V79 COLONY FORMING ASSAY

Experiment Name : ^{131}IUdR toxicity (Crossed dose from 10% 10X labeled cells); **Exp. # :** 1;
Investigator: A. Bishayee **Date:** 08/13/98

1. Set the shaker at 37°C incubator with 5% CO_2, set the Coulter Counter, wash cells (from one 75 cm^2 flusk, subcultured 1:2, 24h before) with PBS, trypsinize cells, resuspend in 7 ml MEMB for each flusk, pool, vortex, pass five times through 3 cc syringe with 21 gauge needle, perform cell count by transfering 100 ul in Coulter cup containing 20 ml isotone (Coulter balanced electrolyte solution)
2. Dilute to ~400,000 cells/ml in MEMB (final volume 11 ml) [Actual count : 470,000 cells/ml)
3. Transfer 1 ml of cell suspension into ten 6 ml tubes (Falcon plastic test tube, 12x175 mm) labeled 1-10 both on cap and wall
4. Shake the tubes for 3-4 h at 37°C, 5% CO_2 **Date/Time:** 08/13/98; 3-30 p.m.
5. Prepare MEMB containing radioactivity in hood
   83 µl ^{131}IUdR (prepared on 08/13/98) +0.5ml MEMB
6. After 3-4 h, remove test tubes from shaker and add MEMB with or without radioactivity according to Table below. **Date/Time:** 08/13/98; 7-30 p.m.

Tube #	^{131}IUdR uCi/ml	Cells in MEMB (ml)	MEMB (ul)	MEMB+ ^{131}IUdR [60 uCi/ml] (ul)
1	0	1.0	200	0
2	0	1.0	200	0
3	0.1	1.0	198	2
4	0.25	1.0	195	5
5	0.5	1.0	190	10
6	1	1.0	180	20
7	2	1.0	160	40
8	5	1.0	100	100
9	7.5	1.0	50	150
10	10	1.0	0	200

7. Return test tubes to roller for 12 h. **Date/Time:** 08/13/98; 7-45 p.m.

1.2
2.2
3.2, 3.3
4.2, 4.3
5.2, 5.3
6.2, 6.3
7.2, 7.3
8.2, 8.3, 8.4
9.3, 9.4
10.3, 10.4

12x75 Fisher Brand

8. While test tubes are rolling label 40 (4x10) 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:** 8/14/98; 9-00 a.m.
10. **During centrifugation, obtain ice**
12. Remove buckets from centrifuge and carefully remove 100 µl of supernatant and place in prelabeled gamma-tube.
13. Decant supernatant, click tubes, vortex, resuspend in 3 ml wash MEMA
14. Centrifuge tubes for 10 min at 2000 rpm, 4°C
15. Decant supernatant, click tubes, vortex, resuspend in 3 ml wash MEMA
16. Centrifuge tubes for 10 min at 2000 rpm, 4°C
17. Decant supernatant, click tubes, vortex, resuspend in 3 ml MEMA
18. Centrifuge tubes for 10 min at 2000 rpm, 4°C
19. Decant the supernatant, add 1 ml cell suspension containing 3,600,000 cells/ml to each tube
20. Add 2 ml of MEMA in each tube
21. Centrifuge tubes for 10 min at 4°C
22. Decant supernatant, click tubes, resuspend in 200 ul ice cold MEMA, transfer the cell suspension in polypropylene microcentrifuge tubes with attached caps (Helena Plastics, 400 ul) using pipet tips
23. Again add 200 ul ice cold MEMA, resuspend and transfer the cell suspensions in the same polypropylene microcentrifuge tubes (Total volume ~400 ul)
24. Centrifuge tubes for 5 min at 1000 rpm, 4°C
25. Transfer tubes at 10°C for 72 h. **Date/Time:** 8/14/98) 11-45 a.m.
26. Transfer 10 ul supernatant in three sets of tubes containing small pieces of tissue paper from 100 ul supernatant removed earlier and count them for radioactivity

    Date/Time: 8/17/98; 2-00 p.m.
27. After 72 h, carefully remove the supernatant from the top, resuspend pellet in 200 ul wash MEMA and 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 wash MEMA by using pasteur pipet

    Date/Time: 8/17/98; 9-30 a.m.
28. Again add 200 ul wash MEMA in microcentrifuge tubes, resuspend and transfer the cell suspensions in 12 ml tubes
29. Centrifuge the tubes for 10 min at 2000 rpm, 4°C (***precooled centrifuge***)
30. Labeling and preparation of dilution tubes and colony dishes
    - load 69 60 mm petri dishes with 4 ml MEMA
    - load 36 T-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.
31. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA

32. Centrifuge tubes for 10 min at 2000 rpm, 4°C
33. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
34. Centrifuge tubes for 10 min at 2000 rpm, 4°C
35. Decant supernatant, click tubes, vortex, resuspend in 2 ml wash MEMA, pass five times through 3 cc syringe with 21 gauge needle
36. Determine cell concentration by transfering 100 µl to Coulter cup
37. Vortex tube, transfer 0.5 ml into dilution tube X.5, vortex tube X.5 and transfer 0.5 ml to tube X.4, vortex tube X.4 and transfer 0.5 ml to tube X.3 and vortex tube X.3 and transfer 0.5 ml to tube X.2. Keep tubes on ice.
38. 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.
39. Transfer 300 µl of cell suspension (in triplicate) to gamma tubes for each tube
40. Incubate petridishes for 1 week
41. Count gamma tubes for radioactivity **Date/Time :** 08/17/98; 3-30 P.M.
42. After 1 week, wash colonies 3 times with normal (1X) saline,and 2 times with methanol. Stain colonies with 0.0 5% crystal violet
43. Count colonies. There must be between 25 and 250 colonies for the flask to be a valid data point.

Expt #1 08/13/98

Initial Cell count = 8788, 8710, 8593
Avg. Cell count = 8697
Cell Conc. = 8697 X 400
= 3,478,800 cells/ml

For dilution,
Vol. of cell suspension required = $\frac{4400000}{3,478,800}$
= 1.26

Take 1.26 ml of cell suspension + 9.74 ml MEMB = 11 ml

After dilution,

Final Cell count = 1175, 1159, 1191
Avg. cell count = 1175
Cell Conc. = 1175 X 400
= 470,000 cells/ml

Expt #1

08/13/98

Prepare ^{131}I/UdR in MEMB (60 μCi/ml)

Prepare 550 ml of 60 μCi/ml ⇒ 33 μCi required

Stock : 08/13/98 ~~29 μCi/ml~~ 0.398 μCi/ml

$$\text{Stock required} = \frac{33}{0.398} = 82.9 \text{ ml}.$$

① Take 83 ml Stock
② Keep it in Room temp for ~2-3 h
③ Add 550 ml MEMB

Expt. #1

08/14/98

Initial Cell Count = 12137, 12089, 12167

Avg. Cell Count = 12131

Cell Conc. = 4852400

We need 11 ml of 3,600,000 cells/ml = 39,600,000 cells

For dilution,

vol. of original cell suspension required = $\frac{39,600,000}{4852400}$

= 8.1

Take 8.1 ml cells + 2.9 ml MEMB = 11 ml

After dilution,

Final Cell Count = 8415, 8409, 8437

Avg. Cell Count = 8420

Cell Conc. = 3,368,133 cells/ml

Efficiency = 0.18
Yield = 0.82

TABLE-1

Expt. # : 1 **Date/Time :** 08/17/98; 2-00p.m.

	Tube #	Medium count for 10 ul (cpm)	Avg. cpm	dpm [cpm/0.7056] 0.147	µCi/ml (A_t) on counting [dpm/22200]	µCi/ml (A_o) on addition [$A_t/e^{-\lambda t}$]
0	1	2, 1, 2				
0	2	3, 1, 2				
0.1	3	148, 122, 182	150.6	1024.9	0.0461	0.0635
0.25	4	439, 433, 459	443.6	3018.14	0.1359	0.1872
0.5	5	790, 837, 817	814.6	5541.9	0.2496	0.3438
1	6	1519, 1576, 1541	1545.3	10512.4	0.4735	0.6522
2	7	2819, 2913, 3212	2981.3	20281.1	0.09135	1.2583
5	8	8390, 8948, 8563	8633.6	58732.1	2.6456	3.644
7.5	9	13165, 14080, 13929	13724.6	93365.0	4.2056	5.7928
10	10	18693, 18971, 19486	19116.6	130045.3	5.8578	8.0687

08/13/98; 7-45pm

$e^{-\lambda t}$

$= e^{-\frac{0.693}{193.2} \times 89.25}$

$= 0.7260$

72h + 17h + 5.25h

~~89.25~~

89.25h

TABLE-2

Expt. # : 1 **Date/Time :** 08/17/98; 3-30 p.m.

Tube #	Radioactivity for 300 ul cell suspension (cpm)	Avg. cpm	dpm [cpm/0.~~7056~~ 0.147]	µ Ci/ml (A_t) on counting [dpm/666000]	µ Ci/ml (A_o) after 12 h incubation [$A_t/e^{-\lambda t}$]
1	1, 2, 1				
2	2, 1, 2				
3	834, 949, 837	873.3	5941.0	0.0089	0.0118
4	2780, 2748, 2571	2699.6	18365.0	0.0027	0.0365
5	5381, 5672, 5311	5454.6	37106.5	0.0557	0.0738
6	10809, 10847, 10645	10767	73244.8	0.1099	0.1457
7	19671, 19415, 19399	13595	924829	0.1388	0.1840
8	40231, 40912, 40040	40394.3	274791	0.4125	0.5468
9	47082, 47633, 47996	47570.3	~~3236077 / 255586~~	0.4857	0.6439
10	57246, 58161, 58001	578026	393215	0.5904	0.7825

$e^{-\lambda t}$

$= e^{-\frac{0.693 \times 78.5}{193.2}}$

$= 0.7545$

8/14/98 — 9:00 a.m.

72h + 6.5

$= 78.5$

0.3763

TABLE-3

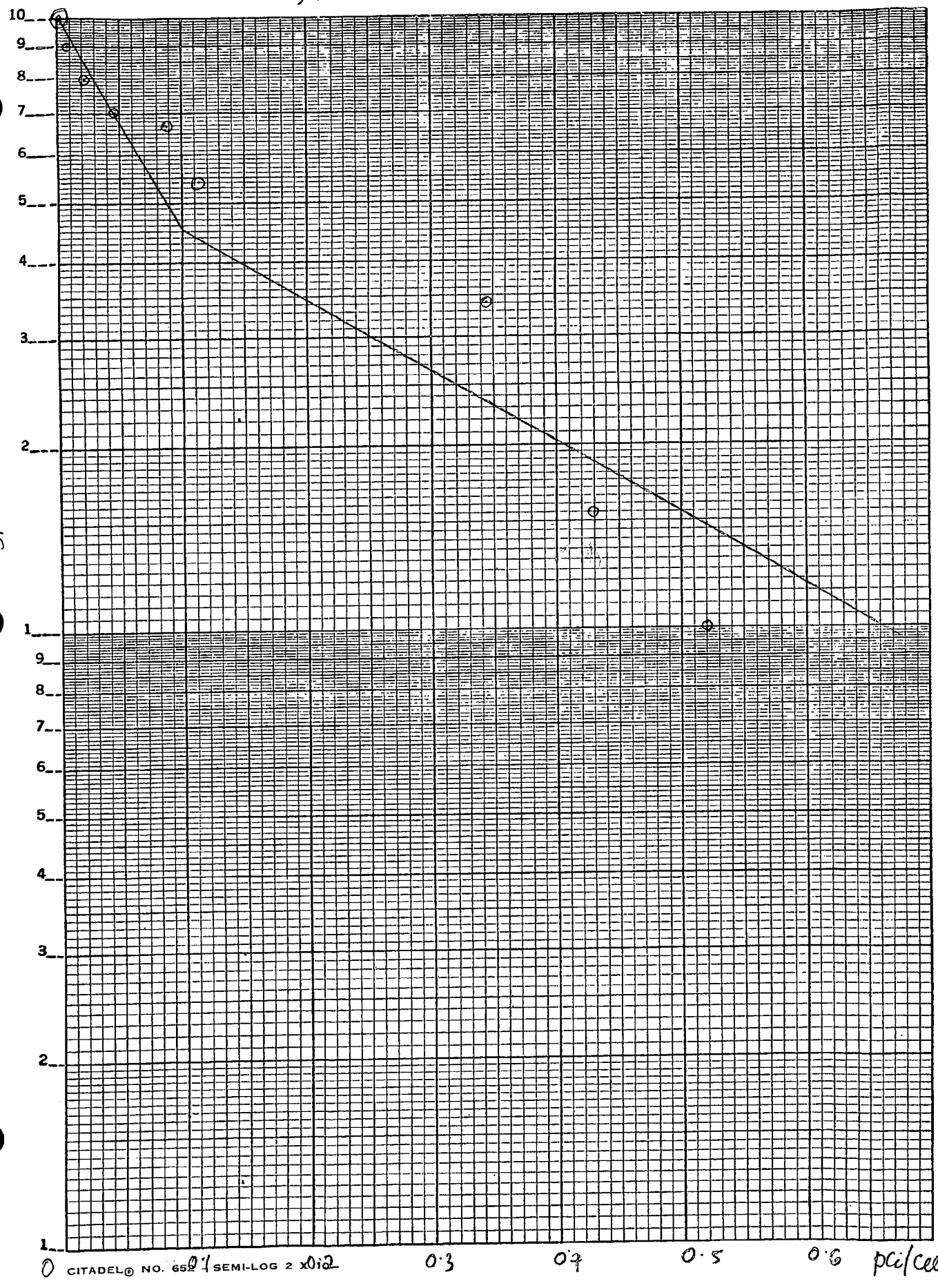
Expt. # : 1 **Date/Time :** 08/17/98; 10-30 a.m.

µCi/ml	Tube #	Coulter count for 100 ul cell suspension MS = 50 µl	Avg. count	Cells/ml [Avg. count x 4000]	pCi/cell [uCi/ml x 10^6 / Cells/ml]
	1	443, 441, 410	431.3	1725333.3	
	2	443, 432, 408	427.6	1710666.6	
0.1	3	371, 374, 370	371.6	1486666	0.00793
0.25	4	428, 452, 409	429.6	1718666	0.02123
0.5	5	386, 393, 400	393	1572000	0.04694
1	6	428, 411, 404	414.3	1657333	0.08791
2	7	418, 403, 393	404.6	1618666	0.1136
5	8	393, 415, 379	395.6	1582666	0.3454
7.5	9	378, 370, 375	374.3	1497333	0.4300
10	10	345, 388, 393	375.3	1501333	0.5212

TABLE-4

Expt # : 1 **Date :** 8/24/98

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony	SF
1·2	134	131	142	140·16	
2·2	153	142	139		
3·2	125	130	127	127·33	0·9084
4·2	105	120	108	111	0·7919
5·2	108	101	93	100·66	0·7181
6·2	96	86	100	94	0·6706
7·2	75	74	79	76	0·5422
8·2	44	49	52	48·33	0·3448
9·3	227	240	238	23·5	0·1676
10·3	150	159	131	14·66	0·1046


Expt. #1
10% labeling
10
9
8
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SF
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