Chapter 4

An Intercomparison of Commonly Used Liquid Scintillation Counting Vials

K. Painter[a] and M.J. Gezing

Amersham/Searle Corporation, Arlington Hts., Illinois, U.S.A.

INTRODUCTION

The science of liquid scintillation spectrometry has probably advanced more rapidly than any other instrumental technique. From the beginnings of the early 1950's to the present we have witnessed tremendous progress in instrumentation and sample chemistry. The liquid scintillation analyst is now confronted with push button instrumentation and premixed cocktails which seemingly make the technique as simple to perform as operating the ordinary television set.

Unfortunately, these technological advances have removed the necessity for many users to become familiar with the technique. All too often we have seen users place their samples in vials, add premixed cocktail, push a button on the counter and read a number off a computer tape. Due to the pitfalls and idiosyncrasies of the technique, however, the novice can frequently find himself in considerable difficulty. The liquid scintillation literature is replete with warnings related to instrumentation and sample chemistry. We can only add our voices to those who have cautioned newcomers in this field to review the literature and be aware of the limitations of the liquid scintillation technique.

ADVANTAGES AND DISADVANTAGES OF COMMON COUNTING CONTAINERS

The choice of liquid scintillation vial is of utmost importance in critical counting applications. A recent paper[1] has reviewed in detail the advantages and disadvantages of various counting containers. To summarise the findings the disadvantages of counting vials are pointed out in Table 1.

Several types of counting bottles found in routine use are shown in Fig. 1. The disadvantages of each counting vial are keyed to Tables 2 and 3 and are provided as a warning of the most frequent disadvantages to which a particular vial may be prone.

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[a] Present address: Micromedic Diagnostics, Fort Collins, Colorado, U.S.A.

Table 1. Disadvantages of common counting vials.

A.	High background count rate
B.	Low counting efficiency
C.	Limited volume capacity
D.	Breakage
E.	Swelling
F.	Reactive to solvents/solubilisers
G.	Inability to inspect samples visually
H.	High cost
I.	Incompatibility with external standard
J.	Adsorption of sample
K.	Permeability to solvent/sample
L.	Photoluminescence
M.	Static electricity
N.	Acute volume:efficiency dependency

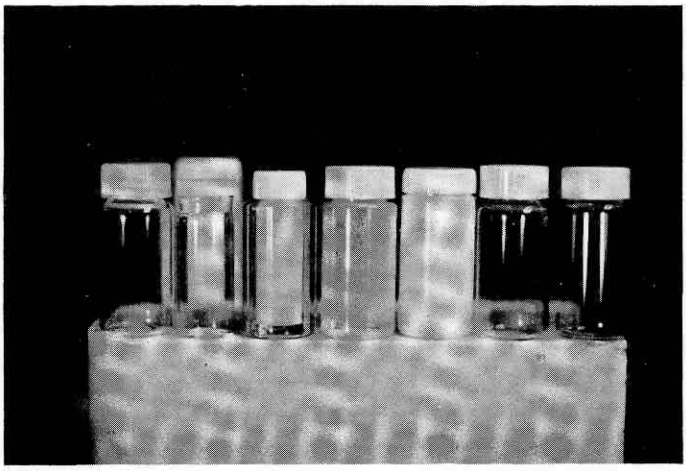

Fig. 1. Various liquid scintillation vials, Left to right: borosilicate glass, general purpose glass, polyethylene, nylon, Mini-vial™, 3 dram mini-vial and quartz. Not shown, Sterilin mini-vial.

INTERCOMPARISON OF COMMON COUNTING VIALS

Tables 2 and 3 provide intercomparison data for the counting vials shown in Fig. 1 with respect to two figures of merit for Carbon-14 and tritium. E^2/B (where E is efficiency squared divided by background count rate) figure of merit gives a good measure of the optimum performance of a liquid scintillation spectrometer for unquenched or very slightly quenched samples. The E^2/B figure of merit was determined using an air quenched toluene solution containing 6% Spectrafluor[a] PPO-POPOP.

Although EV (where E is efficiency and V is volume) has frequently been used as a sample figure of merit,[2-4] a more practical figure of merit is $EV/\sqrt{B}$.[2,5] The latter takes into account efficiency, volume and background. The $EV/\sqrt{B}$ figure of merit is calculated on a 40% (v/v) water sample in PCS[a] liquid scintillation cocktail. Relative

[a] Trademark Amersham/Searle.

Table 2. Intercomparison of various liquid scintillation vials — tritium

	Relative E^2/B	Relative $EV\sqrt{}/B$	Disadvantages
Borosilicate glass	35.9	67.5	DJ
General purpose glass	16.7	47.8	ADJ
Polyethylene	88.9	100.0	EFGIK
Nylon	60.5	71.4	EFGIL
Mini-vial™	100.0	37.7	CEFGIKMN
Sterilin mini-vial	51.2	24.9	CEFGIKN
3 Dram mini-vial	58.6	39.9	DIJN
Quartz	65.0	77.6	DHJ

Table 3. Intercomparison of various liquid scintillation vials — Carbon-14

	Relative E^2/B	Relative $EV\sqrt{}/B$	Disadvantages
Borosilicate glass	64.7	87.0	DJ
General purpose glass	42.9	73.2	ADJ
Polyethylene	100.0	100.0	EFGIK
Nylon	87.4	82.1	EFGIL
Mini-vial™	99.3	34.7	CEFGIKMN
Sterilin mini-vial	91.4	29.4	CEFGIKN
3 Dram mini-vial	85.2	50.4	DIJN
Quartz	89.0	93.3	DHJ

figures are given to maximise vial comparison.

It can be seen from the tables that for samples with little quench, the Mini-vial™[a] and polyethylene bottles appear to be superior; unfortunately, unquenched samples of this type are rare in bioanalytical research. For larger samples where more quenching is present, 20 ml vials are superior to any of the miniature vials.

Percent counting efficiencies in this study did not vary much with the type of counting vial, but backgrounds varied considerably. In applications where sample count rates are greater than 100 times background, background count rate need not be considered,[6] and economical general purpose glass bottles can be used. It is interesting to note that relatively high figures of merit are obtained for general purpose glass vials when counting beta-emitters other than tritium in moderately to highly quenched systems.

A WORD OF CAUTION IN USING THE EXTERNAL STANDARD RATIO METHOD OF QUENCH CORRECTION

It is not convenient to prepare a quenched standard calibration set in screw cap, polyethylene or polypropylene vials, as the permeability of these containers renders the set useless after several days. Many laboratories use commercial quenched standard sets in 20 ml flame-sealed glass bottles to prepare an external standard ratio calibration curve which is then applied to other types of counting vials, such as nylon, polyethylene and miniature vial combinations. Figure 2 shows the gross error which can be introduced in the experiment in such cases. A commercial quenched standard set in 20 ml flame-sealed glass borosilicate

[a] Trademark Nuclear Associates.

vials was compared with the internal standard method to determine actual counting efficiencies.

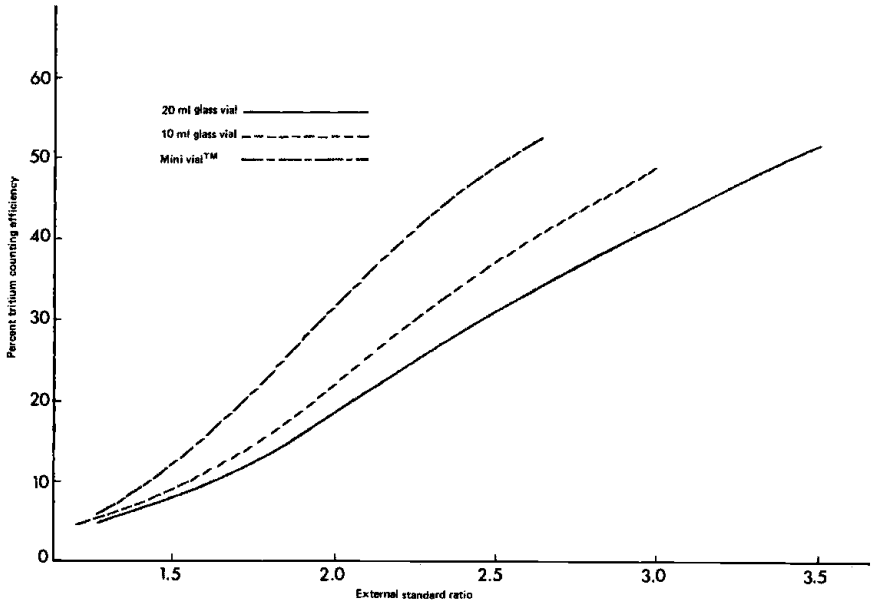

Fig. 2. External standard channels ratio calibration curves for vials of different composition and shape.

We have found the external standard ratio method of quench correction to be impractical for any counting bottle other than 20 ml glass vials. This is not surprising since one would not expect to obtain the same external standard gamma interaction with plastic and glass, and hence a different Compton continuum or simulated beta spectrum will result. Despite claims to the contrary from one supplier, we have found this variability to be true of instruments from all commercial manufacturers.

We have shown in Fig. 3, however, that with the use of the sample channels ratio method[7] of quench correction quite satisfactory results can be obtained using a commercial glass quenched standard set to construct a quench correction curve for many different types of counting containers.

For those who have access to on-line computers, Glass[8] has suggested an excellent method of simultaneously computing and comparing both sample channels ratio and external standard ratio. We strongly recommend this approach.

WASHING AND RE-USING COUNTING VIALS

When one considers the cost of washing scintillation vials, i.e. technician time, counter time and the chance of a contaminated bottle affecting the precision of data, re-using standard counting bottles is false economy. Polyethylene, polypropylene and nylon vials should never be re-used, and caps should be discarded after a single use.

Rummerfeld and Goldman[9] have discussed the economics of vial washing versus the accuracy required for the experiment. Harris and Friedman,[10] Kushinsky and Paul,[11] Radin[12] and Drosdowsky and Egoroff[13] have described various scintillation vial washing devices. A word of caution, however; in several of the

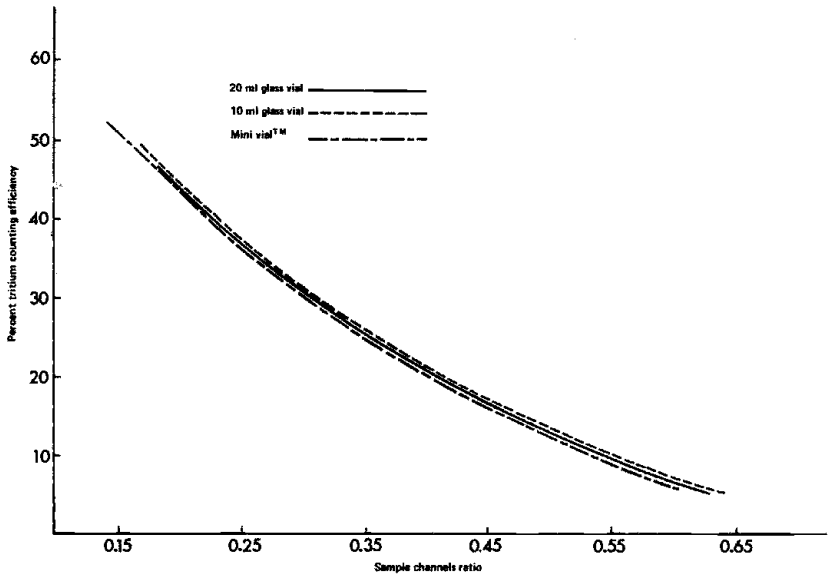

Fig. 3. Sample channels ratio calibration curves for vials of different composition and shape.

studies mentioned above radioactive steroids were employed to test the effectiveness of the washing device. Since steroids are known to be fairly soluble in non-polar aromatic solvents and tend not to adsorb to glass,[14,15] those contemplating re-use of counting vials should not presume that similar results will be obtained with other types of samples. Lipids, cations, multivalent anions, proteins and basic amino acids are particularly prone to glass adsorption.

Washing and re-using counting bottles is a risky business unless each vial is filled with fresh scintillant and counted prior to the addition of the radioactive sample.

SUMMARY

A comparison of common counting vials has shown that the optimum counting vial for a particular experiment depends upon volume of sample, counting efficiency or quench and background count rate. Eight commonly used vials including three miniature vials are intercompared with respect to two figures of merit. Users are warned regarding the re-use of scintillation vials and the application of the external standard ratio method of quench correction to vials of different construction. The sample channels ratio technique of quench correction is shown to be independent of vial material or shape.

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DISCUSSION

R. Dierckx: Clean vials are very important for accurate counting; polyethylene vials are especially sensitive in this respect. It is enough to take a clean vial in your hand to change the counting efficiency before and after by a few percent. We clean our vials with trichlorethylene. What do you recommend for cleaning the vials?

K. Painter: Cleaning methods depend entirely on the types of samples being counted. There are three publications in the literature which enthusiastically recommend various cleaning devices, but at least two of the papers describe the use of radioactive steroids which tend not to adsorb to glass. The type of cleaning agent to be used varies with the sample being counted.

B.W. Fox: We have found that ultrasonic cleaning provides an excellent means of cleaning vials used for a variety of sample preparation methods. Have you experienced the formation of a noxious gas resembling formaldehyde when using polypropylene vials? We suspect that it occurs when the Mahin and Lofberg peroxide–perchloric acid procedure is used in these vials. The result of this gaseous emission can be corrosion of the belts and damage to the light collecting system.

K. Painter: Polypropylene is not used to any extent in the States; I have not observed this phenomenon. All digestions, whether acidic, basic or oxidative in nature, should be carried out in glass vials.

D.E. Bowyer: With respect to trying to wash vials, it is obviously important to remember that the washing-up solution must be chosen so as to remove the chemical entity absorbed and does not relate to the isotope used as label.

P. Johnson: You made the point early in your talk that one advantage of the narrow mini-vial over larger vials was that the photomultiplier tube faces in the counter could be closer together, thus reducing light scatter. Therefore, what is the point of using a mini-vial inserted within a larger vial?

K. Painter: The point is that, with the reduced diameter of the mini-vial, photons have a shorter path length within the sample, which is advantageous in a high-quenching situation. Fewer photons will be lost due to quenching (not scattering)

if the path length through the quenching medium is reduced.

P. Johnson: Many of us with biomedical applications have enough trouble dissolving components in much more than 10 ml and the small-volume vials would therefore be disadvantageous. I am not sure that you have really brought out the advantages of the mini-vials — can you re-iterate them?

K. Painter: There is one big advantage — cost. Lower volumes of scintillator mean lower costs. Counting efficiencies are higher and backgrounds are significantly lower, resulting in superior figures of merit. The table shown previously which intercompares the various counting vials demonstrates this point.

M. Ergin: It seems that standard glass vials are most suitable for Carbon-14 counting. In counting natural Carbon-14 activity would you like to comment on the contribution of the vials to the background activity, due to Potassium-40, etc? Does it change from vial to vial?

K. Painter: In our experience the greatest variation in background count rate with glass vials is from different lots, either from the same supplier or from different suppliers. I would not recommend glass vials for counting natural Carbon-14 activity, but rather teflon, nylon or polyethylene in that order.