V79 COLONY FORMING ASSAY

Experiment Name : ^{131}IUdR + 10% DMSO; **Exp. # :** 2; **Investigator:** A. Bishayee
Date: 08/20/98

1. Set the rocker-roller at 37^{0}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 : 416,533 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:** 08/20/98; 4-30 P.M.
5. Prepare MEMB containing radioactivity in hood

   28 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:** 08/20/98; 7-45 PM

Tube #	^{131}IUdR uCi/ml	Cells in MEMB (ml)	MEMB (ml)	MEMB+ ^{131}IUdR [1.2 uCi/ml] (ml)	10 % DMSO in MEMA (ml)	MEMA (ml)	
1	0	1.0	1.0	0	2.0	0	
2	0	1.0	1.0	0	2.0	0	
3	0.2	1.0	0.67	0.33	2.0	0	
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	
7	0	1.0	1.0	0	0	2.0	
8	0.2	1.O	0.67	0.33	0	2.0	
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: 08/20/98; 8-00 P.M.

1·2

2·2

3·2, ~~3·3~~

4·2, 4·3

5·2, 5·3

6·2

7·2

8·2

9·2 9·3

10·3, 10·4

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:** 08/21/98; 9-00 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:** 08/21/98; 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: 08/21/98; 1-00 p.m.

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

    Date/Time: 08/24/98; 11-00 a.m.

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 :** 08/24/98; 7-00 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. # 2

08/20/98

Initial cell count = 7551, 7715, 7633
Avg. cell count = 7633
Cell conc. = 7633 × 400
= 3053200 cells/ml

For dilution,

Vol required = $\frac{4400000}{3053200}$ ml
= 1.44 ml

Take 1.4 ml cell suspension + 9.6 ml MEM8 = 11 ml

After dilution,

Final cell count = 1119, 1012, 993
Avg. cell count = 1041
Cell conc. = 1041 × 400
= 416,533 cells/ml

Expt. #2

08/20/98

Preparation of ^{131}IUdR in MEMB:

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

Stock

on 08/13/98 at 2-pm = 0.398 μCi/μl

on 08/20/98 at 8 p.m = **0.2132** μCi/μl.

7 days + ~~0.25~~ 6 = 174 h

$A_t = A_0 \times e^{-\lambda t}$

$= 0.398 \times e^{-\frac{0.693 \times 174}{193.2}}$

$= 0.398 \times 0.5357$

$= 0.2132$ μCi/μl

Stock required $= \frac{6}{0.2132} = 28.1$ μl

① Take 28 μl Stock ^{131}IUdR

② After 3-4 h, add 5 ml MEMB

TABLE-1

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

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	4, 2, 1				
2	1, 2, 1				
3	498, 515, 511	508	3577.4	0.1611	0.1712
4	989, 978, 1071	1012.6	7131.4	0.3212	0.3414
5	1474, 1520, 1547	1513.6	10659.6	0.4801	0.5102
6	2, 1, 1				
7	1, 0, 1				
8	428, 445, 533	468.6	3300.4	0.1486	0.1580
9	944, 988, 1018	983.3	6924.8	0.3119	0.3315
10	1324, 1495, 1485	1434.6	10103.2	0.4551	0.4837

08/20/98, 8-00 p.m.

12h + 5h = 17h

$e^{-\lambda t}$

$= e^{-\frac{0.693 \times 17}{19.2}}$

$= 0.9408$

TABLE-2

Expt. # : 2 **Date/Time :** 08/24/98 ; 7-00 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, 0				
2	1, 1, 0				
3	3002, 3022, 3049	3024.5	21299.8	0.0319	0.0429
4	5360, 5506, 5325	5397	38007	0.057	0.0765
5	7805, 7796, 7624	7741.6	54518.7	0.0818	0.1098
6	2, 1, 2				
7	1, 1, 0				
8	3081, 3107, 2948	3045.3	21446	0.0322	0.0432
9	5932, 5964, 5868	5921	41699.5	0.0626	0.0840
10	8018, 8269, 8303	8196.6	57723	0.0866	0.1163

$e^{-\lambda t}$

$= e^{-\frac{0.693 \times 82}{193.2}}$

$= 0.7451$

08/21/98 ; 9-00 a.m

72h + 10h

= 82h

TABLE-3

Expt. # : 2 **Date/Time :** 08/24/98; 1-15 p.m

Tube #	Coulter count for 100 ul cell suspension	Avg. count	Cells/ml [Avg. count x 400]	pCi/cell [uCi/cell x 10^6 Cells/ml]
1	655, 615, 645	638.3	255333	
2	639, 673, 656	656	262400	
3	776, 762, 786	774.6	309866	0.1384
4	~~642~~, 556, 551, 518	541.6	216666	0.3530
5	585, 540, 540	555	222000	0.4945
6	568, 529, 527	541	216533	
7	~~579~~, ~~663~~, 734, 756, 712	734	293600	
8	573, 544, 546	554.3	221733	0.1948
9	569, 546, 529	548	219200	0.3832
10	~~643~~, 552, ~~546~~, 509	~~[illegible]~~	~~[illegible]~~	~~0.54827~~
		535.6	214266	0.5427

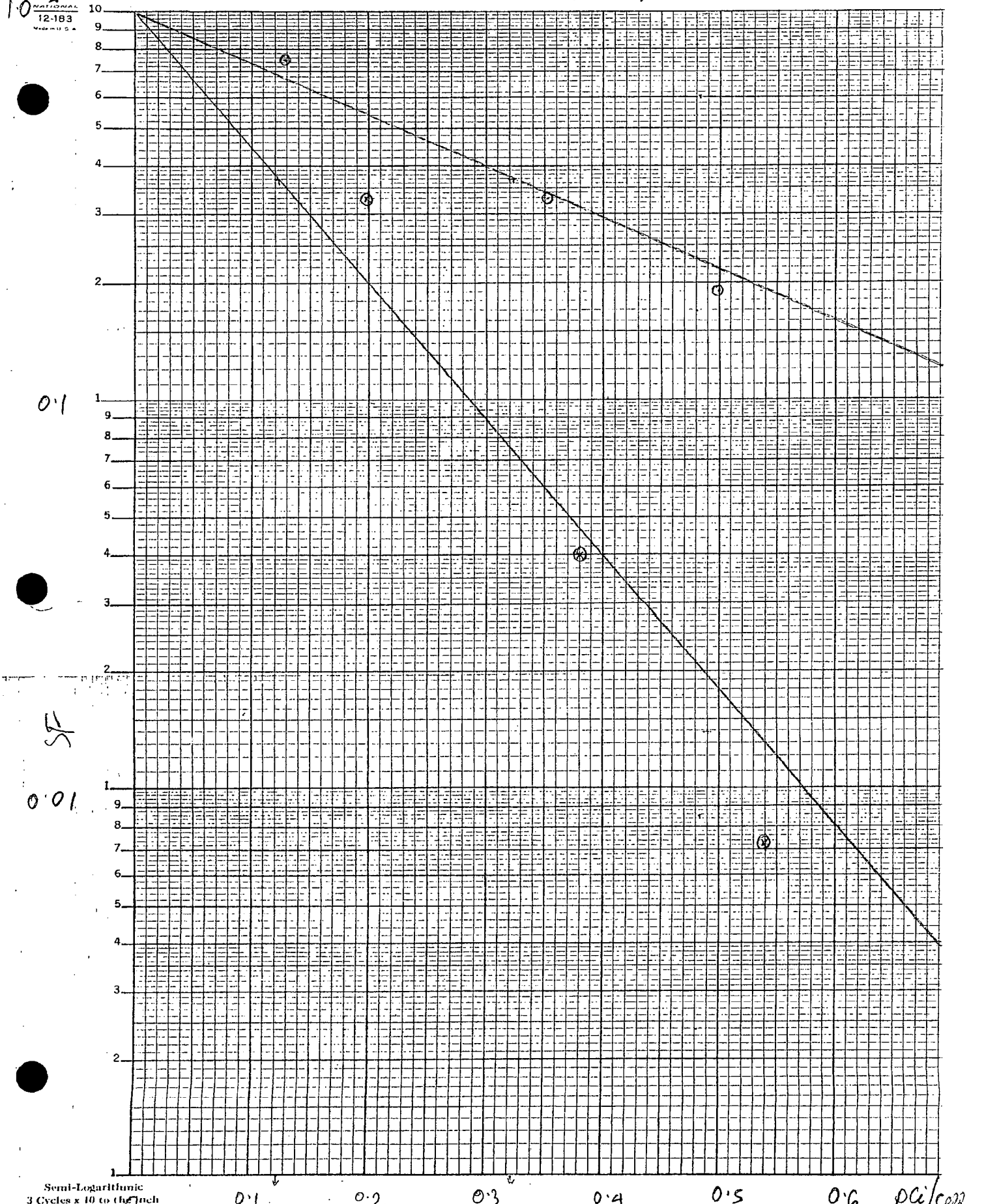
TABLE-4

Expt. #: 2 Date: 08/31/98

Colony Counts and Survival Fraction

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony	SF
1·2	109	118	108	113·16	
2·2	111	106	127		
3·2	85	95	76	85·33	0·7540
4·2	48	38	29	38·33	0·3387
5·3	226	206	240	22·4	0·1979
6·2	134	145	142	132·66	
7·2	115	129	131		
8·2	48	44	41	44·33	0·3341
9·3	59	54	47	5·33	0·0402
10·4	109	99	88	0·98	0·0074

Expt #2 Bilver + 10% DMSO DMF = 2.63



Semi-Logarithmic
3 Cycles x 10 to the Inch

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