CHOICE OF COUNTING VIAL FOR LIQUID SCINTILLATION: A REVIEW

Kent Painter
Amersham/Searle Corporation
Arlington Heights, Illinois, USA

ABSTRACT

Various types of counting containers have been used in liquid scintillation counters. The most commonly used types are standard 20 ml capacity glass, polyethylene and nylon vials. A comparison is made of the various counting parameters, such as background, efficiency, E^2/B, permeability, external standard ratio, wall adsorption, durability and inertness to solvents. A number of new miniature counting vials are described and warnings are given regarding external standard ratio quench correction, volume dependency, static electricity and geometry. The advantages and disadvantages of various types of liquid scintillation caps are discussed. Special emphasis is given to recognizing and avoiding errors produced by poor choice of vial, with specific examples from the literature.

INTRODUCTION

In many ways liquid scintillation counting has become so routine that few papers in bioanalytical research discuss or reference counting procedures or materials. Even papers dealing specifically with liquid scintillation often fail to mention the counting container. Unfortunately, we have encountered many instances where the choice of counting vial was based solely upon habit or economy with little thought given to possible adverse consequences. It has been frequently noted that the counting bottle can be a major factor in liquid scintillation counting accuracy and reproducibility (1-6).

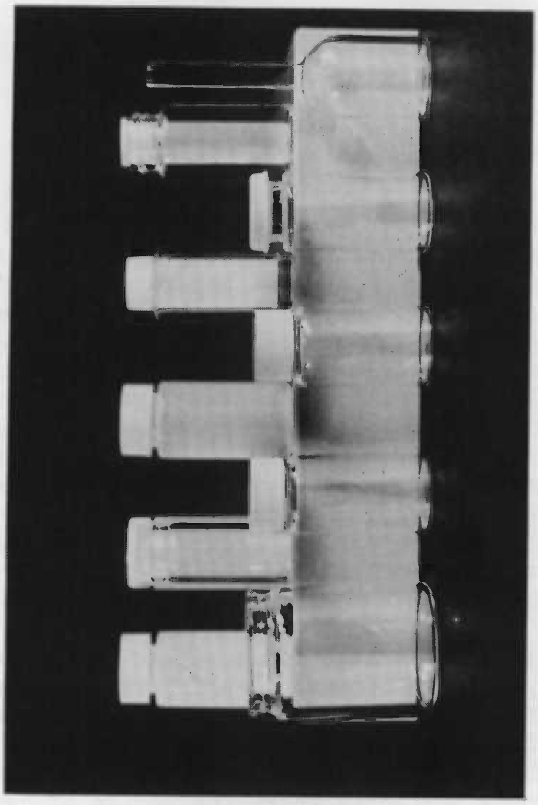


Fig. 1. <u>Liquid Scintillation Counting Vials</u>
Top row left to right: (a) Polyethylene (b) 10 ml Glass Insert for Polyethylene Holder (c) Nylon (d) Minivial™ (e) Polyethylene Insert for Glass Holder. Bottom row left to right: (f) 60 ml Glass (g) Borosilicate Glass (h) General Purpose Glass (i) Quartz (j) Flame-seal Borosilicate.

It is the purpose of this paper to review the history and development of the various counting containers now in use and to compare the relative merits of currently used systems.

HISTORY

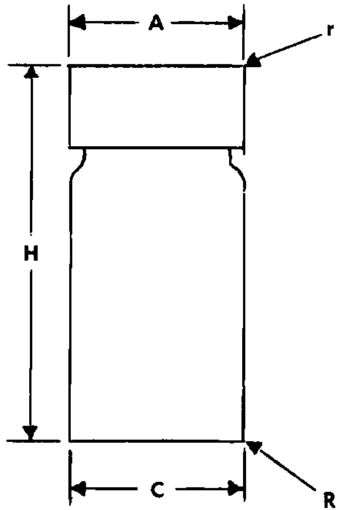
Due to limitations of cocktail capacity for aqueous samples, the first commercial liquid scintillation counters introduced in the early 1950's were designed to accommodate rather large 60-85 ml weighing bottles (7-12) (Fig. 1). In some systems vials were optically coupled to the photomultiplier tube by the use of a silicone fluid (7,10,13,14), but by the mid-1950's reflective counting chambers of polished aluminum proved convenient and superior in performance to fluid coupled systems.

It soon became apparent that the use of smaller counting containers would allow the photomultiplier tubes to be positioned closer together, thereby reducing light path distance losses and resulting in improved counting efficiencies. Backgrounds due to natural radioactivity in glass were also reduced due to the smaller mass of the counting container. The first successful liquid scintillation spectrometer manufacturer introduced in 1953 an ordinary 5 dram (20 ml) medicine bottle which became the accepted standard in the industry (15). Kimble Opticlear medicine vials were used initially (12), followed soon after by vials from Wheaton (10,12,16).

The standard 5 dram medicine bottle has been modified over the years to accommodate various instrument designs. A flat or slightly concave bottom was required and various height and bottom dimensions were necessary for sample sensing switches, group select indicators, and sample-changer elevator mechanisms. A drawing of a vial recently proposed as a standard by the International Electrotechnical Commission (17) is shown in Fig. 2. It should be noted that at present this standard has not been accepted or approved. Use of a counting bottle of improper dimension can result in a costly repair bill (18).

GLASS COUNTING VIALS

Glass counting bottles are of two general types: borosilicate glass and general purpose soda-lime or flint glass. The major differences between the two types are the higher cost and lower background count rate of borosilicate



45(Secretariat)166
March 1973

	Dimension values min mm	max mm
A	25.0	(1)
C	26.0	30
H	58.0	63.0
r	0.4	3.6
R		1.5

(1) Maximum not to exceed bottle diameter C.

Fig. 2. Recommendation Concerning Standard Dimensions of Test Bottles for Liquid Scintillation Counting-International Electrotechnical Commission.

glass (19,20). With general purpose glass vials, tritium backgrounds of 50 cpm are common, but good borosilicate glass bottles are generally in the range of 18-22 cpm. It should be emphasized that background count rates of glass vials differ considerably from manufacturer to manufacturer (21) and, more importantly, from lot to lot (4,20,22 23). Samples of each new shipment of vials should be checked and lot numbers recorded. We recommend purchasing a large quantity from a single lot.

Variations in mass and wall thickness can cause serious reproducibility problems, especially with the external standard ratio method of quench correction (3,24). Laney (29)has reported that background count rate of various containers is a function of mass rather than composition. A value of 1.3 cpm/g was nearly identical for several types of plastic and glass vials studied. Paix (23) has shown that the major variation between different sources of glass is due to natural radium-226 and its decay products. This was demonstrated by placing a crushed glass bottle in a low-level gamma spectrometer. Potassium-40 appears to be of lesser importance in background contribution of glass bottles.

Glass counting vials offer the advantages of inertness to strongly acidic or basic solvents, such as NCS* and Hyamine hydroxide†, resistance to permeability and deformation, low susceptibility to photoluminescence and compatibility to the external standard ratio method of automatic quench correction. Glass vials allow the analyst to be able to visually inspect samples (25). It should be noted that figures of merit for samples containing large volumes of water are not vastly different for general purpose and borosilicate glass vials when counting energetic beta emitters. This is due to the fact that the higher background of the general purpose vial is quenched in the same manner as the sample (26-28) and stresses the importance of using an identical sample minus activity for accurate background determination. We find very often that analysts use an unquenched background standard supplied with instruments, rather than an identical blank sample for determining background count rates. This usually results in a high estimate of background count

*Trademark Amersham/Searle
†Trademark Rohm and Haas

rate, but in some samples the opposite effect has been noted(29,4).

There are three disadvantages to the use of glass counting vials, only one of which can be regarded as serious. The first, which is really a nuisance, is breakage. Many of you, I am sure, have lost a sample from time to time by dropping a bottle. At one time a rather large vial manufacturer had difficulty with a borosilicate vial which would snap off at the neck when the slightest pressure was used to tighten the cap. Fortunately, the problem only occurred in about 5% of the bottles, but the problem did exist for several years.

The second disadvantage of glass bottles is cost. However, one must balance the value of glass versus the disadvantages of other types of containers, particularly with regard to accurate quench correction, and with the accuracy required for the experiment. We feel that glass vials are the vial of choice for critical counting applications where accuracy and reproducibility are required. For most counting applications standard glass vials can be used rather than the more expensive borosilicate glass vials.

The third and only serious disadvantage of glass is vial wall adsorption.

Recognizing vial wall adsorption can be a problem in itself. Sometimes it would be manifest in a decreasing count rate, but chemiluminescence, phase separation or precipitation can also be the cause. Chemiluminescence can be identified by comparing single phototube versus coincidence counts. Companies now offer instrument options which automatically compare such data and reject a sample which is chemiluminescent, but not one which is adsorbing or precipitating.

Usually an adsorbing sample can be distinguished from a precipitating sample by comparing glass to nylon or polyethylene counting vials which do not have polar surfaces (30). Another method is to shake vigorously or vortex the vial to see if the count rate increases by redissolving the sample. It should be emphasized that the mass of labelled sample is usually quite small, and one cannot rely on a visual check to ensure that precipitation is not occurring.

Phase separation can be a serious problem for the newer detergent cocktails which are quite temperature/phase dependent, particularly in cases where samples are prepared under ambient conditions and counted in refrigerated systems (31). We feel much more comfortable with glass bottles where we can "see" the sample, even though visual evidence is not the only criterion one must use for determining the homogeneity of a sample.

Another simple check for adsorption is to pour the scintillator solution from the original vial to a second vial, fill the original vial with fresh scintillant and recount. If adsorption is occurring, the original vial will retain a good percentage of the original counts.

The use of a double ratio method of quench correction (32,25) is the preferred screening method to check for precipitated, phased, adsorbed, or any non-homogeneous sample. If a sample is adsorbing, the external standard ratio will usually indicate a higher counting efficiency than the sample channels ratio. D. S. Glass (34) has shown that modern spectrometers with on-line computers can simply and routinely perform this check for the analyst.

Certain types of compounds, such as lipids, cations, multivalent anions, proteins and basic amino acids have been frequently reported to adsorb (30,35-44).

Several methods have been used to overcome vial-wall adsorption problems. Use of polyethylene or nylon containers usually prevents adsorption. Adding an excess of the corresponding non-radioactive carrier compound (30,35,37) results in a competition for the binding sites on the vial wall (45). If sufficient carrier material is present to saturate the vial walls, adsorption is minimized. For inorganic radioactivity standards, The Radiochemical Centre has found that 100 μg of carrier material per ml is needed to prevent appreciable adsorption.

Another method to overcome the problem of adsorption is to siliconize the vial walls (30,37,46,47). It should be noted that if a sample adsorbs to the counting vial, it has probably adsorbed to walls of flasks, pipettes and all other glass surfaces with which it has been in contact. All glassware should be siliconized or carrier added at the earliest possible stage in the assay.

Use of complexing agents (NCS*, Di(2-ethylhexyl)phosphate, EDTA) can prevent vial wall adsorption, particularly of the inorganic ions, such as $^{22}Na^{+}$ and $^{32}PO_4^{-3}$.

*Trademark Amersham/Searle

Although photoluminescence has been reported as a problem with glass counting bottles (10,48), we have not experienced any difficulties, as it is standard practice for samples to remain in the counter for four hours before counting. In our experience vials prone to photoluminescence are:

nylon>>>>polyethylene>glass

We always like to use a glass vial, but one must be aware of the problem of vial wall adsorption.

Two additional glass vials should be mentioned. Quartz counting vials were recognized many years ago because of the improved properties of light transfer or resultant higher counting efficiency and also lower backgrounds (14, 20). Although quartz vials can still be obtained from Packard, their cost ($12.00 each) is prohibitive for most applications.

Etched or sandblasted glass vials have been used to improve pulse heights and counting efficiency (49-51). A 3-5% increase in counting efficiency using frosted vials has been reported (52). Although frosted vials were commercially available from Beckman, presently there is no known commercial source. The $0.25 cost of the vials apparently was not worth the extra 3-5% in performance.

POLYETHYLENE/POLYPROPYLENE VIALS

Bell (53) noted in 1957 that chlorotrifluoropolyethylene bottles counted with the same efficiency as glass, but lowered the background 3- to 4-fold. Horrocks and Studier (54) and Rapkin and Gibbs (50,55,56) evaluated the application of polyethylene counting containers, pointing out several advantages and a number of disadvantages.

Polyethylene has the advantage of highest counting efficiency and lowest background of any commercial 20 ml bottle (55,57,58). Other advantages include low cost, combustibility for disposal purposes, non-breakability, non-polar surfaces which resist adsorption, and low susceptibility to photoactivation by strip lighting or sunlight.

The most serious disadvantage of polyethylene counting vials is permeability of the vial walls to many chemicals, but in particular, toluene and xylene. Rapkin and Gibbs (55) indicated that the leakage rate for toluene was approximately .7%/day. Lieberman and Moghissi (58) found a somewhat similar weight loss rate for xylene and toluene (but little loss of water and dioxane), and further pointed out that there was considerable variation from manufacturer to manufacturer. One polypropylene counting bottle tested in our laboratories showed a 47% weight loss in six days at 22°C.

Leakage of toluene and xylene poses a serious environmental problem for workers continually exposed to vapors. It is recommended that polyethylene bottles be discarded immediately after use. A further hazard is fire, particularly when quantities of filled bottles are stored in poorly ventilated areas, such as storerooms or closets (22).

A radiological hazard also exists, particularly when penetrating substances such as toluene and n-hexadecane are being used as in internal standards.

The permeation of other radioactive substances through polyethylene containers has been reported to us many times. As an example, certain enzymatic assays are carried out directly in liquid scintillation bottles. A carboxyl-^{14}C labelled substrate and a decarboxylase enzyme are used to measure a reaction rate by the liberation of $^{14}CO_2$. When polyethylene bottles are used, $^{14}CO_2$ tends to diffuse into the vial wall while the blank reaction is being carried out. When scintillator solution is added, $^{14}CO_2$ diffuses back into solution, resulting in high blanks and non-reproducible results (59).

Hansen (38) reported that when polyethylene vials, some with cocktail but no activity and some with cocktail plus Toluene-^3H, were placed in a liquid scintillation counter, the "background" count rate in the vials containing no activity rapidly increased with time.

Many polyethylene bottles swell appreciably as toluene permeates the vial wall. Serious instrument repair bills can result if such a vial jams the sample-changer mechanism. Heating polyethylene bottles usually softens the vial to the point where it is not useable, and polyethylene bottles are not recommended for use with strongly basic solubilizers, like Hyamine hydroxide* and NCS†. Opacity of most polyethylene bottles prevents direct inspection of the sample -- a serious disadvantage.

A more important effect of permeability noted by Bush(25)

*Trademark Rohm and Haas
†Trademark Amersham/Searle

and further studied by Laney (4) and others (60,61) is that once toluene or xylene has begun to permeate the vial walls, changes occur in the external standard ratio. The vial wall becomes more "efficient" when it is saturated with primary solvent. Although one study indicates that this variation stabilizes after 5 hrs. (61), another recommends two days (4). It should be noted that the time required for the external standard ratio to stabilize with polyethylene vials varies considerably from manufacturer to manufacturer and from batch to batch. If polyethylene vials must be used, the sample channels ratio method (33) of quench correction should be used.

Polyethylene and polypropylene vials differ considerably in wall thickness and the density used in manufacture. The physical characteristics vary from nearly transparent to opaque, and from soft and pliable to rigid.

In general, we have not found that the rate of permeation through vials from various suppliers is very different. It takes longer for the solvents to permeate the thick-walled, high-density bottle initially, but once the molecules have permeated the outer surface, the rate of solvent loss is not very different from manufacturer to manufacturer.

Curtis (61) has studied relative transpiration rates for a number of solutions stored in polyethylene, polypropylene, high-density polyethylene, teflon and glass containers.

We have not seen any real advantage of polypropylene as opposed to polyethylene, although one report (61) prefers polypropylene. In our experience the significant difference in plastic vial performance is the source of supply and not the plastic employed.

Spurious counts due to photoluminescence do not appear to be a serious problem with polyethylene bottles, providing they are placed in a counter several hours prior to counting (18,57). Static electricity has been reported with polyethylene bottles (4,57,63)but we have never found this to be a serious problem except in very dry climates. Treating vials with an anti-static agent, avoiding rubbing vials with cloth and preventing vials from continuously circulating through the counter can prevent photoluminescence due to static electricity.

In Cerenkov counting polyethylene vials are preferred to glass because of significant background reduction (64-67); nylon vials are unsuitable for the aqueous samples normally used.

NYLON VIALS

Nylon counting vials offer all the advantages of polyethylene vials -- unbreakable, disposable, extremely low background (68), adsorption resistance -- without the disadvantage of toluene or xylene permeability.

One major disadvantage of a nylon vial is that it softens and deforms when used with strongly polar solvents, such as cocktails containing large amounts of methanol, Triton X-100, water and some of the pre-mixes (58). Any commercial cocktail that is designed to hold large amounts of water probably will not work well with nylon vials.

A second disadvantage of nylon is its high susceptibility to photoluminescence. Nylon vials when exposed to the same light source result in 2-3 orders of magnitude higher luminescence than polyethylene or glass and the decay time is considerably longer.

Nylon vials are useful in applications where the cocktail contains minimum amounts of polar solvents, providing precautions are taken to protect the bottles from exposure to sunlight and fluorescent lighting.

TEFLON VIALS

Calf (69,70) has used teflon counting vials to achieve higher E^2/B than polyethylene for low-level counting, and teflon is much more resistant to leakage than polyethylene (62).

There is no known commercial source of teflon vials, but one would expect them to be very expensive, perhaps more so than borosilicate glass.

MINIATURE VIALS

Recent improvement in sample preparation, which allows the analyst to count up to 40 per cent aqueous sample, has generated considerable interest in the use of smaller counting vials. The first of the commercial versions, the Mini-vial™*, consists of a holder the size of a standard 20 ml counting vial which has been bored down the center to accommodate a 7 ml polyethylene vial.

The Mini-vial™ has a history of difficulties. The first holder design was simply a Lucite tube with an open

*Trademark Nuclear Associates

bottom. The shape of the holder caused serious jamming problems in some counters, and the holder was so short it passed beneath many sample sensing devices.

A second version was produced which featured an enclosed bottom, but the radius of curvature at the bottom edge of the holder would not trip some sample sensing switches. Further difficulties with spurious luminescence due to static electricity have been reported to us many times.

A third version said to overcome the static electricity and geometry difficulties is now being produced. Although we have not tested the newer version, we are not aware of any difficulties with the latter.

A further difficulty with the Mini-vial™ is destruction of the rather expensive holder by toluene permeating the polyethylene vial. If a vial is left in holder more than a few hours, it fuses to the holder, and the holder frequently deforms. The polyethylene insert is subject to all the disadvantages described for standard 20 ml polyethylene bottles.

Two novel miniature vial combinations are shown in Fig. 1. The first used a standard 3 dram (10 ml) glass vial and a 24 mm polyethylene vial as a holder. This combination is very inexpensive and offers all the advantages of a glass system. The glass vial fits snugly in the polyethylene holder when the cap is screwed on.

Another miniature vial combination employs a standard 22 mm glass vial as a holder, for which Sterilin designed a 5 ml polyethylene insert vial. Although this combination is economical, it possesses all the inherent disadvantages of a polyethylene counting vial and its use is not recommended.

COUNTING BOTTLE CAPS

Several types of bottle caps are available and the choice can seriously affect results. It was discovered quite early that aluminum foil-lined caps would increase counting efficiency 1-2% by reflecting photons escaping from the solution surface back down towards the photomultiplier tubes. It should be noted, however, that foil and cork-lined caps are incompatible with strongly basic cocktails

*Trademark Nuclear Associates

containing NCS* or Hyamine†. Teflon or polyethylene lined caps are preferred in these applications.

It was noted in 1965 that liquid scintillation counting vials with white screw caps resulted in higher, more erratic background count rates than liquid scintillation counting vials containing black caps (71). Further studies have shown that the higher background count rates are due to photoactivation of the caps by sunlight and laboratory fluorescent lighting (48).

As a typical example, when an empty 20 ml glass counting vial with white cap was allowed to drop into the counting chamber while the lid of the counter remained open, 1500 counts were recorded in the tritium channel during the first 0.01 minute, even with the coincidence mode of the spectrometer in operation. Under the same conditions, black-capped vials gave only 140 counts.

The phosphorescence of white caps appears to decay to normal levels very quickly, usually within two minutes (72). However, with the increasing popularity of multi-user counters, it is possible that while one individual's samples are counting, a second user may be opening the lid to unload or load samples. It is recommended that the counting cycle be stopped before opening the counter, and that the counter be reset 2-3 minutes after the lid has been closed.

The use of black caps on liquid scintillation vials, however, will minimize the problem of spurious counts due to photoluminescence (73).

Snap-on or push-in polyethylene caps are available for certain vials, but these almost inevitably cause fluid to be squeezed out of the vial when inserted. These caps can only be conveniently used one time and should not be used with the internal standard method of quench correction. For reasons of protection from radiation contamination, we do not allow snap-on or push-in plastic caps in our laboratories.

Complete sealing of commercial screw-cap counting vials is nearly an impossible task (71,74) mainly due to an irregular top edge. All vials leak primary solvent vapors, polyethylene, as previously discussed, being the worst offender. We find taping the space between the lower edge

*Trademark Rohm and Haas
†Trademark Amersham/Searle

of the cap and the shoulder of the bottle with teflon convenient for samples we may wish to re-count at a later date or for samples of long counting times. Care should be taken not to use thick tape which may cause jamming of the sample changer mechanism if the diameter becomes too large. Alternatively, flame-sealable low-background liquid scintillation glass bottles are commercially available (Fig. 1) but the laborious task of sealing the bottles precludes routine use.

EFFECT OF VIAL GEOMETRY IN COUNTING CHAMBER

Garfinkel (71), Stanley (5) and Laney (4) have described significant error introduced in the assay by the differential positioning of a counting vial in the counting chamber. When using mini-vials, it is imperative that holders are of reproducible dimensions both in terms of concentricity and height of sample vial.

VIAL FILLING DEVICES

Filling large numbers of counting vials can be a tedious, and sometimes hazardous, task. Several devices are available which reduce exposure to solvent vapors and speed bottle filling.

The least expensive filling device is the tilting dispenser offered by many suppliers in adjustable and fixed volumes. Cost is generally about $15.00. A more accurate and rapid filling mechanism is the Repipet*, which sells for about $75.00. This device screws on a standard 4 1 bottle and can fill a counting bottle accurately in a few seconds.

A much more elaborate (and expensive) device is the Multi-Jet 100†, which fills ten vials simultaneously by one stroke of a lever, or an entire case of vials in less than one minute. The cost, however, is $595.00 per unit.

EFFECT OF VIAL ON PULSE HEIGHT SPECTRUM

While it is commonly recognized that the shape of a beta energy spectrum changes with sample composition, it should be noted that energy spectrum shifts when using vials of different composition can cause an increase or decrease in counting efficiency, changes in ratio and changes in spill-

*Trademark Labindustries
†Trademark Isolab

over factors (50,60,66,75). Optimal gain and discriminator settings should be established with the type of vial to be used in the assay.

EFFECT OF VOLUME ON COUNTING EFFICIENCY

Several investigators have shown that optimum counting efficiency in a 20 ml counting vial plateaus between 10-15 ml where the efficiency is very little affected by volume (4,60,66,67,76-78). Rummerfeld and Goldman (3) have shown efficiency of mini-vials to be much more sensitive to small volume changes. In the above studies if height of sample in vial had been plotted against counting efficiency, nearly identical curves would have been obtained.

SPECIAL TYPES OF VIALS

Schram (79) has reviewed counting vials which can be used as flow cells in conventional liquid scintillation spectrometers. Ashcroft (80) constructed a glass vial to count gamma emitting nuclides by an external sample method, and these vials are now commercially available. Schram (81) has suggested a modified counting bottle for bioluminescence assays.

WASHING AND REUSING COUNTING VIALS

Washing and reusing counting bottles is risky business, unless each vial is filled with fresh scintillant and counted prior to the addition of the radioactive sample. We have investigated reusing scintillation bottles several times and have found that if one considers the cost of technician time, counter time and the chance of a contaminated bottle affecting the precision of data, reusing standard counting bottles is false economy. In no case should polyethylene, polypropylene or nylon vials be reused, and all caps should be discarded after a single use.

Rummerfeld and Goldman (76) have discussed the economics of vial washing versus the accuracy required for the experiment. Harris and Friedman (82) have described a simple rinsing and cleaning apparatus for use with glass counting bottles. Drosdowsky and Egoroff (83) designed a simple decontamination unit which can clean up to 55 vials per hour. Several vial washing units are available commercially, among them the Refluxowasher (Buchler Inst.) which has been evaluated by Kushinsky and Paul (84).

It is interesting to note that in the studies mentioned above radioactive steroids were employed. Since steroids are known to be fairly soluble in non-polar aromatic solvents and tend not to adsorb to glass (30,85), those contemplating reuse of counting vials should not presume that similar results will be obtained with other types of samples.

SUMMARY

The choice of counting container can drastically affect not only the optimum conditions of the assay, but also the accuracy and reproducibility of counting data.

For the majority of applications we recommend the use of a glass counting vial, and the sample channels ratio method of quench correction. The exception is extreme low level counting where polyethylene or teflon must be used to achieve high figures of merit.

In the future I believe the ability to count large volumes of aqueous samples and the high specific activity labelled compounds now available will spur manufacturers to develop, with only minor modifications to counting chamber geometry and sample changer, counters which will be designed to accommodate 5-10 ml bottles.

ACKNOWLEDGEMENTS:

I should like to acknowledge the invaluable assistance of Bart Laney and Dave Hansen (Nuclear-Chicago); Jim Gibbs (Packard); Murray Vogt (Isolab); and Mike Gezing, Dorothy Gabbei and JoAnn Jacobsen (Amersham/Searle).

BIBLIOGRAPHY

1. Birks, J.B., and Poullis, G.C., in Liquid Scintillation Counting (Crook, M.A., Johnson, P., and Scales, B., Eds.) Heyden, London, 1972, p. 1.
2. Kalbhen, D.A., in Liquid Scintillation Counting, (Dyer, A., Ed.) Heyden, London, 1971, p. 127.
3. Cavanaugh, R., in The Current Status of Liquid Scintillation Counting (Bransome, E.D., Ed.) Grune and Stratton, New York, 1970, p. 293.
4. Horrocks, D.L., in Tritium (Moghissi, A.A., and Carter, M.W., Eds.) Messenger Graphics, Phoenix, 1973, p. 30.
5. Stanley, P.E., in Liquid Scintillation Counting, (Crook, M.A., Johnson, P., and Scales, B., Eds.) Heyden, London, 1972, p. 285.
6. Spratt, J.L., in Liquid Scintillation Counting, (Crook, M.A., Johnson, P., and Scales, B., Eds.) Heyden, London, 1972, p. 245.
7. Rapkin, E., in The Current Status of Liquid Scintillation Counting, (Bransome, E.D., Ed.) Grune and Stratton, New York, 1970, p. 45.
8. Utting, G.R., in Liquid Scintillation Counting, (Bell, C.G., and Hayes, F.N., Eds.) Pergamon, New York, 1958, p. 67.
9. Guinn, V.P., in Liquid Scintillation Counting, (Bell, C.G., and Hayes, F.N., Eds.) Pergamon, New York, 1958, p. 166.
10. Davidson, J.D., and Feigelson, P., Int. J. Appl. Radiat. Isotopes, 2, p. 1 (1957).
11. Packard, L.E., in Liquid Scintillation Counting, (Bell, C.G., and Hayes, F.N., Eds.) Pergamon, New York, 1958, p. 50.
12. Davidson, J.D., in Liquid Scintillation Counting, (Bell, C.G., and Hayes, F.N., Eds.) Pergamon, New York, 1958, p. 88.
13. Hodgson, T.S., Gordon, B.E., and Ackerman, M.E., Nucleonics, 16, p. 89 (1958).
14. Bernstein, W., Bjerknes, C., and Steele, R., in Liquid Scintillation Counting (Bell, C.G., and Hayes, F.N., Eds.) Pergamon, New York, 1958, p. 74.
15. Rapkin, E., in Liquid Scintillation Counting, (Crook, M.A., Johnson, P., and Scales, B., Eds.) Heyden, London, 1972, p. 61.

16. Radin, N.S., in Liquid Scintillation Counting, (Bell, C.G., and Hayes, F.N., Eds.) Pergamon, New York, 1958, p. 108.
17. International Electrotechnical Commission, Technical Committee No. 45 (Nuclear Instrumentation), Draft 45 (Secretariat) 166, March 1973.
18. Horrocks, D.L., in Survey of Progress in Chemistry, Vol. 5, (Scott, A.F., Ed.) Academic Press, New York, 1969, p. 185.
19. Agranoff, B.W., in Liquid Scintillation Counting, (Bell, C.G., and Hayes, F.N., Eds.) Pergamon, New York, 1958, p. 220.
20. Agranoff, B. W., Nucleonics, 15, P. 106 (1957).
21. Kasida, Y., and Iwakura, T., Radioisotopes (Japan), 11, p. 257 (1962).
22. Kobayashi, Y., and Maudsley, D.V., in Methods in Biochemical Analysis (Glick, D., Ed.), 17, p. 31 (1969).
23. Paix, D., Int. J. Appl. Radiat. Isotopes, 19, p. 162 (1968).
24. Hagashimura, T., Int. J. Appl. Radiat. Isotopes, 13, p. 308 (1962).
25. Mueller, E, in The Current Status of Liquid Scintillation Counting, (Bransome, E.D., Ed.) Grune and Stratton, New York, 1970, p. 181.
26. Schram, E., Organic Scintillation Detectors, Elsevier, New York, 1963.
27. Scales, B., Anal. Biochem., 5, p. 489 (1963).
28. Wang, C. H., in The Current Status of Liquid Scintillation Counting (Bransome, E.D., Ed.) Grune and Stratton, New York, 1970, p. 305.
29. Laney, B. H., in Tritium, (Moghissi, A.A., and Carter, M.W., Eds.) Messenger Graphics, Phoenix, 1973, p. 156.
30. Litt, G. J., and Carter, H., in The Current Status of Liquid Scintillation Counting, (Bransome, E.D., Ed.) Grune and Stratton, New York, 1970, p. 156.
31. Turner, J.C., Int. J. Appl. Radiat. Isotopes, 19, p. 557 (1968).
32. Bush, E.T., Int. J. Appl. Radiat. Isotopes, 19, p. 447 (1968).
33. Bush, E.T., Anal. Chem., 35, p. 1024 (1963).
34. Glass, D.S., in Organic Scintillators and Liquid Scintillation Counting, (Horrocks, D.L., and Peng, C.T., Eds.) Academic Press, New York, 1971, p. 803.

35. Davison, P.F., and Andersson, L.P., Anal. Biochem., 47, p. 253 (1972).
36. Allison, J.M., Monro, A.M., and Offerman, J.L., Anal. Biochem., 47, p. 73 (1972).
37. Robel, E., Anal. Biochem., 51, p. 137 (1973).
38. Hansen, D.L., Nuclear-Chicago Corporation, DesPlaines, Illinois, U.S.A., personal communication.
39. Johnson, E.P., and Lowenthal, G.C., Int. J. Appl. Radiat. Isotopes, 23, p. 196 (1972).
40. Brandreth, Dale A., and Johnson, R.E., Science, 169, p. 864 (1970).
41. Hayes, F.N., U.S.A.E.C. Document LA-1639 (1963).
42. Blanchard, F.A., and Takahashi, I.T., Anal. Chem., 33, p. 975 (1961).
43. Dobbs, H.E., in Organic Scintillators and Liquid Scintillation Counting, (Horrocks, D.L., and Peng, C.T., Eds.) Academic Press, New York, 1971, p. 669.
44. Snyder, F., and Stephens, N., Anal. Biochem., 4, p. 128 (1962).
45. Tuck, A.G., and McNair, A., Int. J. Appl. Radiat. Isotopes, 23, p. 395 (1972).
46. Petroff, C.P., Nair, P.P., and Turner, D.A., Int. J. Appl. Radiat. Isotopes, 15, p. 491 (1964).
47. Parkinson, P.I., and Medley, E., in Liquid Scintillation Counting, (Crook, M.A., Johnson, P., and Scales, B., Eds.), Heyden, London, 1972, p. 109.
48. Scales, B., in Liquid Scintillation Counting, (Crook, M.A., Johnson, P., and Scales, B., Eds.) Heyden, London, 1972, p. 101.
49. Gordon, B.E., and Curtis, R.M., Anal. Chem., 40, p. 1486 (1968).
50. Rapkin, E., and Packard, L.E., Proc. Univ. New Mexico Conf. on Organic Scintillation Detectors, U.S. Govt. Printing Office, Washington, 1961, p. 216.
51. Schwerdtel, E., Int. J. Appl. Radiat. Isotopes, 17, p. 479 (1966).
52. "Frosted Vials", Beckman Instrument Company, Fullerton, Calif., S70462-1069-150A.
53. Bell, C.G., in Liquid Scintillation Counting, (Bell, C.G., and Hayes, F.N., Eds.) Pergamon, New York, 1958, p. 157.
54. Horrocks, D.L., and Studier, M.H., Anal. Chem., 33, p. 615 (1961).
55. Rapkin, E., and Gibbs, J.A., Int. J. Appl. Radiat. Isotopes, 14, p. 71 (1963).

56. Rapkin, E., and Gibbs, J.A., Packard Instrument Company Bulletin, Number 9, 1965.
57. Moghissi, A.A., Kelley, H.L., Regnier, J.E., and Carter, M.W., Int. J. Appl. Radiat. Isotopes, 20, p. 145 (1969).
58. Lieberman, R., and Moghissi, A.A., Int. J. Appl. Radiat. Isotopes, 21, p. 319 (1970).
59. Berry, J.A., Carnegie Institution of Washington, Stanford, California, U.S.A., personal communication.
60. Haviland, R.T., and Bieber, L.L., Anal. Biochem., 33, p. 323 (1970).
61. Johanson, K.J., and Lundqvist, H., Anal. Biochem., 50, p. 47 (1972).
62. Curtis, G.J., Rein, J.E., and Yamamura, S.S., Anal. Chem., 45, p. 996 (1973).
63. Moghissi, A., in The Current Status of Liquid Scintillation Counting, (Bransome, E.D., Ed.) Grune and Stratton, New York, p. 86 (1970).
64. Lauchli, A., Int. J. Appl. Radiat. Isotopes, 20, p. 265 (1969).
65. Parker, R.P., and Elrick, R.H., Int. J. Appl. Radiat. Isotopes, 17, p. 361 (1966).
66. Gould, J.M., Cather, R., and Winget, G.D., Anal. Biochem., 50, p. 540 (1972).
67. Parker, R.P., and Elrick, R.H., in The Current Status of Liquid Scintillation Counting, (Bransome, E.D., Ed.) Grune and Stratton, New York, p. 110 (1970).
68. Kobayashi, Y., and Maudsley, D.V., in The Current Status of Liquid Scintillation Counting, (Bransome, E.D., Ed.) Grune and Stratton, New York, 1970, p. 76.
69. Calf, G.E., Int. J. Appl. Radiat. Isotopes, 20, p. 611 (1969).
70. Calf, G.E., in Organic Scintillators and Liquid Scintillation Counting, (Horrocks, D.L., and Peng, C.T., Eds.) Academic Press, New York, p. 719, 1971.
71. Garfinkel, S.B., Mann, W.B., Medlock, R.W., and Yura, O., Int. J. Appl. Radiat. Isotopes, 16, p. 27 (1965).
72. Laney, B., Nuclear-Chicago Corporation, DesPlaines, Illinois, personal communication.
73. Painter, K., and Gezing, M. (in press).
74. Burleigh, R., in Liquid Scintillation Counting, (Crook, M.A., Johnson, P., and Scales, B., Eds.) Heyden, London, p. 139, 1972.
75. Butterfield, D., and McDonald, R.J., Int. J. Appl. Radiat. Isotopes, 23, p. 249 (1972).

76. Rummerfield, P.S., and Goldman, I.H., Int. J. Appl. Radiat. Isotopes, 23, p. 353 (1972).
77. Price, L.W., Laboratory Practice, April, 1973, p. 277.
78. Clausen, T., Anal. Biochem., 22, p. 70 (1968).
79. Schram, E., in The Current Status of Liquid Scintillation Counting, (Bransome, E.D., Ed.) Grune and Stratton, New York, 1970, p. 129.
80. Ashcroft, J., Anal. Biochem., 37, p. 268 (1970).
81. Schram, E., in The Current Status of Liquid Scintillation Counting, (Bransome, E.D., Ed.) Grune and Stratton, New York, 1970, p. 129.
82. Harris, J.E., and Friedman, L., Anal. Biochem., 30, p. 199 (1969).
83. Drosdowsky, M., and Egoroff, N., Anal. Biochemistry, 17, p. 365 (1966).
84. Kushinsky, S., and Paul, W., Anal. Biochem., 30, p. 465 (1969).
85. "Super Hot Tritiated Steroids", Amersham/Searle Corp., Arlington Heights, Illinois, U.S.A., (1971).