Experiment with Shaker

V79 COLONY FORMING ASSAY

Experiment Name : ^{3}HTdR toxicity (cluster, 100% labeling); **Exp. # :** 1;
Investigator: A. Bishayee **Date:** 11/12/98

1. Set the shaker at 37^{0}C incubator with 5% CO_2, set the Coulter Counter, wash cells (from two 150 cm^2 flusk, subcultured 1:2, 24h before) with PBS, trypsinize cells, each resuspend in 9 ml MEMB, pool, 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 ~4,000,000 cells/ml in MEMB [Actual count : 4,144,000 cells/ml)
3. Transfer 1 ml of cell suspension into ten 6 ml tubes (Falcon plastic test tube, 12x75 mm) labeled 1-10 both on cap and wall
4. Keep the tubes in the shaker (shaking mode : interval) for 3-4 h at 37°C, 5% CO_2

   Date/Time: 11/12/98; 3-00 p.m.
5. Prepare MEMB containing radioactivity in hood

   12 µl ^{3}HTdR (Stock : 1 µCi/µl on 11/12/98) + 3 ml MEMB
6. After 3-4 h, remove test tubes from shaker and add MEMB with or without radioactivity according to Table below. **Date/Time:** 11/12/98; 7-00 p.m.

Tube #	^{3}HTdR uCi/ml	Cells in MEMB (ml)	MEMB (ml)	MEMB+ ^{3}HTdR (ml) [4uCi/ml]
1	0	1.0	1.0	0
2	0	1.0	1.0	0
3	0.01	1.0	0.995	0.005
4	0.05	1.0	0.975	0.025
5	0.1	1.0	0.950	0.050
6	0.2	1.0	0.900	0.100
7	0.5	1.0	0.750	0.250
8	0.75	1.0	0.625	0.375
9	1	1.0	0.500	0.500
10	2	1.0	0	1

1 - 4 µCi
0.74 mBq

7. Return test tubes to shaker for 12 h . **Date/Time:** 11/12/98; 7-30 p.m.
8. Next day, while test tubes are in shaker 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:** 11/13/98; 9-00 a.m.
10. Remove buckets from centrifuge and carefully remove 150 µl of supernatant and place in prelabeled gamma-tube.
11. Decant supernatant, click tubes, vortex, resuspend in 3 ml wash MEMA
12. *Centrifuge tubes for 10 min at 2000 rpm, 4°C*
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 of MEMA
18. Centrifuge tubes for 10 min at 2000 rpm, 4°C
19. Decant supernatant, click tubes,vortex, transfer the cell suspension in polypropylene microcentrifuge tubes with attached caps (Helena Plastics, 400 ul) using 200 ul pipet tips
20. Again add 200 ul ice cold MEMA, resuspend and transfer the cell suspensions in the same polypropylene microcentrifuge tubes (Total volume ~400 ul)
21. Centrifuge tubes for 5 min at 1000 rpm, 4°C
22. Transfer tubes at 10°C for 72 h. **Date/Time:** 11/13/98; 11-30 a.m.
23. Transfer 30 ul supernatant in three sets of 20 ml scintillation vials containing 6 ml liquid scintillation cocktail (Aquasol) from 150 ul supernatant removed earlier (Step 10) and count them for radioactivity **Date/Time:** 11/13/98; 12-00 noon
24. 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:** 11/16/98; 9-30 a.m.
25. Again add 200 ul 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 66, 60 mm petri 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, resuspend in 10 ml wash MEMA
29. Centrifuge tubes for 10 min at 2000 rpm, 4°C

1·2
2·2
3·2
4·2, 4·3
5·2, 5·3
6·2, 6·3, 6·4
7·2, 7·3, 7·4
8·2, 8·3, 8·4
9·3, 9·4
10 3, 10·4

30. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
31. Centrifuge tubes for 10 min at 2000 rpm, 4°C
32. Decant supernatant, click tubes, vortex, resuspend in 2 ml wash MEMA, pass five times through 3 cc syringe with 21 gauge needle
33. Determine cell concentration by transfering 100 µl to Coulter cup
34. Vortex tube, transfer 0.5 ml into dilusion 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 100 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 **Date/Time :** 11/16/98, 8-45 p.m.
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 flask to be a valid data point.

MS = 50 ml

Expt #1

11/12/98

Initial cell count = 2128, 2137, 2122
Avg. Cell Count = 2129
Cell Conc. = 8,516,000 cells/ml

For dilution

Take 5.5 ml cells + 5.5 ml MEMB = 11 ml

After dilution,

Final count = 1012, 1053, 1013
Avg. count = 1036
Cell Conc. = 4,144,000 cells/ml

3 #Tdr in cluster : Expt #1. (1)

F-451 30 µl medium

[redacted]R: 2 ID:WIPE PRESET TIME: 1.00 FRI 13 NOV 1998 11:52
S[redacted]PLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
H#: 1 ABC:N QCF:N RCM:N
CHANNEL 1-LL: 0 UL: 400 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0
CHANNEL 2-LL: 0 UL: 670 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0
CHANNEL 3-LL: 0 UL:1000 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0
DATA CALC: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR:@ 1.00000
HALF LIFE(DAYS):N

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
1	**- 1	1	9.00	66.67	1.00	1.89	98.0	
		2	18.00	47.14				
		3	22.00	42.64				
19	**- 1	1	19.00	45.88	1.00	3.81	53.0	
		2	21.00	43.64				
		3	33.00	34.82				
20	**- 2	1	26.00	39.22	1.00	5.65	57.0	
		2	35.00	33.81				
		3	45.00	29.81				
21	**- 3	1	26.00	39.22	1.00	7.49	58.0	
		2	34.00	34.30				
		3	38.00	32.44				
22	**- 4	1	19.00	45.88	1.00	9.18	59.0	
		2	30.00	36.51				
		3	42.00	30.86				
[redacted]	**- 5	1	28.00	37.80	1.00	10.87	56.0	
		2	35.00	33.81				
		3	50.00	28.28				
24	**- 6	1	19.00	45.88	1.00	12.62	59.0	
		2	28.00	37.80				
		3	33.00	34.82				
25	**- 7	1	187.00	14.63	1.00	14.56	56.0	
		2	193.00	14.40				
		3	210.00	13.80				
26	**- 8	1	170.00	15.34	1.00	16.30	58.0	
		2	181.00	14.87				
		3	191.00	14.47				
27	**- 9	1	180.00	14.91	1.00	18.23	58.0	
		2	191.00	14.47				
		3	201.00	14.11				
28	**-10	1	821.00	6.98	1.00	20.02	58.0	
		2	838.00	6.91				
		3	850.00	6.86				
29	**-11	1	843.00	6.89	1.00	21.72	58.0	
		2	853.00	6.85				
		3	863.00	6.81				
30	**-12	1	811.00	7.02	1.00	23.42	59.0	
		2	829.00	6.95				
		3	845.00	6.88				
31	**- 1	1	1510.00	5.15	1.00	25.22	57.0	
[redacted]		2	1523.00	5.12				
		3	1530.00	5.11				

Efficiency = 0.65
yield = 1.

SCN	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
32	**- 2	1	1640.00	4.94-5M	1.00	27.12	57.0	
		2	1654.00	4.92				
		3	1662.00	4.91				
33	**- 3	1	1407.00	5.33-5M	1.00	29.01	57.0	
		2	1423.00	5.30				
		3	1433.00	5.28				
34	**- 4	1	3023.00	3.64-6M	1.00	30.95	57.0	
		2	3034.00	3.63				
		3	3037.00	3.63				
35	**- 5	1	3192.00	3.54-6M	1.00	32.69	60.0	
		2	3204.00	3.53				
		3	3211.00	3.53				
36	**- 6	1	3159.00	3.56-6M	1.00	34.53	62.0	
		2	3170.00	3.55				
		3	3176.00	3.55				
37	**- 7	1	7292.00	2.34-7M	1.00	36.42	63.0	
		2	7303.00	2.34				
		3	7314.00	2.34				
38	**- 8	1	8121.00	2.22-7M	1.00	38.32	62.0	
		2	8135.00	2.22				
		3	8142.00	2.22				
39	**- 9	1	7868.00	2.25-7M	1.00	40.37	62.0	
		2	7882.00	2.25				
		3	7886.00	2.25				
	**-10	1	10846.81	1.98-8M	0.94	42.24	59.0	
		2	10864.89	1.98				
		3	10877.66	1.98				
41	**-11	1	12105.88	1.97-8M	0.85	43.77	60.0	
		2	12114.12	1.97				
		3	12122.35	1.97				
42	**-12	1	11699.42	1.99-8M	0.87	45.48	63.0	
		2	11715.61	1.99				
		3	11726.01	1.99				
43	**- 1	1	14736.55	1.93-9M	0.72	47.01	56.0	
		2	14753.10	1.93				
		3	14765.52	1.93				
44	**- 2	1	16312.31	1.94-9M	0.65	48.35	59.0	
		2	16336.92	1.94				
		3	16347.69	1.94				
45	**- 3	1	16329.69	1.96-9M	0.64	49.93	59.0	
		2	16345.31	1.96				
		3	16351.56	1.96				
46	**- 4	1	29225.64	1.87-10M	0.39	51.26	58.0	
		2	29266.67	1.87				
		3	29271.80	1.87				
47	**- 5	1	32123.07	1.96-10M	0.33	52.32	58.0	
		2	32153.84	1.96				
		3	32163.07	1.96				
48	**- 6	1	32916.92	1.93-10M	0.33	53.37	56.0	
		2	32978.46	1.93				
		3	32990.77	1.93				

TABLE-1

Expt. # : 1 **Date/Time :** 11/13/98; 12-00 noon

Tube #	Medium count for ~~20~~ 30 ul (cpm)	Avg. cpm	dpm [cpm/0.65] Effi = 0.65 Yield = 1	µ Ci/ml (A_t) on counting [dpm/~~22200~~ 66600]	µ Ci/ml (A_o) on addition [$A_t/e^{-\lambda t}$]
1	19, 26, 26				
2	19, 28, 19				
3	187, 170, 180	179	275.3	~~0.012~~	0.004
4	821, 843, 811	825	1269.2	~~0.057~~	0.019
5	1510, 1640, 1407	1519	2336.9	~~0.105~~	0.035
6	3023, 3192, 3159	3124.6	4807.1	~~0.216~~	0.072
7	7292, 8121, 7868	7760.3	11938.9	~~0.537~~	0.179
8	10846, 12105, 11699	11550	17769.2	~~0.800~~	0.266
9	14736, 16312, 16329	15792.3	24295.8	1.094	0.364
10	29225, 32123, 32916	31421.3	48340.5	~~2.177~~	0.725

$$\mu Ci/ml = \frac{dpm}{60 \times 37999} \times \frac{1000}{30} = \frac{dpm}{66600}$$

3HTRd + m Cluster : Expt #1

USER: 6 ID:H3 HOWELL PRESET TIM=: 1.00 MON 16 NOV 1998 20:43
SAMPLE REPEAT: 1 CYCL: REPEAT 1 SCR:N RS232:N

H#: 1 AQC:N QCF:N RCM:N
CHANNEL 1-LL: 0 UL: 400 2SIGNA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0
DATA CALC: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR:0 1.00000
HALF LIFE(DAYS):N

200 µl cells

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	ERR
1	**- 1	1	12.00	57.74	1.00	1.65	83.0	
2	**- 2	1	1C 24.00	40.82	1.00	3.43	79.0	
3	**- 3	1	19.00	45.88	1.00	5.32	80.0	
4	**- 4	1	14.00	53.45	1.00	7.25	80.0	
5	**- 5	1	2C 28.00	37.80	1.00	8.93	80.0	
6	**- 6	1	20.00	44.72	1.00	10.61	78.0	
7	**- 7	1	294.00	11.66	1.00	12.34	77.0	
8	**- 8	1	3C 263.00	12.33	1.00	14.13	74.0	
9	**- 9	1	279.00	11.97	1.00	15.95	78.0	
10	**-10	1	1410.00	5.33	1.00	17.83	78.0	
11	**-11	1	4C 1563.00	5.06	1.00	19.51	81.0	
12	**-12	1	1545.00	5.09	1.00	21.24	78.0	
13	**- 1	1	3214.00	3.53	1.00	23.08	81.0	
14	**- 2	1	5C 3222.00	3.52	1.00	24.92	80.0	
15	**- 3	1	3147.00	3.57	1.00	26.71	84.0	
16	**- 4	1	4430.00	3.00	1.00	28.68	74.0	
17	**- 5	1	6C 4716.00	2.91	1.00	30.47	76.0	
18	**- 6	1	4427.00	3.01	1.00	32.15	75.0	
19	**- 7	1	12779.39	1.95	0.82	33.71	73.0	
20	**- 8	1	7C 13301.25	1.94	0.80	35.28	77.0	
21	**- 9	1	13503.23	1.96	0.77	36.94	79.0	
22	**-10	1	20126.21	1.96	0.51	38.28	79.0	
23	**-11	1	8C 20982.53	1.92	0.51	39.62	80.0	
24	**-12	1	23273.68	1.90	0.48	40.82	81.0	
25	**- 1	1	24500.00	1.90	0.45	41.99	79.0	
26	**- 2	1	9C 22757.78	1.98	0.45	43.10	75.0	
27	**- 3	1	22655.55	1.98	0.45	44.22	74.0	
28	**- 4	1	62900.00	1.83	0.19	45.32	82.0	
29	**- 5	1	10C 61969.70	1.98	0.17	46.31	84.0	
30	**- 6	1	65335.00	1.75	0.20	47.17	80.0	

TABLE-2

Expt. # : 1 **Date/Time :** 11/16/98; 8-45 p.m.

Tube #	Radioactivity for 200 ul cell suspension (cpm)	Avg. cpm	dpm [cpm/0.65]	μ Ci/ml (A_t) on counting [dpm/444000]	μ Ci/ml (A_o) after 12 h incubation [$A_t/e^{-\lambda t}$]
1	See the attached				
2	Sheet				
3		278	428.7	0.000965	
4		1506	2316.9	0.005218	
5		3194.3	4914.3	0.01106	
6		4524.3	6960.5	0.01567	
7		13 1943	20298.9	0.0457	
8		21460.3	33015.8	0.0743	
9		23304	35852	0.0807	
10		63407	97549	0.2197	

$$\mu Ci/ml = \frac{dpm}{60 \times 37000} \times 5 = \frac{dpm}{444000}$$

TABLE-3

Expt. # : 1 **Date/Time :** 11/16/98

Tube #	Coulter count for 100 ul cell suspension	Avg. count	Cells/ml [Avg. count x 4000]	pCi/cell [uCi/ml x 10^6 / Cells/ml]
1	666, 618, 638	640	2562666	–
2	634, 638, 611	627	2510666	–
3	589, 565, 548	567	2269333	0.00042
4	653, 613, 647	637	2550666	0.00204
5	569, 562, 580	570	2281333	0.00484
6	624, 605, 604	611	2444000	0.00641
7	535, 524, 558	539	2156000	0.02119
8	566, 543, 531	546	2186666	0.03397
9	691, 688, 677	685	2741333	0.02943*
10	637, 628, 618	627	2510666	0.08750

* not reliable

TABLE-4

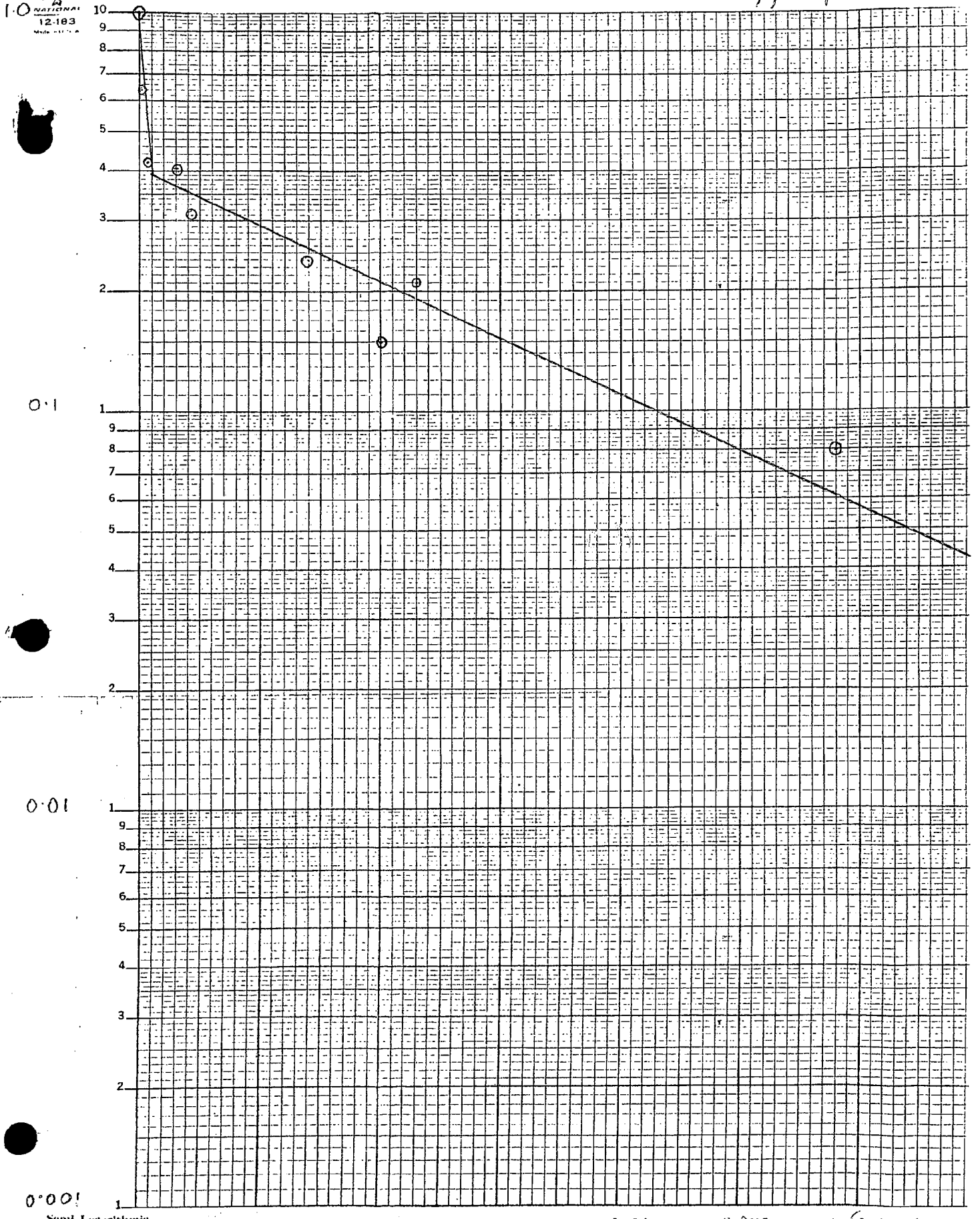
Expt. #: 1

Date: 11/23/98

Colony Counts and Survival Fraction

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony	SF
1·2	112	95	102	94·5	
2·2	89	79	90		
3·2	69	61	55	61·66	0·6524
4·2	36	41	39	38·66	0·4091
5·2	43	40	31	38	0·4021
6·2	33	28	29	30	0·3174
7·2	25	23	21	23	0·2433
8·2	22	24	16	20·66	0·2186
9·3	146	149	139	14·46	0·1530
10·3	90	74	78	8·06	0·0853

3HTAR in Cluster, Eqpt #1



NATIONAL 12-183

Semi-Logarithmic
3 Cycles x 10 to the inch

B001760