

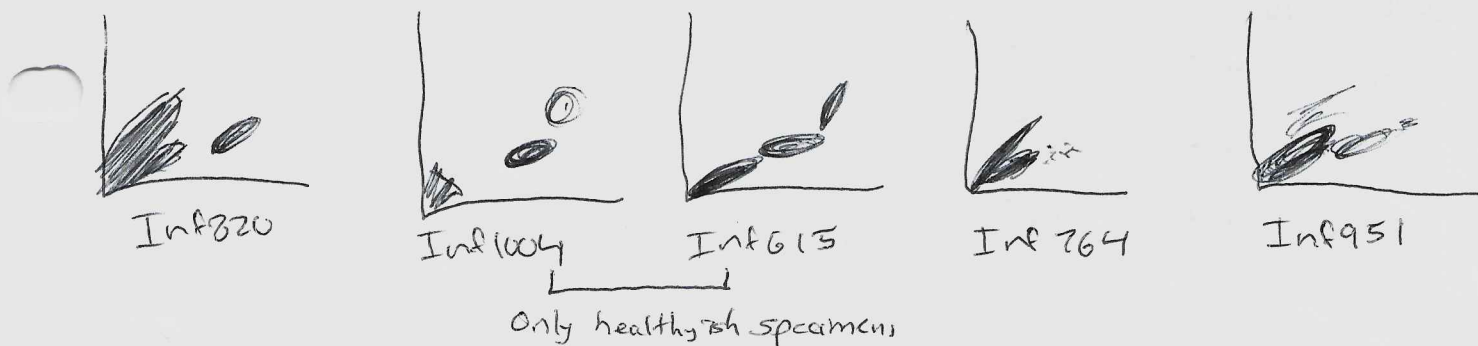
July 28th, 2023

2023_gdConventionalTest / Bookend Box Bs

Specimen	Status	Location	Conc	Date	Notes	Volume	Lym	Lym+Mon	Total
Inf 820-0 a-3	U75L	Box 23A	(4) <29.27		lysed looking pellet large red clump	1		3.59 E6	
Inf 1004 a-3	V4AB	Box 25A	(5) 6.987		Pellet, w/ RBCs HEALTHY	1	12 E6	16.4 E6	
Inf 615-2 a-5	TJ2Q	Box 14 B F.6	(1) 8.567		healthy? pellet HEALTHY	1	6.09 E6	8.99 E6	
Inf 764-4 a-6	TYFZ	Box 17 B A.6	(2) 30.07		healthy? lysed? clumping Lysed	1		0.3 E6	
Inf 951-5 a-9	V193	Box 19 B F.5	(3) 11.67		black? Silly 1/2 red purple medium	1		3.77 E6	
					Mostly unhealthy h				

12:56 throw spin @ 1:05 DNase @ 1:21 pm 1:26 resus
DNase @ 1:33 pm

Count @ 2:03 pm Inf 802: few cells alive (minority)



"Resuspend all cells @ 3.0 M, place into incubator for 6 hrs"

2:56 pm →
Dying blotted
and into ice.

3:05 pm

37°C tubes
(3/4 Inf 1004)

1) 0.84 → 60 pl 2 pl
4) 0.25 → 650 pl 2 pl
2) 0.49 → 410 pl 2 pl
1) 0.80 → 100 pl 2 pl

2
x 770 pl
14 pl
2
x 100
2 pl

9:14 PM (Inf 820/Inf 951 dying
were solid clump, disrupted but
adds > all finished dying)

9:28 pm 1/0 → 43 pm

9:35 pm Sds → 9:55 pm

Spin @ 9:45 pm

Surface @ 9:57 pm → 10:17 pm / 24 pm

Sds spin @ 10:00 pm

Rbc lyse @ 10:26 pm

Spin @ 10:30 pm

Strep @ 10:41 → 56 pm

Spin @ 10:58 pm

11:08 pm Fix Perm → 18 → 22

Spin @ 11:34 2nd @ 11:46 pm

Intra @ 11:56 pm

Final spin @ 12:46

stashed 50 pl
@ 12:53 am

QC:
 No immediate drops LSR-II @ 10:25 pm
 run bleach @ 10:32 pm
 run ^{H₂O} contract @ 10:43 pm

11:19 pm // cleared & warmed up.

No PE, P_{el} or P_{dazzle} signal...

R676	$10^4 \wedge 1/2$	neg @ $10^2 \wedge 1/2$
R780	$10^4 \wedge 1/2$	" " "
B530	"	10^3
B710	"	$10^2 \wedge 1/2$
V450	10^5	10^3
V510	10^4	10^3
V620	$10^4 \wedge 1/2$	10^2
Y590	$10^4 \wedge 1/2$	10^2
Y615	$10^4 \wedge 1/2$	10^2
Y780	$10^4 \wedge 1/2$	10^2

Samples @ 1:28 pm

horrid fite spill on PMT Vd2
 (issue adv on dead cells,

Mirac

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Ex-vivo Gd-P4b

#	Detector	Fluorochrome	Marker	Clone	L/D 15 min (RT)	Surface 20 min @4C	1	Intracellular 40min @RT	1
1	V450	BV421	PD1						
2	V525	BV510	L/D Aqua			2	2		
3	V670	BV650	CD56		<1:500>				
4	B530	Alexa 488	Perforin			1.8	1.8		
5	B710	PerCPeF710	CD3					3	3
6	Y590	PE	GzmB			1.5	1.5		
								1.5	1.5
7	Y780	PE-Vio770	NKG2A						
8	R670	APC	V82			0.6	0.6		
9	R780	APC-Fire750	CD16			1	1		
						1	1		
Antibody Total					7.9	7.9	4.5	4.5	
PBS					12.6	12.6	16	16	
Pipette draw volume / sample					19.5		19.5		

Ex-vivo Gd-P2

#	Detector	Fluorochrome	Marker	Clone	L/D 15 min (RT)	Surface 20 min @4C	1	Streptavidin	Intracellular 40min @RT	1
1	V450	BV421	V82							
2	V525	BV510	CD3			1	1			
3	V670	BV650	NKG2D			1.5	1.5			
4	B530	Alexa 488	Perforin			2	2	1.5		
5	B710	PerCPeF710	CD56						3	3
6	Y590	PE	PD1			1	1			
						1.5	1.5			
7	Y780	PE-Vio770	NKG2A							
8	R670	APC	V81			0.6	0.6			
9	R780	APC-Fire750	L/D Horizon		<1:1000>	1	1			
Antibody Total					8.6	8.6	1.5	3	3	
PBS					11.9	11.9	19	17.5	17.5	
Pipette draw volume / sample					19.5		19.5	19.5		

Ex-vivo Gd-P1

#	Detector	Fluorochrome	Marker	Clone	L/D 15 min (RT)	Surface 20 min @4C	1		
1	V450	BV421	PD1				2		
2	V525	BV510	L/D Aqua		<1:500>				
3	V670	BV650	CD16			1.5	1.5		
4	B530	FITC	V82			1.2	1.2		
5	B710	PerCPeF710	CD25			2	2		
6	Y590	PE	CD28			2	2		
7	Y615	PE-Dazzle	CD27			1.5	1.5		
8	Y780	PE-Vio770	CD3			0.5	0.5		
9	R670	APC	V81			1	1		
10	R780	APC-Fire750	CD45RA			1.8	1.8		
Antibody Total					13.5	13.5			
PBS					7	7			
Pipette draw volume / sample					19.5				

Ex-vivo gd-CK: PMA-ionomycin

#	Detector	Fluorochrome	Marker	Clone	L/D 15 min (RT)	Surface 20 min @4C	2	Intracellular 40min @RT	2
1	V450	BV421	PD1				4		
2	V525	BV510	L/D Aqua		<1:500>	2			
3	V670	BV650	CD27			1.5	3		
4	B530	FITC	V82			1.2	2.4		
5	B710	PerCPeF710	CD3			1	2		
6	Y590	PE	CD56			0.5	1		
7	Y780	PE-Vio770	IFN γ					0.6	1.2
8	R670	Alexa 647	TNF α					2	4
9	R780	APC-Fire750	CD45RO			2	4		
Antibody Total					8.2	16.4	2.6	5.2	
PBS					12.3	24.6	17.9	35.8	
Pipette draw volume / sample					19.5		19.5		

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