

Determination of Statistical Precision of Tritium d.p.m. in Dual Labelled Samples with Variable Isotope Ratios and Quenching

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INTRODUCTION

The statistics of liquid scintillation counting are a function of numerous parameters.¹ In dual isotope counting the principal ones are the isotope ratio and the degree of quenching. These two parameters affect statistics simultaneously. The quantitation of statistics pertaining to the activity of the weaker isotope takes on a special significance when the isotope ratio and quenching vary simultaneously for a given sample series. Many authors^{2,3} have investigated experimental series of dual isotope samples (tritium, carbon-14), allowing the isotope ratio to vary while keeping quenching at a constant level and *vice versa*. This type of study permits the definition of a lower isotope ratio limit below which the weaker isotope may not be counted under statistically significant conditions.

In this communication we have set as our goal the automatic estimation, using a computer, of the relative standard deviation (RSD) of tritium activity in d.p.m. of a series of dual isotope samples (tritium, carbon-14), where isotope ratio and quenching vary simultaneously. The method of estimation is based on the implicit relationship between the RSD of the tritium activity, the count rate (c.p.m.) ratio in the two counting channels A and B, quenching and the counting statistics of channels A and B. This relationship may be estimated graphically while the computer program permits us to estimate in particular the RSD of computed tritium activity of each sample by processing data furnished by a liquid scintillation counter.

The method finds application in the fields of cellular incorporation where two labelled precursors are used simultaneously (thymidine, uridine, etc.). In fact, in these cases the isotope ratio is not known *a priori* and one may observe important variations in quenching from one sample to the next.⁴

MATERIALS AND METHODS

Materials

The following materials were used in our investigations:

1. SL 40 liquid scintillation counter[†]

* Attaché de Recherches.

† Intertechnique, 78 Plaisir, France.

2. PDP-10 computer*
3. Labelled toluene -tritium 2.58×10^6 d.p.m./mg
carbon-14 5.95×10^5 d.p.m./mg
4. Carbon tetrachloride

Methods

Principle of estimating the RSD of tritium activity. The relative standard deviation (RSD) of tritium d.p.m. in the case of dual isotope counting of tritium/carbon-14 by means of screening tritium from the carbon-14 channel is given by the following equation:

$$d.p.m.^3H = \frac{(c.p.m.A - BA) - (c.p.m.B - BB)(E[^{14}C(^3H)]/E[^{14}C])}{E[^3H]}$$

$$\sigma_R [d.p.m.^3H] = \frac{\sigma [d.p.m.^3H]}{d.p.m.^3H} = \frac{\sqrt{\sigma_{RA}^2 \cdot c.p.m.A^2 + \sigma_{BA}^2 BA^2 + (E[^{14}C(^3H)]/E[^{14}C])^2 \cdot (\sigma_{RB}^2 \cdot c.p.m.B^2 + \sigma_{BB}^2 \cdot BB^2)}}{c.p.m.^3H} \quad (1)$$

where:

- $E[^3H]$ = tritium efficiency in channel A
- $E[^{14}C]$ = carbon-14 efficiency in channel B
- $E[^{14}C(^3H)]$ = carbon-14 efficiency in channel A
- σ_{RA} = relative standard deviation of counts per minute (c.p.m.A) in channel A
- σ_{RB} = relative standard deviation of counts per minute (c.p.m.B) in channel B
- σ_{BA} = relative standard deviation of background in channel A
- σ_{BB} = relative standard deviation of background in channel B
- BA = background in channel A
- BB = background in channel B

In Eqn. (1) each factor is known for a given sample, but it is not possible to calculate the value $\sigma_R [^3H]$ d.p.m. from a single value of tritium c.p.m. The count rates in channels A and B express both the activity of each isotope and quenching; we have taken the count ratio A/B as a computation parameter.

To estimate the $\sigma_R [^3H]$ d.p.m. of a dual isotope sample counted once, using Eqn. (1), different values of activity for each isotope are generated to obtain the ratio A/B identical to that of the sample. From then on using Eqn. (1) the $\sigma_R [^3H]$ d.p.m. may be estimated taking into account the sample characteristics ($E[^3H]$, $E[^{14}C]$, $E[^{14}C(^3H)]$), σ_{RA} , σ_{RB} , σ_{BA} , σ_{BB}) and the values of the activity of the two isotopes corresponding to an A/B ratio identical to the sample under study. Thus, the $\sigma_R [^3H]$ d.p.m. estimation is not connected to the computed absolute values of the activity of the two isotopes.

Figure 1 traces the identification process and the calculations. For example, if a tritium d.p.m. value is fixed at 10000, taking account of the efficiencies for each sample measured, the value Y_i (carbon-14 d.p.m.) is found which gives a ratio A/B identical to the sample measured. With the statistical conditions of the sample measured, $\sigma_R [^3H]$ d.p.m. is computed using Eqn. (1).

* Digital Equipment Corporation, Maynard, Massachusetts 01754, U.S.A.

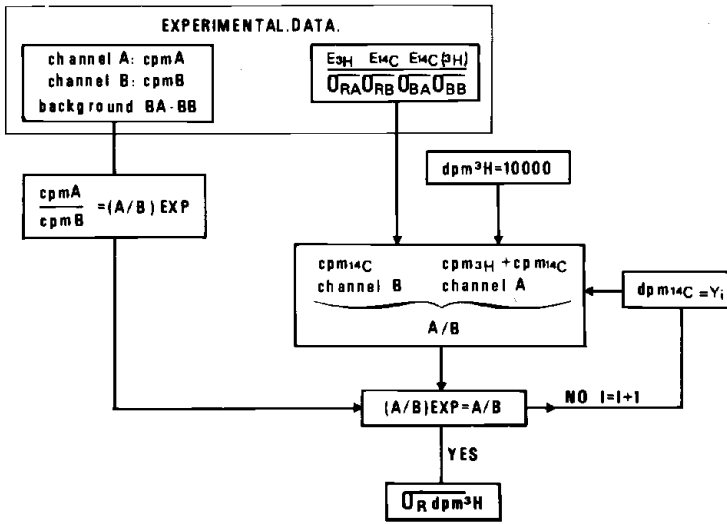


Fig. 1. Iterative calculations for RSD of tritium d.p.m.

Fortran IV program. We have written a Fortran program for computing the RSD of tritium d.p.m. for a series of dual isotope samples. Counting data is provided by the liquid scintillation counter on punched tape while the same tape provides the external standard ratio, E_1/E_2 , from which counting efficiencies are computed. Quench curves (efficiencies versus E_1/E_2) are entered in the computer in the form of a third order polynomial of which the coefficients have been previously established by the method of least squares.

The computing process is shown by Fig. 1 while Fig. 2 illustrates the general flow chart of the program.

The equivalence of the experimental and computed ratios c.p.m.A/c.p.m.B is fixed within an interval of $\pm 0.001 \times (\text{c.p.m.A/c.p.m.B})$ (experimental). This value of 0.001 is that which experimentally corresponds to a minimum identification interval of two (c.p.m.A/c.p.m.B) values and computed, taking into account the slow rate of increase of the value of DHC - (DHC = $^3\text{H d.p.m./}^{14}\text{C d.p.m.}$). In order to reduce the number of iterations, an approximate value of the DHC ratio is calculated from which the iterative process starts.

The computer calculates the RSD of tritium d.p.m. and carbon-14 d.p.m. as well as the ratio $^3\text{H d.p.m./}^{14}\text{C d.p.m.}$

Verification of results. We have experimentally checked the graphs and the results obtained with the Fortran program by repetitive counting of a series of samples with different isotope ratios and quench levels. The calculation of the true RSD based on several measurements has permitted us to evaluate the estimate which we make of $\sigma_R [^3\text{H}]$ d.p.m. from a single measurement. Samples were measured thirty times for each quench level and were prepared according to Table 1. They were quenched progressively with carbon tetrachloride so as to give the efficiencies shown in Table 2.

RESULTS

We established for different quench levels the curves relating the tritium d.p.m. RSD

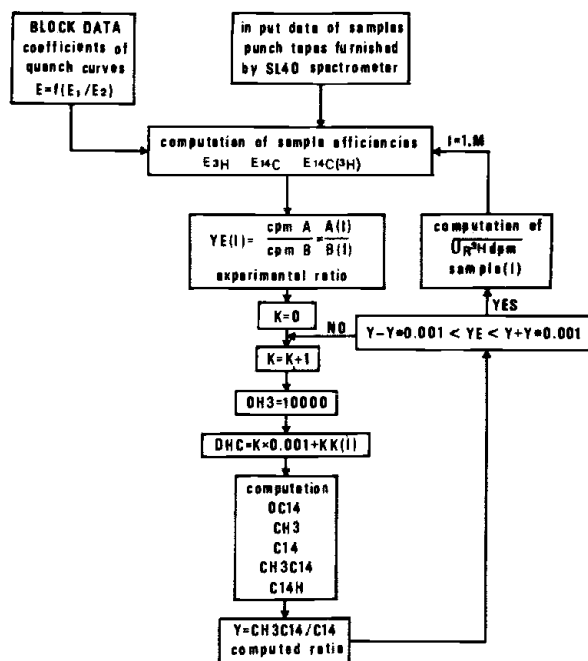


Fig. 2. Flow chart of Fortran IV program.

M number of samples . DHC = DH3/DC14 = ³H d.p.m./¹⁴C d.p.m.

KK: initial value of DHC for the sample (I) compute as a function of YE, E³H, E¹⁴C, E¹⁴C(³H).

c.p.m.A = CH3C14 = C14H + CH3 . c.p.m.B = C14.

Y = c.p.m.A/c.p.m.B (computed).

Table 1. Preparation of experimental samples (tritium, carbon-14).

Number	Toluene standard ³ H (μ l)	Toluene standard ¹⁴ C (μ l)	d.p.m. ³ H/d.p.m. ¹⁴ C
1	0	100	0
2	10	800	0.046
3	10	400	0.101
4	100	800	0.536
5	100	400	1.011
6	100	200	2.066
7	100	100	4.122
8	100	50	7.939
9	100	20	21.899
10	100	0	∞

to the (³H d.p.m./¹⁴C d.p.m.) ratio (see Fig. 3) and to the channels count rate ratio A/B (see Fig. 4). The curves take two parameters into account, quenching and activity ratio. When quench ranges are very wide and the statistical counting conditions of the samples are different, the curves are not useful.

Table 2. Quench and efficiency values of each isotope for the sample series prepared according to Table 1.

E_1/E_2	$E^3\text{H}$	$E^{14}\text{C}$	$E^{14}\text{C}(^3\text{H})$
5.20	0.46	0.68	0.17
3.85	0.34	0.575	0.26
2.91	0.24	0.42	0.34
1.94	0.135	0.175	0.52

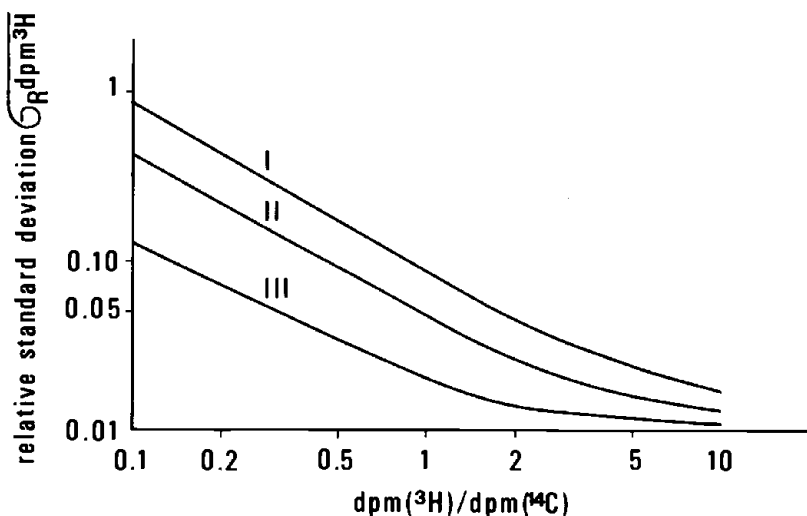


Fig. 3. Relative standard deviation σ_R [d.p.m.³H] as a function of ³H d.p.m./¹⁴C d.p.m. The relative standard deviation of c.p.m. in the channels A and B are equal at 1% ($\sigma_{RA} = \sigma_{RB} = 1\%$). Curve I, quenching $E_1/E_2 = 1.6$; curve II, quenching $E_1/E_2 = 2.23$; curve III, quenching $E_1/E_2 = 3.5$.

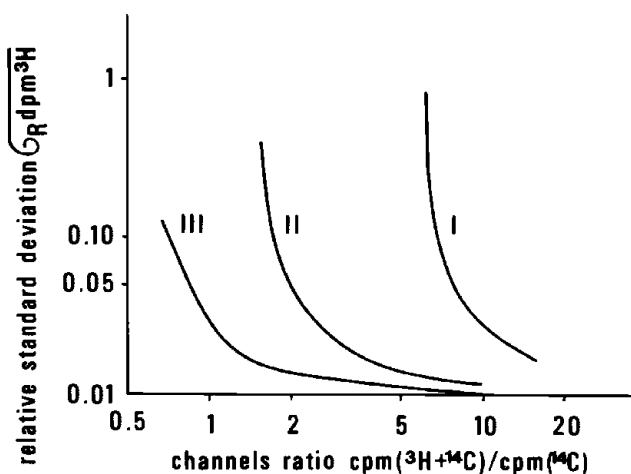


Fig. 4. Relative standard deviation σ_R [d.p.m.³H] as a function of ³H c.p.m./¹⁴C c.p.m. The computation parameters are as those of Fig. 3.

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*****
* N * T * CPH A * RA * CPH B * RB * E1/E2
* * * DPH A * RSD * DPH B * RSD * DPH A/DPH B
*****
* 1 * 0.12* 81975.4 * 1.0* 14245.9 * 2.4* 1.79000
* * * 212526.6 * 6.3* 104225.2 * 2.4* 2.039
*****
* 2 * 0.16* 62317.7 * 1.0* 116971.9 * 0.7* 3.83000
* * * 26484.9 * 8.2* 205908.3 * 0.7* 0.129
*****
* 3 * 0.08* 116880.8 * 1.0* 251647.1 * 0.6* 4.02300
* * * 33399.6 * 11.4* 426472.3 * 0.6* 0.078
*****
* 4 * 0.10* 99830.3 * 1.0* 61207.5 * 1.2* 3.88600
* * * 212178.4 * 1.4* 106506.9 * 1.2* 1.992
*****
* 5 * 0.09* 117948.1 * 1.0* 21816.0 * 2.3* 1.55900
* * * 29370.1 * 87.1* 197787.8 * 2.3* 0.148
*****
    
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Fig. 5. Example of the computer print out for different dual-labelled samples (tritium, carbon-14).

Table 3. Comparison of relative standard deviation σ_R d.p.m. tritium between experimental and calculated values for different isotope ratios and quenching. E = experimental values; C = computed values. $\sigma_{RA} > 0.6\%$; $\sigma_{RB} > 0.6\%$.

No.	$\frac{\text{d.p.m. } ^3\text{H}}{\text{d.p.m. } ^{14}\text{C}}$	σ_R d.p.m. tritium (%)							
		$E_1/E_2 = 5.20$		$E_1/E_2 = 3.85$		$E_1/E_2 = 2.91$		$E_1/E_2 = 1.94$	
		E	C	E	C	E	C	E	C
1	0	78.0	83.0	86.0	873.0	73.2	431.0	—	—
2	0.046	16.4	15.1	18.2	17.0	23.5	31.0	—	—
3	0.101	7.1	6.5	10.7	9.2	13.8	17.2	30.0	87.0
4	0.536	2.4	2.0	2.4	2.5	7.5	5.9	14.5	12.5
5	1.011	1.9	1.6	2.21	1.9	2.4	2.3	9.3	8.0
6	2.066	1.5	1.3	1.5	1.4	1.6	1.3	6.4	6.4
7	4.122	1.2	1.1	1.4	1.2	1.4	1.2	2.3	2.5
8	7.939	1.1	1.0	1.2	1.1	1.2	1.1	1.1	1.1
9	21.899	1.1	1.0	1.1	1.0	1.17	1.0	1.1	1.0
10	∞	1.1	1.0	1.1	1.0	1.14	1.0	1.1	1.0

We have written a computer program to obtain an estimate for each sample of the relative standard deviation of the net count rate of the weaker isotope. Figure 5 shows an example of the print out for different dual-labelled samples (tritium, carbon-14).

We have checked the results of the program for estimating statistical error by a series of samples defined previously and counted repetitively to obtain the true relative standard deviation of computed tritium d.p.m. Table 3 gives the results obtained for various (^3H d.p.m./ ^{14}C d.p.m.) activity ratios and quench levels where tritium efficiencies vary from 0.135 to 0.46.

Measurements made where σ_{RA} and σ_{RB} were less than 0.006 (gross count rate error in channels A and B) resulted in a computed RSD lower than the experimental values obtained in repeat measurements. The experimental RSD includes the total of statistical fluctuations while the computed value includes only the σ_{RA} and σ_{RB} as counting con-

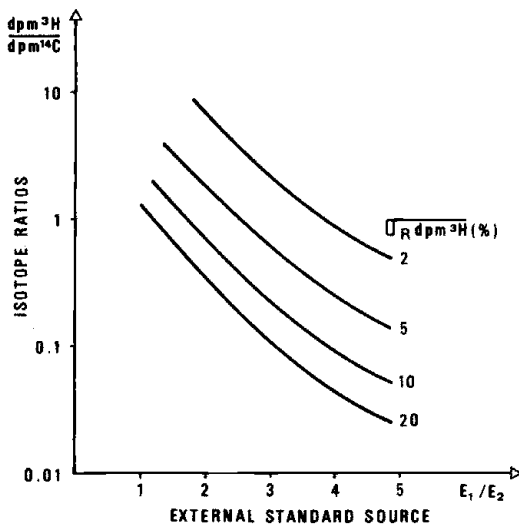


Fig. 6. Isotope ratios ^3H d.p.m./ ^{14}C d.p.m. as a function of quenching for different values of σ_R [d.p.m. ^3H]. $\sigma_{RA} = 1\%$; $\sigma_{RB} = 0.6\%$.

ditions. We have observed that for values of σ_{RA} and σ_{RB} greater than 0.6%, the experimental and computed results for σ_R [^3H] d.p.m. are identical.

Our results permit the construction of a curve network relating the isotope ratio (^3H d.p.m./ ^{14}C d.p.m.) to quenching (E_1/E_2 - count rate ratio of external standard) for different values of σ_R [^3H] d.p.m. (see Fig. 6). While it is not imperative to accompany each measurement by the tritium d.p.m. RSD value, one may use the curves to distinguish between samples having an σ_R [^3H] d.p.m. greater or less than a given value.

DISCUSSION

The estimate of tritium d.p.m. RSD made by the computer does not take into account the quality of quench curve fitting. In practice the fits are performed with a precision of 1% and may certainly be neglected within the framework of our σ_R [^3H] d.p.m. estimates. However, we have verified the accuracy of the estimate as regards variations in values produced by the computer for repetitively counted samples (Fig. 7). The estimate of σ_R [^3H] d.p.m. based on a single measurement remains constant up to a value of about 20%. Above this value, excessive fluctuation occurs. This phenomenon is not a problem when results of tritium d.p.m. obtained with a statistical error much above 20% must be reconsidered.

The experimental results which we have obtained agree with those found in the literature.⁵ Bush² has determined optimum counting conditions to obtain minimal statistical error in dual isotope counting. With large series of samples one is obliged to adopt fixed isotope window settings. Thereafter it becomes necessary to evaluate the statistical precision of the measurement of each sample. This procedure is particularly useful for experiments involving cellular incorporation but also when the order of magnitude of the isotope activities is unknown or when the quench range is very wide. Such is the case when counting plasma dissolved directly in liquid scintillator with the aid of emulsifiers.⁶

In conclusion, the estimate of the relative standard deviation of the activity of the

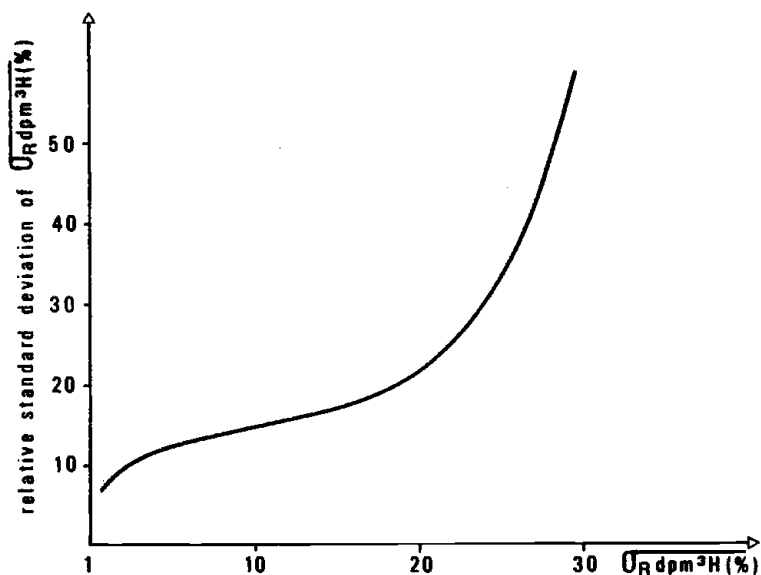


Fig. 7. Relative standard deviation of σ_R [d.p.m.³H] as a function of σ_R [d.p.m.³H].

weaker isotope in each dual isotope sample facilitates and increases the reliability of the dual isotope technique using liquid scintillation counting when activity ratios and quenching vary widely:

(Copy of the program written in Fortran IV is available through the research laboratory INSERM U.90, Hôpital Necker, Paris).

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DISCUSSION

H. E. Barber: Your formula shows all radioactivity counts as c.p.m. Did the calculation of standard deviation of tritium operate on c.p.m. or on total counts, conversion to c.p.m. being made at the end of the statistical calculation thus taking advantage of any prolonged

counting time on the precision of the estimation?

J. Assailly: Of course, the relative standard deviation of c.p.m. or total counts is the same. In the formula we have expressed the relative standard deviation of d.p.m. tritium activity, and we can simplify the denominator by replacing $E[{}^3\text{H}]$ d.p.m. tritium with c.p.m. tritium. In this formula, all the factors are experimental data. But, of course we cannot calculate the $\sigma_{\text{R}}[{}^3\text{H}]$ d.p.m. by means of a single measurement, it is the estimation program which performs this.

F. E. L. ten Haaf: I could not follow your explanation of your last slide (Fig. 7). Can you explain this again?

J. Assailly: This slide shows the variations of the relative standard deviation of $\sigma_{\text{R}}[{}^3\text{H}]$ d.p.m. as a function of $\sigma_{\text{R}}[{}^3\text{H}]$ d.p.m. In fact this curve is a graphical picture of the stability of our estimation program of the d.p.m. tritium precision.