

“FILTERSCINT”: A HIGH EFFICIENCY SCINTILLATION TECHNIQUE FOR ³H-LABELED HARVESTED SAMPLES

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ABSTRACT. Replacement of vial liquid scintillation counting (LSC) technology by the flatbed scintillation counter for filtration assays has led to a rapid increase in sample throughput, ease of preparation and reduction of scintillant waste. Disposal of this waste becomes more important each year, not only because of the radioactivity present, but also the organic solvent content. Alternatives to liquid scintillant involve meltable solid scintillant (Meltilex™), meltable solid scintillant filters (Fluoromats) and solid scintillant particles on a filter matrix (Ready Filter™). Filtration of ³H-labeled cells using any of these techniques gives counting efficiencies near 75% of di-isopropylnaphthalene (DIN)-based liquid scintillant, which is, itself, estimated to give true counting efficiency of 54% for filtered cell samples (Potter & Warner 1986). I describe a new technique using an aliquot of a solid scintillant material in suspension (yttrium silicate), which is placed into the wells of a microtitration plate along with the cells before harvesting. After filtration and drying, the cells become intimately surrounded by solid scintillant that, because of its high intrinsic efficiency, gives count rates at or just above those with DIN-based scintillant. In addition, if liquid scintillant is added so that it soaks into the solid scintillant, cells and filter, the counting efficiency rises to 140–150% of liquid scintillant alone, *i.e.*, about 75% true counting efficiency for ³H in a heterogeneous system.

INTRODUCTION

Usually, LSC samples are dissolved in scintillant or, if insoluble and finely divided, may be suspended in scintillant gels. Particulate samples may also be filtered onto a filter support layer; this technique is often used where unincorporated isotopes must be washed from the sample, *e.g.*, from biological cells by semi-automated harvesting methods. The samples can be placed in separate vials with liquid scintillant before counting. The flatbed scintillation counter (Betaplate™ system) was introduced recently in which multiple samples deposited on a filter support are placed together in scintillant without being separated (Warner *et al.* 1985; Potter *et al.* 1986, 1987). This allows a considerable reduction in preparation time and in the amount of scintillant required. The scintillant, however, usually contains an organic solvent, which poses disposal problems, even in small amounts. The presence of liquid scintillant is also a problem for some samples containing small labeled molecules, which may dissolve in scintillant and diffuse away from the correct sample area.

One solution to this problem is to use a special sheet of solid scintillant, which is heated until it melts and soaks into the filter (Meltilex™, Wallac Oy). When the composite cools, the scintillant solidifies, halting further potential diffusion of the sample. Figure 1 shows the results of harvesting two whole plates of 96 samples of cells labeled with ³H-thymidine. Here, the Meltilex™ was melted either using a hot plate or by a special heater to heat just those areas bearing the samples. The means were not significantly different and the standard deviations were small. The overall efficiency was about 70% of BetaplateScint™. A method described by Fujii & Roessler (1991) uses melted paraffin scintillant poured onto and infiltrating a filtered sample. For the Betaplate™, this method would cause problems of sample movement during infiltration; it is probably best suited to single samples for standard LSC.

As another solution to diffusion problems, the filter itself may be composed of scintillant, so that the close proximity of the sample particles to the filter yields countable scintillations, as described in the original flatbed LSC patent (Warner & Potter 1981). Another technique, reported by Wundlerly & Threadgill (1991) is that the filter may support solid scintillant particles (such as yttrium

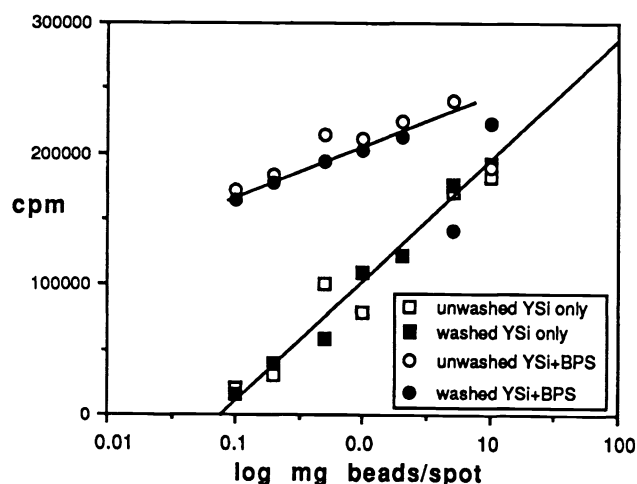


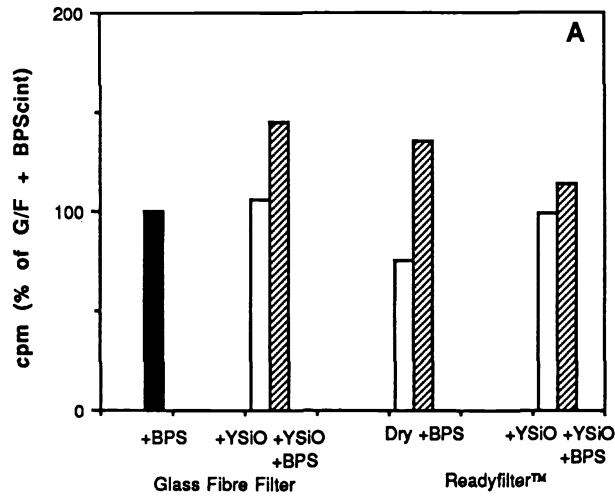
Fig. 3. Effect of different amounts of yttrium silicate loading per sample well on counting efficiency with and without additional BetaplateScint™, and for either washed cells or cells without removal of unincorporated isotope (unwashed)

TABLE 2. Relative Counting Efficiencies for Harvested Cells and Malarial Parasites using "Filterscint" Solid Scintillant

K562 cells - (n = 12)	× 1000 cpm	St. dev.	CV%	% of G/F
<i>Experiment A</i>				
Glass fiber + liquid scintillant	662.0	18.6K	(2.8)	100.0
Y ₂ SiO ₃ "Filterscint"	703.2	34.4K	(4.9)	89.0
Y ₂ SiO ₃ + liquid scintillant	954.8	59.5K	(6.2)	144.2
<i>Experiment B</i>				
Glass fiber + liquid scintillant	753.7	18.9	(2.5)	100.0
Y ₂ SiO ₃ "Filterscint"	798.3	31.0	(3.9)	105.9
Y ₂ SiO ₃ + compaction by heated rollers	853.0	32.3	(3.8)	113.2
<i>Malarial parasites - (N + 12)</i>				
Glass fiber + liquid scintillant	19.28	0.42	(2.19)	100.0
Y ₂ SiO ₃ "Filterscint"	17.64	1.37	(7.78)	91.5
Y ₂ SiO ₃ + liquid scintillant	29.96	1.05	(3.51)	155.4

The crosstalk produced by laterally emitted light and detected in a neighboring sample position was also measured for the different techniques (Table 3). All methods were satisfactory, but less so for Ready Filters™. This may have been caused by the continuity of high-efficiency scintillant between the samples; also Ready Filters™ do not have the printed grid used in the Betaplate™ glass fiber filters to reduce crosstalk (Warner & Potter 1988). In the future, it should be possible to approach "Filterscint" + liquid scintillant counting efficiency by adding a small amount of particulate meltable solid solvent to the scintillant particles, which may itself be, or contain, a scintillant. This will improve energy transfer and achieve maximum counting efficiency after heating by heated rollers. This material will also serve to bind the particulate scintillant and sample on the filter to prevent accidental movement. It would be useful for some applications to include, in the binder component, a substance that could stop the incorporation of isotope at the time of its addition. For example, for cells incorporating ³H-thymidine into their DNA in a proliferation assay, the incorporation is reduced greatly if non-radioactive thymidine is added in considerable excess of the ³H-thymidine. It is expected that 100 μg thymidine/well is sufficient. For special purposes, metabolic inhibitors or detergent, to lyse the outer membrane, could be added.

K562 cells



Malarial parasites

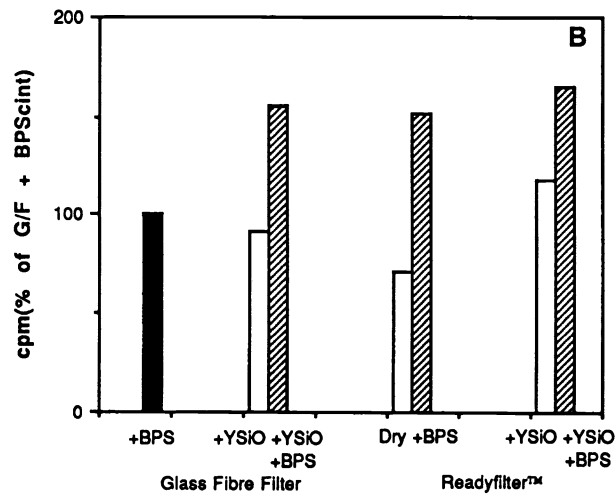


Fig. 4. Relative counting efficiencies of "Filterscint" for (A) K562 cells and (B) malarial parasites compared with Ready Filter™

TABLE 3. Crosstalk and CV for Different Filters and Scintillants (n = 12)

Filter	Crosstalk (%)	CV (%)
Glass fiber + BpScint™	0.002	2.5
Meltilex™	0.001	3.9
G/F + YSiO(Filterscint)	0.013	2.5
Ready Filter™	0.161	2.5
Ready Filter™ + YSiO	0.115	4.6

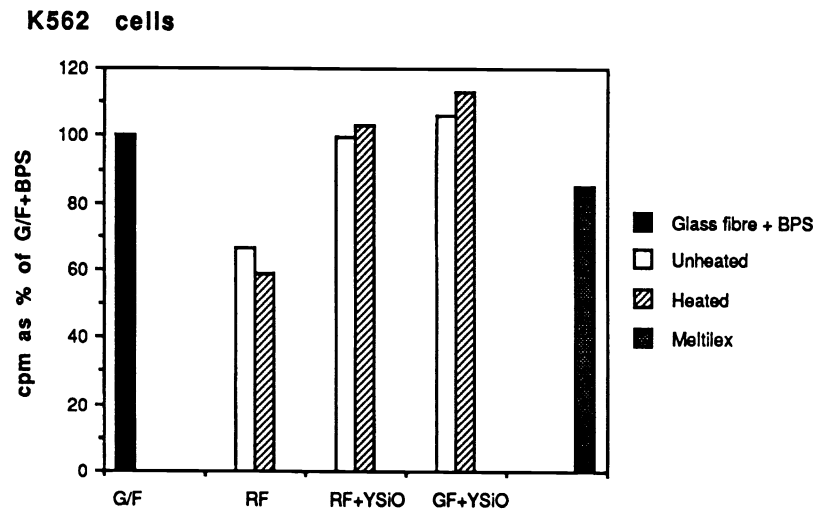


Fig. 5. Effect of compaction by heated rollers on counting efficiency of "Filterscint"

Sometimes it is necessary to include a binder in order to form a composite pellet. Such a binder must be inert enough not to affect the assay, although some suitable substances may already be present as sample components. One example is glucose, which is present in the growth media containing cells that may be incorporating labeled compounds. In the future, a dispenser will be used for the convenient addition of "Filterscint" to the wells of plates before harvesting.

ACKNOWLEDGMENTS

I wish to thank Dr. B. Elford for the gift of labeled parasites, Dr. D. Ferguson for electron microscopy, Beckman Ltd. for the gift of Ready Filters™ and Wallac for material support.

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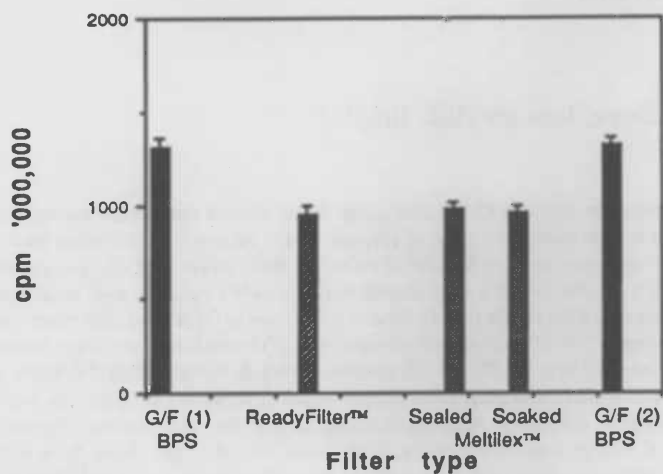


Fig. 1. Relative counting efficiency for harvested cells using Meltilex™, Ready Filter™ and BetaplateScint™ liquid scintillant

silicate) on its surface, placed there by filtration, scattering or spraying onto appropriate adhesive (Wunderly & Quint 1989); samples are then filtered onto this surface. Figure 1 shows results from my tests of these filters; counting efficiency was about 70% of BetaplateScint™. This corresponds well with the 36% true counting efficiency reported by Wunderly. For these two types of filter/scintillant configurations, about half of the beta particles emitted from the radioisotopes will not encounter the scintillant, resulting in a maximum efficiency of 50%. The surface complexity of a yttrium silicate particle (Fig. 2) may account for the higher counting efficiency actually measured. Theoretically, the maximum counting efficiency for ^3H is about 72% ($2 \times 36\%$), assuming all electrons strike the scintillant. Although these Ready Filters™ are intended for punching out into vials, they could be suitable for the flatbed counter.

To improve (theoretically) the previous techniques, the fibers of the filter may consist of, or be coated with, a meltable scintillant. After filtration and drying, the filter may be heated so that the scintillant flows all around the particles of sample and the counting efficiency is thereby increased

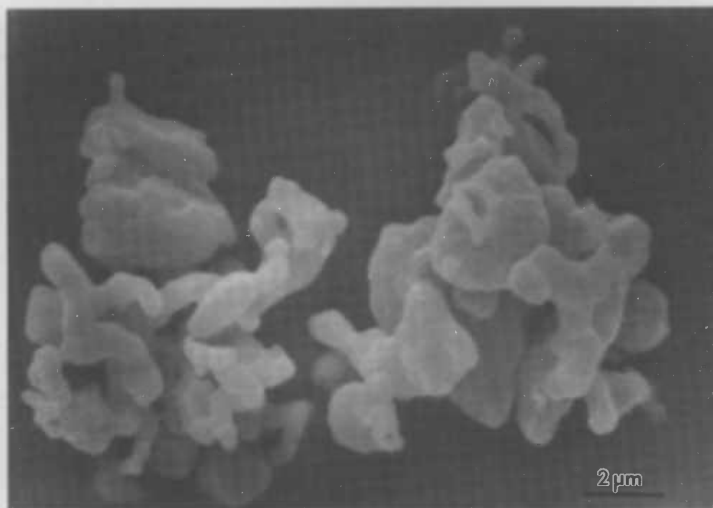


Fig. 2. Scanning electron micrograph of yttrium silicate particles

TABLE 1. Relative counting efficiency of "Fluoromat" with di-isopropylnaphthalene solid solvent, PPO and bis-MSB for harvested cells (K562 cells at a density of $1 \times 10^6 \text{ ml}^{-1}$, 200/well)

Filter	Sample 1% (CV)*	Sample 2% (CV)	Mean % (CV)
<i>Control - G/F</i>			
+ BetaplateScint™	100.0 (± 3.0)	100.00 (± 6.2)	100.00 (± 4.4)
<i>Fluoromat</i>			
Before heat	50.6 (± 2.7)	43.9 (± 14.0)	47.3 (± 9.9)
After heat	78.4 (± 3.5)	69.7 (± 7.4)	73.1 (± 5.2)

* CV = coefficient of variation: σ expressed as percentage of mean

considerably (Potter & Warner 1990). Because flow occurs on a microscopic scale, there is, again, no significant diffusion of soluble sample components away from the sample area. Previous tests (Potter & Warner 1991) used durene as a melttable solid solvent for the fluors. At the suggestion of staff at Fisons Scientific Equipment, Leicester, UK, I have now used solid isomers of DIPN containing fluors as coatings on polypropylene depth filters; after passing through heated rollers, these give a maximum counting efficiency of 73.1% relative to BetaplateScint™ (Table 1). Thus, the solid melttable scintillant, the yttrium silicate filters and the melttable scintillant-coated filter all have a similar maximum counting efficiency compared with the best liquid scintillant methods.

FILTERSCINT

Here I present preliminary data for yet another solution to the various problems, whereby the scintillant, in the form of small particles or beads, such as yttrium silicate, is aliquotted as a slurry or as a dispersible pellet into a vessel that holds the sample together with any unincorporated isotope. Sample and scintillant are then filtered together onto any suitable retaining filter support in such a way that every sample particle is surrounded on all sides and packed in by solid scintillant. Unincorporated isotope will pass through the filter as usual. After drying, the counting efficiency of this method is considerably greater than the method that has solid scintillant already in place on the surface of the filter. As described above, particles composed of scintillants, such as yttrium silicate, are intrinsically more efficient at detecting low-energy electrons than liquid or melttable solid scintillants. Thus, it might be possible to obtain higher counting efficiency with this "Filterscint" technique than to use the best liquid scintillants, although the poor energy transfer between the sample and scintillant particles will probably reduce counting efficiency to a lower level than the theoretically possible maximum. In a preliminary experiment, ^3H -thymidine-labeled cells were harvested in this way, with different amounts of yttrium silicate in the wells. A marked dependence on this quantity was observed (Fig. 3). The "washed" cells were used to compare with the more usual harvesting when unincorporated isotope is present, to confirm that significant non-specific binding did not occur. In further experiments, similar counting efficiencies were obtained with large K562 cells and ^3H -hypoxanthine-labeled malarial parasites (Fig. 4A, B). Counting efficiency was similar to that using BetaplateScint™. When Ready Filter™ was used as a base, we noted little improvement over the glass fiber filter alone (Table 2). Counting efficiency increases further with both Ready Filter™ and glass fiber using the "Filterscint" technique upon the addition of liquid scintillant (BetaplateScint™) to such a filter (Fig. 3, Table 2). This is likely due to further detection of electrons by the liquid scintillant and better optical properties of the sample. Little improvement was shown if the filters were compacted by heated rollers, which should have also enhanced coupling between emitted electrons and scintillant (Fig. 5). Thus, the Filterscint technique probably gives 70–75% true counting efficiency for ^3H as labeled particulate matter (DNA in lysed cells) counted under heterogeneous counting conditions.