

LIQUID SCINTILLATION COUNTING
RECENT APPLICATIONS AND DEVELOPMENT
VOLUME II. SAMPLE PREPARATION AND APPLICATIONS

HIGH EFFICIENCY GAMMA RADIATION DETECTION
IN CLINICAL RADIOIMMUNOASSAYS
BY LIQUID SCINTILLATION COUNTING
WITH "LSC GAMMA VIALS"

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A polycrystalline sodium iodide detector (LSC Gamma Vial) has been developed which allows high efficiency counting of gamma emitting radioisotopes in liquid scintillation counters. The adaptor will fit any standard liquid scintillation counter. The LSC Gamma Vials allow I-125 and Co-57 to be detected in a standard liquid scintillation counter with counting efficiencies of 74% and 54% respectively, as compared to that of conventional gamma counting equipment. Even higher comparative efficiencies (> 100%) were obtained when the

standard LSC coincidence circuitry was negated. This detector offers counting efficiency that is relatively independent of volume, has good count rate linearity, and exhibits low background count rates.

INTRODUCTION

Radioreceptor assay is a well established technique being increasingly utilized in both research and clinical medicine (1)(2). Details of this technique have been described in many publications, and for our purposes it is sufficient to say that these assays involve a competitive binding reaction between two major components, the binding material (antibody, binding protein, or membrane receptors) and the substances to be measured (hormones, drugs, enzymes, etc.).

The sensitivity of radioimmunoassay and competitive protein binding assays depends on the fact that each individual molecule can be labeled with a radioactive atom. Once tagged, the progress of the tagged molecule in a competitive binding reaction can be followed and ultimately used to determine the concentrations of the substances of interest.

The most commonly used isotopic labels for such assays are I-125, Co-57, H-3, and C-14. However, since I-125 and Co-57 are gamma emitters, while H-3 and C-14 are beta emitters, two basically different types of nuclear counting equipment are required to detect both types of radiation. These gamma emitters are most conveniently counted with a sodium iodide well detector, while beta emitters must be detected in a liquid scintillation counter.

Efforts have been made to develop liquid scintillation cocktails that can be utilized to count low energy gamma emitters in a liquid scintillation counter, but these require careful preparation, have been generally inefficient with volumes greater than 3 cc, require quench correction, and are expensive (3)(4)(5)(6)(7). Since both beta and gamma emitters are commonly employed to label antigens in radioimmunoassay procedures, the ability to utilize both isotopes has obvious economic advantages.

A solid state scintillation device has been developed into which gamma isotope labeled samples can be placed for subsequent counting in a liquid scintillation counter. With these adapters, a liquid scintillation counter has dual capabilities in meeting the needs of the clinical laboratory using RIA techniques. The use of these adapters is cost effective since they provide (a) an economic method of using a single instrument to do all radioassay tests regardless of system for counting the more predominant gamma emitters in

the event of instrument failure in laboratories where both liquid scintillation and gamma counters are available, and (c) the convenience of counting one or two samples in an LSC counter without interrupting programming of the high volume automated gamma counters.

MATERIALS

The LSC Gamma Vial was designed expressly for counting gamma emitting nuclides in a liquid scintillation counter. Vial configuration and composition of the polycrystalline NaI material was originally conceived and patented by two authors (8) (9). The actual detectors were developed and fabricated by Bicron Corporation.

The detector material is a polycrystalline sodium iodide (Bicroguard). The density of the material is similiar to that

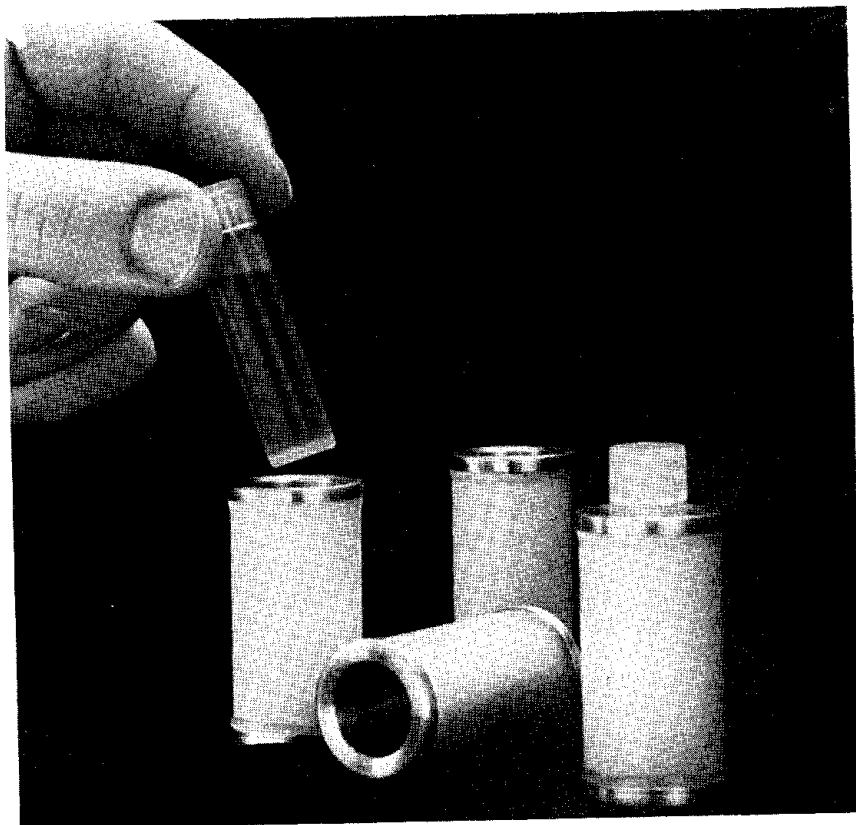


Figure 1. Gamma vial detectors and sample vials.

of pure sodium iodide, but because of the polycrystalline structure it is insensitive to thermal or mechanical shock.

The dimensions of the gamma vial detectors are similar to those of standard liquid scintillation counting vials currently in use, and accommodate samples up to 17 mm diameter by 50 mm long (Figure 1).

PROCEDURE

In order to evaluate the LSC gamma vials for gamma counting efficiency, count rate linearity, sample volume insensitivity and sample count rate reproducibility, the following measurements were carried out on I-125 labeled samples. The procedure followed was to first count the samples in a multi-sample Nuclear Chicago gamma counter, and then to repeat the measurements of the same samples on a manual Nuclear Chicago 4539 LS counter. Liquid scintillation counting was done in two modes, first counting with the pulse summation and coincidence gate active and secondly with the coincidence gate negated. The efficiency of this particular instrument for H-3 was approximately 60%.

The following measurements were made:

(a) *Count rate linearity.* In order to determine the count rate linearity of the LSC Gamma Vials with increasing radioactivity, serial dilutions of I-125 were prepared in 1 cc saline.

(b) *Comparison of LSC vs. well detector at different volumes.* In order to determine the clinical usefulness of the Bicon vials and to determine the effect of volume differences on the reproducibility of the counting rates, eighteen samples of volumes of approximately 0.05 cc, and twenty-three samples of volumes of 2 cc were measured. The 0.05 cc volumes consisted of the bound fraction from a routine RIA. The 2 cc volumes represented the volume routinely measured in a T-4 assay.

(c) *Single sample reproducibility.* To determine the reproducibility of consecutive measurements of a single sample, I-125 activity in 1 cc saline was determined in the sample 40 consecutive times with the liquid scintillation counter in the coincidence mode.

(d) *Background.* The background of ten different solid state scintillators was measured. In each case, the empty vials were counted for five minutes.

TABLE 1. Count Rate Linearity

Dilution Tube	Gamma Well Detector	LSC Coincidence Mode	Ratio $\frac{B}{A}$
	A	B	
1	95,447	71,548	.75
2	24,658	18,675	.76
3	6,849	5,240	.77
4	2,390	1,829	.77
5	623	495	.79
		Mean	.77
		Std. Dev.	.014

RESULTS

The results of the count rate linearity are shown in Table 1. The activity of I-125 varied from 623 CPM to 95,447 CPM (Nuclear Chicago gamma counter). There was a corresponding increase in the I-125 activity measured in the liquid scintillation counter using the Gamma Vials. The mean of the ratios of the activity of I-125 as measured by the liquid scintillation counter to the gamma counter was .77 (SD .014, range .75 to .79).

A comparison of sample count rate using a LSC and well detector with varying sample volumes is shown in Table II. Columns A & B represent a comparison between the results of two radioassays performed with a standard well counter and with an LSC using the Bicon Gamma Vials. Sample volumes of 0.05 cc and 2 cc were used. There was no significant difference in the ratios of the activity of I-125 as determined in the liquid scintillation counter using the Bicon Gamma Vials to that measured by the gamma counter.

As can be seen, however, there is a difference in the efficiency of counting I-125 in the liquid scintillation counter according to whether the instrument was operated in the coincidence or summation only mode. As compared to the

TABLE II-A. Volume/Detection Method Comparison (0.05 cc)

Tube No.	Gamma Well Detector		LSC		Coincidence Mode		Ratios		
	CPM	Sum Mode	Sum Mode	LSC	Coincidence Mode		B	A	C
	A	B	C						
1	7,162	7,739	5,352			1.08		.75	
2	8,552	9,220	5,770			1.08		.67	
3	8,934	9,752	6,260			1.09		.70	
4	8,625	9,608	6,087			1.11		.71	
5	8,005	8,761	5,830			1.11		.73	
6	8,524	9,804	6,455			1.08		.76	
7	9,086	10,082	6,481			1.11		.71	
8	8,954	9,804	6,412			1.09		.73	
9	8,509	8,989	6,180			1.06		.73	
10	8,477	9,398	6,270			1.11		.74	
11	9,157	9,998	6,963			1.09		.76	
12	8,245	9,213	5,994			1.12		.73	
13	8,889	9,711	6,429			1.09		.72	
14	7,759	8,355	5,387			1.08		.69	
15	7,394	7,918	5,432			1.07		.73	
16	5,859	6,277	4,182			1.07		.71	
17	3,795	4,330	2,713			1.14		.71	
18	3,983	4,147	2,645			1.04		.66	
			Mean			1.09		.72	
			Std. Dev.			.02		.03	

TABLE II-B. Volume/Detection Method Comparison (2 cc)

Tube No.	Nuclear Chicago Gamma CPM	Nuclear Chicago Liquid Sum Mode	Nuclear Chicago Liquid Sum Coincidence Mode	Ratios	
	A	B	C	$\frac{B}{A}$	$\frac{C}{A}$
1	14,233	16,410	11,071	1.15	.78
2	7,062	7,823	5,474	1.11	.78
3	16,542	18,760	12,959	1.13	.78
4	12,134	13,759	9,095	1.13	.75
5	11,498	13,012	8,837	1.13	.77
6	26,676	29,773	19,967	1.12	.75
7	16,748	18,788	12,818	1.12	.77
8	9,965	11,207	7,670	1.12	.77
9	11,470	12,877	8,889	1.12	.77
10	14,467	16,014	10,997	1.11	.76
11	9,358	10,408	7,018	1.11	.75
12	14,964	16,967	11,415	1.13	.76
13	18,920	21,213	14,349	1.12	.76
14	17,844	20,423	13,740	1.14	.77
15	8,496	9,731	6,698	1.15	.79
16	6,399	7,211	5,946	1.13	.78
17	12,958	14,171	9,523	1.09	.73
18	9,513	10,615	7,232	1.12	.76
19	13,827	15,485	10,756	1.12	.78
20	23,519	26,361	18,389	1.12	.78
21	10,939	12,178	8,400	1.11	.77
22	12,008	13,480	9,142	1.12	.76
23	24,988	28,327	19,142	1.13	.77
			Mean	1.12	.77
			Std. Dev.	.013	.04

TABLE III. Sample Reproducibility

Count	Count Rate (cpm)	Count	Count Rate (cpm)
1.	45,774	23.	45,135
2.	45,818	24.	45,694
3.	45,655	25.	45,765
4.	45,292	26.	45,598
5.	45,500	27.	45,504
6.	45,659	28.	45,111
7.	45,568	29.	45,533
8.	45,560	30.	45,500
9.	45,684	31.	45,118
10.	45,460	32.	45,358
11.	46,085	33.	45,459
12.	45,237	34.	45,278
13.	45,828	35.	45,278
14.	45,571	36.	45,629
15.	46,112	37.	46,237
16.	45,488	38.	46,114
17.	45,342	39.	45,543
18.	45,494	40.	45,291
19.	45,506		
20.	45,425		Mean = 45,528.97
21.	45,531		
22.	45,425		Std. Dev. = 244.57

gamma counter, the mean ratio of activities in the coincidence mode was .72 (S.D. .03, range .66 to .76) for the 0.05 cc volumes and .77 (S.D. 0.04, range .73 to .79) for the 2 cc volumes. In the summation mode, the mean ratios were 1.09 (S.D. .02, range 1.04 to 1.14) for the 0.05 cc volumes and 1.12 (S.D. .02, range 1.09 to 1.15) for the 2 cc volumes.

Data collected in measuring single sample reproducibility is shown in Table III. The average counting rate for 40 consecutive measurements of the same sample was 45,529, Sigma = 244.57.

The background level of the liquid scintillation counter with a H-3 window setting in the normal mode of counting was 18 cpm. In the pulse summation mode only, it was 70 cpm.

TABLE IV. Comparison of Searle 1190 Gamma Counter
and Searle Isocap LSC with Bicron Gamma Vials

<i>Digoxin I-125 Assay</i> 7/19/77		
<i>Sample</i>	<i>Searle 1190 Gamma Counter</i>	<i>Searle Isocap LSC with Bicron Vials</i>
<i>Control I</i>	0.92 ng/ml	0.84 ng/ml
<i>Control II</i>	1.56	1.70
<i>Control III</i>	3.44	3.52
<i>Patient</i>		
1	1.16 ng/ml	1.21 ng/ml
2	<0.2	0.32
3	1.00	0.99
4	<0.2	<0.2
5	1.80	1.81
6	0.92	0.89
7	0.63	0.60
8	2.09	2.20
9	1.07	0.97
10	2.00	1.82
<i>Total cpm</i>	20,834	8,244

A digoxin I-125 assay performed with a commercial kit (Kallestad) was counted in a 300 sample gamma counter (Searle Model 1185 or Model 1190). Reduction was carried out by an on-line computer (Searle PDS-3). The exact same samples were then placed in the Bicron Gamma Vials and counted in a liquid scintillation counter (Searle Model Isocap 300). A H-3 window was used which covers the energy range 20 to 17000 KeV (H-3 EFF \approx 60%). Samples were counted for one minute using the gamma counter and two minutes using the LSC adapters. Data reduction was carried out by the same on-line computer as above.

A Vitamin B 12 Co-57 assay performed with a commercial kit (BioRad Quant Count) was counted following the same procedure as the Digoxin I-125 assay above. Counting time using the Bicron Gamma Vials was 20 minutes per sample.

DISCUSSION

TABLE V. Comparison of Searle 1185 Gamma Counter
and Searle Isocap LSC with Bicron Gamma Vials

<i>Digoxin I-125 Assay</i> 7/20/77		
<u>Sample</u>	<u>Searle 1185 Gamma Counter</u>	<u>Searle Isocap LSC with Bicron Vials</u>
<i>Control I</i>	<i>0.81 ng/ml</i>	<i>0.78 ng/ml</i>
<i>Control II</i>	<i>1.48</i>	<i>1.63</i>
<i>Control III</i>	<i>3.81</i>	<i>3.68</i>
 <i>Patient</i>		
<i>1</i>	<i>0.78 ng/ml</i>	<i>0.69 ng/ml</i>
<i>2</i>	<i>1.78</i>	<i>1.04</i>
<i>3</i>	<i>0.58</i>	<i>0.57</i>
<i>4</i>	<i>0.27</i>	<i><0.2</i>
<i>5</i>	<i>0.69</i>	<i>0.59</i>
<i>6</i>	<i>0.33</i>	<i>0.33</i>
<i>7</i>	<i>1.33</i>	<i>1.38</i>
<i>8</i>	<i>0.92</i>	<i>0.85</i>
<i>9</i>	<i>2.95</i>	<i>2.66</i>
<i>10</i>	<i>2.39</i>	<i>2.32</i>
<i>11</i>	<i>1.65</i>	<i>1.46</i>
<i>12</i>	<i>0.41</i>	<i>0.33</i>
<i>13</i>	<i>0.52</i>	<i>0.44</i>
<i>14</i>	<i>2.28</i>	<i>2.10</i>
<i>15</i>	<i>2.34</i>	<i>2.31</i>
 <i>Total cpm</i>	 <i>24,764</i>	 <i>8,535</i>

In this study, we have demonstrated the feasibility of using a solid state gamma detector adapter to perform clinical studies with I-125 and Co-57 labeled radioassay material in a liquid scintillation counter.

Other methods suggested for counting these gamma isotopes in liquid scintillation counting equipment have a number of drawbacks, such as:

1. Limited sample volume.
2. Loss of efficiency with increasing sample volume.
3. Expensive cocktail preparation.
4. Dependence of efficiency upon concentration of fluor and electron density increasers.
5. Necessity of applying quench correction techniques.

TABLE VI. Comparison of Searle 1185 Gamma Counter and Searle Isocap LSC with Bicron Gamma Vials

Vitamin B 12 Co-57 Assay 7/20/77		
Sample	Searle 1185 Gamma Counter	Searle Isocap LSC with Bicron Vials
1	1157 pg/ml	1222 pg/ml
2	539	608
3	834	9555
4	2200	2054
5	849	917
6	514	582
7	509	464
8	371	382
9	416	513
10	656	627
	Total cpm 19,824	1427

The Bicron Gamma Vials on the other hand, were shown to be largely volume independent, highly efficient and reproducible. These parameters are of some import, since in many cases both total and bound activity must be determined in radiimmunoassay. For example, in some cases a 20 fold difference in volume is involved in counting the bound vs. free fractions. In addition, precoated tubes such as those used in the Abbot Australian Kit and resin filled syringes such as those commonly found in T-3 and T-4 kits may also be readily handled by the gamma vial adapters. These kits, which require specialized test tubes, may not be counted with either the "microfuge or special cocktail techniques."

Cocktail preparation difficulties and expense are also eliminated when using the detectors as opposed to standard liquid scintillation techniques in which such problems as chemiluminescence and quenching are often encountered (10).

The only disadvantages with the gamma vials are their relatively high initial cost and the fact that prolonged exposure to direct sunlight or intense fluorescent lighting will result in an intense phosphorescence which can take several hours to decay. However, incandescent lighting or brief exposure to limited fluorescent lighting has little or no effect on the devices.

SUMMARY

This paper describes a solid state scintillator device into which gamma isotope labeled radioassay samples can be placed for subsequent counting in a liquid scintillation counter.

These adapters add a dual capability to the liquid scintillation counter in meeting the requirements in the clinical laboratory using RIA techniques. They provide:

(1) an economic method of using a single type of instrument (LSC) to do all radioassay tests regardless of isotope.

(2) an economic method of providing a "back-up" for counting the more predominant gamma emitters in the event of instrument failure in laboratories where both liquid scintillation and standard gamma counters are available, and;

(3) a convenient means of counting one or two samples in an LSC counter without interrupting programming of the high volume automated gamma counters.

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