

## HETEROGENEOUS COUNTING ON FILTER SUPPORT MEDIA

E. Long, V. Kohler and M.J. Kelly  
Beckman Instruments Inc.  
Scientific Instruments Division  
Irvine, CA 92713

### Abstract

Many investigators in the biomedical research area have used filter paper as the support upon which radioactive samples are counted. This means that a heterogeneous counting of sample sometimes results. The count rate of a sample on a filter will be affected by positioning, degree of dryness, sample application procedure, the type of filter, and the type of cocktail used. Positioning of the filter (up or down) in the counting vial can cause a variation of 35% or more when counting tritiated samples on filter paper.

Samples of varying degrees of dryness when added to the counting cocktail can cause nonreproducible counts if handled improperly. Count rates starting at 2400 CPM initially, can become 10,000 CPM in 24 hours for  $^3\text{H}$ -DNA (deoxyribonucleic acid) samples dried on standard cellulose acetate membrane filters. Data on cellulose nitrate filters shows a similar trend.

Sample application procedures in which the sample is applied to the filter in a small spot or on a large amount of the surface area can cause nonreproducible or very low counting rates. A tritiated DNA sample, when applied topically, gives a count rate of 4,000 CPM. When the sample is spread over the whole filter, 13,400 CPM are obtained with a much better coefficient of variation (5% versus 20%).

Adding protein carrier (bovine serum albumin-BSA) to the sample to trap more of the tritiated DNA on the filter during the filtration process causes a serious beta absorption problem. Count rates which are one-fourth the count rate applied to the filter are obtained on calibrated runs.

Many of the problems encountered can be alleviated by a proper choice of filter and the use of a liquid scintillation cocktail which dissolves the filter. Filter-Solv has been used to dissolve cellulose nitrate filters and filters which are a combination of cellulose nitrate and cellulose acetate. Count rates obtained for these dissolved samples are very reproducible and highly efficient.

Quantitative results can only be obtained by dissolving or emulsifying the filter and the sample in the cocktail. The normal quench monitors of external standard channels ratio or sample channels ratio are valid for dissolved filters. When the filter is transparent or opaque in the vial there are too many variables to be assured that a stable, constant efficiency count rate is obtained.

### Introduction

In pursuing biomedical literature these days, one is struck by the relative frequency with which one finds that the authors are counting samples on filter paper and measuring the disintegrations per minute (DPM) from these samples. For instance, the book "DNA Synthesis In Vitro" (1) has numerous articles on acid precipitated deoxyribonucleic acid (DNA) counted on filter support media in which the counts per minute are translated into specific activities. Recent journal articles in such prestigious journals as *Biochemistry* and *Journal of Biological Chemistry* have also contained articles in which specific activities on filter support media are claimed. All of these articles bring up the question as to how are these counts as obtained in the liquid scintillation counter corrected for quench, self-absorption, and beta absorption. Many authors appear to assume constant quench, constant self-absorption, and constant beta absorption, as well as other factors for each of their samples. This fact prompted us to reiterate some previously known experimental evidence on the position of the filter paper (2-4), the solubility of samples in cocktails which leach the material off of the filter paper and its inherent problems (5-9) and the problem of reproducibility of sample spot size and the use of carrier material to increase filtration efficiency. These problems can be alleviated if the filter and solution are able to be dissolved in a proper cocktail configuration. In the subsequent discussion, we will present evidence to show why it is necessary to dissolve the filter in order to obtain the true activity of the sample and to give the magnitude of error encountered for each experimental condition tried.

### Filters and Cocktails

There are two general types of filters used in biochemical analysis. The first type is a "depth" or crude filter which is used in routine separations. They are generally made of coarse fibers which are pressed together to form flow channels of random spacing and variable size. Filtered samples are trapped throughout the matrix of the filters, and

## HETEROGENEOUS COUNTING

due to the random orientation of the fibers, there is no absolute pore size. Paper filters and glass fiber filters are examples of this type.

The second type of filter is a "screen" or membrane filter that has definite ranges of particle retention. These filters are commonly composed of mixed esters of cellulose acetate, cellulose nitrate, and other polymers. These filters have a uniform microporous matrix. Millipore\* and Sartorius\*\* membrane filters are common representatives of this class.

A wide variety of cocktails have been used in counting radioactive material on filter papers. One of the most common cocktail formulations is a toluene/PPO cocktail formulation (6 grams PPO per liter of toluene) (7). Others have reported the use of similar toluene or xylene formulations with the presence of solubilizers or emulsifiers (Triton X-100\*\*\*\*, Bio-Solv III\*\*\*\*) (8,9).

There are two widespread uses for the depth and screen filters in radiochemical analysis. Many compounds of biochemical interest such as proteins and nucleic acids will precipitate out of solution in the presence of acids. Common acid solutions which cause this precipitation are: trichloroacetic acid (TCA) (10%), or perchloric acid (5 to 10%). The precipitated material will not pass through the pores of the filters and can be effectively collected on the filter. If the compound of interest has been radiochemically labeled, the filter containing precipitate can be counted by liquid scintillation. Many depth filters commonly serve this purpose, and the collection is generally more rapid than centrifugation.

### Materials and Methods

<sup>3</sup>H DNA (human) was a generous gift from D. Kingsbury, University of California, Irvine. Millipore filter, type HAWP (.45 u) was obtained from Millipore Corporation. Whatman No. 1 Filter Paper, Glass Fiber Filter (Reeve Angel Grade 934AH, 2.1 cm), and Sartorius SM111 and SM113 (pore size 0.45 u) filters were obtained from Science Essentials, Fullerton, California. Bovine Serum Albumin (Fraction V) was obtained from Pentex, Inc., Kankakee, Illinois.

- 
- \* Registered trademark, Millipore Corporation
  - \*\* Registered trademark, Sartorius-Membranfilter GMBH
  - \*\*\* Registered trademark, Beckman Instruments, Inc.
  - \*\*\*\* Registered trademark, Rohm and Haas

Samples were filtered on a standard Millipore filtration apparatus with a fritted glass disc support. Samples were measured in standard glass liquid scintillation vials on a Beckman LS-350 Liquid scintillation counter. The gain was set so that the tritium efficiency in a wide open window was over 60%.

Multichannel analyzer scans were performed on a Nuclear Data Model 1100 (10). All pipetting was done with a Clay Adams dispensing microliter pipette. Cocktail supplies were Beckman liquid scintillation grade.

### Results and Discussion

Oftentimes, the type of filter used in a biochemical experiment is determined by cost rather than by performance. Depth filters of the paper or glass fiber type are generally less expensive than that of the membrane type. The coarse nature of the paper and glass fiber type, however, increased the likelihood of beta absorption. Counting efficiencies with these systems are generally less than that of the membrane type.

To compare the efficiencies of various filtering media, [<sup>3</sup>H]-amino acid serum (195,600 dpm) was placed onto Whatman paper filters and glass fiber filters, allowed to dry for one hour, and counted in three cocktails: toluene/PPO, a non-emulsifying cocktail; Brays solution, a dioxane-based cocktail; and Filter-Solv, an emulsifying and dissolving cocktail. The results, shown on Table I, are compared to the same sample counted in Filter-Solv, but without a filter paper.

Table I

Filter	Cocktail	Toluene/PPO	Filter-Solv	Brays Solution
Whatman Paper	Mean CPM <sup>1</sup>	5083±2.7%	4222±6.2%	3769±1.9%
	ESCR <sup>2</sup>	.775	.720	.725
	SCR <sup>3</sup>	.726	.626	.635
Glass Fiber	Mean CPM <sup>1</sup>	14,223±.7%	22,934±1.4%	12,026±4.0%
	ESCR <sup>2</sup>	.768	.715	.724
	SCR <sup>3</sup>	.712	.672	.626
Without Paper	Mean CPM <sup>1</sup>	-	72,651±1.5%	-
	ESCR <sup>2</sup>	-	.706	-
	SCR <sup>3</sup>	-	.702	-

1. Duplicates were for each cocktail.
2. ESCR based on an LS-350 liquid scintillation counter with a cesium 137 source for ESCR.
3. SCR based on Channel settings of: A=0.300, B=80,300.

## HETEROGENEOUS COUNTING

A considerable loss in count rate is seen when paper or glass fiber filters are used, mainly due to beta absorption by the intact filter. This causes approximately 95% loss with paper filters and 82% loss with glass fiber filters as compared to the same sample counted without a filter (Table I). None of the cocktails can dissolve these types of filters. Emulsifying type dissolving cocktails (Filter-Solv) can remove some of the material from the glass fibers and suspend it in solution, resulting in a higher counting rate.

### Basic Problems in Filter Counting

The measured count rate obtained from a sample on a filter paper can be used to determine the amount of material trapped on the filter. Certain inherent problems with filters in cocktails can affect the measured count rate, irrespective of the actual radioactive material on the filter. This section will illustrate the nature and extent of these problems.

Heterogeneous counting, a term frequently applied to counting a sample on a filter, occurs when the filter remains intact upon exposure to the cocktail. The radioactive sample may thus remain fully imbedded on the filter or be jointly distributed into cocktail and filter. Thus, the radioactive sample can be distributed into two phases and be counted at different efficiencies. Such a situation is commonly encountered with cocktails that have an emulsifier. If this occurs, it may be difficult or impossible to determine the actual effect of quenching in the cocktail.

Heterogeneous counting of samples on filters also poses a further problem in that the filter can absorb some of the beta particles so that the emissions do not produce a measureable excitation of the solvent. This type of quenching is called beta absorption, and can result in a decrease in potential counts from the sample without any direct method of quantitating the degree of quenching. The extent of beta absorption will depend on the energy of the isotope, the type of filter and cocktail used, the degree of heterogeneity in the system, as well as other factors. Whenever a filter remains intact or partially intact in the cocktail, some degree of beta absorption is to be expected.

Intact filters also show geometry and time effects, dependence on the method of application of the sample to the filter, and poor precision. With some filters (usually the screen type), the filters are dried to remove water and make them less opaque than wet filters once cocktail is added. Near transparent filters can often be counted at higher

Table II

<u>Filter Cocktail</u>	<u>Paper</u>	<u>Glass Fibre</u>	<u>Millipore (Mixed Esters)</u>	<u>Cellulose Nitrate</u>	<u>Cellulose Acetate</u>
Toluene (Non- Emulsifier)	H	H	H	H	H
Dioxane	H	H	D	D	D
Dissolving Cocktails (Filter-Solv)	H	H	D	D	T

H - Heterogeneous System; Filter remains intact

D - Dissolved filter

T - Transparent filter

efficiencies than opaque filters, because there is less beta absorption in the system.

Some cocktails are able to chemically dissolve certain filters. While true dissolution of the filter does remove some of the concerns of heterogeneous counting, the aqueous sample must now be rendered into a stable emulsion. Filter-Solv\* is a typical example of this class of special cocktails.

Table II summarizes some of the characteristics which arise with common filters and cocktail formulations.

This report will comment extensively on the use of dissolving cocktails such as Filter-Solv\*. While chemical dissolution of the filter alleviates problems with heterogeneous counting, the sample must still be placed into intimate physical contact with the solvent. Dioxane-based systems (Brays solution) can dissolve some membrane-type filters, but biological precipitates are not emulsified and heterogeneity can still occur. It is imperative that the filter be dissolved and that the sample be finely emulsified in order to obtain valid counting results, 125-I labeled thyroid stimulating hormone (TSH) was precipitated onto Millipore (HAWP) filters and counted in toluene/PPO where the filter was intact, and in dissolving cocktails (Filter-Solv and Brays). Results are summarized in Table III.

The count rate is higher in Filter-Solv cocktail than in Brays, although both filters are dissolved. While precipitate is finely emulsified in Filter-Solv, the sample (not the filter) precipitates out of solution in the dioxane

\* Trademark, Beckman Instruments, Inc.

HETEROGENEOUS COUNTING

Table III  
Millipore Filters

<u>Cocktail</u>	<u>Mean CPM<sup>1</sup></u>	<u>ESCR</u>	<u>SCR<sup>2</sup></u>
Toluene : PPO	3574 ± 3.9%	.671	.846
Filter-Solv	7652 ± 5.7%	.592	.812
Brays	3902 ± 3.3%	.599	.847

1. Values based on duplicate values
2. SCR based on channel settings of: A = 0.300,  
B - 80 300.

based Brays. Therefore, heterogeneity still occurs, and the count rate is lower due to beta absorption. In fact, the amount of beta absorption is comparable to the sample counted in toluene/PPO, where the filter is intact.

Geometry and Position Effects

The actual counting rate of the sample will depend on the position or orientation of the filter in the vial. To demonstrate the effect of positioning, <sup>3</sup>H DNA (human) was TCA precipitated onto cellulose acetate membrane filters. The filters were counted in a toluene/PPO cocktail in two positions--0° and 180°. 0° was taken to be a filter at the bottom of the vial with the sample face up; 180° for the sample face down. Filters were either measured immediately when wet or were dried for 1 hour. Dried filters were visually less opaque than wet ones. The counting rates, as a function of position, are shown in Table IV.

A companion set of filters was counted in a dissolving cocktail (Filter-Solv). A higher count rate was seen in this system as the cocktail penetrated the filter further than with toluene/PPO system to increase the contact between radioactive sample and cocktail solvent.

Drying the membrane filters to make them less opaque upon addition of cocktail greatly alleviates the geometry effect. Dry filters gave essentially identical results whether counted at 0° or 180°. Opaque filters, however, generate considerable geometry effects. The measured count rate would decrease by nearly 40% (Table IV) without any significant change in the External Standard Channels Ratio (ESCR).

In the case of opaque filters, a change in the SCR was artificially induced upon measuring the 180° oriented filter. This drop in SCR occurred solely by positioning the filter at

Table IV  
Geometry Effects with Cellulose Acetate Filters

<u>Appearance</u>	<u>Position</u>	<u>CPM</u>	<u>ESCR</u>	<u>SCR<sup>1</sup></u>
Opaque	0°	9,818	.668	.617
(Toluene/PPO)	180°	6,545	.661	.465
Transparent	0°	26,574	.650	.626
(Toluene/PPO)	180°	26,542	.645	.609
Transparent (Filter-Solv)	-	29,651.6±2.9% <sup>2</sup>	.595	.543

1. SCR based on channel settings of: A = 0.300, B = 80.300
2. Duplicate average

180° - there was no increase in quenching in the system. The ESCR also does not appreciably change to reflect the loss in counting efficiency due to positioning of the filter.

There is nearly a 12% CPM difference between filters that are effectively "transparent" or dried and those counted in a suitable dissolving cocktail.

In a separate experiment, another batch of [<sup>3</sup>H] DNA (human) was TCA precipitated onto cellulose nitrate membrane filters (Sartorius-SM113). Similar effects were found which are summarized in Table V.

As in the case of cellulose acetate membrane filters, the cellulose nitrate filters show that positioning the filter affects the observed counting rate. There is

Table V  
Geometry Effects with Cellulose Nitrate Filters

<u>Appearance</u>	<u>Position</u>	<u>CPM</u>	<u>ESCR</u>	<u>SCR<sup>1</sup></u>
Opaque	0°	20,276	.672	.622
(Toluene/PPO)	180°	13,247	.696	.486
Transparent	0°	25,685	.681	.592
(Toluene/PPO)	180°	25,869	.669	.580
Dissolved (Filter-Solv)	-	36.831±1.1% <sup>2</sup>	.584	.632

1. SCR based on channel settings of: A = 0.300, B = 80.300
2. Duplicate Average

## HETEROGENEOUS COUNTING

approximately a 40% increase in count rate between "transparent" cellulose nitrate filters and dissolved filters. This suggests that filters which are transparent or near transparent to the naked eye can still experience extensive quenching by beta absorption.

### Changing counting rates

Opaque filters will commonly show a changing count rate for the same sample with time. In some cases where an emulsifying cocktail is used, material slowly elutes off the filter and is emulsified into the cocktail solution. The emulsified material is in better physical contact with the cocktail and will be counted at a higher efficiency than that which remains on the filter; hence, the count rate will rise with time.

A changing count rate has also been found with non-emulsifying cocktails (toluene/PPO). In these cases, the rise in counts stems from a progressive penetration of the dried filter by the cocktail--better penetration leads to better physical contact between the sample and cocktail, and a higher counting rate results.

To illustrate this, radioactive material in the form of [<sup>3</sup>H] DNA (human) was filtered on cellulose acetate membrane filters (Sartorius SM 111) and positioned at 0° in a vial. The samples were counted for various time intervals in a standard scintillation vial with 10.0 ml of non-emulsifying toluene/PPO cocktail. Two counting channels were used--the first channel covering the entire tritium energy range, and the second channel covering the upper energy tritium events. A ratio of the counting rate in the second channel divided by the first is a typical form for the SCR method. The counting rates of the filter sample as a function of time of exposure to cocktail are shown in Table VI.

While the count rate continues to increase as the cocktail continues to penetrate the filter, the ESCR does not change. The SCR, however, does undergo an initial change by decreasing .041 units to erroneously suggest a decrease in counting efficiency even though the count rate has increased.

The initial decrease in SCR stems from an increased sensitivity to low energy tritium decays as the cocktail penetrates the filter. This increased penetration of cocktail improves the intimate contact between sample and cocktail solvent so that weak beta decays which were previously absorbed can now produce a measurable pulse of light. Essentially, all of these pulses are from low energy decays and the second counting channel (which forms the numerator of the SCR) does not count as many of these low

Table VI

<u>Time of Counting</u> <u>(hours)</u>	<u>Gross CPM</u> <u>Channel A</u>	<u>Gross CPM</u> <u>Channel B</u>	<u>ESCR</u>	<u>SCR<sup>1</sup></u>
0	2409	1608	.669	.668
1	3351	2101	.665	.627
2	5706	3491	.666	.612
3	7212	4473	.668	.620
24	9818	6057	.668	.617

1. SCR based on channel settings of: A = 0.300, B = 80.300.

energy decays as the first channel. After 1 hour, this penetration stabilizes. It is important to mention that these weak beta decays were occurring in the sample as soon as cocktail was added--they were just absorbed by the filter to prevent the production of any light pulse.

The initial change in SCR is, of course, not due to any genuine change in chemical or color quenching. In fact, the ESCR which effectively measures the cocktail solution for quench content is the same for all measurements. After low energy penetration by the cocktail, further penetration produces a uniform increase in pulses throughout the entire LS spectrum to render the SCR unchanged. The count rate, however, increases accordingly.

In Table VII, results are shown which demonstrate the time effects with cellulose nitrate membrane filters.

Table VII

## Time Effects

CPM<sup>1</sup>

<u>Time</u>	<u>Channel A</u>	<u>Channel B</u>	<u>ESCR</u>	<u>SCR<sup>2</sup></u>
0	8,930	6,854	.785	.768
30 min	10,757	7,982	.782	.742
60 min	11,252	8,292	.786	.734
90 min	12,049	8,666	.783	.719

1. Sample applied to cellulose nitrate filters and counted in a toluene/PPO cocktail.
2. SCR based on channel settings of: A = 0.300, B = 80.300.

HETEROGENEOUS COUNTING

Table VIII

	Application	
	<u>Spread Over Filter</u>	<u>Topically Applied</u>
Mean CPM on Filter <sup>1</sup>	13,400 ± 642	4,131 ± 821
Mean CPM of Effluent	138 ± 31	206 ± 10
Mean ESCR	.767 ± .001	.768 ± .001
Mean SCR <sup>2</sup>	.709 ± .010	.702 ± .004

1. Based on average of five filters
2. SCR based on channel settings of : A=0.300, B=80.300.

Dry filters do not show such a strong dependence on time of exposure to cocktail. Apparently, the removal of water from the filter facilitates a rapid penetration of filter by the cocktail, and a stable count rate is obtained.

Application of Sample to Filter

The counting rate of samples on filters will be affected by the surface area of exposed sample. Samples which are spread over the entire filter have a greater exposed surface area to the cocktail than samples which are applied topically.

A set of seven identical samples containing the same amount of [<sup>3</sup>H] DNA (human) were precipitated and applied onto Millipore filters. Four were finely spread over the entire surface of the filter, while the remaining three were topically applied onto a small surface of the filter. All samples were dried and made transparent in a toluene/PPO cocktail. The counting rates of the two samples are shown in Table VIII.

High coefficients of variation on filters

Intact filters typically show a high coefficient of variation for replicates. Samples counted on a filter paper often show count rates which depend greatly on the geometry of counting, the method of application, the degree of dryness, and other physical parameters. All of these parameters contribute to produce a coefficient of variation outside of the expected experimental deviation of pipetting and counting statistics.

An example of the high coefficient of variation that is encountered with typical filters is shown in Table IX. In this example, 5 filters were prepared by TCA precipitating

Table IX

<u>Cocktail</u>	<u>Mean CPM<sup>4</sup></u>	<u>Mean ESCR</u>	<u>Mean SCR<sup>5</sup></u>
Toluene: PPO, wet <sup>1</sup>	6,671.9±39%	.777±9.2%	.711±1.3%
Toluene: PPO, dry <sup>1</sup>	27,925.6±11%	.774±1.6%	.719±1.0%
Filter-Solv <sup>2</sup>	39,056.1±2.8%	.706±1.0%	.692±.8%

1. Millipore filters, 5 replicates.
2. Cellulose nitrate filter, triplets.
3. Coefficient of variation (percent).
4. All samples counted to a 2% error.
5. SCR based on channel settings of: A = 0.300, B = 80.300.

tritiated DNA onto standard cellulose nitrate filters. The filters were collected and counted in toluene/PPO. A second set of 5 filters was prepared in the same way but was dried for 1 hour before adding cocktail. The physical parameters that affect the count rate exert a strong effect on wet filters. Drying the filters does reduce this coefficient of variation, but it is nevertheless quite high. As a baseline for comparison, identical filters were dissolved in a special dissolving cocktail (Filter-Solv) and counted.

#### Beta Absorption on Filters

Beta absorption occurs when filters remain intact, or partially intact upon addition of cocktail. Beta emissions can occur within the matrix of the filter and sample without exciting the cocktail to produce light scintillations. When an intact filter is counted, the observed count rate is due to radioactive decays that actually leave the filter paper and travel into the cocktail.

The degree of absorption is, in part, related to the amount of material on the filter--the more material on the filter, the greater the probability that some of the decays will be absorbed. It is common practice to add an unlabeled carrier substance to the radioactive material, and precipitate the entire mixture with an acid solution (trichloroacetic acid is commonly used). The carrier facilitates the trapping of the radioactive material on the filter by coprecipitation. If an insufficient amount of carrier is used, however, the radioactive material will pass through the filter and not be counted. If too much carrier is used, the degree of absorption increases.

To illustrate in a typical application, tritiated DNA (Human) was TCA precipitated on a standard Millipore filter

## HETEROGENEOUS COUNTING

(type HAWP). Varying amounts of an unlabeled carrier (Bovine Serum Albumin) were used to facilitate trapping the DNA on the filter. After drying the filters for over 60 minutes, duplicates were counted in a toluene/PPO system with an ESCR (External Standard Channels Ratio) as well as an SCR (Sample Channels Ratio) taken for each sample. Upon addition of cocktail to the dried filters, they became relatively transparent to the naked eye.

For comparison, an identical series was prepared, but the samples were dissolved in a special dissolving cocktail (Filter-Solv). The 50 microliter aliquot of tritiated DNA was also counted directly in a cocktail without the use of a filter. Its count rate was approximately 44,000 counts per minute. Results are summarized in Table X.

Note, that with relatively small amounts of carrier (under 200 micrograms) a certain amount of the DNA will not be trapped on the filter. Filters dissolved without any carrier, have nearly the same count rate as the dried filters, but nearly 14,000 of the 44,000 total counts per minute in the DNA aliquot are lost through the filter. If carrier is included with the precipitation, the count rate increases as more radioactive material is trapped on the filter. A plateau of nearly 40,000 counts per minute is trapped on the filter from 200 to 700 micrograms of carrier. This is not seen, however, in filters that are counted heterogeneously (transparent but undissolved) as the level of beta absorption increases as the amount of unlabeled carrier is increased. With this type of DNA and this type of Millipore filter, the plateau is not reached until 200 micrograms of carrier is used. The filter really contains approximately 40,000 counts per minute of DNA, but the highest count rate with transparent filters is less than 25% of that of the dissolved filter. Over 75% of the material on the 200 microgram BSA filter has been absorbed. Moreover, this level of absorption increases as more carrier is used.

Conventional instrumental methods of assessing quenching (the loss in the counting efficiency) do not reflect the reduction in counts due to beta absorption. While the ESCR (External Standard Channels Ratio) and the SCR (Sample Channels Ratio) between the transparent filters and the dissolved filters cannot be compared directly, since different cocktails are used, the ESCR and the SCR for all transparent filters remain relatively unchanged even though the count rate has decreased from 28,320 to 3,351 counts per minute.

The ESCR is not expected to change, as the loss in count rate stems from the heterogeneous distribution of

Table X  
Effect of Carrier on Absorption of Sample

<u>Appearance of Sample</u>	<u>Micrograms</u>			
	<u>BSA</u>	<u>CPM</u>	<u>ESCR</u>	<u>SCR<sup>1</sup></u>
Transparent Filter <sup>2</sup>	0	28,320±1.149	.776	.731
Dissolved Filter	0	29,950	.715	.706
Transparent Filter	100	22,832±50	.774	.739±.001
Dissolved Filter	100	34,433	.704	.670
Transparent Filter	200	9,656±316	.772	.744±.002
Dissolved Filter	200	39,449	.708	.691
Transparent Filter	300	7,247±742	.775±.001	.736±.005
Dissolved Filter	300	39,157	.709	.698
Transparent Filter	500	4,781±664	.773±.001	.733±.005
Dissolved Filter	500	37,947	.697	.680
Transparent Filter	700	3,351±469	.773±.001	.733±.001
Dissolved Filter	700	39,705	.703	.694

1. SCR based on channel settings of: A = 0.300, B = 80.300.
2. Duplicates were made for each set.

radioactive material in a non-scintillating phase (the matrix of the filter). In the samples where the filter is dissolved, the ESCR values are lower than the ESCR values of the transparent filters because some chemical quenching has been introduced into the system through the dissolution of the filter and the emulsification of the sample.

The SCR is based on the energy distribution of the tritiated sample. If chemical or color quenching is present in the sample, the resulting distribution of the tritiated sample will change. Two preselected counting channels of the LS counter, which are set to measure discrete portions

## HETEROGENEOUS COUNTING

of the sample's LS spectrum, will show a change when the two channels are expressed as a dimensionless ratio. When quenching is present, the counting rate will change, but the distribution of the LS spectrum will also change and the SCR will decrease to reflect the effect of quench. In the case of the filter, however, the SCR does not change in accordance to the increase in absorption.

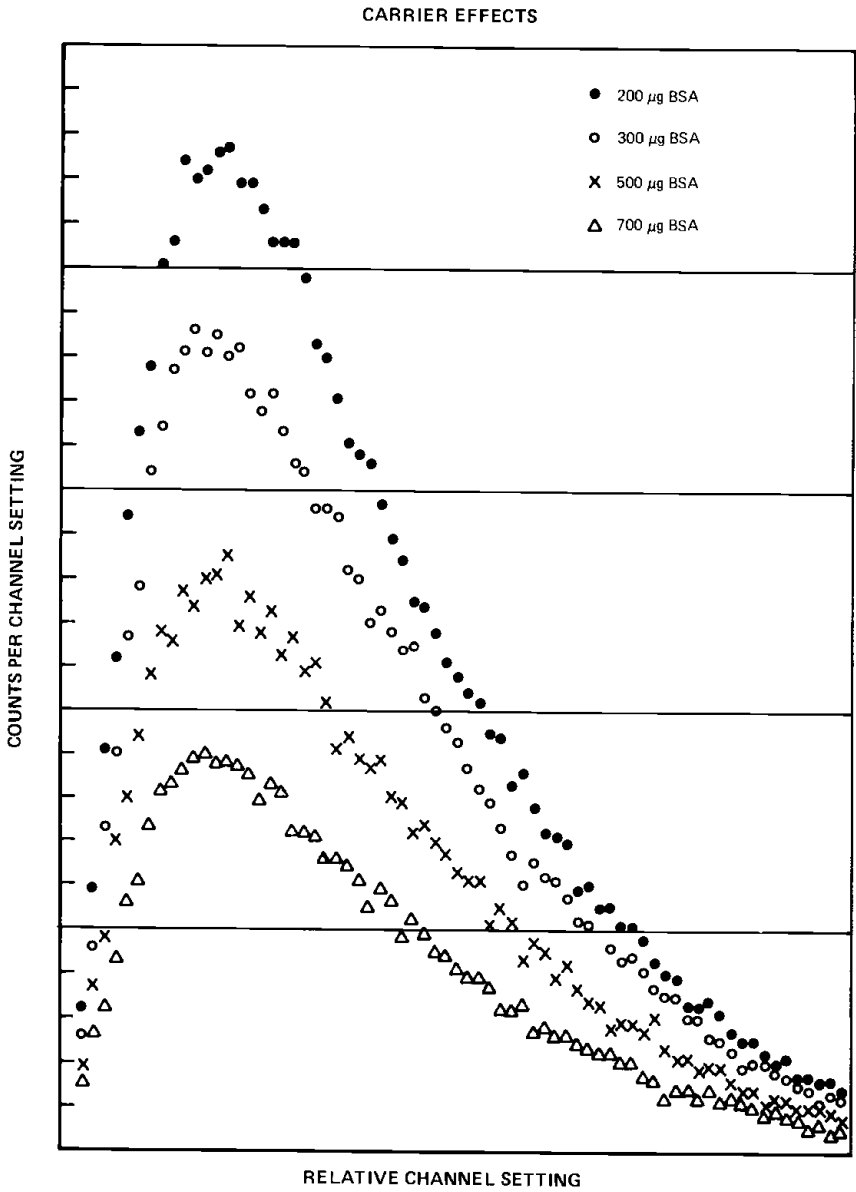
To demonstrate this, after counting the samples on an LS counter, the samples were placed in a multichannel analyzer which effectively counts the samples for time increments over small energy ranges to obtain an LS spectrum of the radioactive sample.

As more carrier is added to the filters, the absorption increases and the counting rate decreases accordingly. The distribution of the sample's LS spectrum, however, does not change, and therefore, the SCR for these samples does not change either. Figure 1 shows the multichannel scans of these filtered samples. Increasing beta absorption merely causes a diminution in the total LS spectrum; the distribution is not affected, and the SCR, therefore, does not change. Figure 2 shows a comparison of a transparent filter and an identical aliquot in a dissolved filter. The difference in area between the two curves is due to the absorption of the radioactive material on the transparent filter.

In general, heterogeneity produces beta absorption which can lower the potential count rate for a sample on a filter without changing the ESCR or the SCR. In addition to inducing heterogeneity in a system by the sample carrier (this type of beta absorption is more appropriately called "self absorption"), heterogeneity can originate from other ways.

Wet filters will have more absorption than filters which are dried. Heterogeneity is also a function of the length of time a filtered sample is allowed to dry. Cellulose nitrate filters (Sartorius SM 113) were used to trap TCA precipitated DNA (tritiated, approximately 44,000 counts per minute applied to each filter) and each filter was dried for a different length of time before counting in a toluene/PPO cocktail. To determine the true amount of activity trapped on the filter, a similarly prepared filter was counted in a special dissolving cocktail (Filter-Solv). Both the ESCR and the SCR was taken for the filters and the results are shown in Table XI.

As the filters are dried for longer times, the counting rate rises. Both the ESCR and the SCR, however, remain unchanged. As the filters are dried longer, less water remains on the filter to inhibit cocktail contact with the

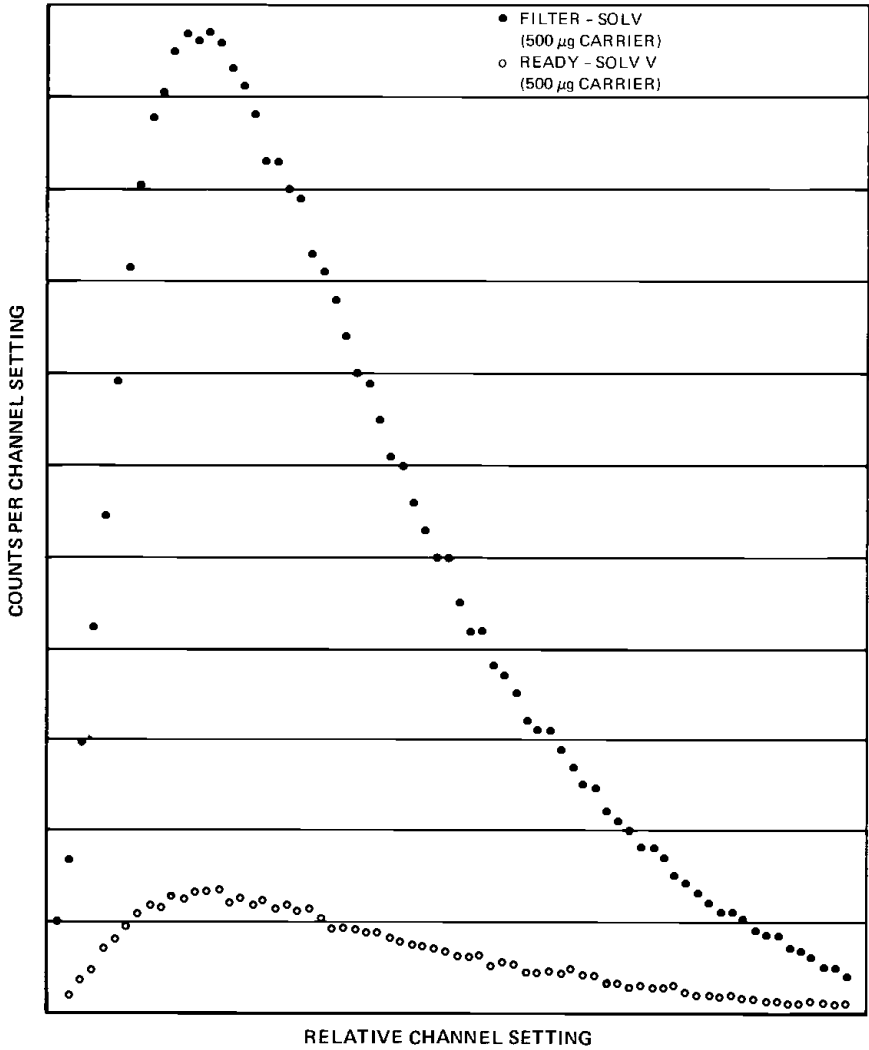


Carrier Effects on DNA Samples

FIGURE 1

HETEROGENEOUS COUNTING

CARRIER EFFECTS



Comparison of Absorption Effects on Dissolved Versus Undissolved Filter

FIGURE 2

Table XI

<u>Sample</u>	<u>Drying Time</u>	<u>Appearance</u>	<u>CPM</u>	<u>ESCR</u>	<u>SCR<sup>1</sup></u>
D	0	Opaque	12,325	.782	.703
A	15 min	Opaque	16,880	.777	.716
B	60 min	Opaque	26,539	.778	.705
C	1 day	Transparent	32,785	.773	.711
Filter-Solv	-	Dissolved	36,881	.697	.692

1. SCR based on channel settings of: A = 0.300, B = 80.300.

sample. The improved penetration of cocktail on the filter reduces the amount of beta absorption on the filter. As beta absorption merely affects the total LS spectrum of the sample and not the energy distribution, the ESCR and the SCR should not change to reflect the improved counting efficiency of the dried samples. At least 36,000 counts per minute were present on each filter, but none of the intact filters indicate the true activity on the filters. Even the filter dried for 1 day experiences some degree of beta absorption.

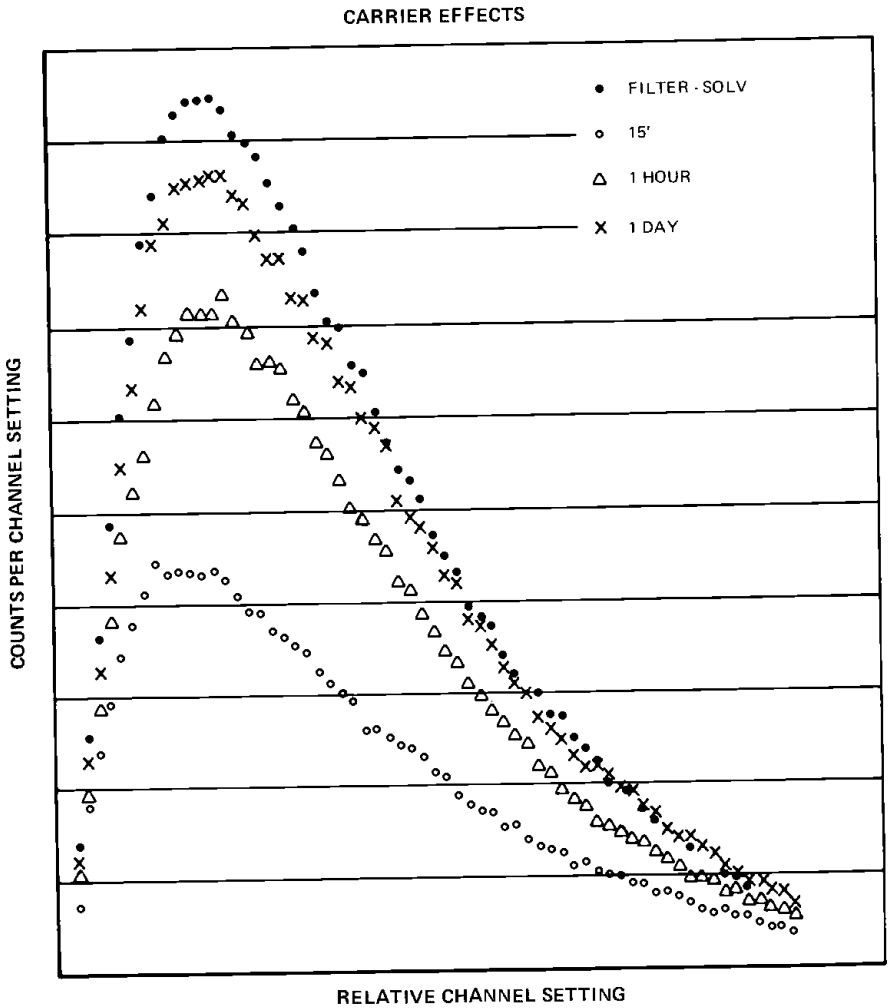
Multichannel scans were taken of the samples and their spectra are shown in Figure 3. The decrease in absorption results in an increase in counting rate, but the SCR and ESCR fail to show this effect.

As long as absorption can occur, the true activity on the filter can be "masked" or hidden, and interpretation of results based solely on the count rate with filter heterogeneous systems can lead to many erroneous conclusions.

Consider a series of tritiated DNA samples of varying activity which was TCA precipitated in the presence of different amounts of carrier protein (Bovine Serum Albumin). The precipitates were collected on Millipore filters (HAWP) and dried for over one hour to increase their transparency once cocktail (toluene/PPO) was added. Companion sets were counted in a dissolving cocktail (Filter-Solv) and the results are shown in Table XII. The actual counts per minute applied to the filter was determined by adding the tritiated aliquots directly without any filtration to an emulsifier cocktail. The filtered effluents were also counted to ensure that the material was not passed through the filter. The average activity of the effluents was determined to be approximately 303 cpm.

While some filters have up to 100% more radioactive material on them than others, the count rates with the heterogeneous systems are clearly unreliable. The ESCR and

HETEROGENEOUS COUNTING



Drying Time Effects Versus Dissolved Filter

FIGURE 3

Table XII

Actual CPM <u>Applied</u>	$\mu\text{g}$ <u>BSA</u>	Mean <sup>1</sup> CPM <u>of Filter</u>	Mean <u>ESCR</u>	Mean <u>SCR<sup>2</sup></u>	Mean CPM Dissolved <u>Filter</u>
41,191	200	7667±552 (374) <sup>3</sup>	.771±.001	.717±.007	39,842±748
63,653	300	8833±437 (458)	.771±.002	.722±.008	59,623±738
82,544	500	8696±261 (178)	.769±.001	.721±.001	80,547±1382

1. Based on duplicate samples.
2. SCR based on settings of: A = 0.300, B = 80.300.
3. CPM of wash.

the SCR remain unchanged because the count rate on the filters depends solely on the extent of beta absorption. Previous experiments have shown that beta absorption can greatly affect the counting rate without producing any change in the Sample Channels Ratio or the External Standard Channels Ratio.

Dissolved filters can be expected to give a true reflection of the actual material on the filter. They all experience an average loss of about 4% of the total activity (CPM) applied to the filter. Several factors such as chemical quenching caused by dissolution of the filter, and loss of material through the filter contribute to this 4% reduction in the counting rate.

This is not a 4% loss in true activity in the sense of disintegrations per minute (DPM), but rather a loss in maximal counting rate (CPM). In many biochemical experiments (such as labeling DNA), it is not possible to know the actual DPM due to uncertainties in the labeling process and all values are expressed as a "relative counting efficiency". On this basis, the filters dissolved in Filter-Solv (ESCR of .696, SCR of .686) give a 96% relative counting efficiency and the actual corrected count rate can be obtained by dividing the observed count rate by .96.

### Conclusions

The examples discussed in this report illustrate many of the difficulties in counting radioactive material on filters. The observed count rate will depend on a number of physical parameters in the preparation of the samples for

## HETEROGENEOUS COUNTING

counting. Moreover, the heterogeneous nature of the sample counted will cause absorption of the radioactive emissions. The absorption seen on many of the samples occurred with filters which were effectively "transparent". Due to variations in sample handling, it may be impossible to determine the extent of absorption on each sample that is filtered.

The cocktail used for these filter samples cannot be lightly regarded. Toluene/PPO has many attractive advantages. Its ease in preparation and inexpensive cost lends itself well to counting filters. The use of xylene or other fluors may improve the scintillation efficiency of the cocktail somewhat, but in general, these non-emulsifying cocktails will result in a heterogeneous sample to count. The absorption generated by these conditions can cause the count rate of the sample to be unindicative of the true activity on the filter.

Drying the screen type filters (membrane) before the addition of the cocktail is recommended, but this procedure is time consuming and may not always alleviate all of the difficulties. Geometry effects are, of course, lessened as the dry filters are less opaque than moist ones, but the counting rate is greatly dependent on the surface area of exposure. Absorption is still present on filters even after extended drying times.

Many common commercial emulsifying systems, however, are reported to produce heterogeneity with depth or screen filters (11). Frequently, the cocktail that dissolves the filter will be counted at an apparently lower efficiency than with a heterogeneous system. The organic based components which can dissolve a cellulose polymer will usually be unsuitable scintillation solvents and the ESCR and SCR for such samples are generally lower than with heterogeneous samples. Unlike heterogeneous systems, however, the ESCR and the SCR of dissolving cocktails truly reflects the quenching that has occurred in the system.

By appropriate use of standards, the actual activity on the filter can be determined by an appropriate "correction factor". This correction factor is slightly different than conventional chemical or color quenched corrections as a finite amount of material can pass through the filter. The loss in count rate due to amount of material loss through the filter (assumed to be constant for given type of filter under a given set of conditions) can be included with the conventional loss in count rate due to quenching to produce this correction factor.

Based on studies of sulfur-35, Bush has suggested the use of a "double ratio" of the ESCR and the SCR to indicate

heterogeneity in samples. The tacit assumption in this technique is that the ratio of the SCR divided by the ESCR changes with absorption because beta absorption will affect the beta spectrum (12). The method was originally intended to be a qualitative indication of sample heterogeneity. Certainly, heterogeneous samples can show changes in their SCR values, but such a generalization is not always valid.

It appears that the weak isotopes, such as tritium, do not always show a change in their beta spectrum. In the examples cited in this report, neither the SCR or the ESCR show the extent of absorption or heterogeneity, and the value of a double ratio for these samples is limited. With the weak beta emitters, the count rate depends on the material in surface contact with the cocktail and the beta spectrum cannot always reflect the absorption within the filter.

#### Acknowledgements

The authors would like to thank Beckman Instruments for its support of this work. We would also like to thank Dr. D. Kingsbury of the University of California at Irvine for his donation of samples and many useful discussions. We would especially like to thank Dr. Donald Horrocks for his insight and help throughout this work.

#### Bibliography

1. "DNA Synthesis In Vitro" (Well, R.D. and Inman, R.B. ed.) University Park Press, Baltimore (1973).
2. J.W. Geiger and L.B. Wright, *Biochem. Biophys. Res. Comm.* 2, 282 (1960).
3. R.B. Loftfield, *Atomlight*, No. 13, New England Nuclear Corp., Boston Mass. (1960).
4. J.D. Davidson, *Proc. Conf. Organic Scintillation Detectors*, Univ. of New Mexico, 1960 (G.H. Daub, F.N. Hayes, and E. Sullivan, eds.), TID-7612, p. 232, U.S. At. Energy Commission, Washington, D.C. (1961).
5. N.B. Furlong *in* *The Current Status of Liquid Scintillation Counting* (E.D. Bransome Jr., ed.), p. 201, Grune and Stratton, New York, 1970.
6. E.D. Bransome Jr. and M.F. Grower, *Analytical Biochemistry* 38, 401 (1970).
7. R.E. Johnsonbaugh, *Analytical Biochemistry* 54, 490-4 (1973).
8. R.M. McKenzie, *Analytical Biochemistry* 54, 17-31 (1973).
9. A. Chakravarti, *Analytical Biochemistry* 40, 484 (1971).
10. D.L. Horrocks, *Nucl. Instr. Meth.* 117, 589 (1974).
11. *Multiple Sample Filtration and Scintillation Counting*, Millipore AB 304, p. 12.
12. E.T. Bush, *Internat. J. of Applied Radiation and Isotopes* 19, 447 (1968).