V79 COLONY FORMING ASSAY

Experiment Name : ^{125}IUdR + MEA; **Exp. # :** 3; **Investigator:** A. Bishayee
Date: 05/11/98

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 : 403466 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:** 05/11/98; 4-30 p.m.
5. Prepare MEMB containing radioactivity in hood
   3.75 µl ^{125}IUdR (prepared on 04/09/98) + 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:** 05/11/98; 8-00 p.m.

Tube #	^{125}IUdR uCi/ml	Cells in MEMB (ml)	MEMB (ml)	MEMB+ ^{125}IUdR (ml) [1.0 uCi/ml]	MEMA+ MEA 100 ug/ml (1.3 mM)	MEMA	
1	0	1.0	1.0	0	2.0	0	
2	0	1.0	1.0	0	2.0	0	
3	0.01	1.0	0.98	0.02	2.0	0	
4	0.02	1.0	0.96	0.04	2.0	0	
5	0.03	1.0	0.94	0.06	2.0	0	
6	0	1.0	1.0	0	0	2.0	
7	0	1.0	1.0	0	0	2.0	
8	0.01	1.0	0.98	0.02	0	2.0	
9	0.02	1.0	0.96	0.04	0	2.0	
10	0.03	1.0	0.94	0.06	0	2.0	

7. Return test tubes to roller for 12 h, increase the elevation angle of the roller.
   Date/Time: 05/11/98; 8-15 p.m.

1.2
2.2
3.2
4.3
5.3
6.2
7.2
8.2
9.3
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:** 05/12/98; 9-15 a.m.
10. **During centrifugation, move roller to 10°C and obtain ice**
11. Prepare 11 ml of sterile MEA (100 ug/ml) 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 100 ug/ml of MEA as per Table. Keep on ice!
20. Transfer tubes to roller at 10°C for 72 h. **Date/Time:** 05/12/98; 11-00 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: 05/12/98; 12-00 noon
21. After 72 h, remove tubes and **place on ice**, add 8 ml ***ice cold*** wash MEMA.

    Date/Time: 05/15/98; 2-30 p.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 :**
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 dish to be a valid data point.

Expt #3

05/11/98

Initial Cell count = 6415, 6580, 6320

Avg. Cell count = 6438.3

Cell conc = 400 × 6438.3

= 2575333.3 Cells/ml

For dilution,

vol of cell suspension required = $\frac{4400000}{2575333.3}$

= 1.70

Take 1.7 ml of cells + 9.3 ml MEMB = 11 ml.

After dilution,

Final count = 1071, 988, 967

Avg. count = 1008.6

Cell conc. = 400 × 1008.6

= 403466 Cells/ml

Expt #3

05/11/98

Preparation of ^{125}I/UdR in MEMB

Prepare 5 ml of 1 μCi/ml ^{125}I/UdR = 5 μCi required

Stock on 04/09/98 1·93 μCi/μl

on 05/11/98 1·93 × 0·69
= 1·33 μCi/μl.

Stock required = $\frac{5}{1.33}$ = 3·75 μl.

① Take 5 ml of MEMB
② Add 3·75 ml of stock ^{125}I/UdR

Name of the Expt # : ^{125}I/UdR + 1.3mM MEA

Expt # : 3

Date/Time : 05/12/98; 12-00 noon

cpm for 10µl medium onto tissue

POWER-UP. MODEL 9455 VERS. 1.0 PRINTER UPDATE.

1	1.00	Back { 24 } 25	244
2	1.00	Back { 27 } 25	270
3	1.00	1M { 30	250
4	1.00	1M { 22	287
5	1.00	1M { 22	250
6	1.00	2M { 14	247
7	1.00	2M { 25	236
8	1.00	2M { 21	240
9	1.00	3M { 226	479
10	1.00	3M { 253	488
11	1.00	3M { 259	490
12	1.00	4M { 409	626
13	1.00	4M { 454	711
14	1.00	4M { 412	640
15	1.00	5M { 568	817
16	1.00	5M { 607	848
17	1.00	5M { 613	816
18	1.00	6M { 27	252
19	1.00	6M { 27	232
20	1.00	6M { 29	259
21	1.00	7M { 18	210
22	1.00	7M { 17	227
23	1.00	7M { 15	232
24	1.00	8M { 196	415
25	1.00	8M { 231	474
26	1.00	8M { 246	430
27	1.00	9M { 377	603
28	1.00	9M { 434	671
29	1.00	9M { 385	621
30	1.00	10M { 580	809
31	1.00	10M { 617	852
32	1.00	10M { 574	830

TABLE-1

Expt. # : 3 **Date/Time :** 05/12/98; 12-00 noon

Tube #	Medium count for 10 ul (cpm)	Avg. cpm	dpm [cpm/0.7056]	µCi/ml (A_t) on counting [dpm/22200]	µCi/ml (A_o) on addition [$A_t/e^{-\lambda t}$]
1	5, −3, −3	0	0	0	0
2	11, 0, −4	0	0	0	0
3	201, 228, 234	221	313.2	0.0141	0.0142
4	384, 429, 387	400	566.8	0.0255	0.0257
5	543, 582, 588	571	809.2	0.0364	0.0367
6	2, 2, 4	0	0	0	0
7	−7, −8, −10	0	0	0	0
8	171, 206, 221	199.3	282.5	0.0127	0.0128
9	409, 360, 352	373.6	529.5	0.0238	0.0240
10	555, 592, 549	565.3	801.2	0.0360	0.0363

05/11/98; 8-00 p.m.

12h + 4.25h

= 16.25h

$e^{-\lambda t}$

$= e^{-\frac{0.693 \times 16.25}{1440}}$

$= 0.9922$

05/15/98
4-00p.m.

300µl cells
for MEA#3

1	1.00	26	249
~~2~~	~~1.00~~	~~38~~	~~274~~
3	1.00	19	232
4	1.00	28 } 1C	261
5	1.00	24	252
6	1.00	22	262
7	1.00	21 } 2C	253
8	1.00	14	251
9	1.00	198	413
10	1.00	182 } 3C	444
11	1.00	184	395
12	1.00	299	498
13	1.00	329 } 4C	544
14	1.00	347	559
15	1.00	521	726
16	1.00	538 } 5C	790
17	1.00	515	747
18	1.00	18	264
19	1.00	24 } 6C	218
20	1.00	22	277
21	1.00	23	252
22	1.00	18 } 7C	221
23	1.00	16	249
24	1.00	243	493
25	1.00	249 } 8C	505
26	1.00	270	518
27	1.00	296	518
28	1.00	310 } 9C	535
29	1.00	304	541
30	1.00	733 } 10C	965
		723	
		743	

POWER-UP. MODEL 9455 VERS. 1.0 PRINTER UPDATE.

TABLE-2

Expt. # : 3 **Date/Time :** 05/15/98 ; 4-00 p.m.

Tube #	Radioactivity for 300 ul cell suspension (cpm)	Avg. cpm	dpm [cpm/0.7056]	μ Ci/ml (A_t) on counting [dpm/666000]	μ Ci/ml (A_o) after 12 h incubation [$A_t/e^{-\lambda t}$]
1	-7, 2, -2	0	0	0	0
2	-4, -5, -12	0	0	0	0
3	172, 156, 158	162	229.59	0.00034	0.000358
4	273, 303, 321	299	423.75	0.00063	0.00066
5	495, 512, 489	498	706.72	0.00106	0.001102
6	-8, -2, -4	0	0	0	0
7	-3, -8, -10	0	0	0	0
8	217, 223, 244	231.3	327.85	0.00049	0.000511
9	270, 284, 278	277.3	393.04	0.00059	0.00061
10	707, 697, 717	707	1001.9	0.0015	0.00156

05/12/98 ; 9-15 a.m

72h + 6.75

= 78.75

$e^{-\lambda t}$

$= e^{-\frac{0.693 \times 78.75}{1440}}$

$= 0.9628$

TABLE-3

Expt. # : 3 **Date/Time :** 05/15/98; 2-30 P.M

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	298, 252, 244	264.6	105866.6	0
2	300, 285, 279	288	115200	0
3	370, 338, 351	353	141200	0.00253
4	274, 236, 250	253.3	101333.3	0.00651
5	286, 232, 221	246.3	98533.3	0.01118
6	465, 421, 449	445	178000	0
7	474, 384, 416	424.6	169866.6	0
8	424, 369, 378	390.3	156133.3	0.00327
9	235, 211, 205	217	86800	0.00702
10	348. 333, 317	332.6	133066.6	0.01172

298 252,

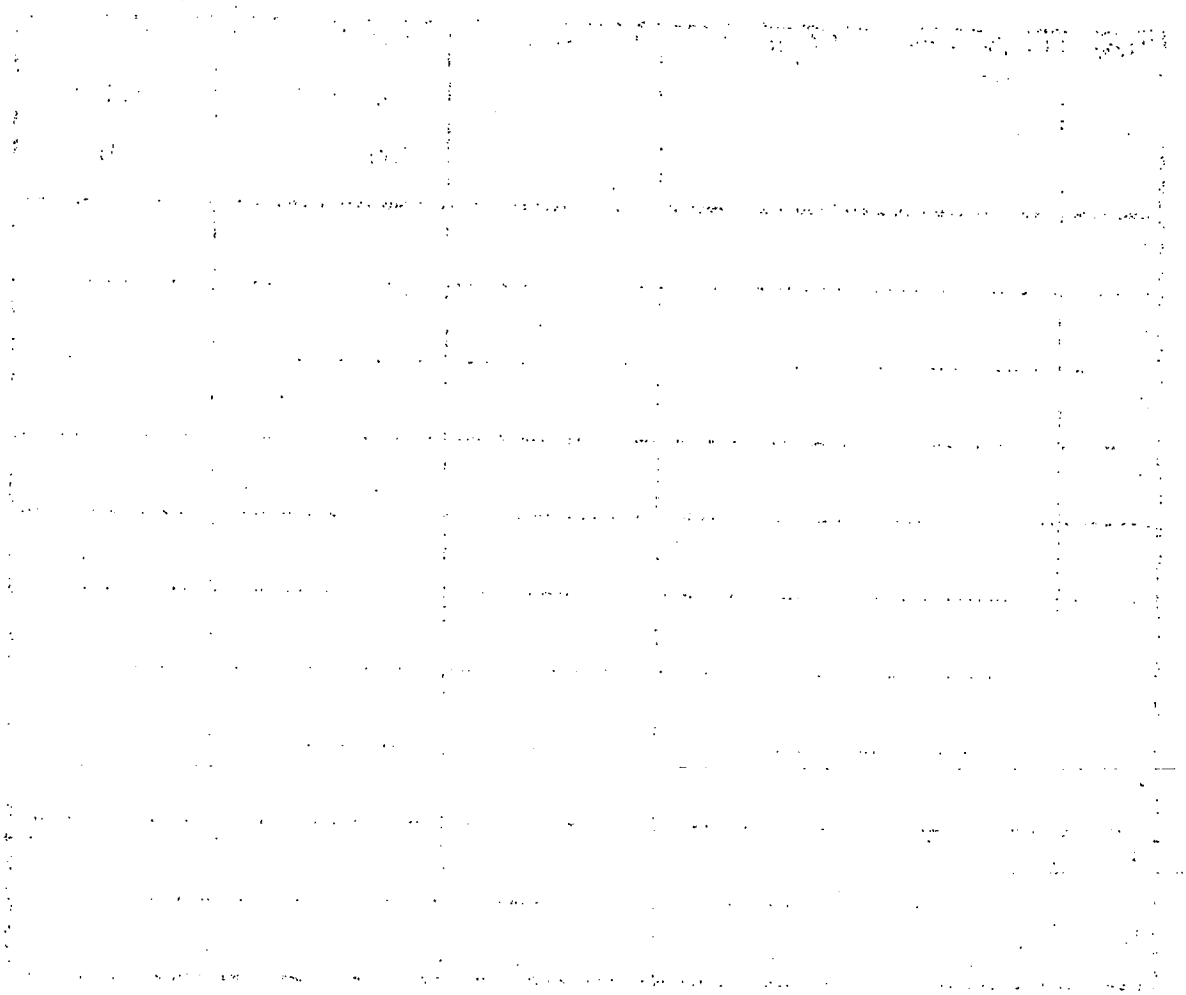


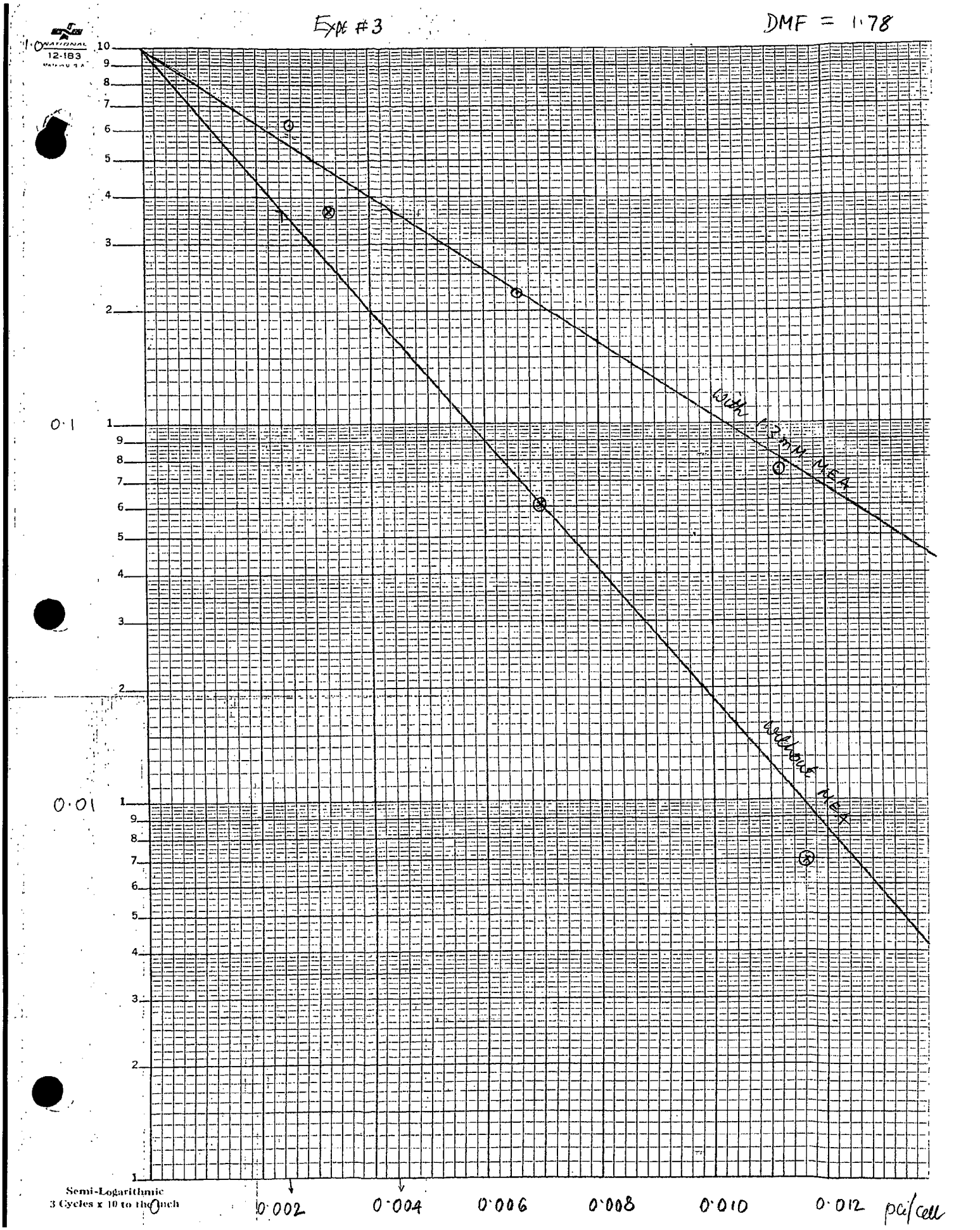
TABLE-4

Expt. #: 3

Date: 05/22/98

Colony Counts and Survival Fraction

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony	SF
1·2	59	55	57	58·83	
2·2	56	62	64		
3·2	33	42	36	37	0·6289
4·3	120	135	140	13·16	0·2236
5·3	38	45	50	4·43	0·0753
6·2	77	70	62	68	
7·2	65	63	71		
8·2	26	23	28	25·66	0·3773
9·3	35	42	49	42	0·0617
10·4	48	52	42	0·4733	0·00696


Expt #3
DMF = 1·78
10
9
8
7
6
5
4
3
2
0·1
1
0·01
0·002
0·004
0·006
0·008
0·010
0·012
pa/cell
Semi-Logarithmic
3 Cycles x 10 to the Inch