

10

AL-N cell survival & Mutagenesis  
CX-10 (HTdR, 50 % cluster)

Experiment Name : <sup>3</sup>HTdR toxicity (cluster, 50% labeling);

Exp.;

Performed by: M.Lenarczyk

Date: 03/08/2001/

1. Set the rocker-roller at 37°C incubator with 5% CO<sub>2</sub>, wash cells (from three 175 cm<sup>2</sup> flask, sub-cultured 1:2, 24h before) with HBSS, trypsinize cells, each re-suspend in 10ml RPMI, pool, pass five times through 10 cc syringe with 21 gauge needle, count cells

[Actual count ALN-2,018,400  
CX10-2,090,000]

1A. 200 cells/PGd's  
were plated  
for PE at  
37°C

1B. ALN & CX10  
10 cells  
↓  
Pl assay.

2. Prepare cell suspension with final concentration of 2000000/ml
3. Transfer 1 ml of cell suspension into 20 12 ml tubes (Falcon polypropylene TC) labeled 1-10 both on cap and wall.
4. Roll the tubes in standard culture condition
5. Prepare RPMI1640/FCS8 containing radioactivity

Date/Time: 03/08, 2001 / 16:00 - 18:15

640 µl HTdR (Stock : 1 µCi/µl on Feb., 15, 2001) + 3.4 ml RPMI 1640

6. Remove test tubes from roller and add RPMI/FCS8 with or without HTdR according to the table:

Date/Time: 03/08/01 / 18:30

106, 81, 114 - ALN  
110, 88, 98, - CX10

Tube #	<sup>3</sup> HTdR µCi/ml	Cells in RPMI (ml)	RPMI (ml)	RPMI + <sup>3</sup> HTdR [stock -160 µCi/ml] (ml)
1	0	1.0	1.0	0
2	0	1.0	1.0	0
3	5	1.0	0.940	0.060
4	10	1.0	0.875	0.125
5	20	1.0	0.750	0.250
6	30	1.0	0.625	0.375
7	40	1.0	0.500	0.500
8	50	1.0	0.375	0.625
9	60	1.0	0.250	0.750
10	80	1.0	0	1

14:30 (20h)  
19:40

160 µCi/ml

x 14 ml

x = 640 µCi

640 µCi (1 µCi/ml) + 3.4 ml RPMI/1640/FCS8 → 160 µCi/ml

7. Return test tubes to roller for 20-24 h. **Date/Time:** March 9/01 ~~18:30~~ 18:30
8. Next day, prepare and label 10 gamma-tubes (13 X 100 mm VWR glass test tube)
9. Remove tubes and centrifuge at 2000 rpm at 4°C for 10 min (precooled centrifuge).  
**Date/Time:** 1 March 09, 2001 / 14:30 (20 hrs)
10. Remove buckets from centrifuge and carefully remove 150 µl of supernatant and place in pre-labeled gamma-tube.
11. Decant supernatant, click tubes, vortex, re-suspend in 10 ml HBSS/CS8
12. Centrifuge tubes for 10 min at 2000 rpm, 4°C
13. Decant supernatant, click tubes, vortex, re-suspend in 10 ml HBSS/CS8
14. Centrifuge tubes for 10 min at 2000 rpm, 4°C
15. Decant supernatant from 10 unlabeled tubes (set B – without H-3), click tubes, vortex, re-suspend the pellet in 5 ml RPMI/FCS8, & transfer cell suspension into set A tubes ( e.g. 1 B into 1A into 1B ... 10A into 10B)
16. Add 5 ml RPMI1640/FCS8 and centrifuge tubes for 10 min at 2000 rpm, 4°C
17. Decant supernatant, click tubes, resuspend in 2 ml – syringe them 5x,
18. Centrifuge tubes, decant supernatant, vortex.
19. Transfer the cell suspension in polypropylene micro-centrifuge tubes with attached caps (Helena Plastics, 400 µl) using 200 µl pipet tips
20. Again add 200 µl ice cold RPMI, re-suspend and transfer the cell suspensions in the same polypropylene micro-centrifuge tubes (Total volume ~400 µl)
21. Centrifuge tubes for 5 min at 1000 rpm, 4°C
22. Transfer tubes at 10°C for 72 h. **Date/Time** March 10/2001 / 16:30
23. Transfer 30 µl supernatant in three sets of 20 ml scintillation vials , then add 6 ml liquid scintillation cocktail (EcoLume) 150 µl supernatant removed earlier (Step 10) and count them for *medium* radioactivity **Date/Time:** /
- ✓ 24. After 72 h, carefully remove the supernatant from the top, re-suspend pellet in 200 µl HBSS/CS8 & transfer the content to ten 12 ml tubes (Falcon plastic test tube, 17x100 mm, labeled 1-10 both on cap and wall) containing 10 ml RPMI/CS8 by using Pasteur pipet **Date/Time:** March 12, 2001 / 17:00
- ✓ 25. Again add 200 µl RPMI in micro-centrifuge tubes, re-suspend and transfer the cell suspensions in 12 ml tubes
- ✓ 26. Centrifuge the tubes for 10 min at 2000 rpm, 4°C (precooled centrifuge)

Cells were plated for SF in F12/FCS8

Medium activity  $A_2-CX10$ , M3 03/09/2001

PAGE: 1

USER: 6 ID: H3 HOWELL PRESET TIME: 1.00 SAT 10 MAR 2001 21:26  
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR: N RS232: N  
 H#: 1 ADC: N QCF: N RCM: N  
 CHANNEL 1-LL: 0 UL: 400 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0  
 DATA CALC: CFM, UNKNOWN REPLICATES: 1 NORM FACTOR: 1.00000  
 HALF LIFE (DAYS): N

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
1	**	1	8.00	70.71	1.00	1.42	85.0	
2	**	2	14.00	53.45	1.00	3.00	81.0	
3	**	3	16.00	50.00	1.00	4.58	77.0	
4	**	4	14.00	53.45	1.00	6.13	81.0	
5	**	5	14.00	53.45	1.00	7.76	75.0	
6	**	6	9.00	66.67	1.00	9.38	78.0	
7	**	7	92693.33	1.70	0.15	10.08	78.0	
8	**	8	99719.99	1.64	0.15	10.79	77.0	
9	**	9	96353.33	1.66	0.15	11.50	78.0	
10	**	10	188350.00	1.46	0.10	12.21	79.0	
11	**	11	178670.00	1.50	0.10	12.92	78.0	
12	**	12	183646.66	1.21	0.15	13.63	77.0	
13	**	13	394693.31	0.82	0.15	14.35	78.0	
14	**	14	387093.31	0.83	0.15	15.08	79.0	
15	**	15	379110.00	1.03	0.10	15.80	79.0	
16	**	16	565206.62	0.69	0.15	16.54	81.0	
17	**	17	568006.62	0.69	0.15	17.28	78.0	
18	**	18	587620.00	0.83	0.10	18.03	80.0	
19	**	1	856613.31	0.56	0.15	18.85	78.0	
20	**	2	836633.31	0.56	0.15	19.63	80.0	
21	**	3	816173.31	0.57	0.15	20.39	81.0	
22	**	4	994913.31	0.52	0.15	21.18	79.0	
23	**	5	964413.31	0.53	0.15	21.95	77.0	
24	**	6	950793.31	0.53	0.15	22.73	80.0	
25	**	7	1178990.00	0.58	0.10	23.53	78.0	
26	**	8	1167686.62	0.48	0.15	24.33	77.0	
27	**	9	1170646.62	0.48	0.15	25.13	78.0	
28	**	10	1554613.25	0.41	0.15	25.97	77.0	
29	**	11	1454120.00	0.43	0.15	26.78	79.0	
30	**	12	1602026.62	0.41	0.15	27.62	78.0	
32	**	14	130162.86	1.95	0.35	28.58	4.0	

Std M-3  
 DPH=98200

IN TABLE

Medium count (2nd) ~~EN-080~~ (H3)

USER: 6 ID:H3 HOWELL PRESET TIME: 1.00 SUN 11 MAR 2001 15:52  
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N  
 H#: 1 AGC:N GCF:N RCM:N  
 CHANNEL 1-LL: 0 UL: 400 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0  
 DATA CALC: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR:Q 1.00000  
 HALF LIFE(DAYS):N

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
1	29- 1	1	12.00	57.74	1.00	1.42	84.0	
2	29- 2	1	13.00	55.47	1.00	3.00	81.0	
3	29- 3	1	10.00	63.25	1.00	4.63	78.0	
4	29- 4	1	14.00	53.45	1.00	6.25	81.0	
5	29- 5	1	8.00	70.71	1.00	7.82	76.0	
6	29- 6	1	10.00	63.25	1.00	9.44	78.0	
7	29- 7	1	95193.33	1.67	0.15	10.20	78.0	
8	29- 8	1	100050.00	2.00	0.10	10.90	77.0	
9	29- 9	1	97760.00	1.65	0.15	11.67	78.0	
10	29-10	1	192226.66	1.18	0.15	12.38	79.0	
11	29-11	1	179746.66	1.22	0.15	13.08	77.0	
12	29-12	1	157870.00	1.46	0.10	13.79	77.0	
13	29-13	1	401260.00	1.00	0.10	14.53	78.0	
14	29-14	1	392120.00	0.82	0.15	15.26	79.0	
15	29-15	1	383320.00	0.83	0.15	15.99	78.0	
16	29-16	1	574846.62	0.68	0.15	16.74	81.0	
17	29-17	1	577380.00	0.68	0.15	17.48	78.0	
18	29-18	1	592473.31	0.67	0.15	18.23	79.0	
19	**- 1	1	857173.31	0.56	0.15	19.05	78.0	
20	**- 2	1	841926.62	0.56	0.15	19.82	80.0	
21	**- 3	1	822653.31	0.57	0.15	20.58	80.0	
22	**- 4	1	1016386.62	0.51	0.15	21.37	79.0	
23	**- 5	1	998266.62	0.52	0.15	22.15	77.0	
24	**- 6	1	966846.62	0.53	0.15	22.93	79.0	
25	**- 7	1	1196786.62	0.47	0.15	23.73	79.0	
26	**- 8	1	1190273.25	0.47	0.15	24.53	78.0	
27	**- 9	1	1193580.00	0.58	0.10	25.33	79.0	
28	**-10	1	1590286.62	0.41	0.15	26.18	77.0	
29	**-11	1	1503540.00	0.42	0.15	27.00	79.0	
30	**-12	1	1420966.62	0.41	0.15	27.84	79.0	
32	**-14	1	[29968.57]	1.95	0.35	28.81	4.0	

Std H-3  
 DPM-98200

TABLE-3

Expt. #:  $A_2N + C \times 10$  (50%, H3)

START ON:  
Date/Time: 03/12/01

+ Survival for  $A_2N$ , (7, 10.5, 12°C)  
or  $C \times 10$  (10.5°C)

Tube #	Coulter count for 100 $\mu$ l cell suspension	Avg. count	Cells/ml [Avg. count x 400 ]	Total # of cells ( $\times 10^6$ )	% Recovery
1	6720, 6923, 6789	6791	2 716 267	5.43	264
2	6410, 6399, 6251	6333	2 533 333	5.07	247
3	5598, 5562, 5452	5517	2 206 933	4.40	109
4	5281, 5260, 5395	5292	2 116 800	4.23	109
5	4981, 5265, 5281	5156	2 062 267	4.12	102
6	4970, 5006, 5084	5000	2 000 000	4.00	99
7	4883, 4898, 4934	4906	1 962 533	3.93	97
8	5198, 5198, 5379	5238	2 095 333	4.19	103
9	4783, 4782, 4706	4737	1 894 800	3.79	94
10	4709, 4669, 4605	4641	1 856 400	3.71	92

2  
No. of cells in dish

A N  
7°C 5426, 5509, 5363  
10.5°C 4298, 4411, 4367  
12°C 4302, 4260, 4361  
37°C  
C x 10  
10.5°C 5136, 5120, 5229  
37°C

Bdys - 20  
Mode - 500  $\mu$ l

5346 2 138 400 4.28  
4332 1 732 200 3.47  
4288 1 715 066 3.43  
5142 2 056 667 4.11

Colonies

106, 81, 114  
200  
119, 88, 98  
200

cell activity,  $A_{cell} \times 10^4$  (Hz)  
(1st count)

USER: 6 ID:H3 HOWELL      PRESET TIME: 1.00      TUE 13 MAR 2001 09:19  
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N      RS232:N  
 H#: 1 ADC:N QCF:N RCM:N  
 CHANNEL 1-LL: 0 UL: 400 ZSIGMA: 2.00 BKG SUB: 0.00 BKG ZSIG: 0.00 LSR: 0  
 DATA CALC: CPM, UNKNOWN REPLICATES: 1      NORM FACTOR: 0 1.00000  
 HALF LIFE(DAYS):N

SAM	POS	CH	CPM	ZSIG%	TIME	EL TIME	AVG H#	ERR
1	**	1	68206.66	1.98	0.15	0.56	87.0	
2	**	2	73946.66	1.90	0.15	1.27	88.0	
3	**	3	74740.00	1.89	0.15	1.97	88.0	
4	**	4	57060.00	1.87	0.20	2.77	90.0	
5	**	5	44176.00	1.90	0.25	3.63	87.0	
6	**	6	52970.00	1.94	0.20	4.43	91.0	
7	**	7	27212.50	1.92	0.40	5.45	88.0	
8	**	8	24842.22	1.89	0.45	6.46	86.0	
9	**	9	36666.66	1.91	0.30	7.31	94.0	
10	**	10	11571.11	1.96	0.90	8.78	86.0	
11	**	11	12072.94	1.97	0.85	10.19	88.0	
12	**	12	10657.89	1.99	0.95	11.77	88.0	
13	**	13	9.00	66.67	1.00	13.33	86.0	
14	**	14	13.00	55.47	1.00	14.90	84.0	
15	**	15	4.00	100.0	1.00	16.47	93.0	
16	**	16	144.00	16.67	1.00	18.03	87.0	
17	**	17	111.00	18.98	1.00	19.59	88.0	
18	**	18	108.00	19.25	1.00	21.22	87.0	
19	**	19	85955.00	1.74	0.20	22.07	95.0	
20	**	20	102359.99	1.61	0.15	22.78	90.0	
21	**	21	65325.00	1.75	0.20	23.53	86.0	
22	**	22	143260.00	1.36	0.15	24.24	96.0	
23	**	23	176986.66	1.23	0.15	24.95	89.0	
24	**	24	133380.00	1.41	0.15	25.66	89.0	
25	**	25	162493.33	1.28	0.15	26.37	95.0	
26	**	26	140210.00	1.69	0.10	27.07	89.0	
27	**	27	142606.66	1.37	0.15	27.78	92.0	
28	**	28	256860.00	1.25	0.10	28.50	93.0	
29	**	29	240580.00	1.29	0.10	29.22	91.0	
30	**	30	157133.33	1.30	0.15	29.92	85.0	
32	**	32	29448.57	1.97	0.35	30.89	1.0	

AB  
A dead Ecolumn

Standard  
DPH-98200

~~IN~~ TABLE

cell activity (2nd count)  
 AN + CK10, H3, 50%<sup>26</sup>)

USER: 6 ID: H3 HOWELL PRESET TIME: 1.00 TUE 13 MAR 2001 12:30  
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR: N RS232: N  
 H#: 1 AGC: N DCF: N RCM: N  
 CHANNEL 1-LL: 0 UL: 400 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0  
 DATA CALC: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR: 0 1.00000  
 HALF LIFE(DAYS): N

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
1	28- 1	1	93.00	20.74	1.00	1.42	87.0	
2	28- 2	1	107.00	19.33	1.00	3.06	87.0	
3	28- 3	1	136.00	17.15	1.00	4.68	85.0	
4	28- 4	1	12.00	57.74	1.00	6.29	92.0	
5	28- 5	1	11.00	60.30	1.00	7.87	85.0	
6	28- 6	1	8.00	70.71	1.00	9.43	84.0	
7	28- 7	1	10381.00	1.96	1.00	10.99	88.0	
8	28- 8	1	11563.33	1.96	0.90	12.53	86.0	
9	28- 9	1	11050.53	1.95	0.95	14.04	86.0	
10	28-10	1	35386.66	1.94	0.30	14.95	92.0	
11	28-11	1	24157.78	1.92	0.45	16.01	86.0	
12	28-12	1	26152.50	1.96	0.40	17.01	87.0	
13	28-13	1	50950.00	1.98	0.20	17.76	93.0	
14	28-14	1	42936.00	1.93	0.25	18.56	87.0	
15	28-15	1	54890.00	1.91	0.20	19.31	92.0	
16	28-16	1	72226.66	1.92	0.15	20.07	89.0	
17	28-17	1	68173.33	1.98	0.15	20.77	88.0	
18	28-18	1	64065.00	1.77	0.20	21.52	88.0	
19	**-- 1	1	63250.00	1.78	0.20	22.38	94.0	
20	**-- 2	1	99433.33	1.64	0.15	23.08	90.0	
21	**-- 3	1	63175.00	1.78	0.20	23.89	86.0	
22	**-- 4	1	140070.00	1.69	0.10	24.60	97.0	
23	**-- 5	1	172553.33	1.24	0.15	25.31	89.0	
24	**-- 6	1	129199.99	1.44	0.15	26.02	88.0	
25	**-- 7	1	158950.00	1.59	0.10	26.73	93.0	
26	**-- 8	1	133646.66	1.41	0.15	27.43	89.0	
27	**-- 9	1	136350.00	1.71	0.10	28.14	91.0	
28	**--10	1	251113.33	1.03	0.15	28.86	94.0	
29	**--11	1	236810.00	1.30	0.10	29.58	91.0	
30	**--12	1	152350.00	1.62	0.10	30.28	86.0	
32	**--14	1	30136.00	1.88	0.38	31.33	2.0	

Standard  
 DPM = 88200

IN TABLE

March 9, 2001

Challenge for CD59, HPRT - 1st

(T<sub>LN</sub> - 100%, M<sub>2</sub>)

Div.	Count (Hemucyt)	Total	$\frac{2 \times 10^5}{P 100's}$ CD59 <sup>+</sup>	HPRT <sup>+</sup>
2.2	1:5 74, 84 $\frac{10^4}{79}$		$\frac{253}{5}$ 50.6	253
3	1:5 29, 32 30.5		$\frac{667}{5}$ 133.4	667
5	1:5 20, 23 21.5		$\frac{930}{5}$ 186	930
7	1:1 57, 48 52.5		381	381
8	1:1 57, 71 64		313	313
10	1:1 100, 101 100.5		199 μl to plate	199 μl to plate

1. CD59 C<sup>+</sup> - 2% × 7.5 ml = 150 μl × 18 = 2,700 ml  
 2. PE C<sup>+</sup> - 2% × 1.5 ml = 30 μl × 18 = 0,540 ml  
 3. SI Anti - 0.5% × 7.5 ml = 37.5 μl × 18 → + 9 ml mix → 0.5 ml / P 100's

3,24 ml } 3.6 ml C<sup>+</sup>  
 20 ml RPMI  
 ↓ 0.5 / P 35's

CD59 2 × 2 × 10<sup>5</sup> / P 100's  
 HPRT 5 × 2 × 10<sup>5</sup> / P 100's

PREPARE "HPRT" medium  
 6 × 5 P 100's × 10 ml = 300 ml  
 precise total vol. = 350 ml  
 350 ml + 2.3 ml 6TG (stock)  
 ↓  
 10 μl / P 100's

6-TG - Stock sol  
 2.5 mg + 10 ml H<sub>2</sub>O  
 ↓  
 0.25 mg/ml  
 = 1.5 × 10<sup>-3</sup> M

VERTE for SF & HPRT mutant data.



|||  
0 0 0

SURVIVAL DATA

↓

1. Cells were plated in 4 60's, 3 dishes/dose point for 200/dish
2. Cells were in F12 medium (~~HPRT~~ we were out of HPRT)

↓!!!

NO ANY COLONIES after 7 days of incubation.

HPRT<sup>-</sup> → NO ANY HPRT<sup>-</sup> mutants

~~1. I plated 10 2x.~~

						# of colonies	
						+ C	- C
Y5	2.2	74,84	79	2	$4.0 \times 10^6 / m$	$\frac{0.05}{0.01} \rightarrow 2 \times 10^5 \times 6$	83,71,94
Y5	3	29,32	305		$1.5 \times 10^6$	$\frac{0.133}{0.027} \rightarrow 4 \times 10^4$	85,92,82
Y5	5	20,23	21.5		$1.08 \times 10^6$	$\frac{0.185}{0.037} \rightarrow 4 \times 10^4$	84,75,25
*1	7	57,48	52.5		$0.525 \times 10^6$	$\frac{0.321}{0.076} \rightarrow 4 \times 10^4$	75,54,73
*2	8	57,71	64		$0.64 \times 10^6$	$\frac{0.313}{0.063} \rightarrow 4 \times 10^4$	53,36,37
	10	104,04	100.5		$1.0 \times 10^6$	$\frac{0.199}{0.040} \rightarrow 4 \times 10^4$	70,73,69

131.  
133/ml  
1.5  
200  
x6

200

PE - 133 cells / P 35's x 6  
CD59 } 2 x 10<sup>5</sup> / P 100's  
HPDT }

\*1 Ad7 - 0.1 ml x + 3 ml → 3 x P/100's = 1.75 x 10<sup>4</sup>  
\*2 Ad8 - 0.25 ml x + 3 ml → 2 x P/100's = 8 x 10<sup>4</sup>

111  
080

Chall = C<sup>+</sup> - 2% x 7 ml = 140 μl x 18 = 2520 μl.  
Anti-SI E7.1 - 0.5% x 7 ml = 35 μl x 18 = 630 μl.

PE + C C<sup>+</sup> - 2% x 1.5 ml = 30 μl x 6 x 3 = 540 μl

Total - 1. C<sup>+</sup> - 2520 + 540 → 3060 → 31.5 μl/well  
2. Anti-SI - 630 μl  
3.

VERTE!

Challenge<sup>3</sup> results

Results for PE:	# of colonies	Average	# of cells plated / P35	Abs. PE.	PE
+C	-C				
2.2	0	83, 71, 94	82.67	131	0.63
3	0	85, 92, 82	86.33	131	0.66
5	0	94, 75, 75	81.33	131	0.62
7	0	75, 54, 73	67.33	131	0.51
8	0	53, 36, 37	42.00	131	0.32
10	0	70, 73, 49	64.00	131	0.49 <del>0.48</del>

CD59<sup>-</sup> colonies:

2.2	318	420	360
3	790		
5	472	458	472
7			
8	602	426	
10	420	380	368

March 12 - Challenge 1st for A<sub>2</sub> (100%)

SAMPLE	COUNT	$\bar{x}$				
4	44,56	$50 \times 10^4 / ml$	$0.5 \times 10^6 / ml$	$\frac{0.4 ml}{1 ml} \rightarrow 2 \times 10^5 \text{ cells}$	$\frac{0.23}{7} \rightarrow 133 / ml$	$\rightarrow 1.5 ml / P100$
9	41,28	$39.5 \times 10^4 / ml$	$0.395 \times 10^6 / ml$	$\frac{0.5 ml}{1 ml} \rightarrow 2 \times 10^5 \text{ cells}$	$\frac{0.23}{7} \rightarrow 133 / ml$	$\rightarrow 1.5 ml / P100$

FOR MPRT

6T0 - stock ( $1.5 \times 10^3 M$ ) 80 P100's  $\times 10 ml = 100 ml$   
 $120 ml - x \rightarrow 0.79$   
 $350 - 2.3 \text{ 6T0}$

6 P100's -

For challenge C<sup>+</sup> -  $2\% \times 8 ml = 0.16 ml \times 6 = 0.96 ml$

MARb -  $0.5\% \times 8 ml = 0.4 ml \times 6 = 2.4 ml$

For PE C<sup>+</sup> -  $2\% \times 1.5 ml = 0.03 \times 6 = 0.18 ml$

C<sup>+</sup>  $0.96 + 0.18 = 1.14 + (8 + 3) ml \text{ RPMI}$

MARb  $2.4 ml +$

C<sup>+</sup> Take

- 3.6 ml C<sup>+</sup> + 20 ml RPMI/FCS
  - Mix - add 0.5 ml in 6 wells 35 mm dishes  
marked C<sup>+</sup>
  - Add to C<sup>+</sup> + RPMI/FCS 0.625 ml MARb - mix  
add 0.5 ml per P100's
- $1.18 ml \text{ C}^+ + 11 ml \text{ RPMI} \rightarrow \text{mix} \rightarrow 1 ml / P100 \times 6$

# CD59<sup>-</sup> Challenge

## Results for PE

PE:

Sample	# of CD59 <sup>-</sup> colonies	# of colonies for PE -C	AVERAGE
4	123, 288, 347	62, 64, 71	65.7
9	48, 78, 79	25, 40, 30	35.0

CD59 <sup>-</sup> AVERAGE	$\frac{\text{CD59}^-}{10^5}$	
4 253/2	127	193
9 68/2	34	97

