V79 COLONY FORMING ASSAY

Experiment Name : ^{137}Cs toxicity (4X 10^6 Cells cluster, Chronic, 10.5° C); **Exp. # :** 1;
Investigator: A. Bishayee **Date:** 10/13/98

1. Set the Coulter Counter, wash cells (from two 150 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 ~4,000,000 cells/ml in MEMB (final volume 11 ml) [Actual count : 4,770,000 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. Add 2 ml MEMA, vortex and centrifuge at 2000 rpm at 4℃ for 10 min (***precooled centrifuge***).
5. Decant the supernatant, click tubes and vortex
6. Transfer the cell suspension in prelabeled (1-5 and C) polypropylene microcentrifuge tubes (Helena Plastics, 400 ul) with attached caps using 200 µl pipet tips
7. Again add 200 ul ice cold MEMA, vortex to resuspend and transfer the cell suspensions in the same polypropylene microcentrifuge tubes.(Total volume in each tube ~400 ul)
8. Centrifuge tubes for 5 min at 1000 rpm, 4℃
9. Place tubes at different distances (positions #1-5) from ^{137}Cs source (placed inside a 6 ml plastic centrifuge tube in a styrofoam platform) at 10℃ for 72 h. Place 2 control tubes away from the source inside the 10.5° C incubator. **Date/Time:** 10/13/98; 7-00 p.m.
10. After 72h of chronic irradiation, remove the tubes from incubator, carefully remove the supernatant from the top, resuspend pellet in 200 ul wash MEMA and transfer the content (using pasteur pipet) to 12 ml tubes (Falcon plastic test tube, 17x100 mm, labeled both on cap and wall) containing 10 ml wash MEMA Date/ Time : 10|16|98; 5-00 p.m.
11. Again add 200 ul wash MEMA in microcentrifuge tubes, resuspend and transfer the cell suspensions in 12 ml tubes
12. Centrifuge the tubes for 10 min at 2000 rpm, 4℃ ***(precooled centrifuge)***
13. Labeling and preparation of dilution tubes and colony dishes
    - load 60 mm petri dishes with 4 ml MEMA
    - load test 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.
14. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
15. Centrifuge tubes for 10 min at 2000 rpm, 4℃

16. Decant supernatant, click tubes, vortex, resuspend in 2 ml wash MEMA, pass five times through 3 cc syringe with 21 gauge needle
17. Determine cell concentration by transfering 100 µl to Coulter cup
18. 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.
19. 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.
20. Incubate petridishes for 1 week
21. After 1 week, wash colonies 3 times with normal (1X) saline,and 2 times with methanol. Stain colonies with 0.0 5% crystal violet
22. Count colonies. There must be between 25 and 250 colonies for the ~~flask~~ dish to be a valid data point.

CS-137 in Chamber
(4×10⁶ cells)

Expt #1

10/13/98

Take 2 × 150 cm² flask (80-90% confluent)
Total vol. = 16 ml

Initial cell count = 1407, 1397, 1422

Avg. cell count = 1408·6

Cell conc. = 1408·6 × 4000 = 5,634,666 Cells/ml

we need 8 ml of 4,000,000 Cells/ml = 32,000,000 cells.

40,000,000 / ml = 32,000,000

Vol. required = 32,000,000 / 5634666·6

= 5·67 ml.

Take 6 ml cells + 2 ml MEM = 8 ml

Final cell count = 11921, 11909, 11945

Avg. count = 11925

Cell conc. = 11925 × 400

= 4,770,000 cells/ml

= 4.7 million/ml.

6/30 p.m
10/13/98

SETUP

Calibration of Cs-137 Cluster Irradiation

UNIT:

LOCATION: 10mCi Cs-137

ENERGY: 1/76

SSD:

GANTRY ANGLE:

COLLIMATION: On 4:30 10/19

FIELD SIZE: Read 8:52 10/20

EXPOSURE: 16.37h

Calibration of Low Dose Rate Cs-137 Irradiation of V79 Clusters irradiated at 10.5°C

Source NEN 10 mCi Cs-137 pellet calibrated on 1/76.

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* 16:30 OCT 19/98 Source in
ZERO SENSORS

A1 TOTAL: 1.024 mV HIGH RESOLUTION
A2 TOTAL: 1.137 mV HIGH RESOLUTION
A3 TOTAL: 986 mV HIGH RESOLUTION
A4 TOTAL: 881 mV HIGH RESOLUTION
A5 TOTAL: 1.173 mV HIGH RESOLUTION

A1 08:52 OCT 20/98 position #1

DOSE: 298 RAD 18.2 rad/h
TOTAL: 1.322 mV

A2 08:52 OCT 20/98 #2

DOSE: 161.3 RAD 9.86 rad/h
TOTAL: 1.298 mV

A3 08:52 OCT 20/98 #3

DOSE: 107.6 RAD 6.57 rad/h
TOTAL: 1.094 mV

A4 08:52 OCT 20/98 #4

DOSE: 99.9 RAD 6.10 rad/h
TOTAL: 981 mV

A5 08:52 OCT 20/98 #5

DOSE: 55.6 RAD 3.40 rad/h
TOTAL: 1.229 mV

THOMSON & NIELSEN

MULTI DOSIMETER SYSTEM

REV 2.0

CAL FACTOR SETTINGS

A1: 1.00mV/RAD	C1: 1.00mV/RAD
A2: 1.00mV/RAD	C2: 1.00mV/RAD
A3: 1.00mV/RAD	C3: 1.00mV/RAD
A4: 1.00mV/RAD	C4: 1.00mV/RAD
A5: 1.00mV/RAD	C5: 1.00mV/RAD
B1: 3.15mV/RAD	D1: 1.00mV/RAD
B2: 3.15mV/RAD	D2: 1.00mV/RAD
B3: 3.15mV/RAD	D3: 1.00mV/RAD
B4: 3.15mV/RAD	D4: 1.00mV/RAD
B5: 3.15mV/RAD	D5: 1.00mV/RAD

NOTES

Calibration of ^{137}Cs irradiator in 10°5°C incubator

12:00 OCT 13/98 — 4.75 h irradiation
ZERO SENSORS

A1 TOTAL: 983 mV	HIGH RESOLUTION	1
A2 TOTAL: 1,122 mV	HIGH RESOLUTION	
A3 TOTAL: 963 mV	HIGH RESOLUTION	
A4 TOTAL: 865 mV	HIGH RESOLUTION	
A5 TOTAL: 1,179 mV	HIGH RESOLUTION	

2

A1 16:46 OCT 13/98

DOSE: 37.7 RAD
TOTAL: 1,021 mV

7.9 rad/h

570

3

A2 16:46 OCT 13/98

DOSE: 23.8 RAD
TOTAL: 1,146 mV

5.0 rad/h

360

A3 16:46 OCT 13/98

DOSE: 25.1 RAD
TOTAL: 988 mV

5.3 rad/h

4

A4 16:46 OCT 13/98

DOSE: 20.4 RAD
TOTAL: 885 mV

4.3 rad/h

310

5

A5 16:46 OCT 13/98

DOSE: 8.5 RAD
TOTAL: 1,187 mV

1.8 rad/h

130

100 µl Cell count

MS = 50 µl

NOTES

C	485, 502, 498
1	447, 462, 444
2	411, 399, 436
3	428, 409, 425
4	389, 389, 374
5	495, 472, 458

NOTES

TABLE-2

Expt # : 1

Date : 10/23/98 ; 10-00 a.m.

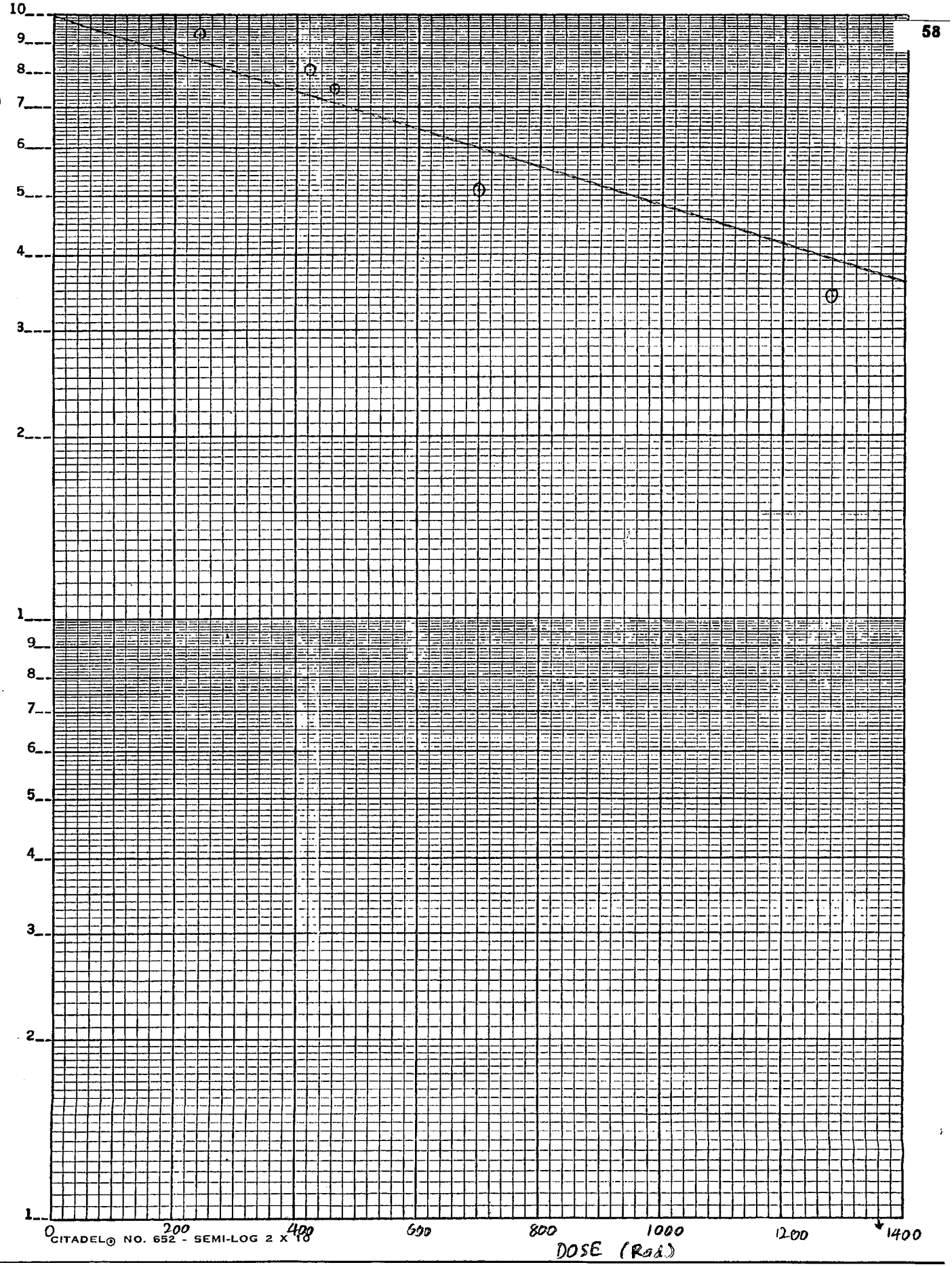
Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony	SF
1·2	73	83	75	77	—
2·2	24	26	29	26·33	0.342
3·2	39	37	43	39·66	0·515
4·2	58	49	67	58	0·7532
5·2	61	60	68	63	0·818
6·2	68	69	78	71·66	0·931

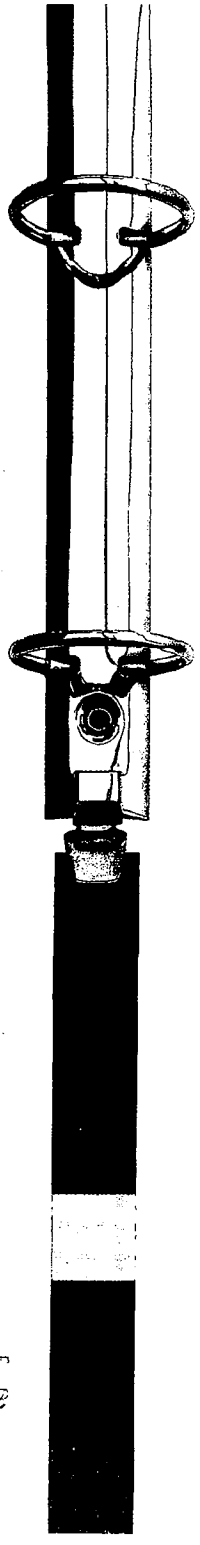
Chronic Cs-137 toxicity : 4×10^6 Cells cluster, 10.5°C for ~70h 10/23/98

Summary of Results

Tube # (Position #)	Total Dose (Rad)	Dose rate (Rad/h)	Time (h)	SF
C	0	0	0	-
C	0	0	0	-
1	1274	18.2	70	0.342
2	690	9.8	70	0.515
3	460	6.5	70	0.753
4	427	6.1	70	0.818
5	238	3.4	70	0.931
6				
7				
8				

Chronic Cs-137 : 4×10^6 Cells cluster at 10°5°C for ~70h : $D_{37} = 1360$ R





Check out these WilsonJones differences!

☑ **Cure GAPiTIS!** Conventional rings can become spread or misaligned—we call it GAPiTIS! Cure it with DublLock! Rings stay closed and aligned—sheets won't tear or hang-up on rings.

☑ **Locking Rings!**
WilsonJones patented ring mechanism actually locks the outside rings. Our 3 position trigger lets you lock, unlock, and open rings—sheets won't fall out!

☑ **EasyFlow Sheet Lifters!**
We've got a new angle on sheet lifters! The patented angle shape of WilsonJones EasyFlow lifter won't catch or tear papers stored in pockets, won't take up valuable ring capacity like conventional sheet lifters! Available in 1½, 2 & 3 inch capacities.

☑ **Extra-Wide Covers!**
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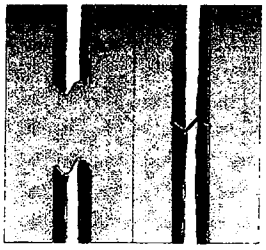
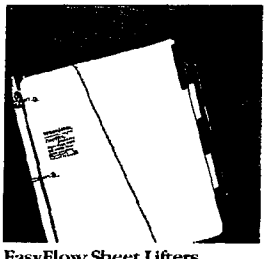
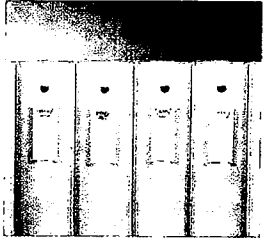


Cure GAPiTIS! DublLock

Sheets won't fall out



EasyFlow Sheet Lifters



Identify and organize

Color coding made easy! Type or write in titles. Tear along perforations and insert into label holders.

WILSON JONES 118468

B007947