CHAPTER 12

An Introduction to Flat-Bed LSC: The Betaplate Counter

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INTRODUCTION

The use of liquid scintillators was first described in 1950.[1,2] Neither paper refers to the use of liquid scintillators for internal sample counting, but describe their use for alpha, gamma, and neutron counting. Reynolds shows a block diagram of a two PM tube coincidence circuit, and thus was the first to suggest this arrangement for liquid scintillation counting reduction of what he calls, "the well known inconvenience of PM tube background." Both papers refer to the use of benzene, xylene, and toluene, and to the addition of anthracene. Kallman reports the effect of napthalene and the use of terphenyl. The paper usually cited as the first reference to internal sample counting was published in 1953.[3] However a paper in 1952,[4] described a method of counting $^{14}CO_2$ in toluene-PPO solution by condensing the gas at -80°C, and thus may have a prior claim.

The first commercial liquid scintillation counter (LSC) is quoted as being marketed in 1954,[5] by Packard, and the earliest reference found to the evaluation of the standard vial was 1957,[6] although this may not have been the first. In this paper, the Wheaton 5 dram vial was compared for background and CPM against 85 mL weighing bottles containing between 10 to 50 mL of scintillant. The Wheaton vial, having a lower background, was shown to be the container of choice. By 1957, Packard seemed to have adopted the Wheaton vial as standard, and their first model in 1954 may also have used a small sized vial. In the U.K. as late as 1968, equipments using the large weighing bottles were still being marketed on the basis of a larger sample volume incorporation.

To reduce thermal noise from the PM tubes, the Packard LSC of 1957, in addition to counting coincidence, used a domestic deep-freeze to house the counting head. Even so, this particular counter had a background for ^3H of 32 cpm and an efficency of 20%, giving a figure of merit of 12.5. However, these figures were very good as a counter marketed in 1968 by a British company, 9 years after the Packard, it had a figure of merit of less than one (0.67). While the Packard counter represents an early version of a liquid scintillation counter, modern equipment is of course based on the same use of vials to contain the scintillant and sample. Up until recently, cooling of the PM tube was a standard feature. The only significant change has been in the instrumentation.

THE FLAT-BED GEOMETRY COUNTER (THE BETAPLATE)

The Betaplate concept was designed in 1976,[7] when it was recognized that the procedure for counting the ^3H labeled DNA of cells filtered onto glass fiber discs could be improved. The principle use of this technique was in the mixed lymphocyte assay where the sample preparation proceedure is based on a cell harvester. Suspensions of ^3H labeled cells are aspirated from a microtitre plate, filtered onto glass fiber sheets and washed using the harvester. The glass fiber filter is 10 cm wide by 25.5 cm long, and with one particular harvester (Skaatron Inc.), the samples are aspirated 12 at a time, each sample occupying an area 1 cm in diam, a whole filter holding 96 samples in a 6×16 matrix.

Conventionally, after the sheet is dried, the discs bearing the samples are individually removed from the sheet with forceps, placed in a standard or minivial and combined with a few milliliters of scintillant. It takes about 20 minutes to prepare 96 samples and load them into a standard LSC, and it is a very tedious operation. Recently, some degree of automation has been available from some manufacturers.

The proposed design (Figure 1) did away with the vial, replacing it with a flat plastic container in which the whole filter sheet could be placed with the 96 samples intact. Scintillant would be added to the whole container and the PM tubes brought up close to each sample in turn; a flat-bed geometry.

In the original drawing, the plate was to be scanned in a raster fashion. The problem of cross talk (that is adjacent sample interference caused by light conduction) was considered and some black material in the form of lines or circles printed on the glass fiber filter between the samples was thought might be an effective method of reducing this problem. It has now been shown [8,9] that the printing reduces cross talk by two orders of magnitude for Tritium, and this forms an important part of the Betaplate concept.

RESULTS AND DISCUSSION

Some basic experiments designed to test the concept were performed using a metal jig made of two sandwiched together aluminium plates, each with a 1 cm diam hole at one end. This device is fitted into a standard vial while a small piece of glass fiber filter bearing ^3H labeled cells could be placed between the

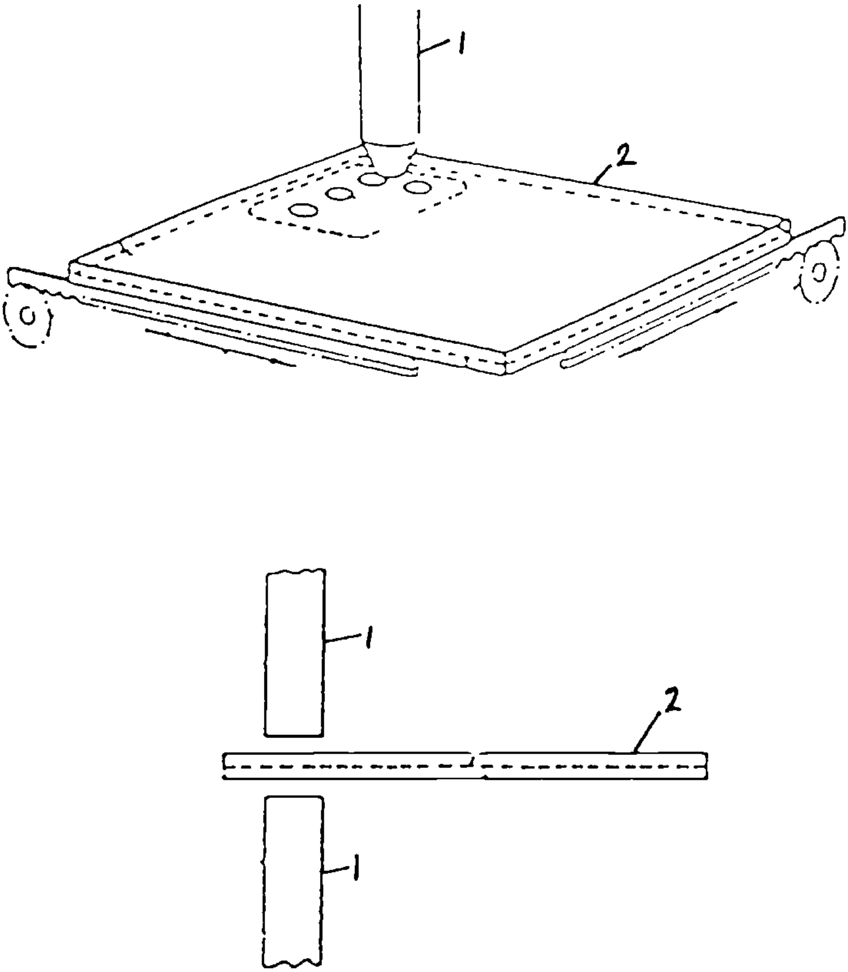

Figure 1. Layout drawing of the flat-bed counter. The photomultiplier tubes (1), are placed either side of the sample container (2) which could be moved in a raster fashion.

metal plates, either opposite the holes to measure sensitivity, or not opposite, and thus masked, to test for cross talk.

With just a wetting of scintillant, the background countrate in the ^3H channel was <10 cpm, and the efficiency was about 50% of that obtained in a standard vial with 2mL of scintillant. After some experimentation, the cross talk was reduced to $<0.1\%$; thus, this crude representation of the principle, showed that cross talk and sensitivity were certainly good enough for work with ^3H labeled cells, although at that time cross talk was considered to be too high with ^{14}C.

A simple, manually operated prototype constructed by Wallac Oy of Finland gave efficiency values of 28% for ^3H and 78% for ^{14}C, showing that reasonable efficiences could be obtained. Their design staff suggested a sealed plastic bag, rather than a flat plastic box, as the sample container and a metal cassette as a carrier and a means of further cross talk reduction.

A second fully automatic prototype (Figure 2) having a 2016 sample capacity was constructed. In our laboratory, the background is low, but not untypical of sites away from high radiation sources, or high enviromental background; however, the prototype was fitted with quartz tubes so the background figures are lower than the standard production Betaplate, although that is also available with quartz tubes if required. The production 1205 Betaplate is shown in Figure 3.

With the Betaplate system, harvesting is the same save the glass fiber filters. They are especially formulated to be strong enough to prevent the sample areas from easily breaking away, and they are printed with a grid to reduce cross talk. Having harvested the samples and dried the filter, it is then slid into the special plastic bag, 10 mL of scintillant added which, for standard glass fiber sheets is sufficent for all 96 samples. The scintillant is gently encouraged to permeate the filter by a hand roller, and the remaining edge is heat sealed. It is then placed in a metal cassette for counting. Time taken about 2 minutes. Glass fiber filters and the special heat-sealable plastic bags are shown in Figure 4.

In addition to glass fiber filters, nylon membranes are available for DNA work and the figures for background, efficiency, and cross talk, include data on nylon membranes (Tables 1–3).

The low values of cross talk (Table 3), show that filter printing not only

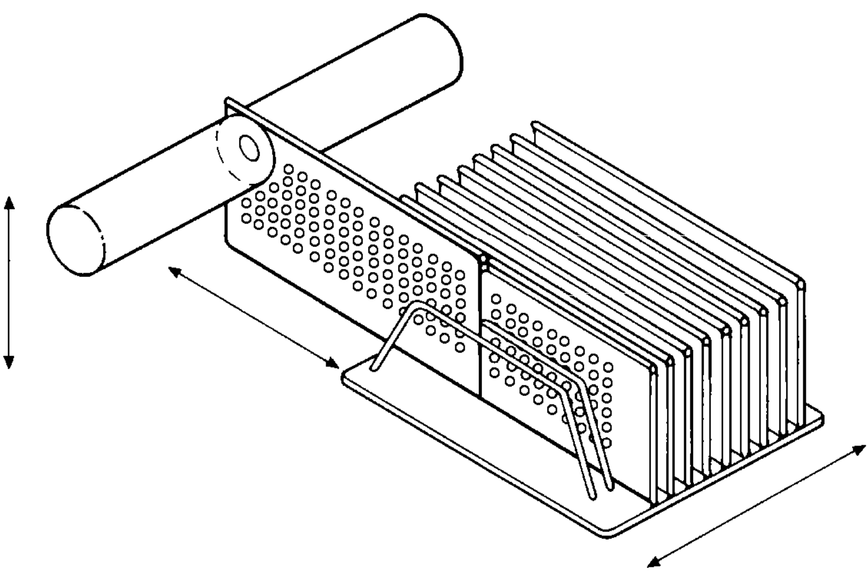

Figure 2. The layout of the MK II prototype.

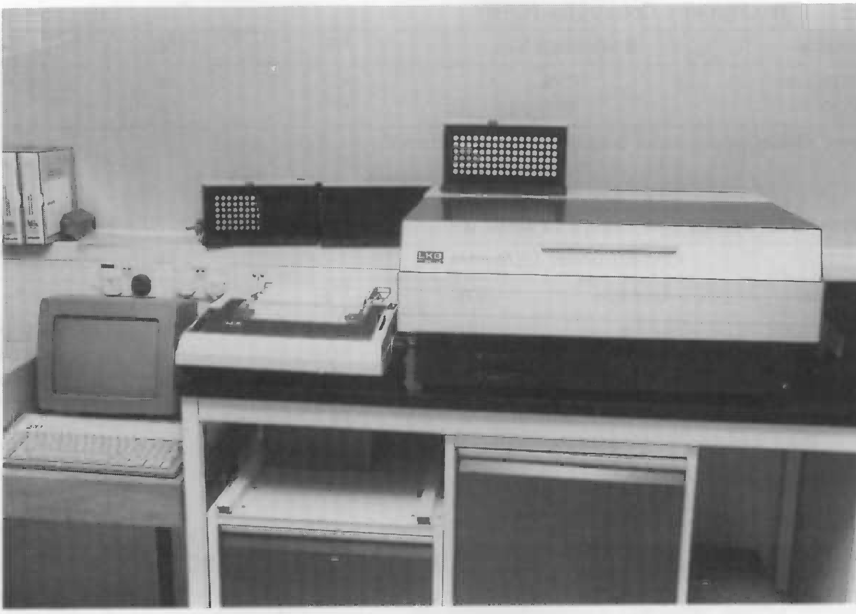

Figure 3. Production model Betaplate (Model 1205 Pharamacia/Wallac). The counter has six counting heads and a loading capacity of 1920 samples.

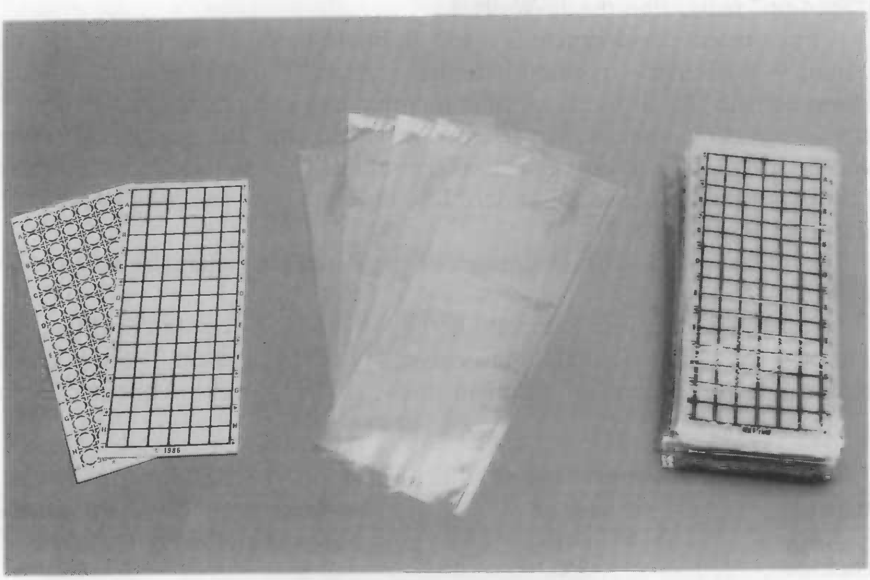

Figure 4. The filter mats with a grid pattern are the standard type used for filtration and the "tile pattern" are the "Spot on" mats taking volumes up to 30 μL.

Table 1. Background Countrates (CPM)

Material	Scintillant Vol.	^{3}H	^{14}C	^{32}P
Glassfiber	10mL	<2	<6	<7
Nylon	4mL	<1	<5	<6

Note: These values were obtained using quartz photomultiplier tubes.

Table 2. Efficiency and Figure of Merit (F)

	Conventional LSC			Flatbed Counter	
Material	**Isotope**	**Eff.**	**F**	**Eff.**	**F**
Glassfiber	^{3}H	47%	110	54%	>2000
Glassfiber	^{14}C	96%	180	97%	>1750
Nylon	^{32}P	90%	324	84%	>1000

Note: The samples used were labeled cells and therefore show "working" efficiencies rather than theoretical maximums.

reduces cross talk for low-energy isotopes, but also works well with higher energy emmitters such as ^{14}C and ^{35}S. It has also been found that good results are obtainable with gamma emmitters that have low-energy electron emmission, such as ^{51}Cr and ^{125}I. Specific cytotoxicity and receptor binding assays, therefore, can be performed using the Betaplate.

While the Betaplate was developed for use with filtered cells, plastic backed filters have been developed for spotting small volumes of up to 30 μL, which then can be dried onto the filter. This technique is used for the cytotoxic assay ("Spot-on" mats, Pharmacia-Wallac).

A more recent development is a 400 μL liquid-holding tray (Figure 5), containing 96 wells in the standard Betaplate format ('T' tray Pharmacia-Wallac). It was designed for use with scintillation proximity assay (SPA) kits requiring a 400 μL sample container (Amersham International). The background count and efficiency with a liquid sample are similar to a standard vial geometry LSC. Correlations between standard LSC and Betaplate counting methods are shown in Figures 6 & 7.

The advantages and disadvantages of the Betaplate system are summarised in Table 4.

Another aspect of the Betaplate is the low bulk of disposables (Figure 8). The bags containing filtered samples save 95% of the plastic bulk and a similar reduction in the volume of scintillant used.[10] The 'T' trays, while not having

Table 3. Cross Talk (Nearest Sample)

Material	Isotope	No lines	Printed lines
Glassfiber	^{3}H	0.600%	0.006%
Glassfiber	^{14}C	3.25%	0.080%
Nylon	^{32}P	0.16%	0.015%
Glassfiber	^{51}Cr	0.36%	0.015%
Glassfiber	^{125}I	2.0%	0.3%

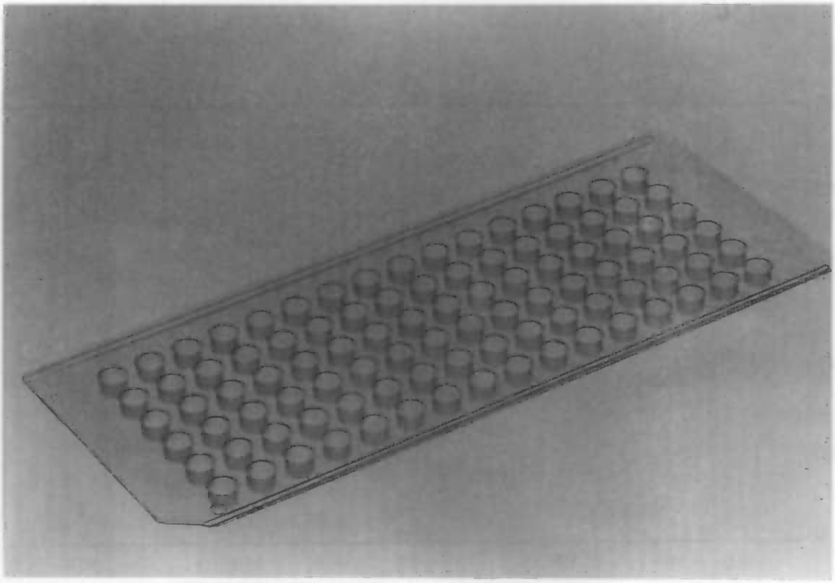

Figure 5. The 'T' tray for liquid samples. (Well capacity 400 μL.)

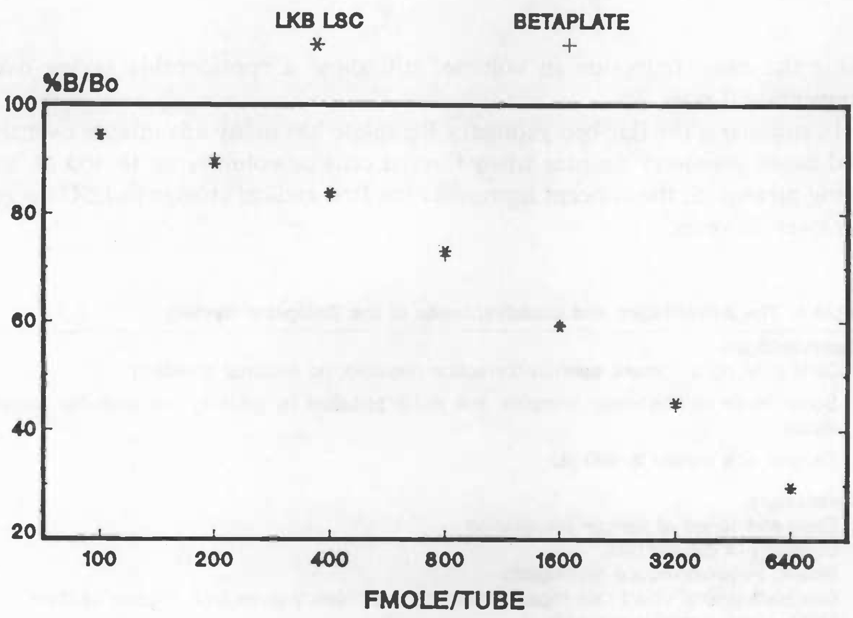

Figure 6. Correlation between a standard LSC and a Betaplate counter: ^{125}I cAMP Scintillation Proximity Assay. (Data kindly supplied by Dr. Nigel Bosworth, Amersham International.)

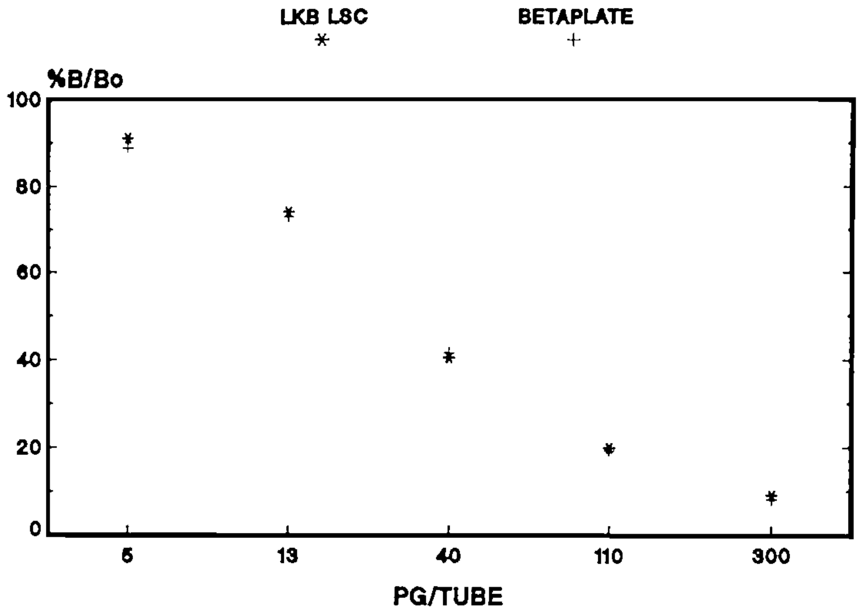

Figure 7. Correlation between a standard LSC and a Betaplate counter: ^{3}H Thromboxane B2 Scintillation Proximity Assay. (Data kindly supplied by Dr. Nigel Bosworth, Amersham International.

quite the same reduction in volume, still show a considerable saving over conventional vials.

In summary, the flat-bed geometry Betaplate has many advantages over the vial based geometry counter when filtered cells or volumes up to 400 μL are being measured; the concept represents the first radical change in LSC design for over 35 years.

Table 4. The Advantages and Disadvantages of the Betaplate System

Disadvantages
1. CPM only, no automatic quench correction possible, no external standard
2. Some cross talk between samples, but much reduced by printing and sensible sample layout
3. Sample size limited to 400 μL

Advantages
1. Ease and speed of sample preparation
2. Economy of disposables
3. Sealed samples reduce biohazards
4. Low background count rate together with good efficiency gives high Figures of Merit
5. Lightweight, compact machine
6. High sample loading capacity
7. Six counting heads gives high sample throughout
8. Low volume of waste for disposal

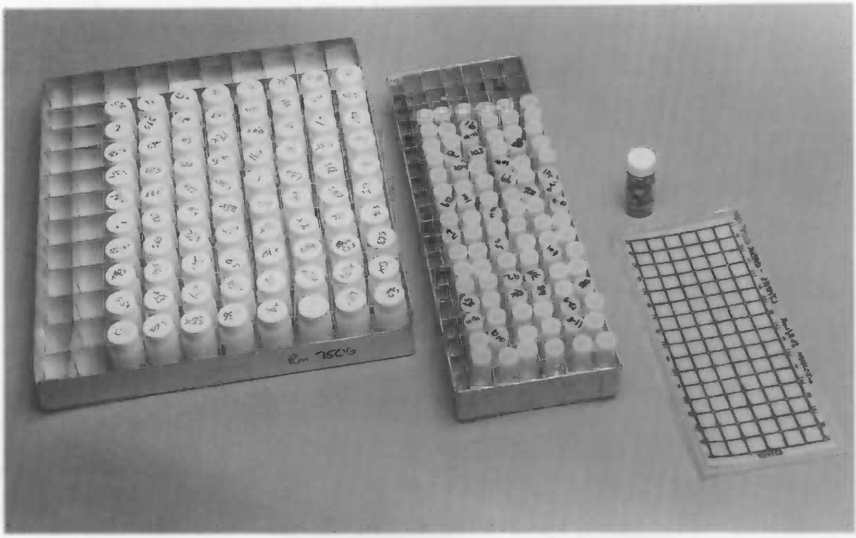

Figure 8. There is a 95% reduction in the volume of disposables when using filter mats. A 96 sample filter can be contained in a single standard vial.

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