THE NEED FOR ACCESS TO RAW DATA USED IN REPORTS AND PUBLICATIONS ~ *QUI TAM,* A RADIOBIOLOGY CASE IN POINT

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Abstract

Recent discussions in the scientific literature have focused on the need for access to raw data that forms the background of scientific publications, reports and grant applications. *Qui tam* is a law suit brought by a "relator" on behalf of the Federal Government charging violation of the False Claims Act. During the "Discovery" phase of such a law suit, documents are exchanged. We had the opportunity to access PDF files containing images of radiobiological experiments, results from which were reported in as many as 8 publications, an R01 grant application, and its renewal. Over 200 experiments performed by 9 individuals in the laboratory in question were analysed. Analysis of over 2500 data entries of 8 individuals were consistent with the expectations of randomness or uniformity using 5 separate statistical tests. Analysis of over 5000 data entries by one individual failed to support the expected null hypothesis for randomness or uniformity. Most of the experiments performed by the questioned individual involved radioisotopes and more than 40 of those experiments involved survival of Chinese hamster V79 cells after incubation with tritiated thymidine. The tritiated thymidine results were of two types: 100% experiments in which all of the cells were exposed to the radionuclide for one cell cycle then incubated in the cold for 3 days, and 50% (bystander) experiments in which the washed, exposed cells were mixed and incubated with an equal number of unexposed cells during the 3 days. The laboratory chief and one post-doctoral fellow were unable to reproduce the questioned fellow's results in 22 attempts to do so, even though they followed exactly the same two protocols. The reported survivals by the questioned fellow were exponential while the survivals in the 22 repeat attempts were all biphasic. The 22 results were all consistent with earlier reports of biphasic survival in the literature for tritiated thymidine in the absence of deoxycytidine, which reverses the known cell cycle blocking effect of tritiated thymidine. The questioned individual's exponential survivals conflicted markedly with those earlier survival reports in which deoxycytidine was absent. Our studies emphasize the need for access to raw data for re-analysis by the scientific population, we hope that ***Photochemistry and Photobiology*** will learn from our experience and archive the raw results that form the background of their publications.

Examples of Triplicate Coulter Counts

These were reported in an experiment by laboratory investigator AB and in an experiment by another laboratory investigator (OI). The terminal digits are shown in bold. The terminal duplicates are shown in red. There are 10 doubles in AB's samples (23.8%) and 4 in the other investigator's samples (9.5%).

Sample #	AB Triplicate Counts			OI Triplicate Counts		
1	57**7**	59**2**	56**3**	8**9**	9**7**	8**6**
2	61**1**	60**7**	65**3**	33**1**	31**6**	32**9**
3	58**1**	59**3**	61**7**	37**8**	33**0**	37**5**
4	63**3**	64**5**	61**9**	33**3**	40**4**	36**7**
5	51**1**	53**7**	54**9**	39**6**	38**2**	40**8**
6	54**4**	56**2**	57**3**	34**2**	33**1**	34**4**
7	66**6**	67**2**	69**3**	34**0**	34**9**	34**4**
8	60**1**	57**2**	63**3**	32**5**	34**7**	30**4**
9	51**1**	52**9**	54**1**	31**5**	29**1**	28**3**
10	53**2**	55**5**	56**2**	30**7**	33**9**	32**3**
11	51**3**	54**9**	56**2**	28**5**	31**4**	32**3**
12	56**2**	53**9**	54**7**	26**0**	26**2**	28**4**
13	56**0**	54**2**	52**2**	36**1**	31**5**	29**8**
14	68**0**	66**9**	67**1**	35**5**	32**4**	35**6**

The Terminal Digit Counts from Above Table and The Chi-Squared Probability of Uniform Distribution

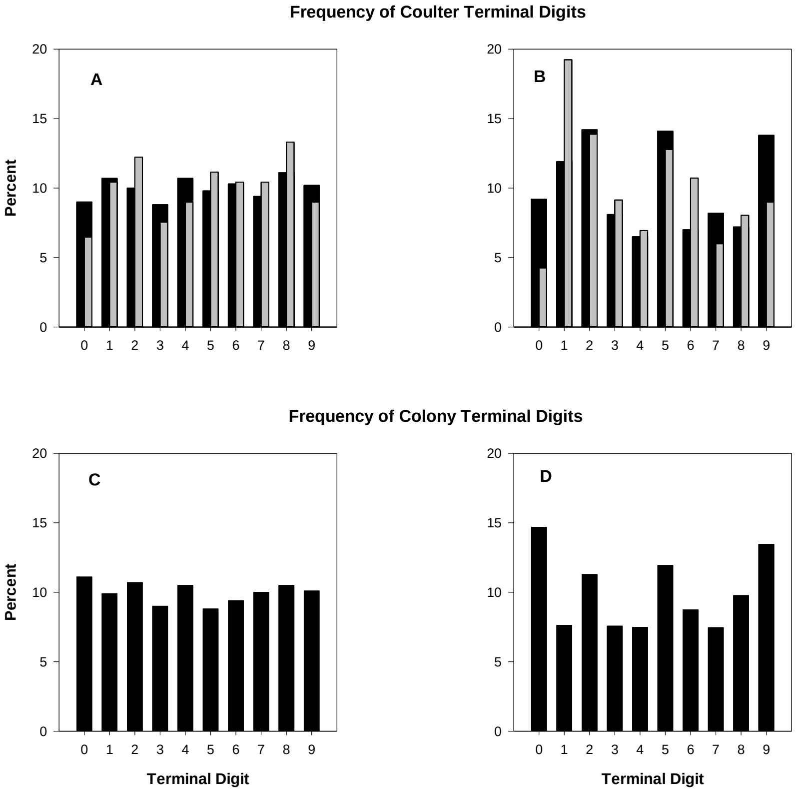
The chi-squared goodness of fit was determined in Microsoft Excel (9 degrees of freedom) for the digit frequencies of AB and OI compared with the control uniform distribution.

Digit	0	1	2	3	4	5	6	7	8	9	Total	Chi Sq	Chi sq p for uniform
AB Freq	2	7	9	8	1	2	2	5	0	6	**42**	**21.8**	**0.0095**
OI Freq	3	4	3	4	7	6	4	4	3	4	**42**	**3.7**	**0.93**
Ctrl Freq	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2	**42**		

Terminal Digit Analysis

The Coulter Counter is a device that counts particles (in this case mammalian cells) as they pass randomly through an orifice. The terminal digits of the counts are expected to be randomly distributed amongst the 10 digits. In Figure A, black bars, the distribution of terminal digits of 2759 Coulter counts recorded by 7 members of the laboratory are shown. The grey bars show the distribution of terminal doubles in this set. Figure B shows similar results for 5155 Coulter counts recorded by post-doc AB.

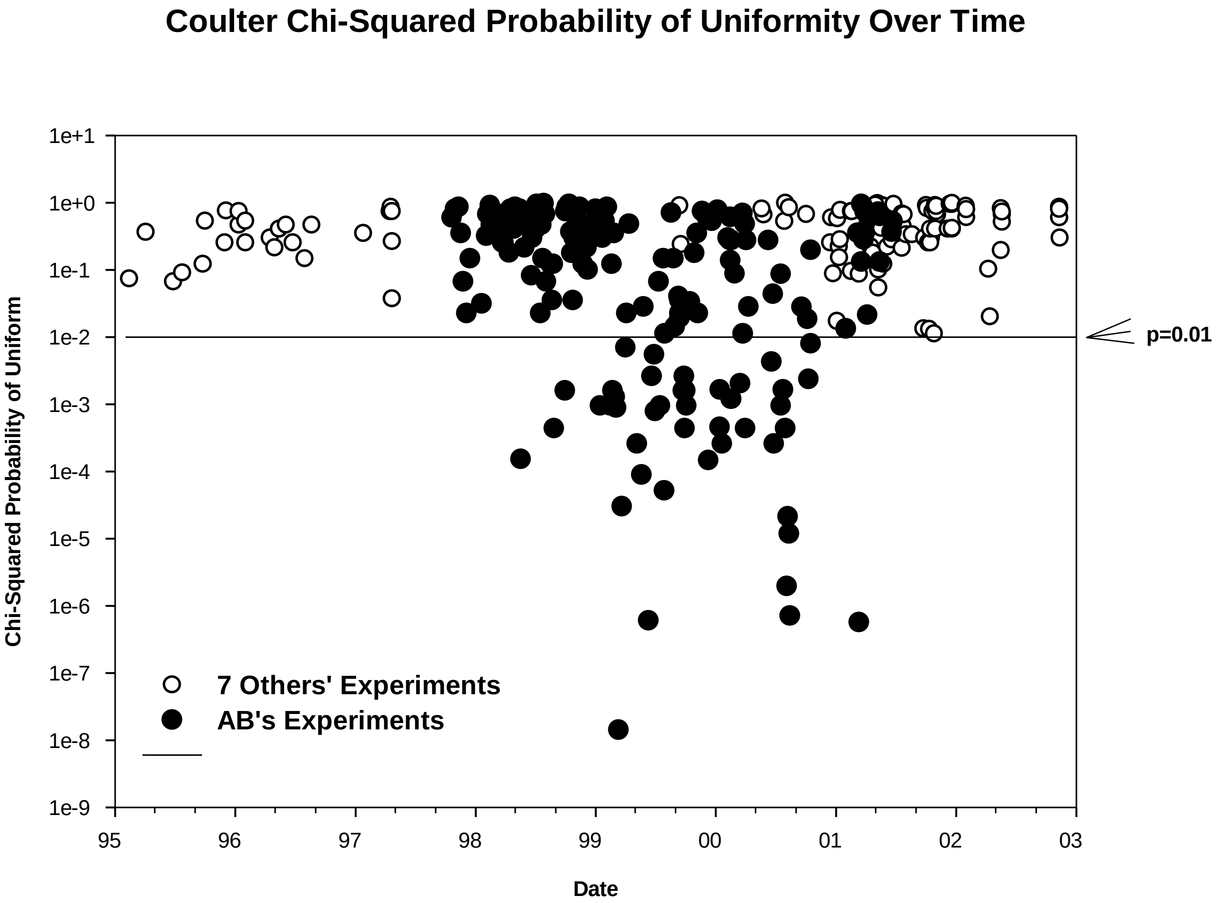
Figure C shows the distribution of the terminal digits of 1556 colony counts recorded by 7 members of the lab. Figure D shows the colony terminal digit distribution for post-doc AB. Note the similarity of the patterns in B and D.



Terminal Digit Analysis of Coulter and Colony Counts

Chi Squared p-values were calculated using the program available in Excel by comparing the actual digit counts to the uniform distribution. The Coulter and colony distributions by others in the lab and from outside are quite likely due to chance. AB’s distributions are extremely unlikely to be due to chance.

		Digit												
Type	**Investigator**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**Total**	**Chi-sq**	**P-value**
Coulter	**AB: 171 experiments**	472	612	730	416	335	725	362	422	370	711	5155	456.4	**1.22x10^{-92}**
Coulter	**AB: terminal doubles**	27	124	88	58	43	81	68	38	52	57	636	113.1	**3.40x10^{-20}**
Coulter	**7 Others: 99 experiments**	249	294	276	244	296	270	284	258	306	282	2759	13.9	**0.13**
Coulter	**7 Others: terminal doubles**	18	29	34	21	25	31	29	29	37	25	278	10.6	**0.30**
Coulter	**Outside lab 1: 11 experiments**	28	34	29	24	27	36	44	33	26	33	314	9.9	**0.36**
Coulter	**Outside lab 2: 17 experiments**	34	38	45	35	32	42	31	35	35	33	360	4.9	**0.84**
Colonies	**AB 114 experiments**	514	267	395	265	262	418	306	261	342	471	3501	228.4	**3.56x10^{-44}**
Colonies	**7 Others: 59 experiments**	173	154	166	140	163	137	147	156	163	157	1556	7.6	**0.57**



Chi squared p-values were calculated over time. AB joined the lab in October, 1997 and left in July, 2001. For 45 experiments the hypothesis of uniformity would be rejected at the 0.01 level, a stringent testing condition. All of the improbable results were seen in experiments performed by AB.

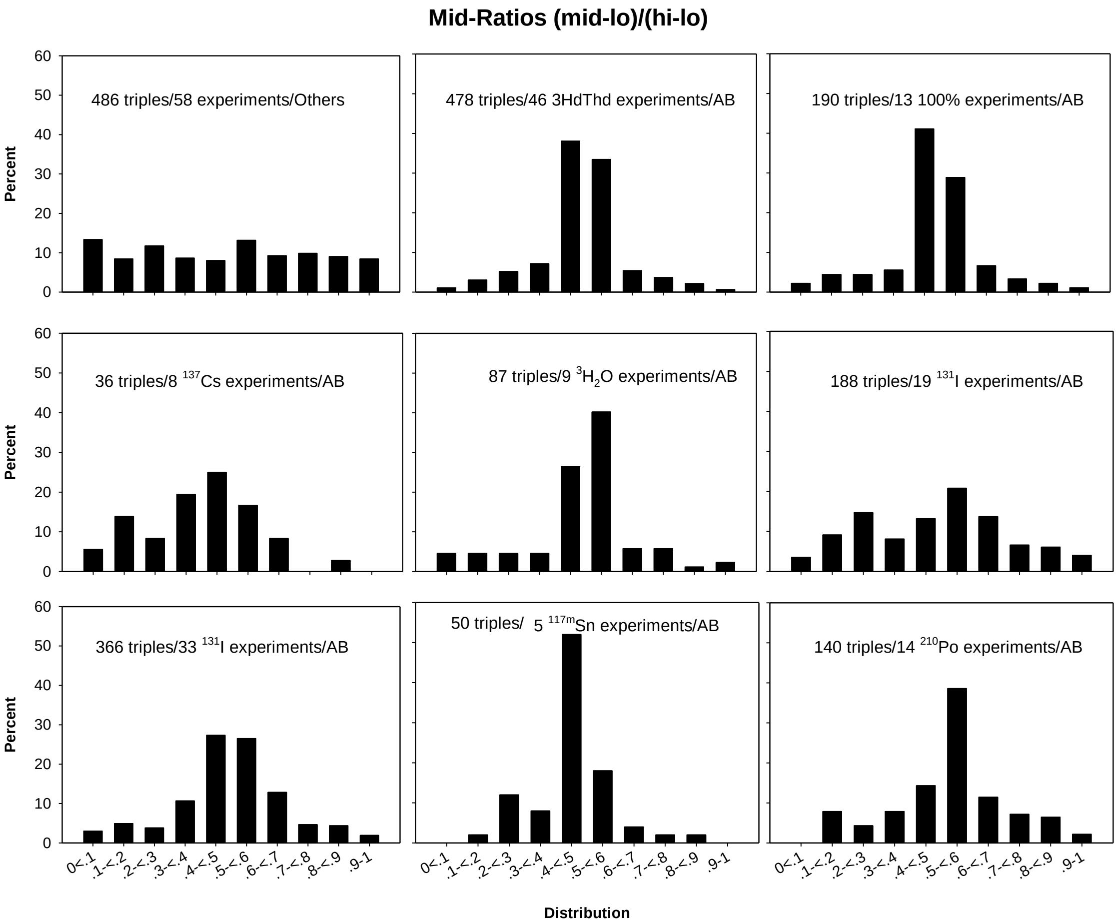
The Average of 3 Colony Counts Appears at an Unusually High Frequency in AB's Results

In these experiments, tissue culture samples were plated in triplicate.

In the colony counts in AB's experiments, the average or middle value appears as one of the triples at an unusually high frequency. This is a graphic representation of that phenomenon.

The Mid-Ratio is the difference between the middle colony count and the lowest colony count divided by the highest count minus the lowest. When one of the counts in the triple is close to the triple average, its mid-ratio will be close to 0.5

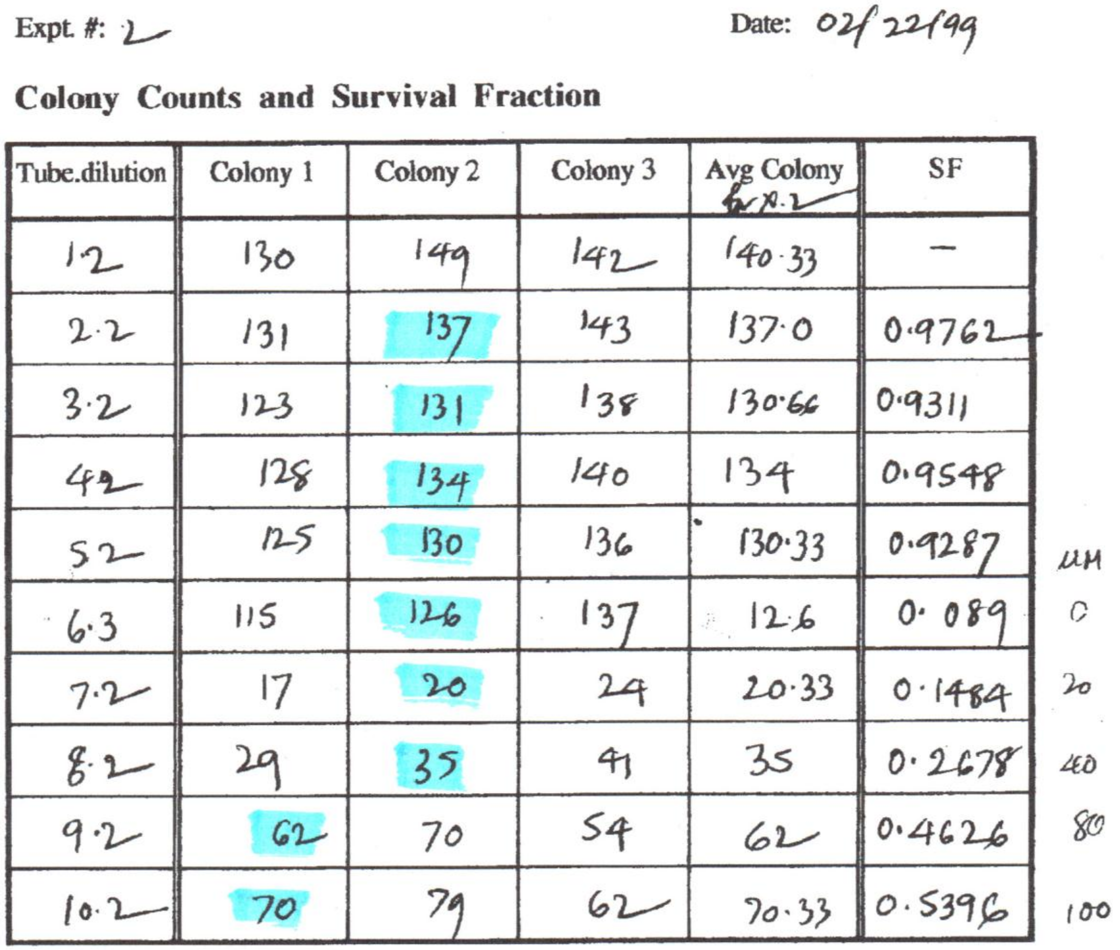
In the triples of others in the lab, there is a fairly uniform distribution of the mid-ratios throughout the various classes ~ top left-hand graph.



In AB's experiments, the mid-ratios cluster close to 0.5

Colony Counts ~ PDF Copy from AB's Notebook

The average appears as one of the triplicate counts in 9 of the 10 samples (high-lighted in aquamarine). SF stands for Surviving Fraction, assumed to be 1.00 in Sample 1.2



Expt. #: 2

Date: 02/22/99

Colony Counts and Survival Fraction

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony for X.2	SF	
1·2	130	149	142	140·33	–	
2·2	131	137	143	137·0	0·9762	
3·2	123	131	138	130·66	0·9311	
42	128	134	140	134	0·9548	
5·2	125	130	136	130·33	0·9287	µM
6·3	115	126	137	12·6	0· 089	0
7·2	17	20	24	20·33	0·1484	20
8·2	29	35	41	35	0·2678	40
9·2	62	70	54	62	0·4626	80
10·2	70	79	62	70·33	0·5396	100

Appearance of the Rounded Average in Some of the Triple Colony Counts

Sample #	AB Triplicate Counts			Average	Mid-ratio (b-a)/(c-a)	OI Triplicate Counts			Average	Mid-ratio (b-a)/(c-a)
1	130	149	142	140.33	0.63	92	111	119	107.33	0.70
2	131	**137**	143	137	0.5	78	85	74	79	0.36
3	123	**131**	138	130.66	0.53	142	126	120	129.33	0.27
4	128	**134**	140	134	0.5	120	129	121	123.33	0.11
5	125	**130**	136	130.33	0.45	64	68	79	70.33	0.27
6	115	**126**	137	126	0.5	92	101	78	90.33	0.61
7	17	**20**	24	20.33	0.43	74	62	94	76.67	0.38
8	29	**35**	41	35	0.5	89	69	67	75	0.091
9	**62**	70	54	62	0.5	85	87	97	89.67	0.17
10	**70**	79	62	70.33	0.47	71	58	55	61.33	0.19

This table presents two experiments, one performed by AB (raw data are shown in Poster #8), the other by another investigator (OI) in the lab. The rounded average, shown in aquamarine, appears in 9 of AB's 10 samples, but does not appear at all in the OI's samples.

Statistical Analysis Of The Number Of Triples That Contain Their Rounded Mean

We have developed a method to estimate the likelihood that a given number of triples in a collection contain their own rounded mean. To do so, we needed to evaluate the probability that a given triple contains its own rounded mean. It seems reasonable that this probability varies with the size of the gap, i.e., the difference between the largest and smallest colony counts of the triple, e.g., c - a. However, this probability depends on whether the gap is even or the gap is odd. If the value of the gap is even, there will only be one middle value that could complete it as a triple containing its own mean, if the gap is odd there will be two middle values. For example, for colony counts with a =10 and c =20, the gap is 10 (even), and the only possible middle value that would create a triple that contains its mean is 15. The rounded mean of the triples 10, 14, 20 (mean=14.7), 10, 15, 20 (mean=15) and 10, 16, 20 (mean=15.3) is 15 for all three triples. However, only (10, 15, 20) actually contains the rounded mean. For colony counts with a = 10 and c = 21, the gap is 11 (odd), and there are 2 middle values: 15 and 16. The mean of the triple 10, 15, 21 is 15.33, which rounds (down) to 15, whereas the mean of 10, 16, 21 is 15.67, which rounds (up) to 16.

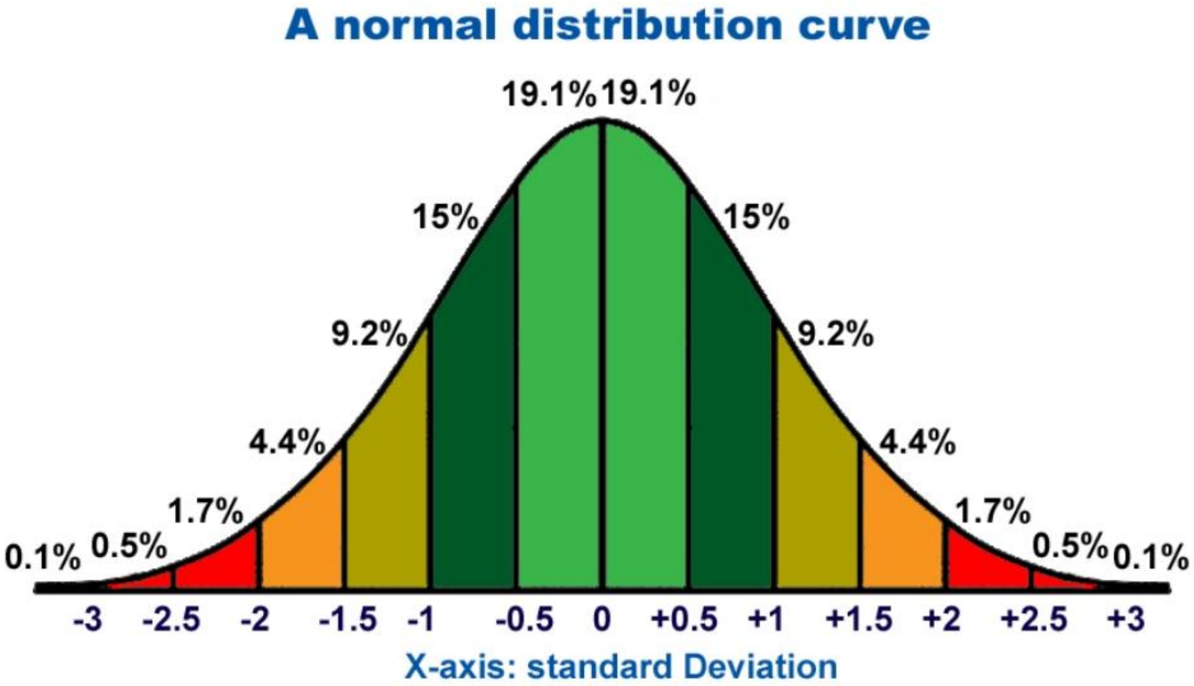
We can now calculate the probability of hitting the mean by chance for any given gap.

If the gap is even, the probability of hitting the mean is 1/(1 + c - a). If the gap is odd, the probability of hitting the mean is 2/(1 + c – a).

Demonstration of Z-Scores

Z-Score

“a standard score [that] indicates how many standard deviations an observation or datum is above or below the mean.” *Wikipedia*



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Example Calculations to Determine the Expectation of the Number of Rounded Means in a Given Set of Gaps and the Probability Calculated from the Z-Score that the Actual Number Could be Contained in That Set

This table illustrates the calculation of the Z-score for the 2 experiments depicted in the preceding table on Poster # 9. The values in column 4 were calculated for even gaps using the expression 1/(1+gap) and for odd gaps 2/(1+gap). The variances in column 5 were calculated from the values in column 4: variance =

(col 4 value)*(1-col 4 value).

The Z-score is the actual number minus the expected number divided by the expected standard deviation.

	Sample #	Gap (c-a)	Expected Number Rounded Means in Triples	Variances of Expected	Standard Deviation	Actual Number of Rounded Means in Triples	Z-Score for Actual Number	Probability of Z-Score
AB	**1**	19	0.100	0.090				
	2	12	0.077	0.071				
	3	15	0.125	0.109				
	4	12	0.077	0.071				
	5	11	0.167	0.139				
	6	22	0.043	0.042				
	7	7	0.250	0.188				
	8	12	0.077	0.071				
	9	16	0.059	0.055				
	10	17	0.111	0.099				
	Sum		**1.086**	**0.934**	**0.967**	**9**	**8.19**	**<1e-9**
OI	**1**	27	0.071	0.066				
	2	11	0.167	0.139				
	3	22	0.043	0.042				
	4	9	0.200	0.160				
	5	15	0.125	0.109				
	6	23	0.083	0.076				
	7	20	0.048	0.045				
	8	22	0.043	0.042				
	9	12	0.077	0.071				
	10	16	0.059	0.055				
	Sum		**0.917**	**0.806**	**0.898**	**0**	**-1.02**	**0.154**

Z-Scores and Probabilities That Triples Contain Their Rounded Means Grouped by Isotope Experiments

Investigator	Isotope	# Experiments	# Triples	Expect # Triples Containing Rounded Means	Actual # Triples Containing Rounded Means	Z-Score for Actual Number	Probability of Z-Score or Higher
AB	^{117m}Sn	5	50	6.1	28	9.7	<< 1×10^{-9}
AB	^{210}Po	14	140	19.7	67	12.0	<< 1×10^{-9}
AB	$^{3}H_2O$	9	90	13.2	28	4.8	1×10^{-6}
AB	^{137}Cs External Beam	4	36	4.8	14	4.8	1×10^{-6}
AB	^{125}I	33	375	67.9	174	18.4	<<< 1×10^{-9}
AB	^{131}I	20	198	33.6	61	5.6	1×10^{-8}
AB	^{3}HdThd	44	478	65.0	301	35.1	<<<< 1×10^{-9}
OI	Various	59	534	86.4	95	1.2	0.123

Many of the survival experiments we examined involved compounds that contained different isotopes. In AB's hands, the actual number of triples that contain their rounded means far exceeds expectations based on expectations of randomness and independence. For other investigators (OI) using the same methods as AB, the frequency of rounded means is within the predicted expectation

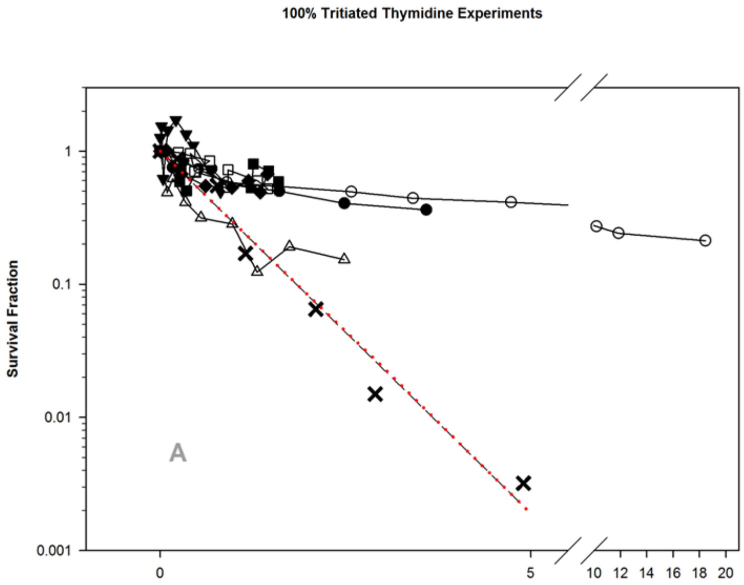
AB's ^{3}HdThd Survival Results Could Not be Replicated

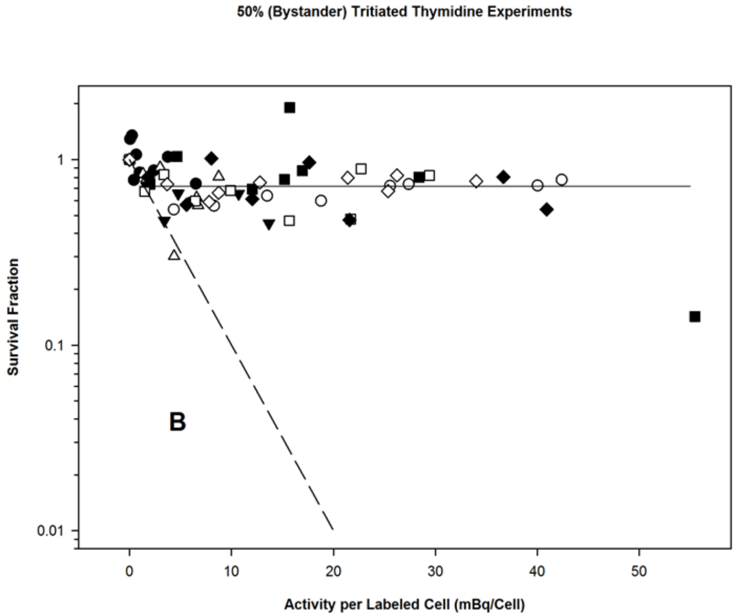
The exponential survival s of V79 cells incubated with ^{3}HdThd reported in 2 publications could not be reproduced in 22 attempts to do so. In A, 100% of the cells were incubated with ^{3}HdThd. The dashed line and X's represents results published in the two papers based on experiments performed by AB. The red line is the theoretical survival based on radiobiological principles. Ten attempts (symbols) to repeat AB's results were made by the PI and a second post-doctoral fellow.

Graph B shows 12 experimental attempts to repeat the bystander (50% experiments) results of AB (dashed line from the two papers). In these experiments, radioactive cells were incubated with non-radioactive cells.

AB's results would argue for a bystander effect, the 12 experiments by the PI and a second post-doc (symbols) would argue against such an effect, at least under these conditions.

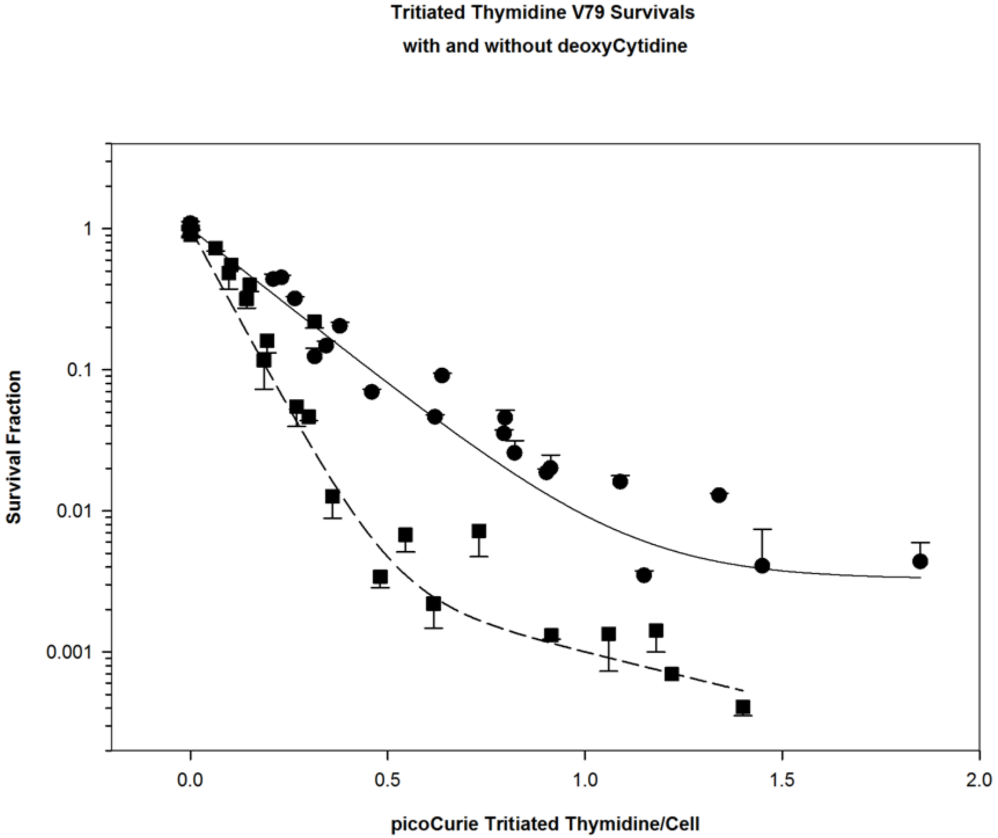
The results of these attempts to replicate are consistent with radiobiological literature that demonstrates ^{3}HdThd blocks the cell cycle unless deoxycytidine is present, which it was not in any of these experiments.





Deoxycytidine Reverses the Cell Cycle Blocking Effect of ^{3}HdThd: No deoxycytidine was present in the experiments described in the preceding posters

These are 6 experiments performed by another investigator in the lab – 3 with deoxycytidine (squares) and 3 without (circles) that demonstrate the reversal by deoxycytidine of the cell cycle blocking effect of ^{3}HdThd. No deoxycytidine was present in any of the other experiments described in these posters so AB's survival results are expected to be biphasic – but they are not.



Summary

- The analysis reported here was only made possible because all the notebooks in the laboratory in question were made available through *subpoena*. Ordinarily, the raw data that underlies experiments is opaque to all but a few. We believe this was a rare and unique opportunity.
- We were fortunate to be able to compare data produced by several other individuals using the same instrument and techniques to that of a single individual, whose results alone could not be explained based on assumptions of randomness, uniformity or chance.
- We used the following tests, all of which are simple and could be put to use in any laboratory
  - Terminal digits of Coulter counts: AB's digit distributions diverged markedly from uniform, while others distributions were consistent with the assumption of uniformity*
  - Terminal digits of colony counts: AB's distributions were highly unlikely to be uniform or random, whereas others' colony terminal digit distributions are likely to have come about by chance*
  - Double terminal digits in the Coulter counts: AB's doubles are not likely to be due to chance, others' doubles are close to the expected 10%
  - Presence of the average as one of the triples in colony counts: AB's results had an inordinately high frequency of the rounded average occurring as one of the triplicate counts, results of others were consistent with the predicted frequency based on gap size
- The statistical analyses are bolstered by the inability to replicate AB's radiobiological results. The differentials in survivals are astounding – about 1000 fold in the 100% experiments, about 100 fold in the 50% (bystander) experiments

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* Terminal digit analysis is recommended as a forensic tool on the USPHS Office of Research Integrity WebSite.

Conclusions

- The results of the statistical analyses reported here argue strongly for making raw data used in the production of scientific papers, research reports and grant applications available to all researchers, reviewers and granting agencies
- Our analyses also argue for sharing such raw data with other researchers in the field in order for them to understand their own, possibly unexpected, experimental results and/or allowing them to avoid performing unnecessary experiments
- The experiments involved in these studies were designed to alleviate a vexing problem in Nuclear Medicine – that of the non-uniform distribution of radio-isotopes in the human body. These results were planned to be used in setting standards for allowable exposures for healthcare workers and to determine isotope doses to be used for diagnostic and therapeutic procedures
- Miscalculations based on these results could have serious consequences for patients and workers in nuclear medicine: over-estimating doses could lead to tissue and organ damage and even to cancer; under-estimating doses could lead to misdiagnoses and lack of therapeutic efficacy

The data here analyzed were involved in the following publications:

Bishayee, A., H. Z. Hill, et al. (2001). "Free radical-initiated and gap junction-mediated bystander effect due to nonuniform distribution of incorporated radioactivity in a three-dimensional tissue culture model." Radiat Res **155**(2): 335-344.

Bishayee, A., D. V. Rao, et al. (2000). "Protection by DMSO against cell death caused by intracellularly localized iodine-125, iodine-131 and polonium-210." Radiat Res **153**(4): 416-427.

Bishayee, A., D. V. Rao, et al. (1999). "Evidence for pronounced bystander effects caused by nonuniform distributions of radioactivity using a novel three-dimensional tissue culture model." Radiat Res **152**(1): 88-97.

Bishayee, A., D. V. Rao, et al. (2000). "Radiation protection by cysteamine against the lethal effects of intracellularly localized Auger electron, alpha- and beta-particle emitting radionuclides." Acta Oncol **39**(6): 713-720.

Bishayee, A., D. V. Rao, et al. (2000). "Marrow-sparing effects of 117mSn(4+)diethylenetriaminepentaacetic acid for radionuclide therapy of bone cancer." J Nucl Med **41**(12): 2043-2050.

Goddu, S. M., A. Bishayee, et al. (2000). "Marrow toxicity of 33P-versus 32P-orthophosphate: implications for therapy of bone pain and bone metastases." J Nucl Med **41**(5): 941-951.

Howell, R. W. and A. Bishayee (2002). "Bystander effects caused by nonuniform distributions of DNA-incorporated (125)I." Micron **33**(2): 127-132.

Howell, R. W., S. M. Goddu, et al. (1998). "Radioprotection against lethal damage caused by chronic irradiation with radionuclides in vitro." Radiat Res **150**(4): 391-399.

Howell, R. W. (2000-2006). 1R01CA083838-01A1 Effects of Nonuniform Distributions of Radioactivity.

Howell, R. W. (2006-2011). "1R01CA083838-06A1 Effects of Nonuniform Distributions of Radioactivity ".