	May 16th, 2	2023						2023 1	LT 02	
330	Specimen	Status	Location	Conc	Date	Notes	Volume	Lym	Lym+Mon	Total
40	Inf0323	HUY						1.7		Total
024	- 0-3	RRFL	(D)	9.24			0.75	9.33	12.4	11 20
60/1	0-6	RREM	A) 16			0.75	5.58	7.4 =	11.34
900	Inf 148	THE HU					1 22 39	0.00	7. 1	
150	2-5	5706	<u> </u>	6.07				9.34	12.5 -	- 16.69
30/1	a-¢	5707	(6)	7. 23			1	7.35		16.67
930	Inf161-8							(,)	16.7 -	-
300	a-4.	SOM D	2	9.7			I I	9.50	14.6 -	9.5
601	Inf180-9	HEU-NIS	(ii)	100			1	0000		
360 80 H	~ 3	SQNA		10.3				8.04	11. 2 -	1/00
20	a-4	Sq Nq	(5)				1	3.05	10.3 -	16.09
1014	ND050			2016	(/13/23		1	0		
40pl -			(ED)					8.64	11.7	8.69
	NOCCE		(-2)	15E6	1/20/23		((1.)	13.7	16.1
A	NDOCG th	ow 6 e					1211		·	
		@ 1	mar C	l u			1 2 M HE	u-hi In	f180 given h	D. Rollins
	Cord 2nd	d Oi	0.06 7:16 0.07 0.20 Se	" SY	oi n			2		
	patelet	hour Ci	U: 20 Se	cord s	5000		Ø	000		(S)
Î	NASE C	210:27	£_1		SHIT				+	
			Second				000			
¶.							Ø			
	tan cont							2		
ί,	1:24 SU 1:30 C	ccesful	(count (1 cle	mp bel	and I	pTet	3 @ RD:	57->9:	377
·	· 30 C	ound con	pleto			0(-)	A	05 C 9:	11->9:	41-
'	-37 by	1 A to	NDOG/-	M	dded	400pl r	nissing of Si closer 51: Sz	SFIRP	scene 9:00	5-715-775
e-C,	12:52 00	45 DO	epp-1	1	CDIO7	a fran man	dod 31	spha	2 7:30pm	
)(2:58 In	cobate			\	lacttle _	1 3,50	Carol	2	
L	2:58 In		7 31947		6:5	Epm/	S	COMID INC	of some	سيداه
augus	4 bs pres	000	P			(Pty5.5 026d	,	3C 3	stepheol C	9:57
7	7,10 pm sp.	in .	6. 48 P	m (14 R	(PG5.5 0026 de	ese 11	10.0	4pm cold	Samala
7	750m	av i							10:34	Ding
	75 pm 6/1							1011	05050	0:15-7500
	7.34 pm se 7.55 Too	Spin Le sa						1 SC 5	20pl from	29t pippek/
	7:59 Hot	8/2 -	20:=-	50100	0	pm stashed @	000			
	8:07 54	notes	b. 6 = 5	7416	-6:33	om stashed E	8:44 pm	S. cys.	e spince 16.	47 pm
	8:07 Sa 8:18 Sele	A 50's -	> 8: NO	- 3/				Jampies	5 FIX Forme	11:05 75
			0.0	PM			t.	x 5 do	ne@ 11:10	2.5

Samples 1st permissish @ 11:29 am

End Permissish @ 11:41am

Intra@ 11:55 >> 12:35 am

Niloso tet @ 12:02 (10 suc my amant

Firal spin @ 00:41

Done @ 12:57 am

A 150,000 2.03 pm Samples C 3:06 pm Taplet 6/60 taleo Done @ A: 14 pm

no separate mput. our manking. At that point in time, snopshot - Dem markers ~ smlar drouned ord by other flors barring sign over lap.

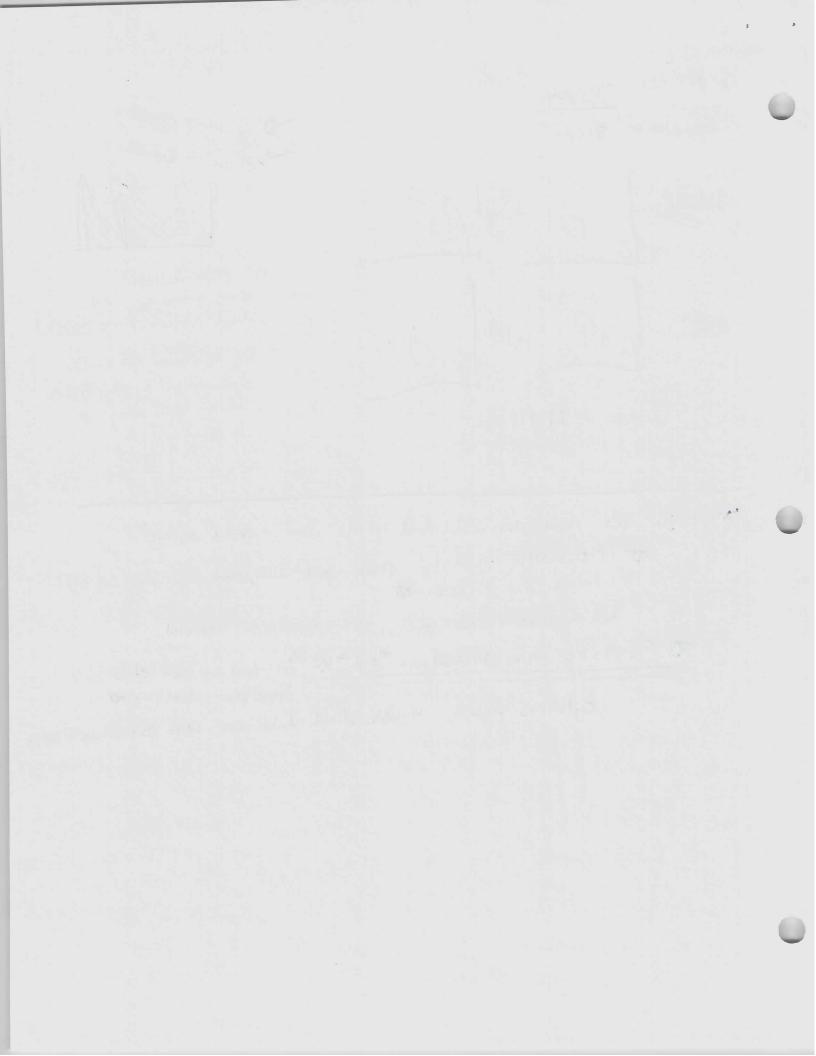
Onniving: Ses monogress model had lysed, low co62 & signal?

TO COIL signal, proa got sumpped into my se etil tobes for NOODG. X

not much cD19 exper, lot morthers imported.

Descriptes also were PMARed... + sight & ait least we have cities

From prior test remains Cytalines present, vanishe effect tetramers, NRTS > resiliency > Miles



Sample INF032 ND050 ND006

1.83 1.83

1.66 1.66

1.321.32

1.16 1.16

0.83

0.66

0.5

0.33

2.25M

1.75M

3.5

4.5

CD107a

INF148 INF161 INF180 ND050

> 4.5 4.5

				1.06	2	** **	S, Z	10 5 Ve	Service 18		2	3		ND006					INF032		
-				-	, & 			5	<u> </u>	1.50			7	11.1	8.69	16.09	9.5	16.69	11.34	Total	
		PMA								R10				Ъ	ר	2	Ъ	2	1.5	Volume	
INF180	INF161	INF148	INF032	Sample		ND006	ND050	INF180	INF161	INF148	INF032	Sample		11.10	8.69	8.05	9.50	8.35	7.56	Concentration	
2	2	2	2	3M		0.73	0.65	0.63	0.68	0.64	0.60	3M		0.27	0.35	0.37	0.32	0.36	0.40	3M	
1.83	1.83	1.83	1.83	2.75M		0.668	0.600	0.574	0.627	0.586	0.552	2.75M		0.25	0.32	0.34	0.29	0.33	0.36	2.75M	
1.66	1.66	1.66	1.66	2.5M		0.608	0.545	0.522	0.570	0.533	0.502	2.5M		0.23	0.29	0.31	0.26	0.30	0.33	2.5M	
1.5	1.5	1.5	1.5	2.25M		0.55	0.49	0.47	0.51	0.48	0.45	2.25M		0.20	0.26	0.28	0.24	0.27	0.30	2.25M	
1.32	1.32	1.32	1.32	2.0M		0.486	0.436	0.417	0.455	0.426	0.401	2.0M		0.18	0.23	0.25	0.21	0.24	0.26	2.0M	
1.16	1.16	1.16	1.16	1.75M		0.425	0.382	0.365	0.399	0.373	0.352	1.75M		0.16	0.20	0.22	0.18	0.21	0.23	1.75M	
Ь	Ь	Н	1	1.5M		0.36	0.33	0.31	0.34	0.32	0.30	1.5M		0.14	0.17	0.19	0.16	0.18	0.20	1.5M	
0.83	0.83	0.83	0.83	1.25M	17.0	0.303	0.272	0.261	0.284	0.266	0.251	1.25M		0.11	0.14	0.16	0.13	0.15	0.17	1.25M	
0.66	0.66	0.66	0.66	1.0M		0.243	0.218	0.209	0.228	0.213	0.201	1.0M		0.09	0.12	0.12	0.11	0.12	0.13	1.0M	
0.5	0.5	0.5	0.5	0.75M		0.18	0.16	0.16	0.17	0.16	0.15	0.75M		0.07	0.09	0.09	0.08	0.09	0.10	0.75M	
0.33	0.33	0.33	0.33	0.5M		0.122	0.109	0.105	0.114	0.107	0.101	0.5M	-	0.05	0.06	0.06	0.05	0.06	0.07	0.5M	

3 3 55

h 11

				59.5		65	olume /sample	Pippette draw volume /sample		19.5	ne /sample	Pippette draw volume /sample	7			1	
	11 187		850	50.0	850	50	t Stain	Brilliant Stain	246.5	14.5		R10 Media	20		SIII	VTRO	And UNSTAINED CONTROLS !!!
	9.5 161.5		212.5	12.5	303	17.8	y Total	Antibody Total	102	6.0		Antibody Total					
					27.2	1.6						НП2	CD38	Arc/rite otto			
					34	2						0323	CD27	APC/Fire 750	Cera		R8
								<1:2500>				N/A	L/D	Zombie NIR			R7
									102	6.0		н4АЗ	CD107a	APC-R700	The B		R6
			11.0	0.1			<2:10/1.2>					3C10	Va7.2/hMR1	AlexaFluor647	12.44	1	RA I
	0.5		11 0	0.7								3G8	CD16	APC	Central		RO
			700	10	4.02	4.4						PD1.3.1.3	PD1	PE-vio770	25.5		DI
					20.4	1 7						M-A251	CD25	PE-Cy5	Abdk A		R12
					10.0	1.0						MAB11	TNFa	PE-Dazzie594	1100400		00
					25.5	15						1011	NKG2D	PE	2m / 1mg	1	B6
1												BASb	CD26	PercP-Cy5.5			84
	01		20 4	12								SK7	CD3	Spark blue 550		1	
	200				-0.0	1.0	<:10/1.5>					6811	Va24/hCD1d	FITC/AF488			83
	C.C7				25.5	15						11A9	CCR6	BV786			B
												827	IFNY	BV750	Libra J		V15
	0.7				17	-						M-T701	CD7	BV711	Pant		¥14
11.					17	1						G043H7	CCR7	BV650			≤ ;
					17	10						5.1H11	CD56	BV605	Cerco	1	< 1 < 1 < 1 < 1 < 1 < 1 < 1 < 1 < 1 < 1
			11.9	0.7					1					MARKET			010
				21	74	4						H1100	CD45RA	BV510	Cord		4.7
			34.0	2.0	24	3						HP-3G10	CD161	BV480			177
			34.0	2.0								\$J25C1	CD19	Pacific Blue		1	×5
			340	200	2.5	T. O. T.						MSE2	CD14	Pacific Blue			V S
8.5	0.5				7 5	15						A019DS	CD127	BV421		-	5
				SCHOOL STREET	3 20	4						SK3	CD4	BUV805			GIAN
	C.F		22.1	1.3								1C6/CXCR3	CXCR3	BUV737			014
17	0.1		11.9	0.7	1	1.00						B6	V82	BUV661		1	
			-		34	20						161	CCR4	GT9And			1170
170	1		8.5	0.5								FNSO	CD69	BUV563	Afraid		UVIO
			11 9	07								RPA-T8	CD8	виучуь	200		UV9
			10.7	-									AF-UV6	AF	C. J.		7VU
	AND DESCRIPTION OF THE PARTY OF		20 4	17								SK11	CD62L	SEEANG		1	
	Spiked 40 min @RT	then Fix/Perm	17	ColdStain 30min @4C	c 17	HotStain 30min @37C	Tetramer 40 min @ RT	(RT)	17	stimill	Vial Lot #	Clone	warker		name UV2		SAD SAD

Simplified Protocol

Collect, count, aliquot cells 2:3.0£+6 Cells R10 / 5ml polystyrene tube Bring volume up to "x" mL. R10, add "y" µL PMA/Ctrl and "z" µl CD107a Cap and incubate at 37°C for 6 hours Thaw cells, DNAse, count.

Wash with 2 ml PBS, spin down 1300rpm 8min 800 ul of LiveDead mix (1:2500) @RT for 15min Wash 2 ml 5% PBS-FBS, spin 1300 rpm, 8min Add HotStain mix, incubate @37C for 30 min Wash 2 ml 5% PBS-FBS 1400 rpm, 6 min

Add Tetramers, incubate @RT for 10 min Wash 2 ml 5% PBS-FBS 1400 rpm, 6 min

Add ColdStain mix, incubate @ 4C for 30min Add 300-500 ul 1x RBC Lysis for 3 minutes Wash 2 ml 5% PBS-FBS 1400 rpm, 6min

300 ul BD FixPerm, incubate @ 4C for 20min

First PermWash: Second Perm Wash: (vortex every 10 minutes)

1 ml PermWash 1500 rpm 6 min 1 ml PermWash 1500 rpm 6 min

Add Intracellular Stain, incubate @ RT for 40min
First PermWash: 2 ml PermWash 1500 rpm 6 min

Resuspend in 70 ul 0.4% PFA-PBS

Cap tubes, wrap rack in foil, store at 4°C

Pippette draw volume/sample:

5

Zombie NIR 0.8

17.5 PBS 7 Zombie

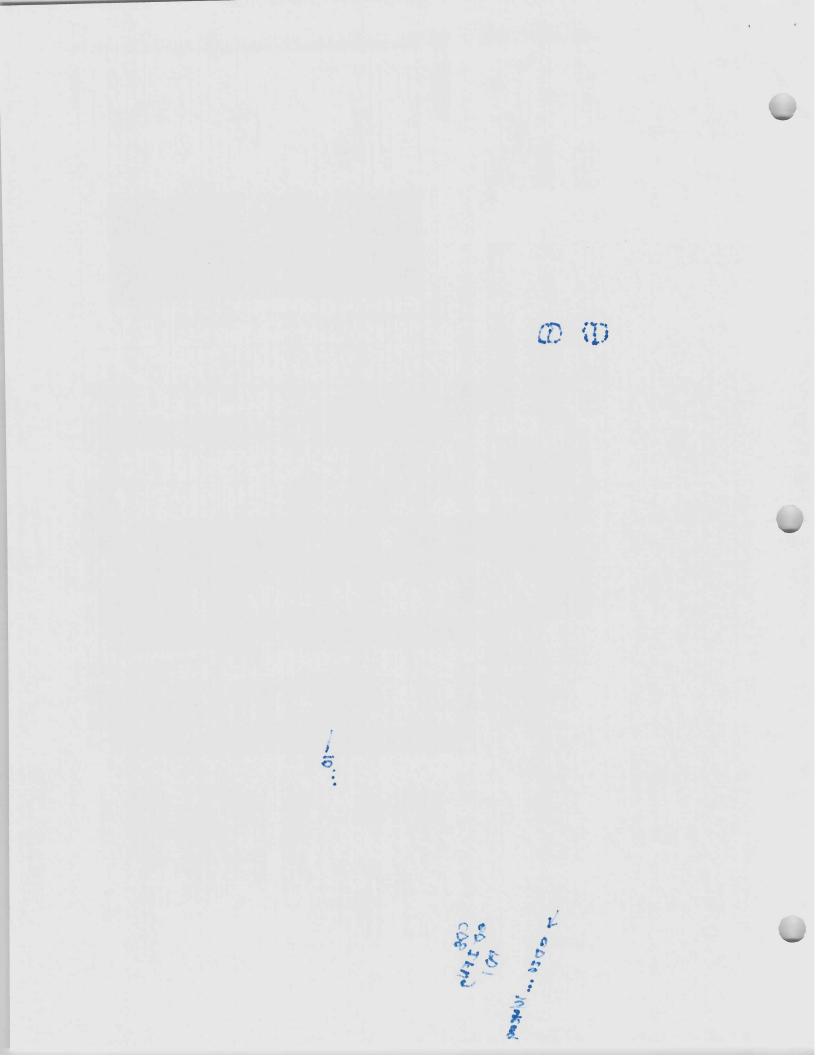
Number Samples
Number Unstained
Number Surface SCs (34)
Number Intracellular SCs

PFA

PFA 0.1915 PBS 1.7235 FBS 150 PBS 150

Perm Water

DOSON ... MARRIED



ILT & NK SFC Panel
Spectrum UV

			-			-	-				_							_
783 812						598	582	542	525	514	508	473	458	443	428	388	373	
UV15 UV16	UV14	UV13	UV12		UV10		0V9	WVU		UV7		DV6	UV5	UV4	UV3	UV2	UV1	ŀ
BUV805	BUV737			0	BUV615		BUV563			BUV496	The second secon	ĄF				BUV395		
[03]	[40]			100	[45]		[56]			[65]	on promoting					[50]	1	
CD4	CXCR3			707	CCR4		CD69			CD8						CD62L		
V15 V16	V14	V13	<1 ₂	V11	V10	6	& S	5 6	ń	ć	Á :	<u>{</u>	ప	S	<u> </u>		1	-
BV786	BV750	BV711		BV650	BV605		BV570	BV510		100	BV/190		PacBlue		BV421			Y CIEC
fool	100	[55]		(00)	[50]		leed a			[cc]	R		55	3	145		\dagger	-
CCR6	IFNγ	CD7		CCR7	CD56		CD45KA			0.191			CD14/19	7	CD437			
B12 B13 B14	B11	B10	B8	В7	B6 5	υ α 4 α	B3	B2		В1							†	
		rercr-cys.s					SparkBlue 550	FITC/AF488									בומפ	5
		(45) CD26					[50] CD3	Vα24/hCD1d										
YG9			YG5		YG3	YG1											T	
Pe-Vio770			PE-Cy5		PE-Dazzle594	PE	ļ										Yellow-Green	
[50]			Hu		75	[50]			- 21								1	
PD1			CD25		TNFα	NKG2D												
R6	R5	R R3	R2	<u>R</u>													T	1
Zombie NIR APC-Fire 750		APC-R700	AF647	APC													Red	
Viability CD27		© CD107a	Va7.2/hMR	CD16														The state of the s

