

## Chapter 20

# Errors in dpm Measurement in Liquid Scintillation Counting with External Standardisation

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### INTRODUCTION

Liquid scintillation counting is not an absolute measuring technique. The results obtained from any system need to be qualified by a determination of the efficiency at which the samples were measured. Basically, samples may be classified as either heterogeneous or homogeneous. Measurements on heterogeneous samples including pieces of filter paper or microcellular filtration discs are affected by self-absorption which is not easy to make allowances for. This paper deals with homogeneous samples and the accuracy of the efficiency measurement in order to obtain correct values of disintegrations  $\text{min}^{-1}$ .

There are three methods of monitoring efficiency available in liquid scintillation counting: (i) internal standardisation – this is the only primary technique but is very slow because it requires double counting, and is relatively expensive; (ii) the sample channels ratio technique – this takes place as the sample is being counted but has several recognised disadvantages, notably the occurrence of statistical inaccuracies when measuring low activity samples; (iii) external standardisation – originally carried out by external standard count (ESC) methods and later by external standard ratio (ESR) measurement. The latter is probably the most maligned of the three techniques because there were a number of instrument problems in earlier years and some confusion among users, but is now certainly the most suitable method for most types of sample.

Since

$$\text{efficiency} = f_1 \text{ (quench)}$$

and  $\text{external standard} = f_2 \text{ (quench)}$

it follows that  $\text{efficiency} = f_3 \text{ (external standard)}$

This relationship forms the basis of a quench correction curve. The idea of using a  $\gamma$ -emitting isotope to produce a Compton electron spectrum in a liquid scintillation counter was first applied to LSC in the early 1960's.<sup>1-4</sup> Any external standardisation system should include the following essential characteristics:

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- (i) wide dynamic range – normally to cover tritium efficiencies down to 0.0%;
- (ii) volume independence – over the designed range, normally 8–18 ml;
- (iii) count rate independence;
- (iv) quencher independence – providing the sample remains stable in solution.

Desirable features are:

- (v) cocktail independence;
- (vi) vial independence.

In current commercially available liquid scintillation counters, three types of radionuclide are used as external standard sources:  $^{133}\text{Ba}$  with a  $\gamma$  photo-electric peak at 360 keV and an X-ray peak at 80 keV;  $^{137}\text{Cs}$  with a  $\gamma$  photo-electric peak at 662 keV and an X-ray peak at 33 keV;  $^{226}\text{Ra}$ , a complex spectrum with a large number of daughter peaks with energy up to 2.8 keV. These spectra are shown in Fig. 1 as they would appear in a standard liquid scintillation counter using unquenched toluene-PPO-dimethyl-POPOP cocktail.

## EXPERIMENTAL

Ten millilitres of cocktail ( $5 \text{ g l}^{-1}$  PPO,  $0.5 \text{ g l}^{-1}$  dimethyl-POPOP) was made up with toluene and counted in standard low-potassium glass vials. A series quenched with carbon tetrachloride was made up for  $^3\text{H}$  and  $^{14}\text{C}$  (NBS certified  $\pm 1\%$   $^3\text{H}$ ,  $\pm 3\%$   $^{14}\text{C}$ ). Figures 2, 3 and 4 show the quench correction curves obtained with these samples on each of three different instruments. In each case the manufacturer's normal wide window counting condition was selected.

In order to assess the dynamic range of an external standard system, the indicated external standard ratio was plotted against the volume of quenching agent employed, as shown in Fig. 5. These results indicate that  $^{133}\text{Ba}$  has too wide a dynamic range, that of  $^{137}\text{Cs}$  is too narrow, while that of  $^{226}\text{Ra}$  is most convenient to use. This ties in with Figs. 2, 3 and 4 which show  $^{133}\text{Ba}$  with efficiency zero at ESR 0.2 and  $^{137}\text{Cs}$  with efficiency 15% and 30% at ESR zero.

To assess the effects of volume on the liquid scintillation counter, a series of standards of from 5–20 ml volume was constructed using the stock cocktail and radioactive label. The effect on counting efficiency of changing volume is shown

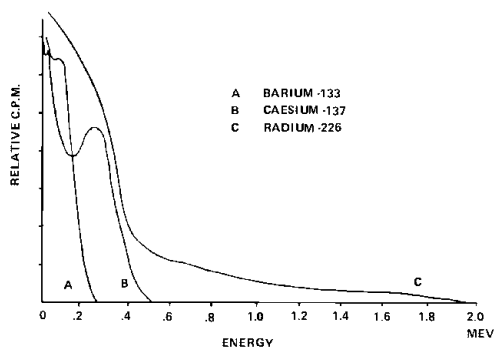


Fig. 1.

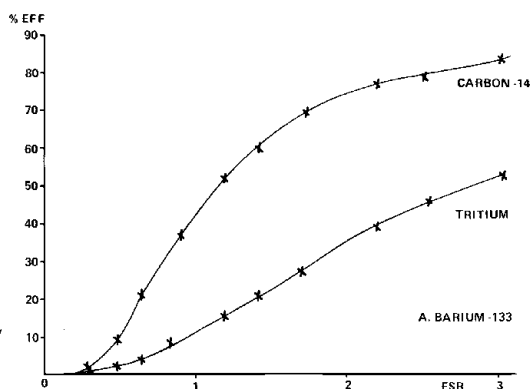


Fig. 2.

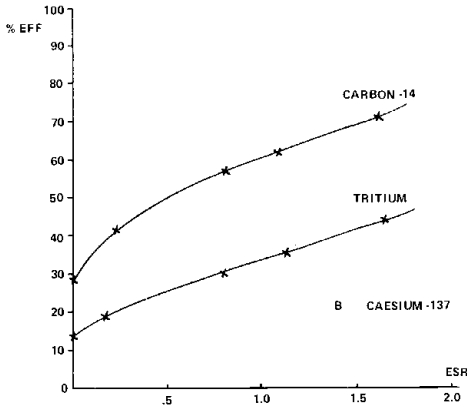


Fig. 3.

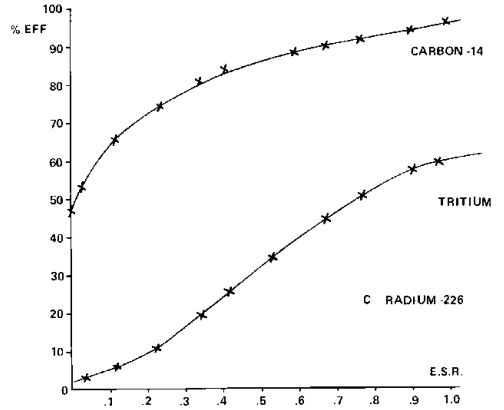


Fig. 4.

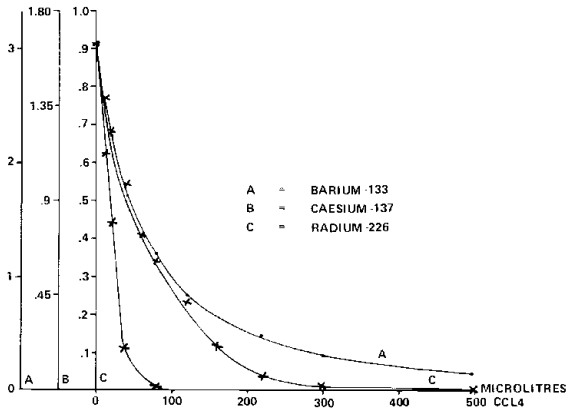


Fig. 5.

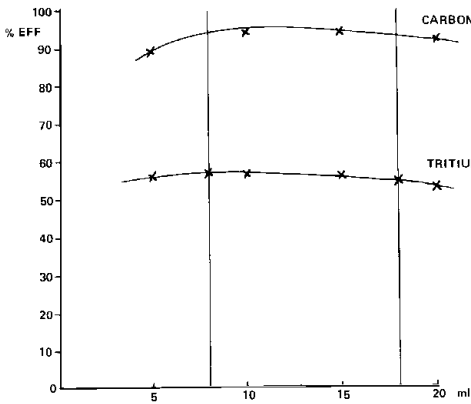


Fig. 6.

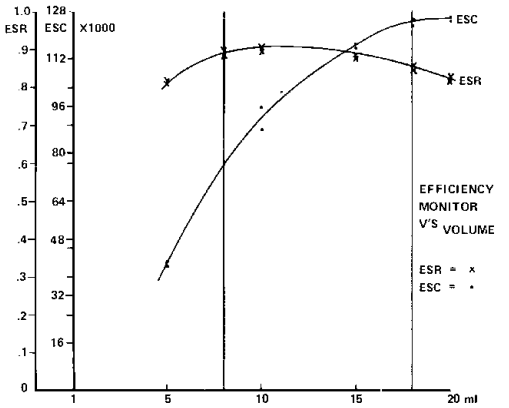


Fig. 7.

in Fig. 6. Between 8-18 ml, the efficiency for  $^{14}\text{C}$  and  $^3\text{H}$  changes by 3%. The effect of volume changes on the two external standard methods is compared in Fig. 7, where it can be seen that the external standard count curve differs considerably from the efficiency volume curve, whereas the external standard ratio curve is very similar to it. The comparative accuracy in disintegrations  $\text{min}^{-1}$  assessment using ESR and ESC with volume change is shown in Fig. 8. Data relating to the indicated counting efficiency was obtained from a standard quench curve using samples of 10 ml volume. It can be seen, therefore, that even small changes in volume or sample height caused by vial imperfection could cause appreciable errors in disintegrations  $\text{min}^{-1}$  accuracy.

The ability of an instrument to be independent of count rate tests not only the external standard system but all of the counting electronics. Figures 9 and 10 illustrate results obtained by increasing activity at constant volume. In the case of  $^3\text{H}$ ,  $^{133}\text{Ba}$  and  $^{226}\text{Ra}$  show very similar results, whereas  $^{137}\text{Cs}$  seems to be less accurate as the count rate increases. In the case of  $^{14}\text{C}$ , both  $^{226}\text{Ra}$  and  $^{133}\text{Ba}$  initially are very similar but then diverge, the results for  $^{226}\text{Ra}$  being similar for both  $^3\text{H}$  and  $^{14}\text{C}$ . In the case of  $^{137}\text{Cs}$ , the results are obviously completely different. (The results for  $^{137}\text{Cs}$  would appear to be completely reversed from those found by Gogan and Gogan,<sup>5</sup> who found that with a caesium external standard source, in the case of  $^3\text{H}$  the error was positive and in the case of  $^{14}\text{C}$  the error was negative.)

	8ml	DPM ERROR	18 ml	DPM ERROR
CARBON -14	ESC $\text{IND}$ EFF	758K / 49%	126K / 62%	+16.3% / -12%
	ACT EFF	57%	54.6%	
	ESR $\text{IND}$ EFF	.88 / 56.5%	.85 / 55%	+ .8% / - .7%
	ACT EFF	57%	54.6	
TRITIUM	ESC $\text{IND}$ EFF	76K / 89%	126K / 97%	+5.6% / -4.7%
	ACT EFF	94	92.5	
	ESR $\text{IND}$ EFF	.88 / 93.5	.85 / 93	+ .5% / - .6%
	ACT EFF	94	92.5	

Fig. 8.

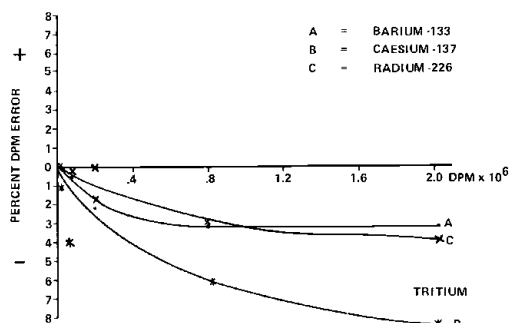


Fig. 9.

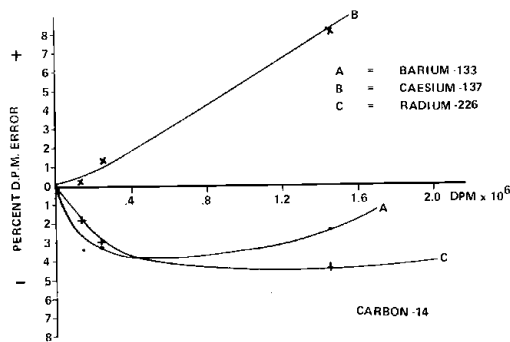


Fig. 10.

The study of the effect of introducing different quenching agents produced the results shown in Fig. 11. Samples were prepared using 10 ml of a standard toluene cocktail and quenched with acetic acid, chloroform, red dye and yellow dye. In each case the amount of quenching agent added was such that all samples should count at a similar efficiency and be measured at approximately 70% of the maximum ESR for that nuclide. It can be seen that the overall accuracy of all three instruments is quite similar in the case of acetic acid, chloroform and yellow dye, but with the red dye the results differ widely. The  $^{133}\text{Ba}$  source appears to show much better accuracy with  $^{14}\text{C}$  samples than with  $^3\text{H}$ . The  $^{137}\text{Cs}$  source appears to give high results for  $^{14}\text{C}$  samples, corresponding with the high results obtained with  $^{14}\text{C}$  in the increasing count experiment. In the case of  $^{226}\text{Ra}$ , the results are consistent for chemical quenchers, but in the case of the  $^3\text{H}$  red sample the answer is very different from that obtained with the other two isotopes. Since the same sample was measured in all three instruments, any error in the original number of known disintegrations  $\text{min}^{-1}$  in the sample should show a similar error with all three isotopes, i.e. they should all be high or low.

The next experiment was designed to test the practicality of using the quench curve obtained for one solvent scintillator system for samples contained in another type of cocktail. Four standard commercial cocktails were used (10 ml): Instafluor<sup>®</sup> (Registered Trade Name, Packard Instrument Co.), a simple xylene-based system designed to count organic samples at high efficiency; Instagel<sup>®</sup>, a complex emulsion cocktail, xylene-based, designed to cope with the presence of large quantities of water; Permafluor V<sup>®</sup>, a toluene-based cocktail with a high scintillator concentration designed for use with sample oxidisers; Permafluor II<sup>®</sup>, a dioxane-based cocktail similar to Bray's solution. The same amount (45  $\mu\text{l}$ itres) of quenching agent was added to each cocktail. From the  $^3\text{H}$  results shown in Fig. 12 it can be seen that the Permafluor II<sup>®</sup> assessments were all low suggesting either that a toluene quench curve cannot be used for dioxane samples or that the original activity in the sample was low. In the  $^{14}\text{C}$  results (Fig. 12) only Instagel appears to give a consistent figure; therefore, it can be assumed that for  $^{14}\text{C}$  a different quench curve is probably needed for each different scintillator system. The overall errors with the  $^{133}\text{Ba}$  source are quite wide, particularly in the case of Instafluor. The

PLOTING TABLE

## CHEMICAL AND COLOUR

$^3\text{H}(1)$	A	B	C
ACETIC ACID	-	- 0.4	- 1.6
CHLOROFORM	- 2.0	+ 1.5	+ 0.8
RED	+ 9.8	+ 7.8	- 6.3
YELLOW	- 1.8	+ 2.4	- 1.4
$^{14}\text{C}(2)$			
ACETIC ACID	- 2.5	+ 0.8	- 1.2
CHLOROFORM	0	+ 8.8	0
RED	- 0.1	+ 8.6	- 1.8
YELLOW	- 1.4	+ 5.8	- 3.7

Fig. 11.

PLOTING TABLE

	A	B	C
$^3\text{H}(1)$			
INSTA FLUOR	+ 4.6	+ 2.4	+ 0.2
INSTA GEL	- 2.7	- 4.5	- 0.2
PERMAFLUOR V	- 7.7	- 6.5	- 0.8
PERMAFLUOR II	- 8.8	- 6.5	- 5.9
$^{14}\text{C}(2)$			
INSTA FLUOR	+ 1.7	+ 1.0	+ 9.1
INSTA GEL	0	+ 0.8	- 0.7
PERMAFLUOR V	- 6.3	+ 4.9	+ 2.0
PERMAFLUOR II	+ 0.4	- 0.9	+ 7.6

Fig. 12.

results from  $^{137}\text{Cs}$  are much more consistent than those of  $^{133}\text{Ba}$  or  $^{226}\text{Ra}$ .  $^{226}\text{Ra}$  shows good consistency with xylene- and toluene-based cocktails, except in the case of Instafluor for  $^{14}\text{C}$ , but does not seem to like dioxane-based cocktails.

## REVIEW OF TWO EARLIER PAPERS

The ability to be able to use vials of different materials is important in some laboratories. Two publications have examined the use of polyethylene vials with toluene-based cocktails. Gogan and Gogan,<sup>5</sup> and Rauschenbach and Simon<sup>6</sup> examined instruments using all three nuclides as external standard sources (Fig. 13). In the case of  $^{133}\text{Ba}$  it was found that the external standard ratio changed by 0.4% per hour. In the case of  $^{137}\text{Cs}$  it was found that the external standard ratio changed by 0.8% per hour. Both groups found that no similar change appeared in an instrument using  $^{226}\text{Ra}$ . The actual error in disintegrations  $\text{min}^{-1}$  assessment would depend on the individual quench correction curve and the level of quench in the sample, but in an overnight run it could be as much as 8% for an instrument using  $^{133}\text{Ba}$  or 16% for an instrument using  $^{137}\text{Cs}$ . When dioxane-based cocktails are used in polyethylene vials no similar drift occurs in an instrument. No instrument shows any change using glass vials with any type of solvent system. Horrocks<sup>7</sup> recently published a method of using  $^{137}\text{Cs}$  for polyethylene vials using toluene-based cocktails which reduces the drift to much lower levels, but this is done by sacrificing dynamic range. With any type of external standard source it is necessary to construct a different quench curve for each type of vial in use — glass, polyethylene, or minivial.

NUCLIDE	G. and G. <sup>a</sup>	R. and S. <sup>b</sup>
$^{133}\text{Ba}$		0.5% $\text{h}^{-1}$
$^{137}\text{Cs}$	0.8% $\text{h}^{-1}$	0.8% $\text{h}^{-1}$
$^{226}\text{Ra}$	<i>M</i>	<i>M</i>

<sup>a</sup> Gogan and Gogan Ref. 5.

<sup>b</sup> Rauschenbach and Simon Ref. 6.

Fig. 13. External standard ratio shift with polyethylene vials.

## CONCLUSION

It has been shown that instruments with different isotopes as external standards differ widely in that all external standard sources do not show the same performance, whereas with two machines using the same isotope as an external standard source it is possible that the characteristics will only differ slightly. Therefore, in their own interest users and prospective users of liquid scintillation counting should thoroughly investigate the suitability of an instrument for their requirements. The fact that many instruments have an external standard does not mean that all such instruments are the same. Its suitability has to be examined closely if disintegrations  $\text{min}^{-1}$  errors are to be kept to a minimum.

## REFERENCES

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7. D. L. Horrocks, *Intern. J. Appl. Radn. Isotopes* 26, 243-56 (1975).

## DISCUSSION

**B. H. Laney:** Did you test various instruments or one instrument with three sources?

**C. G. Horne:** The tests were done on three commercially available instruments using manufacturers' recommended counting conditions and external standard windows.

**B. H. Laney:** The phenomenon of the external standard ratio (ESR) drift with polyethylene vials is caused by additional scintillations produced by the external standard in the vial walls when permeated by the solvent. Consequently, the vial becomes a plastic scintillator producing weak scintillations proportional to the  $\gamma$ -excitation. I reported these observations in *Tritium*, Messenger Graphics (1971) p. 166. In the figure,  $^{226}\text{Ra}$ ,  $^{137}\text{Cs}$  and  $^{133}\text{Ba}$  external standard sources are compared in the same instrument with the same instrument settings. The ESR drifts 15 to 25% within a day when the external standard windows are set to produce statistically significant ESR's over a wide (25:1) change in pulse heights. When the lower level discriminators of both ESR windows are raised to exclude the scintillations produced in the vial walls, the ESR drift with freshly prepared samples is less than 2% with each of the three external standard sources. (Fig. 14).

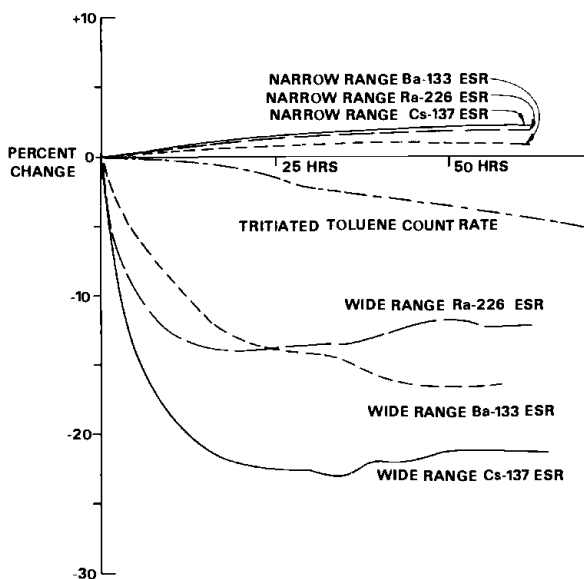


Fig. 14.

**C. G. Horne:** The settings chosen by a manufacturer for his instrument are a compromise to attempt to meet the essential requirements as previously discussed. Therefore, although it is possible that an instrument equipped with any type of external standard source can be adjusted to handle with accuracy one characteristic requirement, it is certainly done to the detriment of the performance of the system to other essential requirements. The slides which Mr Laney has shown have no practical consequence to the man in the laboratory using liquid scintillation counting as another analytical technique. He is not in a position to readjust his instrument daily for every type of sample which he may encounter that day.

**B. Morris:** Having shown that a different vial and/or scintillation cocktail requires a different quench correction curve (external standard channels ratio method), have you investigated the effect of scintillant ageing and batch to batch variation on the application of any one correction curve to any one vial/cocktail system?

**C. G. Horne:** No.

**T. J. Rising:** We have prepared quenched standards for a number of scintillators and biological samples such as urine and plasma and used Radium as the AES source. Over a period of about three months we have checked the curves and found good agreement between scintillators such as Permafluor and Nuclear Enterprise 260.

**P. Gresham:** No curve is reliable in the long term.

**R. P. Parker:** Important samples justify a separate curve generated at the time of the experiment.

**D. J. Barnfield:** Can you explain to me, as a non-physicist, why there are such large discrepancies associated with the use of  $^{137}\text{Cs}$  with samples of high activity?

**C. G. Horne:** I think that in such cases we should look at the instrument as a whole, and consider the electronics involved. Instruments using  $^{137}\text{Cs}$  as external standard tend to utilise logarithmic amplification and those using  $^{133}\text{Ba}$  and  $^{226}\text{Ra}$  tend to utilise linear amplification, the answer to your question lies in that area.

**A. Dyer:** It would be interesting to speculate on what might have happened if you had not put in dimethyl-POPOP.

**C. G. Horne:** We attempted to use materials which are the ones most commonly used in practice.