V79 COLONY FORMING ASSAY

Experiment Name : ^{131}IUdR + 10% DMSO; **Exp. # :** 1; **Investigator:** A. Bishayee
Date: 08/17/98

1. Set the rocker-roller at 37°C incubator, set the Coulter Counter, wash cells (from 75 cm^2 flusk, subcultured 1:2, 24h before) with PBS, trypsinize cells, resuspend in 7 ml MEMB, 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 ~4,00,000 cells/ml in MEMB (final volume 11 ml) [Actual count : 406,933 cells/ml)
3. Transfer 1 ml of cell suspension into ten 12 ml tubes (Falcon plastic test tube, 17x100 mm) labeled 1-10 both on cap and wall
4. Roll the tubes for 3-4 h at 37°C, 5% CO_2 **Date/Time:** 08/17/98; 4:30 p.m.
5. Prepare MEMB containing radioactivity in hood
   22 µl ^{131}IUdR (prepared on 8/13) + 5 ml MEMB
6. After 3-4 h, remove test tubes from roller and add MEMB with or without radioactivity according to Table below. **Date/Time:** 08/17/98; 8-30 p.m.

Tube #	^{131}IUdR uCi/ml	Cells in MEMB (ml)	MEMB (ml)	MEMB + ^{131}IUdR [1.2 uCi/ml] (ml)	40 % DMSO in MEMA (ml)	MEMA (ml)	
1	0	1.0	1.0	0	2.0	0	C(-+)
2	0	1.0	1.0	0	2.0	0	
3	0.2	1.0	0.67	0.33	2.0	0	C(++)
4	0.4	1.0	0.33	0.67	2.0	0	
5	0.6	1.0	0	1.0	2.0	0	
6	0	1.0	1.0	0	0	2.0	C(--)
7	0	1.0	1.0	0	0	2.0	
8	0.2	1.0	0.67	0.33	0	2.0	C(+-)
9	0.4	1.0	0.33	0.67	0	2.0	
10	0.6	1.0	0	1.0	0	2.0	

7. Return test tubes to roller for 12 h. increase the elevation angle of the roller
Date/Time: 08/17/98; 8-45 p.m.

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:** 08/18/98; 9-30 a.m.
10. During centrifugation, **move roller to 10°C and obtain ice**
11. Prepare 11 ml of 10 % DMSO in MEMA, put on 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 10 ml wash MEMA
14. Centrifuge tubes for 10 min at 2000 rpm, 4°C
15. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
16. Centrifuge tubes for 10 min at 2000 rpm, 4°C
17. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
18. Centrifuge tubes for 10 min at 2000 rpm, 4°C
19. Decant supernatant, click tubes, vortex, resuspend in 2 ml ice cold MEMA containing 0 or 5 % DMSO as per Table. Keep on ice!
20. Transfer tubes to roller at 10°C for 72 h. **Date/Time:** 08/18/98; 11-30 a.m.
21. 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: 08/18/98; 12-45 p.m.

21. After 72 h, remove tubes and **place on ice**, add 8 ml ***ice cold*** wash MEMA.

    Date/Time: 08/18/98; 12-00 noon
22. Centrifuge tubes for 10 min at 2000 rpm, 4°C ***(precooled centrifuge)***
23. Labeling and preparation of dilution tubes and colony dishes
    - load 57 60 mm petri dishes with 4 ml MEMA
    - load 30 T-tubes with 4.5 ml MEMA and label them 1.2, 1.3, 1.4, 2.2, 2.3, 2.4, X.2, X.3, X.4, etc.
24. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
25. Centrifuge tubes for 10 min at 2000 rpm, 4°C
26. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
27. Centrifuge tubes for 10 min at 2000 rpm, 4°C
28. Decant supernatant, click tubes, vortex, resuspend in 2 ml wash MEMA, pass five times through 3 cc syringe with 21 gauge needle
29. Determine cell concentration by transfering 100 µl to Coulter cup
30. Vortex tube, 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.
31. 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.

32. Transfer 300 µl of cell suspension (in triplicate) to gamma tubes for each tube
33. Incubate petridishes for 1 week
34. Count gamma tubes for radioactivity **Date/Time :** 08/21/98; 2-00 p.m
35. After 1 week, wash colonies 3 times with normal (1X) saline, and 2 times with methanol. Stain colonies with crystal violet
36. Count colonies (50 or more cells). There must be between 25 and 250 colonies for the flask to be a valid data point.

Expt #1 08/17/98

Initial cell count = 8371, 8432, 8337
Avg. cell count = 8380
Cell conc. = 3352000 cells/ml

For dilution,
vol. of cell suspension required = $\frac{4400000}{3352000}$
= 1.31

Take 1.3 ml cells + 9.7 ml HEMB = 11ml

After dilution,

Final count = 1042, 984, 1026
Avg. count = 1017.3
Cell conc. = 406,933 cells/ml

Expt # 1 08/17/98

Prepare 5 ml of 1.2 μCi/ml ^{131}IUdR in MEMB
= 6 μCi required

Stock

on 08/13/98 at 2-p.m = 0.398 μCi/μl

$A_t = A_0 \times e^{-\lambda t}$ 08/17/98 at 8 pm = 0.276 μCi/μl.

$= 0.398 \times e^{-\lambda t}$ $24 \times 4 + 6$

$= 0.398 \times e^{\frac{-0.693 \times 102}{193.2}} = 102h$

$= 0.398 \times 0.6935$

$= 0.276$

Stock required $= \frac{6}{0.276} = 21.7$ μl.

① Take ~22 μl Stock ^{131}IUdR
② After 2-3h, add 5 ml MEMB

TABLE-1

Expt. # : 1 **Date/Time :** 08/18/98 ; 12-45 pm.

Tube #	Medium count for 10 ul (cpm)	Avg. cpm	dpm [cpm/0.142]	µCi/ml (A_t) on counting [dpm/22200]	µCi/ml (A_o) on addition [$A_t/e^{-\lambda t}$]
1	1, 0, 1				
2	2, 1, 1				
3	662, 647, 639	649·3	4572·7	0·2059	0·2181
4	1369, 1302, 1349	1340	9436·6	0·4250	0·4501
5	2156, 2112, 2089	2119	14922·5	0·6721	0·7118
6	2, 1, 2				
7	2, 0, 1				
8	647, 664, 622	644·3	4537·5	0·2045	0·2164
9	1209, 1234, 1222	1221·6	8603·2	0·3875	0·4104
10	2038, 2199, 2012	2083	14669·0	0·6607	0·6999

08/17/98; 8-45 pm

12n + 4w

16w

$e^{-\lambda t}$

$= e^{-\frac{0.693 \times 16}{193.2}}$

$= e^{-0.057}$

$= 0.9442$

TABLE-2

Expt. # : 1 **Date/Time :** 08/21/98; 2-00 p.m.

Tube #	Radioactivity for 300 ul cell suspension (cpm)	Avg. cpm	dpm [cpm/0.142]	µCi/ml (A_t) on counting [dpm/666000]	µCi/ml (A_o) after 12 h incubation [$A_t/e^{-\lambda t}$]
1	1, 2, 2				
2	1, 1, 1				
3	3347, 3217, 3299	3287	23152.5	0.0347	0.0457
4	5619, 5729, 5712	5686	40046.9	0.0601	0.0791
5	8011, 8099, 7967	8025.6	56518.7	0.0848	0.1116
6	0, 2, 1				
7	0, 0, 1				
8	3445, 3553, 3323	3440.3	24227.6	0.0363	0.0478
9	6003, 6093, 6112	6069	42749.7	0.0641	0.0844
10	8312, 8301, 8297	8303.3	58474.1	0.0877	0.1155

08/18/98; 9-30 a.m

$e^{-\lambda t}$

$= e^{-\frac{0.693 \times 76.5}{193.2}}$

$= 0.76002$

$72h + 4.5h$

$= 76.5$

TABLE-3

Expt. # : (

Date/Time : 08/21/98; 12-00

Tube #	Coulter count for 100 ul cell suspension	Avg. count	Cells/ml [Avg. count x 400]	pCi/cell [uCi/cell x10^6 Cells/ml]
1	514, 536, 490	513·3	205333	
2	626, 606, 612	614·6	245866	
3	678, 634, 652	654·6	261866	0·1745
4	505, 517, 482	501·3	200533	0·3944
5	511, 492, 482	495	198000	0·5636
6	620, 602, 614	612	244800	
7	588, 578, 569	578·3	231333	
8	556, 536, 521	537·6	215066	0·2222
9	456, 478, 477	470·3	188133·3	0·4486
10	461, 452, 442	451·6	180666	0·6392

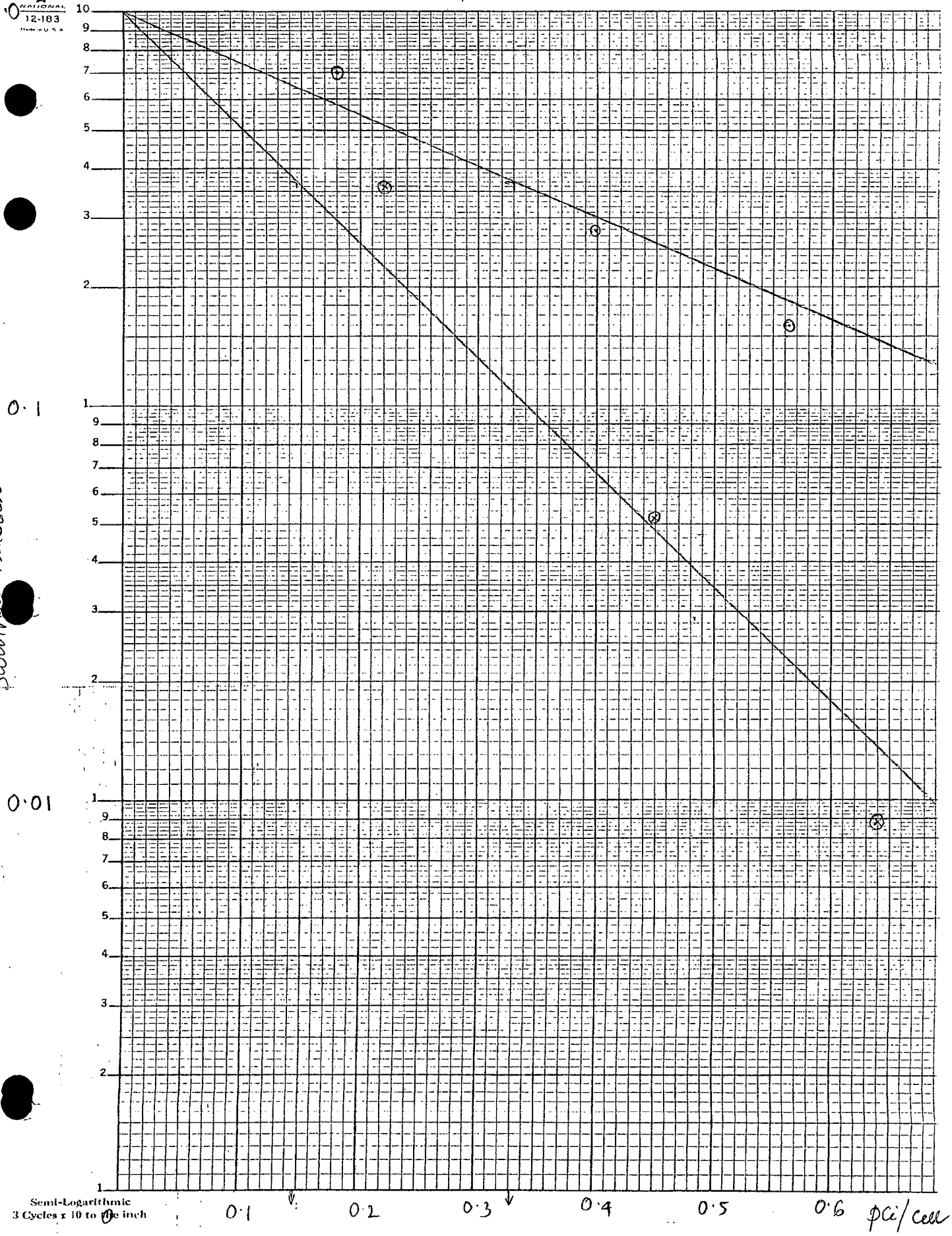
TABLE-4

Expt. #: 1 Date: 08/28/98

Colony Counts and Survival Fraction

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony	SF
1·2	101	92	93	} 93.16	
2·2	89	95	99		
3·2	60	66	73	66·33	0·7119
4·2	24	28	25	25·66	0·2754
5 3	157	148	165	15 66	0·1681
6·2	110	108	102	} 109.83	
7·2	120	112	107		
8·2	40	51	29	40	0·3641
9·3	50	58	63	5·7	0·0518
10·4	100	114	82	0·98	0·0089

Expt #1 131I UdR + 10% DMSO DMF=2.26



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