

- 4 V79/100

### V79 COLONY FORMING ASSAY

**Experiment Name :** <sup>3</sup>HTdR toxicity (cluster, 100% labeling);

**Exp. Dec., 14, 2000**

**Investigator:** R.Howell @ M.Lenarczyk

**Date:**

12/14/00

1. Set the rocker-roller at 37°C incubator with 5% CO<sub>2</sub>, set the Coulter Counter, wash cells (from 3 150 cm<sup>2</sup> flask, sub cultured 1:2, 48h before) with D-PBS, trypsinize cells, each resuspend in 7 ml MEMB, pool, pass five times through 3 cc syringe with 21 gauge needle, count cells by transferring 100 ul in Coulter cup containing 20 ml isotone
2. Dilute to ~4,000,000 cells/ml in MEMB [Actual count : 4 200 000 cells/ml]. *Cells were under HAT condition for 2.5 days.*
3. Transfer 1 ml of cell suspension into ten 10 ml tubes (Falcon polypropylene round bottom tube, 17x100 mm)
4. Keep the tubes in the roller for 3-4 h at 37°C, 5% CO<sub>2</sub> **Date/Time: Dec., 14, 2000 / 16:15**
5. Prepare MEMB containing radioactivity in hood  
40 µl <sup>3</sup>HTdR (Stock :1 µCi/µl on Nov., 6, 2000) + 5 ml MEMB
6. After 3 hra, remove test tubes from roller and add MEMB with or without radioactivity according to Table below. **Date/Time: Dec., 14, 2000 / 19:15**

Tube #	<sup>3</sup> HTdR µCi/ml	Cells in MEMB ( ml )	MEMB+ <sup>3</sup> HTdR ( ml ) [8 µCi/ml]	MEMB ( ml )
1	0	1.0	0	1
2	0	1.0	0	1
3	0.025	1.0	0.062	0.938
4	0.0505	1.0	0.120	0.880
5	0.110	1.0	0.250	0.750
6	0.215	1.0	0.370	0.630
7	0.52	1.0	0.500	0.500
8	0.75	1.0	0.620	0.380
9	1.3	1.0	0.750	0.250
10	2.1	1.0	0	1

$^3\text{HTDR}$  100% labeling

	$\frac{\mu\text{Ci}}{\text{ml}}$	ml cells	ml MEMB $8 \mu\text{Ci}/\text{ml}$	ml MEMB
1	0	1	0	1
2	0	1	0	1
3	0.25	1	0.062	0.938
4	0.5	1	0.12	0.88
5	1.0	1	0.25	0.75
6	1.5	1	0.37	0.63
7	2.0	1	0.5	0.5
8	2.5	1	0.62	0.38
9	3.0	1	0.75	0.25
10	4.0	1	1	0

Prepare  $8 \frac{\mu\text{Ci}}{\text{ml}}$  MEMB (5 ml)

40  $\mu\text{Ci}$   $^3\text{HTDR}$  + 5 ml MEMB

$$\frac{40 \mu\text{Ci}}{5 \text{ml}} = 8 \frac{\mu\text{Ci}}{\text{ml}}$$

7. Return test tubes to roller for 12 h **Date/Time: Dec., 14, 2000 / 19:30**

8. Next day, while test tubes are in roller label 10 gamma-tubes (13 X 100 mm VWR glass test tube)

✓ 9. After ~12 h incubation period, remove tubes and centrifuge at 2000 rpm at 4°C for 10 min (precooled centrifuge). **Date/Time: Dec, 15, 2000 / 9:20**

✓ 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 wash MEMA

✓ 12. Centrifuge tubes for 10 min at 2000 rpm, 4°C *Vortex hard since radioactivity might be on the cap!!!*

✓ 13. Decant supernatant, click tubes, vortex, re-suspend in 10 ml wash MEMA

✓ 14. Centrifuge tubes for 10 min at 2000 rpm, 4°C

✓ 15. Decant supernatant, click tubes, vortex, re-suspend in 10 ml wash MEMA

✓ 16. Centrifuge tubes for 10 min at 2000 rpm, 4°C

✓ 17. Decant supernatant, click tubes, vortex, re-suspend in 7 ml of MEMA

✓ 18. Centrifuge tubes for 10 min at 2000 rpm, 4°C → *cell were clumped on the inner wall!*

✓ 19. Decant supernatant, click tubes, vortex, transfer the cell suspension in polypropylene microcentrifuge tubes with attached caps (Helena Plastics, 400 µl) using 200 µl pipet tips

✓ 20. Again add 200 µl ice cold MEMA, re-suspend and transfer the cell suspensions in the same polypropylene microcentrifuge 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. **Dec., 15, 2000 /** **Date/Time:**

✓ 23. Transfer 30 µl supernatant in three sets of 20 ml scintillation vials containing 6 ml liquid scintillation cocktail (<sup>Ecolumn</sup> Aquasol) from 150 µl supernatant removed earlier (Step 10) and count them for radioactivity **Date/Time: Dec., 15, 2000 / 17:30**

✓ 24. After 72 h, carefully remove the supernatant from the top, re-suspend pellet in 200 µl wash MEMA and transfer the content to ten 12 ml tubes (Falcon polypropylene round bottom test tube, 17x100 mm, labeled 1-10 both on cap and wall) containing 10 ml wash MEMA by using Pasteur pipet *x2* **Date/Time: Dec., 18, 2000 / 10:15**

✓ 25. Again add 200 µl wash MEMA in microcentrifuge tubes, resuspend and transfer the cell suspensions in 12 ml tubes

✓ 26. Centrifuge the tubes for 10 min at 2000 rpm, 4°C (precooled centrifuge)

27. Labeling and preparation of dilution tubes and colony dishes

- load P60's dishes with 4 ml MEMA

- load 40 sterile tubes with 4.5 ml MEMA and label them 1.2, 1.3, 1.4, 1.5; 2.2, 2.3,

2.4, 2.5; X.2, X.3, X.4, X.5 etc.

- ✓ ✓ 28. Decant supernatant, click tubes, vortex, re-suspend in 10 ml wash MEMA
- ✓ ✓ 29. Centrifuge tubes for 10 min at 2000 rpm, 4°C
- ✓ ✓ 30. Decant supernatant, click tubes, vortex, re-suspend in 10 ml wash MEMA
- ✓ ✓ 31. Centrifuge tubes for 10 min at 2000 rpm, 4°C
- ✓ ✓ 32. Decant supernatant, click tubes, vortex, re-suspend in 2 ml wash MEMA, pass 5x through 3 cc syringe with 21 gauge needle
- 33. Determine cell concentration by transfer 100 µl to Coulter cup
- 34. Vortex tube, transfer 0.5 ml into dilution tube X.5, vortex tube X.5, transfer 0.5 ml into dilution tube X.4, vortex tube X.4 and transfer 0.5 ml to tube X.3, vortex tube X.3 and transfer 0.5 ml to tube X.2 and vortex. Keep tubes on ice.
- 35. Transfer 1 ml from dilution tubes into dishes labeled X.2, X.3, X.4 (in triplicate). Only X.2 should be seeded for control T-tubes.
- 36. Transfer 200 µl of cell suspension (in triplicate) to 20 ml scintillation vial containing 6 ml cocktail (Aquasol)
- 37. Incubate petridishes for 1 week
- 38. Count vials for radioactivity  
Dec., 19, 2000 / 15:45
- 39. After 1 week, wash colonies 3 times with normal (1X) saline, and 2 times with methanol. Stain colonies with 0.05% crystal violet
- 40. Count colonies. There must be between 25 and 250 colonies for the dish to be a valid data point.

Dec., 19, 2000 Date/Time : 15:45

1st count

Activity	1st count	2nd count	3rd count	4th count	5th count	6th count
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0.25	0	0	0	0	0	0
0.5	0	0	0	0	0	0
1.0	0	0	0	0	0	0
1.5	0	0	0	0	0	0
2.0	0	0	0	0	0	0
2.5	0	0	0	0	0	0
3.0	0	0	0	0	0	0
4.0	0	0	0	0	0	0

[ ]  
 DPM 98200  
 STANDARD H-3

1st count  
 Medium activity, H3, 100%, U-79

2nd count

7

No.	Date	Particulars	Dr	Cr	Balance
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
21					
22					
23					
24					
25					
26					
27					
28					
29					
30					
31					
32					
33					
34					
35					
36					
37					
38					
39					
40					
41					
42					
43					
44					
45					
46					
47					
48					
49					
50					
51					
52					
53					
54					
55					
56					
57					
58					
59					
60					
61					
62					
63					
64					
65					
66					
67					
68					
69					
70					
71					
72					
73					
74					
75					
76					
77					
78					
79					
80					
81					
82					
83					
84					
85					
86					
87					
88					
89					
90					
91					
92					
93					
94					
95					
96					
97					
98					
99					
100					
101					
102					
103					
104					
105					
106					
107					
108					
109					
110					
111					
112					
113					
114					
115					
116					
117					
118					
119					
120					
121					
122					
123					
124					
125					
126					
127					
128					
129					
130					
131					
132					
133					
134					
135					
136					
137					
138					
139					
140					
141					
142					
143					
144					
145					
146					
147					
148					
149					
150					
151					
152					
153					
154					
155					
156					
157					
158					
159					
160					
161					
162					
163					
164					
165					
166					
167					
168					
169					
170					
171					
172					
173					
174					
175					
176					
177					
178					
179					
180					
181					
182					
183					
184					
185					
186					
187					
188					
189					
190					
191					
192					
193					
194					
195					
196					
197					
198					
199					
200					

100000000

Amount of cells remaining after washing

Exp  
Date Dec, 15, 2000

Mode 500pl  
Background 18

Sample (tube)	Coulter count	Average count $\times 10^4$	Total volume (ml)	L.D.S	% rec
1	158, 160, 175	5.9	5	$2.9 \times 10^5$	70%
2	170, 161, 152	$5.7 \times 10^4/ml$	5	$2.9 \times 10^5$	70%
3	172, 134, 114	$4.9 \times 10^4/ml$	5	$2.4 \times 10^5$	53
4	<del>214</del> , 126, 94, 125	$3.9 \times 10^4/ml$	5	$1.9 \times 10^5$	46
5	150, 124, 81	4.05	5	$2.0 \times 10^5$	47
6	159, 157, 168	5.7	5	$2.9 \times 10^5$	70
7	144, 109, 117	4.25	5	$2.1 \times 10^5$	50
8	150, 125, 146	4.95	5	$2.5 \times 10^5$	60
9	204, 194, 219	7.3	5	$3.8 \times 10^5$	91
10	226, 215, 215	8.0	5	$4.0 \times 10^5$	95

TABLE-3

Expt. #: Dec, 11, 2000

Date/Time: Dec, 18, 2000/15:05

Tube #	Coulter count for 100 ul cell suspension	Avg. count	Cells/ml [Avg. count x 400]	pCi/cell [uCi/ml x 10 <sup>6</sup> Cells/ml]
1	1232, 1209, 1265	1230	492133	
2	2714, <sup>2620</sup> <del>1511</del> , 2702	2679	1069467	
3	4688, 4336, 4734	4586	1832400	
4	3389, 3356, 3387	3377	1,348,933	
5	3674, 3592, 3653	3633	1,451,200	
6	4846, <sup>4822</sup> <del>3534</del> , 4888	4853	1,938,800	
7	4414, 4549, 4327	4430	1,772,000	
8	4116, 4010, 4114	4080	1,632,000	
9	5151, 5125, 5068	5148	2,057,200	
10	4263, 4058, <sup>4013</sup> <del>3877</del>	4111	1,642,533	

X-3 ←  
for SURVIVAL

Biological

~ 25%

~ 50%

X3, X4

Background - 5 counts

4.2 x 10<sup>6</sup>

Coulter Mode - 500ul

① }  
② } samples processed by me  
③ }



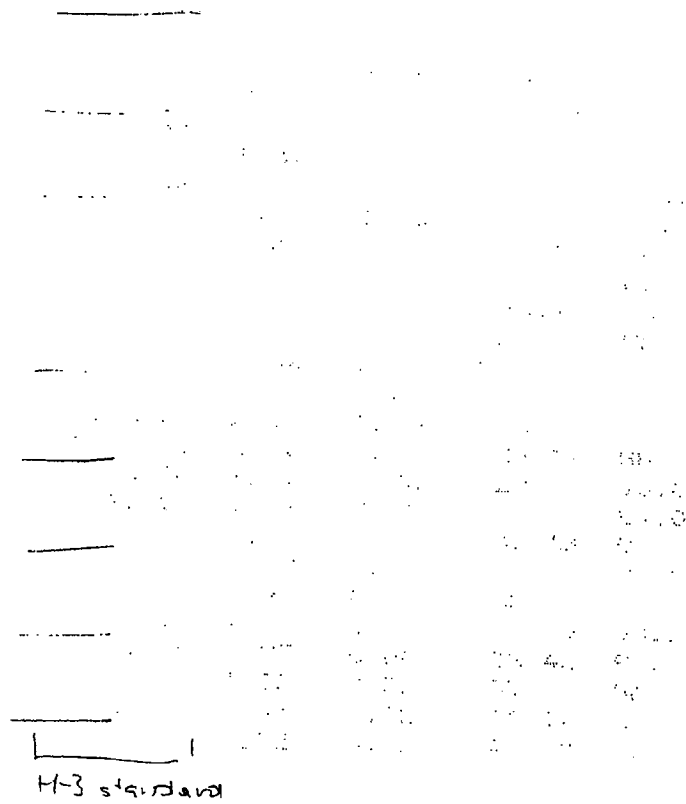
Exp: Dec, 14, 2000. V-79, 100% cluster, <sup>3</sup>H Tair

Cell activity - ~~1st~~ count

Time	Count	Rate	Std. Dev.	Count	Rate	Std. Dev.
0.00	1000	1000	31.6	1000	1000	31.6
0.10	1000	1000	31.6	1000	1000	31.6
0.20	1000	1000	31.6	1000	1000	31.6
0.30	1000	1000	31.6	1000	1000	31.6
0.40	1000	1000	31.6	1000	1000	31.6
0.50	1000	1000	31.6	1000	1000	31.6
0.60	1000	1000	31.6	1000	1000	31.6
0.70	1000	1000	31.6	1000	1000	31.6
0.80	1000	1000	31.6	1000	1000	31.6
0.90	1000	1000	31.6	1000	1000	31.6
1.00	1000	1000	31.6	1000	1000	31.6
1.10	1000	1000	31.6	1000	1000	31.6
1.20	1000	1000	31.6	1000	1000	31.6
1.30	1000	1000	31.6	1000	1000	31.6
1.40	1000	1000	31.6	1000	1000	31.6
1.50	1000	1000	31.6	1000	1000	31.6
1.60	1000	1000	31.6	1000	1000	31.6
1.70	1000	1000	31.6	1000	1000	31.6
1.80	1000	1000	31.6	1000	1000	31.6
1.90	1000	1000	31.6	1000	1000	31.6
2.00	1000	1000	31.6	1000	1000	31.6
2.10	1000	1000	31.6	1000	1000	31.6
2.20	1000	1000	31.6	1000	1000	31.6
2.30	1000	1000	31.6	1000	1000	31.6
2.40	1000	1000	31.6	1000	1000	31.6
2.50	1000	1000	31.6	1000	1000	31.6
2.60	1000	1000	31.6	1000	1000	31.6
2.70	1000	1000	31.6	1000	1000	31.6
2.80	1000	1000	31.6	1000	1000	31.6
2.90	1000	1000	31.6	1000	1000	31.6
3.00	1000	1000	31.6	1000	1000	31.6
3.10	1000	1000	31.6	1000	1000	31.6
3.20	1000	1000	31.6	1000	1000	31.6
3.30	1000	1000	31.6	1000	1000	31.6
3.40	1000	1000	31.6	1000	1000	31.6
3.50	1000	1000	31.6	1000	1000	31.6
3.60	1000	1000	31.6	1000	1000	31.6
3.70	1000	1000	31.6	1000	1000	31.6
3.80	1000	1000	31.6	1000	1000	31.6
3.90	1000	1000	31.6	1000	1000	31.6
4.00	1000	1000	31.6	1000	1000	31.6
4.10	1000	1000	31.6	1000	1000	31.6
4.20	1000	1000	31.6	1000	1000	31.6
4.30	1000	1000	31.6	1000	1000	31.6
4.40	1000	1000	31.6	1000	1000	31.6
4.50	1000	1000	31.6	1000	1000	31.6
4.60	1000	1000	31.6	1000	1000	31.6
4.70	1000	1000	31.6	1000	1000	31.6
4.80	1000	1000	31.6	1000	1000	31.6
4.90	1000	1000	31.6	1000	1000	31.6
5.00	1000	1000	31.6	1000	1000	31.6
5.10	1000	1000	31.6	1000	1000	31.6
5.20	1000	1000	31.6	1000	1000	31.6
5.30	1000	1000	31.6	1000	1000	31.6
5.40	1000	1000	31.6	1000	1000	31.6
5.50	1000	1000	31.6	1000	1000	31.6
5.60	1000	1000	31.6	1000	1000	31.6
5.70	1000	1000	31.6	1000	1000	31.6
5.80	1000	1000	31.6	1000	1000	31.6
5.90	1000	1000	31.6	1000	1000	31.6
6.00	1000	1000	31.6	1000	1000	31.6
6.10	1000	1000	31.6	1000	1000	31.6
6.20	1000	1000	31.6	1000	1000	31.6
6.30	1000	1000	31.6	1000	1000	31.6
6.40	1000	1000	31.6	1000	1000	31.6
6.50	1000	1000	31.6	1000	1000	31.6
6.60	1000	1000	31.6	1000	1000	31.6
6.70	1000	1000	31.6	1000	1000	31.6
6.80	1000	1000	31.6	1000	1000	31.6
6.90	1000	1000	31.6	1000	1000	31.6
7.00	1000	1000	31.6	1000	1000	31.6
7.10	1000	1000	31.6	1000	1000	31.6
7.20	1000	1000	31.6	1000	1000	31.6
7.30	1000	1000	31.6	1000	1000	31.6
7.40	1000	1000	31.6	1000	1000	31.6
7.50	1000	1000	31.6	1000	1000	31.6
7.60	1000	1000	31.6	1000	1000	31.6
7.70	1000	1000	31.6	1000	1000	31.6
7.80	1000	1000	31.6	1000	1000	31.6
7.90	1000	1000	31.6	1000	1000	31.6
8.00	1000	1000	31.6	1000	1000	31.6
8.10	1000	1000	31.6	1000	1000	31.6
8.20	1000	1000	31.6	1000	1000	31.6
8.30	1000	1000	31.6	1000	1000	31.6
8.40	1000	1000	31.6	1000	1000	31.6
8.50	1000	1000	31.6	1000	1000	31.6
8.60	1000	1000	31.6	1000	1000	31.6
8.70	1000	1000	31.6	1000	1000	31.6
8.80	1000	1000	31.6	1000	1000	31.6
8.90	1000	1000	31.6	1000	1000	31.6
9.00	1000	1000	31.6	1000	1000	31.6
9.10	1000	1000	31.6	1000	1000	31.6
9.20	1000	1000	31.6	1000	1000	31.6
9.30	1000	1000	31.6	1000	1000	31.6
9.40	1000	1000	31.6	1000	1000	31.6
9.50	1000	1000	31.6	1000	1000	31.6
9.60	1000	1000	31.6	1000	1000	31.6
9.70	1000	1000	31.6	1000	1000	31.6
9.80	1000	1000	31.6	1000	1000	31.6
9.90	1000	1000	31.6	1000	1000	31.6
10.00	1000	1000	31.6	1000	1000	31.6

std. H-3  
Standard  
DPM-98200

DATA ARE IN TABLE



cell activity

100% , H3, 1-73 (2nd count)

TABLE-4

Expt #: V79, H-3, 100% Date: Dec., 14, 2000

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony	SF
1.2	197	200	202		
2.2	73	69	82		
3.2	62	63	62		
4.2	61	60	55		
5.2	38	47	41		
6.2	49	42	37		
7.2	32	37	36		
8.2.3	220	222	211		
9.2.3	182	175	170		
10.2.3	168	185	174		

For tube:

3.3	401	411	410
4.3	323	347	326
5.3	297	283	272
6.3	296	320	283
7.3	207	217	201

7.4, 8.4, 9.4, 10.4 - too many colonies.

Exp. Dec 14, 2000, H3-cluster, 100%  
 Medium activity

Channel	Count	Rate	Efficiency	Other
1	100	100	100	
2	100	100	100	
3	100	100	100	
4	100	100	100	
5	100	100	100	
6	100	100	100	
7	100	100	100	
8	100	100	100	
9	100	100	100	
10	100	100	100	
11	100	100	100	
12	100	100	100	
13	100	100	100	
14	100	100	100	
15	100	100	100	
16	100	100	100	
17	100	100	100	
18	100	100	100	
19	100	100	100	
20	100	100	100	
21	100	100	100	
22	100	100	100	
23	100	100	100	
24	100	100	100	
25	100	100	100	
26	100	100	100	
27	100	100	100	
28	100	100	100	
29	100	100	100	
30	100	100	100	
31	100	100	100	
32	100	100	100	
33	100	100	100	
34	100	100	100	
35	100	100	100	
36	100	100	100	
37	100	100	100	
38	100	100	100	
39	100	100	100	
40	100	100	100	
41	100	100	100	
42	100	100	100	
43	100	100	100	
44	100	100	100	
45	100	100	100	
46	100	100	100	
47	100	100	100	
48	100	100	100	
49	100	100	100	
50	100	100	100	
51	100	100	100	
52	100	100	100	
53	100	100	100	
54	100	100	100	
55	100	100	100	
56	100	100	100	
57	100	100	100	
58	100	100	100	
59	100	100	100	
60	100	100	100	
61	100	100	100	
62	100	100	100	
63	100	100	100	
64	100	100	100	
65	100	100	100	
66	100	100	100	
67	100	100	100	
68	100	100	100	
69	100	100	100	
70	100	100	100	
71	100	100	100	
72	100	100	100	
73	100	100	100	
74	100	100	100	
75	100	100	100	
76	100	100	100	
77	100	100	100	
78	100	100	100	
79	100	100	100	
80	100	100	100	
81	100	100	100	
82	100	100	100	
83	100	100	100	
84	100	100	100	
85	100	100	100	
86	100	100	100	
87	100	100	100	
88	100	100	100	
89	100	100	100	
90	100	100	100	
91	100	100	100	
92	100	100	100	
93	100	100	100	
94	100	100	100	
95	100	100	100	
96	100	100	100	
97	100	100	100	
98	100	100	100	
99	100	100	100	
100	100	100	100	

H3 Standard  
 OPM-98200 - count

H-3, 0.77, 100%

Parameters

Date	12/14/00
Experiment No.	ML - Dec., 12, 2000
Investigator	R.Howell, M.Lenarczyk
Cell Line	V79
Modifier	None
Radionuclide	H-3
Half-life (days)	4500.45
Radiation Yield	1
Radiochemical	3HTdR
Manufacturer/Lot	NCN/3106-398
Original Calibration Date/Time	11/6/00 / 12:00
Present Calibration Date/Time	12/14/00 / 19:15
Fraction of Cells Labeled	1
Liquid Scintillation Cocktail	EcoLume
Volume of LSC Cocktail (ml)	6
Volume/Type Counting Vial	7/Plastic vial with cup
Model of Counter	Beckman LS5000TD
Counting Efficiency	0.65
Activity Added (Date/Time)	12/14/00 / 19:30
Cells Washed (Date/Time)	12/15/00 / 9:20
Medium Tubes Counted (Date/Time)	12/15/00 / 17:30
Cell Tubes Counted (Date/Time)	12/19/00 / 15:43
Vol. Supernatant Counted (µl)	30
Vol. Suspension Counted Cell Activity (µl)	100
Vol. Suspension Coultier (µl)	100
Coultier Manometer Volume (µl)	500
Average Coultier Background Counts	5
Coultier Calibration Parameter	400
Hemocytometer Counting (Yes or No)?	
	I-125=59.408, H-3=4500.45, Po-210=138.376, I-131=8.02
	I-125=1.47, H-3=1.0, Po-210=1.0, I-131=8.02
	Original Activity Concentration (MBq/ml) 37
	Time Elapsed Since Original Calibration (d) 38
	Present Activity Concentration (MBq/ml) 36.78
	Time Elapsed Between Add and Wash (hr) 14.00
	Time Elapsed Between Add and Count (hr) 22.00
	Time Elapsed Between Wash and Count (hr) 116.00
	Background
	Coultier 1 5
	Coultier 2 5
	Coultier 3 5

MediumActivity

Experiment: ML - Dec., 12, 2000  
 Date: 12/14/2000

Tube #	Medium count (CPM)			CPM Average	CPM corrected for control	DPM CPM(y e)	At $\mu\text{Ci/ml}$ on counting	Ao $\mu\text{Ci/ml}$ at addition	Ao $\text{kBq/ml}$ at addition
	1st	2nd	3rd						
1	13	7	6	9	0	0	0	0	0
2	11	8	8	0	0	0	0	0	0
3	2471	2187	2360	2339	2331	3585	0.0538	0.0538	1.9922
4	4722	4529	4388	4546	4538	6981	0.1048	0.1048	3.8788
5	9332	9191	9353	9292	9283	14282	0.2144	0.2145	7.9355
6	13704	14474	14177	14118	14110	21707	0.3259	0.3260	12.0611
7	18887	18214	18171	18424	18415	28331	0.4254	0.4255	15.7417
8	24124	23073	22522	23240	23231	35740	0.5366	0.5367	19.8582
9	28187	26995	26617	27266	27258	41935	0.6296	0.6297	23.3003
10	36193	34383	34943	35173	35164	54099	0.8123	0.8124	30.0591

CellSuspension

Experiment: ML - Dec., 12, 2000  
 Date: 12/14/00

Tube #	Suspension count (CPM)			CPM Average	CPM corrected for control	DPM CPM/(y e)	A <sub>i</sub> μCi/ml on counting	A <sub>o</sub> μCi/ml after uptake	A <sub>o</sub> kBq/ml after uptake
	1st	2nd	3rd						
1	9	9	11	10	0	0	0.0000	0	0.0000
2	9	9	11	0	0	0	0.0000	0	0.0000
3	698	594	736	676	666	1025	0.00462	0.00462	0.1710
4	985	959	744	896	886	1364	0.00614	0.00615	0.2274
5	2145	1849	1797	1930	1921	2955	0.01331	0.01332	0.4928
6	5609	3588	3274	4157	4147	6381	0.02874	0.02876	1.0642
7	6843	7024	6292	6720	6710	10323	0.04650	0.04653	1.7218
8	14321	11233	7680	11078	11068	17028	0.07670	0.07676	2.8401
9	10189	11626	9433	10416	10406	16010	0.07212	0.07217	2.6703
10	17452	13093	17143	15896	15886	24441	0.11009	0.11017	4.0765

CoulterSurvival

Experiment: ML - Dec., 12, 2000  
 Date/Time: #####

Tube #	Coulter count			Average Cells/ml	Hemocytometer Count in Grid				
	1st	2nd	3rd		1st	2nd	3rd	4th	
1	2714	2620	2702	2679	1069467				
2	2714	2620	2702	2679	1069467				
3	4688	4336	4734	4586	1832400				
4	3389	3356	3387	3377	1348933				
5	3674	3592	3633	3633	1451200				
6	4846	4822	4888	4852	1938800				
7	4414	4549	4327	4430	1770000				
8	4116	4010	4114	4080	1630000				
9	5151	5125	5068	5115	2043867				
10	4263	4058	4013	4111	1642533				

*1=2 since recovery for sample #1  
 was very low (~25%)*

*Real count for sample #1 was*

*1st - 1232  
 2nd - 1209  
 3rd - 1265 }  $\bar{x} = 1235 \rightarrow 0.49 \times 10^6 / ml$   
 total ~  $2 \times 10^6 / ml$   
 25%*

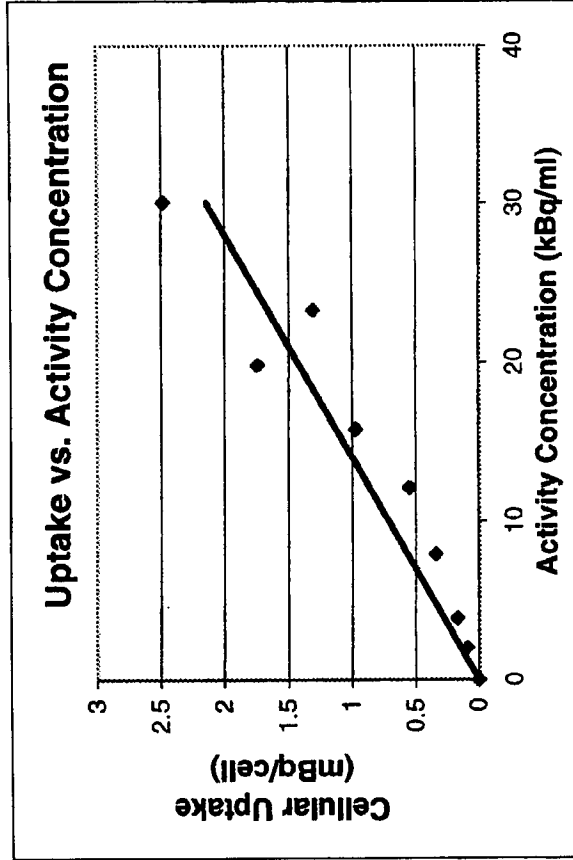
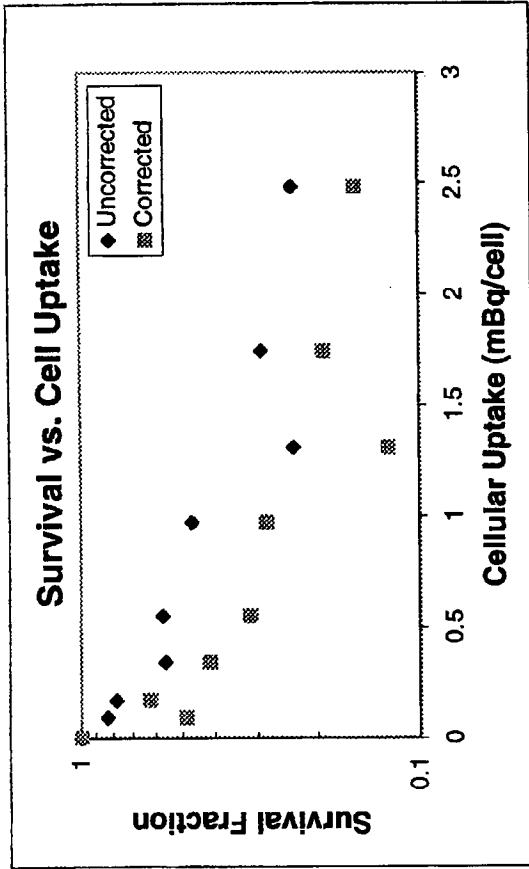
Tube #	Predicted # Cells Seeded	Actual # Cells Seeded	Colony count			Average	PE (%)	SF Uncorrected	SF Corrected
			1st	2nd	3rd				
1	200	107	73	69	82	75	69.817	1.00	1.0000
2	200	107	73	69	82				
3	200	183	62	63	62	62	34.017	0.8348	0.4872
4	200	135	61	60	55	59	43.491	0.7857	0.6229
5	200	145	38	47	41	42	28.942	0.5625	0.4145
6	200	194	49	42	37	43	22.007	0.5714	0.3152
7	200	177	32	37	36	35	19.774	0.4688	0.2832
8	2000	1630	220	222	211	218	13.354	0.2915	0.1913
9	2000	2044	182	175	170	176	8.595	0.2353	0.1231
10	2000	1643	168	185	174	176	10.695	0.2353	0.1532



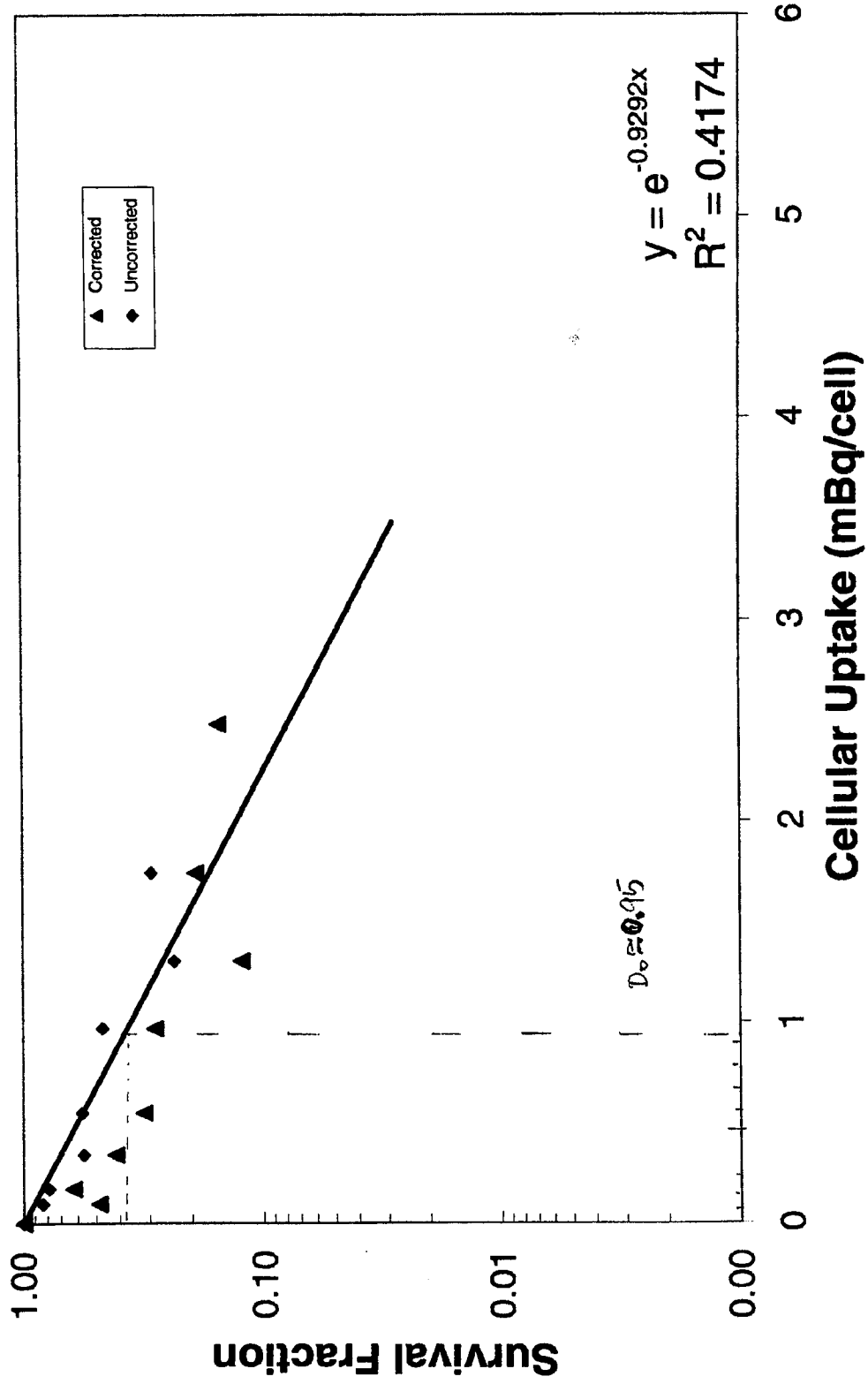
Summary

Experiment: 12/14/00  
 Date/Time:

Tube #	Activity Conc. (kBq/ml)	Activity/Cell (mBq/cell)	Survival Uncorrected	Survival Corrected
1	0.000	0	1.0000	1.0000
2	0.000	0	0.8348	0.4872
3	1.992	0.093	0.7857	0.6229
4	3.879	0.169	0.5625	0.4145
5	7.935	0.340	0.5714	0.3152
6	12.061	0.549	0.4688	0.2832
7	15.742	0.973	0.2915	0.1913
8	19.858	1.742	0.2353	0.1231
9	23.300	3.106	0.2353	0.1231
10	30.059	4.2482	0.2353	0.1532



# Survival vs. Cell Uptake



Parameters

Date	12/14/00
Experiment No.	ML - Dec., 12, 2000
Investigator	R. Howell, M. Lenarczyk
Cell Line	V79
Modifier	None
Radionuclide	H-3
Half-life (days)	4500.45
Radiation Yield	1
Radiochemical	3HTdR
Manufacturer/Lot	NCN/3106-398
Original Calibration Date/Time	11/6/00 / 12:00
Present Calibration Date/Time	12/14/00 / 19:15
Fraction of Cells Labeled	1
Liquid Scintillation Cocktail	EcoLume
Volume of LSC Cocktail (ml)	6
Volume/Type Counting Vial	7/Plastic vial with cup
Model of Counter	Beckman LS5000TD
Counting Efficiency	0.65
Activity Added (Date/Time)	12/14/00 / 19:30
Cells Washed (Date/Time)	12/15/00 / 9:20
Medium Tubes Counted (Date/Time)	12/15/00 / 17:30
Cell Tubes Counted (Date/Time)	12/19/00 / 15:43
Vol. Supernatant Counted (µl)	30
Vol. Suspension Counted Cell Activity (µl)	100
Vol. Suspension Coultter (µl)	100
Coultter Manometer Volume (µl)	500
Average Coultter Background Counts	5
Coultter Calibration Parameter	400
Hemocytometer Counting (Yes or No)?	

I-125=59.408, H-3=4500.45, Po-210=138.376, I-131=8.02  
 I-125=1.47, H-3=1.0, Po-210=1.0, I-131=8.02

Original Activity Concentration (MBq/ml) 37  
 Time Elapsed Since Original Calibration (d) 38  
 Present Activity Concentration (MBq/ml) 36.78

Time Elapsed Between Add and Wash (hr) 14.00  
 Time Elapsed Between Add and Count (hr) 22.00  
 Time Elapsed Between Wash and Count (hr) 89.50 — 116

Background			
Coultter 1	5	5	5
Coultter 2			
Coultter 3			

MediumActivity

Change  
↓

Experiment: ML - Dec., 12, 2000  
Date: 12/14/2000

Tube #	Medium count (CPM)			CPM Average	CPM corrected for control	DPM CPM/(y e)	At $\mu\text{Ci/ml}$ on counting	AO $\mu\text{Ci/ml}$ at addition	AO kBq/ml at addition
	1st	2nd	3rd						
1	10	7	8	8	0	0	0	0	0
2	6	6	10	0	0	0	0	0	0
3	2432	2194	2393	2340	2332	3587	0.0539	0.0539	1.9933
4	4514	4370	4211	4365	4357	6703	0.1007	0.1007	3.7246
5	9177	8996	9145	9106	9098	13997	0.2102	0.2102	7.7773
6	13339	13585	13111	13345	13337	20519	0.3081	0.3081	11.4009
7	17777	17422	17760	17653	17645	27146	0.4076	0.4077	15.0835
8	22469	22190	22056	22238	22231	34201	0.5135	0.5136	19.0031
9	26395	26263	23176	25278	25270	38877	0.5837	0.5838	21.6015
10	33390	30922	30871	31728	31720	48800	0.7327	0.7328	27.1148

CellSuspension

Experiment: ML - Dec., 12, 2000  
 Date: 12/14/00

Tube #	Suspension count (CPM)			CPM Average	CPM corrected for control	DPM CPM/(y e)	A <sub>i</sub> μCi/ml on counting	A <sub>o</sub> μCi/ml after uptake	A <sub>o</sub> kBq/ml after uptake
	1st	2nd	3rd						
1				10	0	0	0.00000	0	0.0000
2	9	9	11		0	0	0.00000	0	0.0000
3	698	594	736	676	666	1025	0.00462	0.00462	0.1710
4	985	959	744	896	886	1364	0.00614	0.00615	0.2274
5	2145	1849	1797	1930	1921	2955	0.01331	0.01332	0.4928
6	5609	3588	3274	4157	4147	6381	0.02874	0.02876	1.0640
7	6843	7024	6292	6720	6710	10323	0.04650	0.04653	1.7215
8	14321	11233	7680	11078	11068	17028	0.07670	0.07675	2.8397
9	10189	11626	9433	10416	10406	16010	0.07212	0.07216	2.6698
10	17452	13093	17143	15896	15886	24441	0.11009	0.11016	4.0758

CoulterSurvival

Experiment: ML - Dec., 12, 2000  
 Date/Time: #####

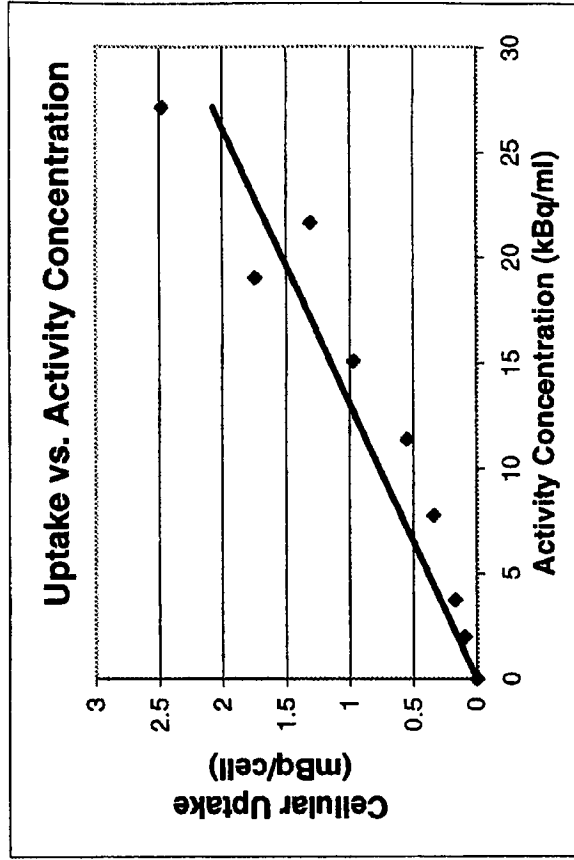
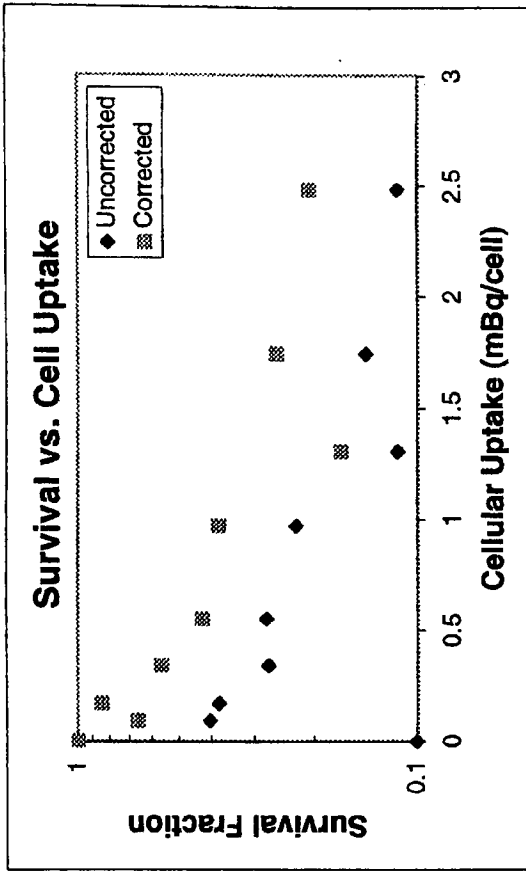
Tube #	Coulter count			Average Cells/ml	Hemocytometer Count in Grid			
	1st	2nd	3rd		1st	2nd	3rd	4th
1	1232	1209	1265	1235	492	133		
2	2714	2620	2702	2679	1069	467		
3	4688	4336	4734	4586	183	2400		
4	3389	3356	3387	3377	134	8933		
5	3674	3592	3633	3633	145	1200		
6	4846	4822	4888	4852	193	8800		
7	4414	4549	4327	4430	177	0000		
8	4116	4010	4114	4080	163	0000		
9	5151	5125	5068	5115	204	3867		
10	4263	4058	4013	4111	164	2533		

Tube #	Predicted # Cells Seeded	Actual # Cells Seeded	Colony count			Average	PE (%)	SF Uncorrected	SF Corrected
			1st	2nd	3rd				
1	2000	492	297	200	202	154	51.357	0.10	1.0000
2	200	107	73	69	82				
3	200	183	62	63	62	62	34.017	0.4052	0.6624
4	200	135	61	60	55	59	43.491	0.3814	0.8468
5	200	145	38	47	41	42	28.942	0.2730	0.5635
6	200	194	49	42	37	43	22.007	0.2774	0.4285
7	200	177	32	37	36	35	19.774	0.2275	0.3850
8	2000	1630	220	222	211	218	13.354	0.1415	0.2600
9	2000	2044	182	175	170	176	8.595	0.1142	0.1674
10	2000	1643	168	185	174	176	10.695	0.1142	0.2082

Summary

Experiment: 12/14/00  
 Date/Time:

Tube #	Activity Conc. (kBq/ml)	Activity/Cell (mBq/cell)	Survival Uncorrected	Survival Corrected
1	0.000	0.000	0.1000	1.0000
2	0.000	0.000	0.4052	0.6624
3	1.993	0.093	0.3814	0.8468
4	3.725	0.169	0.2730	0.5635
5	7.777	0.340	0.2774	0.4285
6	11.401	0.549	0.2275	0.3850
7	15.083	0.973	0.1415	0.2600
8	19.003	1.742	0.1142	0.1674
9	21.601	1.306	0.1142	0.1674
10	27.115	2.481	0.1142	0.2082



# Survival vs. Cell Uptake

