

Chapter 26

Liquid Scintillation Counting of Low-level ^{14}C

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INTRODUCTION

The requirements in radiocarbon dating of an overall precision of ± 80 years or less means that the whole process through sample pretreatment, synthesis and counting must be reproducible to better than $\pm 1\%$. To achieve this, every attempt must be made to minimize the portion attributable to the sample count which, unavoidably, is only accumulated from counting rates in the range of 100 cpm (maximum) to the lowest background that can be obtained from the measurement instrument.

Several papers have been published in recent years describing improvements in instrumentation for low-level liquid scintillation (LS) counting. Hartley *et al.*¹ and Noakes *et al.*² described new designs of LS counters specifically for ^3T and ^{14}C low-level applications. The performances are impressive but as research instruments they are not, as far as is known, available commercially.

At the last LS conference two years ago Noakes³ reviewed the requirements of low-level counting by LS methods and discussed modifications to equipment and techniques which enable high performance to be obtained from standard commercial equipment.

Despite what has been written, however, the setting up from scratch of a low-level LS counting system for radiocarbon dating applications is still a demanding task. It is the intention of this paper to describe a number of practical considerations that have gone into the establishment of the particular working systems used in the radiocarbon dating laboratory at Harwell. This laboratory is now dating in the order of 450 samples per year. Three Packard Tricarb LS spectrometers are employed. Two are standard instruments (3375 and 3255) and one (also 3375), which will be specially described, has been modified along the lines suggested by Noakes.

Because of the time it takes to commission and prove any system concerned with low-level counting, decisions regarding techniques and operating procedure made initially are not easily changed later on. It is therefore most important that the right decisions are made from the outset. Table 1 lists under three main headings a number of the considerations which in retrospect are seen as crucial in this respect. The table is the basis of the discussion to be presented.

SAMPLE MAKE-UP

The first item under this category is the scintillant. Perhaps the most commonly used is a toluene-based cocktail. In the initial setting-up period considerable difficulty was experienced with evaporative losses from the standard screw-cap vials. This swayed the choice to a benzene-based scintillant and one specially produced by Nuclear Enterprises Ltd., NE231A, was chosen. Although losses due to a sealing failure are very much less likely with the modified screw-caps now used (Otlet *et al.*,⁴ tested

Table 1. Factors to be considered in the planning of a low-level liquid scintillation counting system suitable for ^{14}C -dating measurements.

(a)	Sample make-up
	(1) Scintillant, solvent and concentration
	(2) Type of vials
	(3) Total volume (s)
(b)	Instrument settings
	(1) Modifications to reduce background
	(2) Optimization of gain and discriminator ratios
(c)	Operating procedure
	(1) Method of allowing for varying counting efficiencies (quenching)
	(2) Counting sequence; planning to obviate short term variations in background and calibration
	(3) Data analysis

by Burleigh *et al.*⁵), the advantage of the benzene base is that corrections can be applied with confidence should they occur. This also applies to losses which occur during the process (to be recommended) of transferring a made-up solution to a different vial when a recount is required. The ambiguity of a possible differential loss from components of different vapour pressures is removed.

The required concentration is a function of the sample to scintillant solution ratio to be used. Initially the decision was taken to work with a wide range of benzene sample volumes using only scintillant solution to top up to a constant volume. The alternative is to work with a fixed sample:scintillant ratio. This is probably the ideal situation from the point of view of counting. However, in making-up to a constant volume it has the practical problem that if the synthesized sample is too small it must be topped up with 'dead' reagent benzene or, if it is too large, some has to be discarded. NE231A is a high concentration scintillant which allows a wide dilution tolerance.

The type of vial and the total volume used (items (ii) and (iii) in Table 1(a)) are closely linked, since in striving for the lowest background the main advantage of a special vial is that it is designed to hold no more than the chosen maximum volume of the counting cocktail. The background reduction to be gained by restricting the total volume is illustrated in Table 2. The data was derived from observations of 'background' count using standard instrument settings (to be discussed later on) and the following cases: (a) with a fully blackened opaque vial; (b) with no vial; (c) with an empty vial; (d) with a vial containing 14 ml of scintillant. Use of a special vial can at least be expected to reduce the components (b) and (c). Scaling down these contributions for a vial no bigger than the 14 ml referred to predicts a background of, say,

$$0.4 + 2.8 + 1.27 + 2.27 = 6.8 \text{ cpm}$$

and pro-rata for even smaller total volumes (e.g. 7.5 ml may be as low as 3.5 cpm). An alternative to a special vial which is sometimes used, is to shield above the sample volume with black paint or some opaque coating (e.g. Harkness *et al.*⁶).

It should perhaps be stressed that there is no particular significance about the total volume of 14 ml chosen in the example. Archaeological samples come in all kinds of

Table 2. Inventory of contributions to overall background in a typical ^{14}C -counting system.

	cpm
(a) Instrument	0.4
(b) Cross talk	1.9
(c) Empty vial (glass)	3.4
(d) Scintillant (14 ml)	2.8
Total	8.5

(1:6 channel, 70% counting efficiency)

sizes but unless a number of counting schemes for different total volumes are to be employed the volume decided upon is dictated by the largest sample likely to be encountered. Initially the synthesis process employed produced a maximum of 7 ml of sample which with 7 ml of scintillant gave a 50:50 mixture. Nowadays, much larger samples are produced and can be accommodated in counting with much higher sample: scintillant ratios. The 14 ml total has been retained however. (As referred to earlier on, it is not easy to change the initial plans).

To summarize this first category, the system employed is as follows: on the original Tricarb (TRICARB 1), standard low ^{40}K glass vials are used and the higher background is accepted. On the second instrument (TRICARB 2), which is specially dedicated to ^{14}C , standard vials are also used but, by raising the lower platform, the vial rests in the counting cell above the normal position. The 'dead' volume above the liquid level is above the receiving region of the optical system and is satisfactorily cut off, providing an alternative to 'blacking down'.

INSTRUMENT SETTINGS

Strictly, the modification described above encroaches into this next category. On the same Tricarb (TRICARB 2) a number of the suggestions made by Noakes³ have also been incorporated and an even lower background obtained. The results of all the modifications are summarized in Table 3. It is seen that the first and biggest step was obtained by reducing the EHT voltage on the PM tubes and increasing the gain from the electronics to compensate. The voltage was reduced until the optimum gain became about 50% (previously it was approximately 5%). Counting efficiency was lost going down from approximately 75% to 69%, a fall of about 1.08. It is of interest to note that because of this the ϵ/\sqrt{B} remained virtually unchanged. The next most significant reduction (0.5 cpm) came from the addition of the extra shielding over the counting cell. In this case the shielding was a block of boron-loaded polyester, dimensions 650 x 315 x 100 (thickness) mm, borrowed from a gas counter shield where it was previously used as a neutron absorber. When in position the block covers nearly the whole area of the 'pig'. As only this shield was tried it is not known whether the neutron absorption property or simply the γ -absorption property of the dense polyester (ρ approximately 2.5) is most significant.

The second item in this category is the optimization of the gain and discriminator settings. Figure 1 shows two groups of curves obtained by plotting counting efficiency against gain setting for varying channel widths on the standard and modified Packard instruments. The slightly lower gain of the modified instrument is evident at all comparable settings. One advantage also to be seen is the flatter response of the efficiency to relative gain changes. This feature aids gain-stability and reduces the magnitude of quench correction and hence the errors due to residuals in inaccuracies of the correction procedure.

Figure 2 compares the efficiency figures (ϵ) selected from the maximum, or balance point, settings of each channel curve with background (B) values measured in a separate experiment at the same settings. On the criterion ϵ/\sqrt{B} the widely chosen

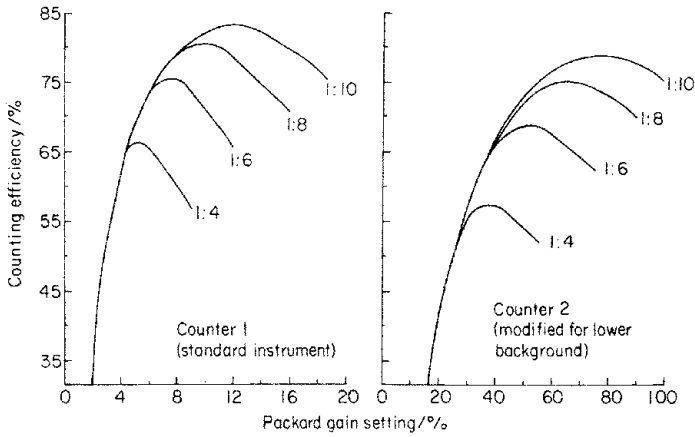


Fig. 1 Variation of counting efficiency with gain and channel width.

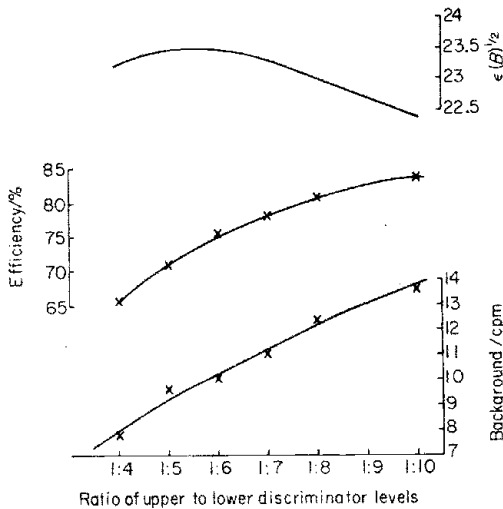


Fig. 2 Optimization of channel width from $\epsilon / B^{1/2}$.

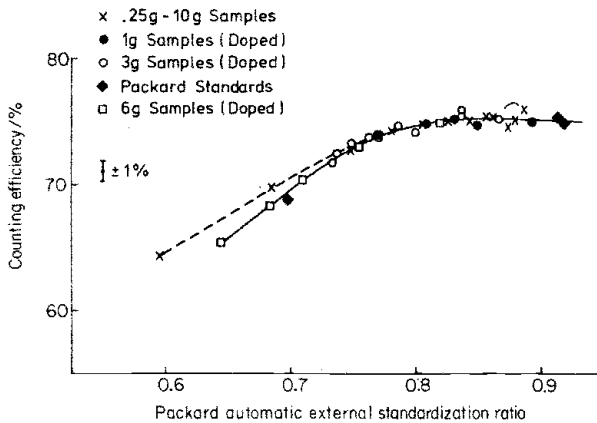


Fig. 3 Benzene counting efficiency correction curve (with NE231A making-up to 14 ml total volume).

Table 3. Data from efficiency correction tests.

(a) Variable benzene quantities				
Benzene ^a weight/g	External ^b standard ratio	Gross sample ^c count rate/cpm	Corrected disintegration rate/dpm	Efficiency /%
0.2464	0.8844	694.5	2776	75.9
0.5080	0.8797	1404	2743	75.0
1.0024	0.8734	2753	2736	74.8
2.0090	0.8605	5542	2754	75.3
3.0103	0.8548	8299	2754	75.3
4.0005	0.8420	11010	2750	75.2
5.0086	0.8223	13739	2741	74.9
6.0105	0.8043	16429	2732	74.7
7.0324	0.7792	19138	2720	74.3
8.0078	0.7488	21358	2666	72.9
9.0121	0.6843	22976	2548	69.7
10.0026	0.5956	23529	2351	64.3
(b) Nominal, 1 g benzene samples with added quench				
Benzene ^a weight/g	External ^b standard ratio	Gross sample ^c count rate/cpm	Corrected disintegration rate/dpm	Efficiency /%
1.0036(0)	0.8914	2765	2745	75.0
0.9979(4)	0.8463	2743	2739	74.8
1.0075(8)	0.8332	2786	2755	75.3
1.0061(12)	0.8055	2769	2742	74.9
0.9964(16)	0.7689	2701	2701	73.8
(c) Nominal, 3 g benzene samples with added quench				
Benzene ^a weight/g	External ^b standard ratio	Gross sample ^c count rate/cpm	Corrected disintegration rate/dpm	Efficiency /%
3.0014(0)	0.8638	8269	2752	75.2
2.9990(2)	0.8379	8305	2766	75.6
3.0173(4)	0.8385	8335	2759	75.4
3.0052(6)	0.7998	8168	2715	74.2
3.0148(8)	0.7838	8214	2721	74.4
3.0132(10)	0.7693	8121	2692	73.6
3.0056(12)	0.7627	8109	2695	73.6
3.0009(14)	0.7479	8025	2671	73.0
2.9952(16)	0.7357	7922	2642	72.2
3.0055(18)	0.7336	7898	2624	71.7
(d) Nominal, 6 g benzene samples with added quench				
Benzene ^a weight/g	External ^b standard ratio	Gross sample ^c count rate/cpm	Corrected disintegration rate/dpm	Efficiency /%
6.0045(0)	0.8191	16465	2740	74.9
6.0088(4)	0.7524	16031	2666	72.9
6.0038(8)	0.7067	15414	2566	70.1
6.0098(12)	0.6822	14997	2494	68.2
6.0024(16)	0.6439	14335	2387	65.2

^a Number of drops of acetone is given in brackets (where applicable).

^b Value given is mean of four determinations.

^c Including background, nominally 10.3 cpm. Value given is mean of four 5 min counts.

channel width of 1 to 6 (100 to 600 on the Packard instruments) is apparently justified. It should be mentioned that for these comparisons ϵ/\sqrt{B} is used rather than the more usual criterion used by liquid scintillation counter manufacturers, ϵ^2/\sqrt{B} . The former is preferred because this value directly relates to the time to be spent counting to achieve given statistics with any sample. This time of count is clearly an important consideration to any laboratory striving to obtain the highest annual throughput of low-level samples. This point leads to the third category: operating procedure.

OPERATING PROCEDURE.

The first consideration here is how to deal with varying sample counting efficiencies. These may arise because of impurities in the sample causing quenching, or simply because of different sample to scintillant solution ratios arising, as mentioned, by keeping to a fixed total volume with varying sample sizes. If an accurate correction is to be made by means of the external standard ratio method it is important to know the range over which a composite quench curve can accommodate variations from both causes. Figure 3 shows the results of tests made to investigate this with four separate groups of samples. These, as listed in Table 3, included one group of varying quantities of pure benzene (0.25-10 g, i.e. 0.3-12 ml) and three groups of specific sizes of sample each doped with increasing amounts of acetone to induce impurity quenching. Two to eighteen drops of acetone is enough to produce the range of values shown. The samples were made up from a benzene stock solution of about 700 dpm g^{-1} (x 50 modern in radiocarbon dating terms) a level which, as seen in Table 3, is well above background in all but the smallest sample sizes.

The $\pm 1\%$ error bar also shown in Fig. 4 highlights the importance of minimizing residual errors due to the quench corrections. The conclusion is drawn that satisfactory allowances can be made using the composite curve with samples giving external standardization ratios (Packard facility) in the range 0.75 to 0.9. In practice a polynomial of power 2 provides an excellent fit within this range for computer calculations of efficiency on specific samples.

The counting sequence is the next step of the operating procedure. The planning of this is necessarily linked to what peripheral support is associated with the particular liquid scintillation counter instrument. The general principle, however, is clearly that the best use should be made of the automatic and programming facilities provided by the instrument and to plan the counting 'runs' in a way which minimizes the uncertainty of short term variations in background and calibration values. The system devised follows the method of Polach of making up a batch, or train, of samples which, loaded into the sample changer belt, can, as a batch, be automatically counted for successive short periods building up to the total count required on each sample over a much longer period. Figure 4 shows schematically the make up of a typical batch-train. In the example four backgrounds and five moderns are interspersed in the set of 15 samples which together make up the full batch. In practice, the actual number of backgrounds and moderns can be varied to achieve whatever balance of counting on each

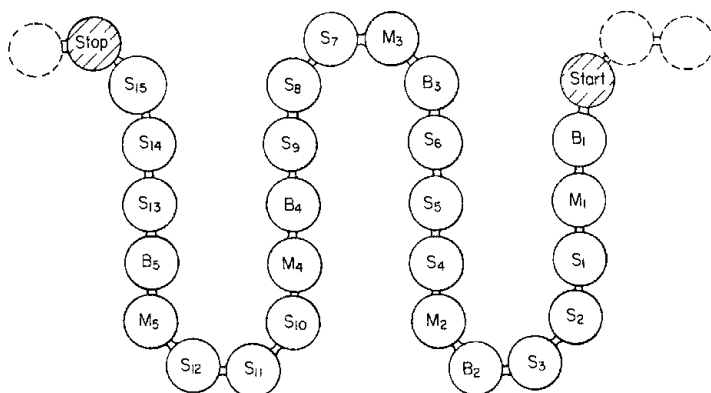


Fig. 4 Schematic make-up of a typical sample train for batch counting. S1-15 represent samples to be dated, M1-5 the modern calibration standards and B1-5 the background samples.

type is required. Counting limits are normally set to trip at either 2000 counts total or 100 min counting time — whichever is reached first. It is arranged that one cycle completes in approximately (but not exactly) 24 h so that in 20 cycles the counting period of every sample moves through the day. Thus most samples realize 40 000 counts ($\pm \frac{1}{2}\%$ standard deviation) and the backgrounds each count for a total of 2000 min. Samples requiring longer counting times can be included in a further counting batch to build up to the number required.

Operating in this way provides quasi-simultaneous observations of background calibration and sample counting. Ambiguities regarding short term variations (i.e. day-to-day variations) are smoothed out over the 20 cycle period.

The control of long term stability is more difficult to achieve and raises the vexed question as to whether the means for the run counting (a run being 20 cycles) for background and calibration samples should be merged into longer-term rolling mean averages. From the analysis of counting data over several years it is believed that long term stability should not be assumed and calculations are always based on the run counting mean values. Further discussion on this point and on the calculation of overall reproducibility obtained is given in an earlier paper.

The final consideration is the analysis of the data. Precise techniques are obviously closely linked to the counting procedure adopted and vary considerably from one laboratory to another. Hall and Hewson of the British Museum have described an on-line computer scheme which perhaps represents the ultimate of what can be achieved. At the other end of the scale most LS systems at least terminate in a teleprinter which, generating paper tape, provides the data in a suitable form for periodic analysis by a remote computer. This is the procedure used at Harwell. The output of completed runs, 20 cycles of counting for each batch, is analysed using specially written programs on the site IBM 360/75. Means and standard deviations for each sample are computed on the merits of the repeated count totals in each cycle assuming a normal or Gaussian distribution. This is done rather than simply taking N^2 which assumes Poisson statistics, although the difference in practice is generally not significant. Rejection of data should be a rare occurrence. Only the very simplest of tests, e.g. 3σ criterion, are used to assess possible rejections. Data so rejected are generally a result of paper tape failure rather than counting errors.

CONCLUSION

The description of the considerations to be given to low-level ^{14}C -counting shows how a balance between the ideal and practical solutions has been achieved to yield a working system which has been operated and proved over several years. It is not intended that the options taken should be regarded as the only ones which can be expected to give satisfactory results. With an ever increasing number of laboratories setting up to count ^{14}C these days, it is hoped, however, that the detailed explanation of this system will provide useful foundation material for anyone faced with the task of formulating new plans from scratch.

REFERENCES

1. P.E. Hartley and V.E. Church, in Liquid Scintillation Counting — Recent Advances (P.E. Stanley and B.A. Scoggins, Eds) Academic Press, London, 1974, p.67.
2. J.E. Noakes, M.P. Neary and J.D. Spaulding, in Liquid Scintillation Counting — Recent Advances (P.E. Stanley and B.A. Scoggins, Eds) Academic Press, London, 1974, p.53.
3. J.E. Noakes, in Liquid Scintillation Counting, Vol 4 (M.A. Crook and P. Johnson, Eds) Heyden, London, 1977, p.189.
4. R.L. Otlet and B. Slade, Radiocarbon, 16, No.2, 178 (1974).
5. R. Burleigh and A. Hewson, in Liquid Scintillation Counting, Vol 4 (M.A. Crook and P. Johnson, Eds) Heyden, London, 1977, p.207.
6. D.D. Harkness and H.W. Wilson, in Proceedings of the Eighth International Radiocarbon Dating Conference, New Zealand, 1972 (T.A. Rafter and T. Grant-Taylor, Eds) Royal Society of New Zealand, Vol 1, p.216.
7. H.A. Polach, At. Energy Aust. 12, No 3, 21 (1969).
8. R.L. Otlet, Proceedings of the Ninth International Conference on Radiocarbon Dating, Los Angeles and San Diego, 1976.
9. J.A. Hall and A.D. Hewson, J. Archaeol. Sci. 4, 89 (1977).

DISCUSSION

B.E. GORDON: ϵ^2/B is a much better criterion than ϵ/B for low count rates since it is the parameter which permits the shortest count time to achieve a desired error.

Since glass vials contribute a major amount to background, could you not employ other vials (i.e. Teflon, quartz)? If these are thin-walled, the background reduction should be substantial.

R.L. OTLET: I think we are at cross purposes here as ϵ/B is certainly not the criterion we use. In the paper I referred to ϵ/B , the parameter derived and plotted in Fig. 3. Optimum ϵ/B is of course entirely consistent with optimum ϵ^2/B . The reasons for preferring to use ϵ/B are already given in the text.

I agree that Teflon vials would probably give lower backgrounds than the low-K glass ones which we use, especially if the restricted volume type I mentioned (described by Noakes) is used. There are, however, two very good reasons why we don't use them.

1. They are not available commercially. To use them we should have to get quite large quantities specially made. With an annual turnover of approximately 450 samples we would need at least one-third of this number on the go at any one time. Individual manufacture would mean that each vial would need its specific background checked before use as nothing comparable to the batch consistency we get from glass vials could be relied upon.
2. Our own tests and those of others⁵ show difficulties in making adequate sealing with this type of vial. Evaporation losses would be totally unacceptable with our counting procedure.

On the last point it is not our experience that the available silica vials actually give significantly lower backgrounds within the counting window we use for ^{14}C (1:6 optimized as explained in the text) sufficient to justify the additional expense and possible individual vial background complications mentioned above.

D.E. CASE: For those of us not too familiar with your techniques could you please indicate to us the order of the count rates with which you have to deal, and their relationship to the ages of the samples? Where is the error factor in counting reflected in age determination?

I believe there has been some divergence of ^{14}C dating from other methods at certain age periods. Would you care to comment on this?

R.L. OTLET: Count rates, sample size and age are of course interlinked. Sample sizes are generally in the range 1-6 g of carbon which, taking a modern ^{14}C level of 13.56 dpm g and about 70% counting efficiency, gives counting rates above background of approximately 14 to 80 cpm maximum. These values of course divide down by a factor of 2 per 5730 years of age and for most purposes our measurement limit is about six to seven half-lives (34 to 40 thousand years) for the larger samples and correspondingly less for smaller ones.

I have described the method we use of overall error assessment in liquid scintillation counting of ^{14}C samples elsewhere. Briefly, however, using our counting procedures the contribution of the sample counting alone amounts to about ± 50 years out of a total of ± 70 years for samples up to about 3000 years old and ± 70 years out of a total of 110 years in the case of a 10 000 year old sample.

Finally, I don't really know what other methods you are referring to. Extravagant claims are often made for some dating techniques which are certainly very much less understood and tested than ^{14}C . It is true, however, that certain assumptions of the original theory of ^{14}C made by W.F. Libby nearly 30 years ago require modification if we are to obtain a true calendar date from a sample. The most difficult of these is the question of constancy of the atmospheric ^{14}C level over all time. Carbon-14 dating of tree rings from long-lived trees (notably Bristlecone pine from California) show the variations which have occurred and provide the data which enables production of a calibration chart to overcome it. Presently available calibration data are far from perfect but work is continuing both here (e.g. G. Pearson, Belfast, on ancient bog oak found in Ireland) and elsewhere. Despite this ^{14}C is still in my opinion the most used and most reliable dating technique available for archaeological times.

G. AYREY: To the layman your deliberate decision to vary the concentration of scintillant seems strange. I wondered why it was not possible to use weighed amounts of solid scintillant and thus make fullest use of your low-level benzene sample?

R.L. OTLET: Briefly the thinking is this: benzene quantities produced from archaeological samples are extremely variable, a range of just over 1-6 ml being quite normal. We felt it was not practical to try to cope with the different counting characteristics and background corrections which varying levels of sample in the vials would cause. In deciding to bring all samples to the same level it is very convenient to top up with scintillant solution only. NE231A was specially prepared by Nuclear Enterprises for use with this in mind. It contains a high concentration of scintillant which gives an approximately constant efficiency over a wide range of dilution by sample benzene. The quench tests I described show this to be the case within the required range. Possible residual errors are reduced to a second order by taking the external standard ratio reading and correcting every sample accordingly.

R. EVANS: Why do you not use internal standardization for measurement of efficiency rather than the usually less accurate external standard measurement?

R.L. OTLET: One important reason is that we want to keep the samples in an uncontaminated state for as long as possible afterwards in case further counting is required. This is certainly the case for the 'modern' calibration standards which are repeatedly used in batch runs over considerably long periods. However, I think it should be stressed that we are not dealing with large degrees of quench. Most of the samples have almost identical counting efficiencies despite the dilution range and to try first to determine the absolute efficiency of each by internal standardization rather than simply to relate the difference in efficiencies by the external standardization source technique may well be a less accurate method of comparing samples and standards.