 100	Lym	Lym + Mon	Total
38	Inf105	HU ♂	IL-2	<83.9>	Not great	5.5	1.69	591 μ L	9.205
350 c.		ZOL	500 cells	<83.9>	enlarged	6.5	0.92	1.086 μ L	5.98
392 c.	Inf105	BCG	4% 20 μ L		Dead or mostly	6.0	0.32		1.92
560 c. 38	Inf041-5	HEU-10 ♀	IL-2	<65.8 μ L>		6.0	2.11	473 μ L	12.66
640 c.		ZOL	<76.9>			6.0	1.67	598 μ L	10.02
437 c.		BCG	30% 25 μ L		Dead or mostly	5.5	0.25		1.325
553 c. 38	Inf064-0	HEU-10 ♂	IL-2	<43.4>	First healthy	5.5	2.98	335 μ L	16.39
600 c.		ZOL				6.5	2.48	403 μ L	16.12
548 c.		BCG				6.5	1.60	625 μ L	10.4
579 c.	Inf356	HEU-6 ♂	IL-2			5.5	2.55	392 μ L	14.02
540 c.		ZOL	<49.9>			6.0	2.51	398 μ L	15.06
		BCG							
52 c. 38	Inf523-0	HEU-10 ♀	IL-2		yellow cells	5.5	1.85	540 μ L	10.175
330 c.		ZOL	<75.0>			6.5	1.91	523 μ L	12.415
		BCG	15% 50 μ L			7.0	0.44		3.08
40 c. 38	Inf251-8	HEU-10 ♀	IL-2	<53>	yellow cells	5.5	2.52	396 μ L	13.84
475 c.		ZOL				6.5	1.43	699 μ L	9.29
585 c.		BCG	<54>			6.5	2.32	431 μ L	15.08
417 c.	Inf274-3	HEU-10 ♀	IL-2	<46>	looks good	5.5	2.51	398 μ L	13.8
432 c.		ZOL				5.5	2.69	371 μ L	14.8
490 c.		BCG				5.5	3.34	299 μ L	18.37

Count @ 5:06 pm \rightarrow 5:51 pm

Flask 6:54
< 8:58 nucleation start >
 \rightarrow 2:58 am

523 x2 IL2
201 } yellow root
BCC pink

- 64 ↑ same

- 251 happy

641 unhappy BCC

- 64 unhappy overall

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	Infl05	IL-2	1.69	592 μ L	
	41-5	IL-2	2.11	473 μ L	→
	41-5	ZOL	1.67	598 μ L	→
↖	64-6	IL-2	2.98	335 μ L	
↖		ZOL	2.48	403 μ L	
↖		BCG	1.6	625 μ L	
	356	IL2	2.55	392 μ L	→
		ZOL	2.51	398 μ L	→
↖	523	IL-2	1.85	540 μ L	
↖		ZOL	1.91	523 μ L	
	251	IL-2	2.53	395 μ L	→
		ZOL	1.43	699 μ L	→
		BCG	2.32	411 μ L	→
↖	274	IL2	2.51	398 μ L	
↖		ZOL	2.69	371 μ L	
↖		BCG	3.34	299 μ L	

11:26 pm resume
Ab spn @ 00:22 am >
Abs prepped @ 1:01 am

$$\begin{array}{r} 15 \\ + 18 \\ \hline 32 \\ \times 1.6 \\ \hline 256 \end{array}$$

1700 mls
→ 51.2 pl

1/2 D Apper
Prepped @ 1:17 am

$$\begin{array}{r} 34 \\ + 15 \\ \hline 49 \\ \times 3 \\ \hline 150 \end{array}$$

150 mls RBC lye

$$\begin{array}{r} 32 \\ \times 4 \\ \hline 128 \text{ mls} \\ \times 9 \\ \hline 106.2 \text{ mls Perm Wash} \end{array}$$

Reagents @ 1:27 prepped

2:39 pm P₁/P₂ spin down

Started Fridge @ 2:55 am

✓ CN spn @ 3:25 am

Scb @ 3:32 am spn @ 3:50 am

Hb @ 3:42 am spn @ 4:09 am

Surfaces @ 4:34 AM → 4:54

spn @ 5:06 am

5:27 am Shep → spn @ 5:45 am

Pi w/ 20 pl 0.4% PFA - PBS

FBS thaw @ 5:40 AM
& cryovial labeling

Fuel @ 6:04 → 14 → 24

Leftovers spn down @ 6:13 am

2nd perm wash @ 6:49 pm

7:15 AM → 7:55 AM
Intact cells

First batch frozen

8:05 Final spn

Done 8:25 am

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D17 Gd-P4b

#	Detector	Fluorochrome	Marker	Clone	L/D 15 min (RT)	Surface 20 min @4C	18	Intracellular 40min @RT	18
1	V450	BV421	PD1*			2.5	45.0		
2	V525	BV510	L/D Aqua		<1:500>				
3	V670	BV650	CD56*			1.8	32.4		
4	B530	Alexa 488	Perforin					3.5	63.0
5	B710	PerCPeF710	CD3			1.5	27.0		
6	Y615	PE-Dazzle							
7	Y780	PE-Vio770	NKG2A			0.6	10.8		
8	R670	APC	V82			1.0	18.0		
9	R780	APC-Fire750	CD16			1.0	18.0		
Antibody Total					8.4	151.2	3.5	63.0	
PBS					12.1	217.8	17.0	306.0	
Pipette draw volume / sample					19.5		19.5		

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D17 Gd-P2

#	Detector	Fluorochrome	Marker	Clone	L/D 15 min (RT)	Surface 20 min @4C	18	Streptavidin	Intracellular 40min @RT	18
1	V450	BV421	V82			1.0	18.0			
2	V525	BV510	CD3			1.5	27.0			
3	V670	BV650	NKG2D			2.0	36.0	1.5		
4	B530	Alexa 488	Perforin						3.5	63.0
5	B710	PerCPVio700	CD56			1.0	18.0			
6	Y590	PE	PD1			1.5	27.0			
7	Y615	PE-Dazzle								
8	Y780	PE-Vio770	NKG2A			0.6	10.8			
9	R670	APC	V81			1.0	18.0			
10	R780	APC-Fire750	L/D Horizon		<1:1000>					
Antibody Total					8.6	154.8	27.0	3.5	63.0	
PBS					11.9	214.2	342.0	17.0	306.0	
Pipette draw volume / sample					19.5		19.5	19.5		

D17 Gd-P1

#	Detector	Fluorochrome	Marker	Clone	L/D 15 min (RT)	Surface 20 min @4C	18
1	V450	BV421	PD1			2.5	45.0
2	V525	BV510	L/D Aqua		<1:500>		
3	V670	BV650	CD16			1.5	27.0
4	B530	FITC	V82			1.2	21.6
5	B710	PerCPeF710	CD25			2.0	36.0
6	Y590	PE	CD28			2.5	45.0
7	Y615	PE-Dazzle	CD27			1.5	27.0
8	Y780	PE-Vio770	CD3			0.5	9.0
9	R670	APC	V81			1.0	18.0
10	R780	APC-Fire750	CD45RA			1.8	32.4
Antibody Total					14.5	261.0	
PBS					6.0	108.0	
Pipette draw volume / sample					19.5		

D17 gd-CK: a-γδTCR

#	Detector	Fluorochrome	Marker	Clone	L/D 15 min (RT)	Surface 20 min @4C	18	Intracellular 40min @RT	18
1	V450	BV421	PD1			2.5	45.0		
2	V525	BV510	L/D Aqua		<1:500>				
3	V670	BV650	CD56			1.5	27.0		
4	B530	Alexa 488	CD107a						
5	B710	PerCPeF710	TNFα				0.0	2.5	45.0
6	Y590	PE	V82			1.0	18.0		
7	Y615	PE-Dazzle							
8	Y780	PE-Vio770	IFNγ					1.1	19.8
9	R670	Alexa 647	CD27			2.0	36.0		
10	R780	APC-Fire750	CD45RO			1.8	32.4		
Antibody Total					8.8	158.4	3.6	64.8	
PBS					11.7	210.6	16.9	304.2	
Pipette draw volume / sample					19.5		19.5		

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< 6:18pm Sunday > clearing LSR, prepping files, freeing up memory

→ Red Laser not reading

7.02 on software

7.22 sc's acquired or how?

BCG works 0.7 7536

Zol works ✓ > Vd1

- 5
- 15
↑
yep

> % BCG?

Done @ 9:10 w/P1

P1 panel clean ✓
~5 min / empty the tubes

M. odelling, CD27

Not as much build up.

Vd ass, whether
marker contact cost time
importance

P2 line (w/ comp errors): ↑ 230 to 750 FSC

9:46pm gradient to perform

< more gd's in HEU's >

< 10:24 post dinner >

< Perform CD56 separation
is struggling >

frequency cells/
total cells

Inf 251 Zol ← clot

11:40pm clearing pre CLK's start.

Subset cells vs debris
over time as
- get downwards?

cytotime to 770 after first 5

IL2 OK4 No stim? ← suboptimal?

Done @ 1:00 AM

$\gamma\delta$ T cell p1									
Spectrum	Violet		Blue		Red				9/9/2023
428	V1	BV421	[4] PD1						
525	V6			B2	FITC	[1.5] Vδ2			
542	V7	BV510	[1.5] L/D Aqua	B3					
582	V8			B4	PE	[4] CD28			
598	V9			B5					
613	V10			B6	PE-Dazzle594	[4] CD27			
664	V11	BV650	[3.5] CD16	B7					
679				B8			R1	APC	[3.5] Vδ1
717	V13			B10	PerCP-eF710		R2	Alexa 647	
783	V15			B13	Pe-Vio770		R4		
812	V16			B14		[3] CD3	R7	APC-Fire 750	[2] CD45RA
							R8		

$\gamma\delta$ T cell pZ								
Spectrum	Violet		Blue		Red			
428	v ₁	BV421 [4]	Vδ2					
525	v ₆			B2 Alexa 488	[1.5] Perforin			
542	v ₇	BV510 [1.5]	CD3	B3 PE	[4] PD1			
582	v ₈			B4 B5				
598	v ₉			B6 PE-Dazzle594	[4]			
613	v ₁₀			B7		R1 APC	[3.5]	Vδ1
664	v ₁₁	BV650 [3.5]	NKG2D	B8		R2 Alexa 647		
679				B10 PerCP-Vio700	CD56	R4		
717	v ₁₃			B13 Pe-Vio770	[3] NKG2A	R7 APC-Fire 750	[2]	L/D Horizon
783	v ₁₅					R8		
812	v ₁₆			B14				

$\gamma\delta$ T cell p4b								
Spectrum	Violet		Blue		Red			
428	V1	BV421	[4] PD1					
525	V6			B2	Alexa 488	[1.5] Perforin		
542	V7	BV510	[1.5] L/D Aqua	B3				
582	V8			B4	PE	[4] GrzmB		
598	V9			B5				
613	V10			B6	PE-Dazzle594	[4]		
664	V11	BV650	[3.5] CD56	B7			R1 APC	[3.5] V δ 2
679				B8			R2 Alexa 647	
717	V13			B10	PerCP-eF710	CD3	R4	
783	V15			B13	Pe-Vio770	[3] NKG2A	R7 APC-Fire 750	[2] CD16
812	V16			B14			R8	

γδ T cell CK									
Spectrum		Violet		Blue			Red		
428	V1	BV421	[4]	PD1					
525	V6				B2	Alexa 488	[1.5]	Vδ2	
542	V7	BV510	[1.5]	L/D Aqua	B3				
582	V8				B4	PE	[4]	CD56	
598	V9				B5				
613	V10				B6	PE-Dazzle594	[4]		
664	V11	BV650	[3.5]	CD27	B7				
679					B8				
717	V13				B10	PerCP-eF710		CD3	
783	V15				B13	Pe-Vio770	[3]	IFN γ	
812	V16				B14				
							R1	APC	
							R2	Alexa 647	[3.5]
							R4		TNF α
							R7	APC-Fire 750	[2]
							R8		CD45RO

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Day 17

Access the Guava, retrieve Hao Ting's cord blood experiments.
- export all fcs files individually + mine
→ < Several hundred cord profiles Malawian infants > ←
+ CD45 info

~ Model Cell Viability? % 45 - FSC SSC
Lymphocyte vs Monocyte
↔

→ as a changing % ←
