

CHAPTER 13

A Plastic Scintillation Detector with Internal Sample Counting, and Its Applications to Measuring ^3H -Labeled Cultured Cells

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INTRODUCTION

Liquid scintillation counting has the advantage of homogenous 4π counting geometry and resultant high counting efficiency, but it frequently requires complex sample preparation and produces excessive amounts of radioactive organic waste.¹ As a result, Cerenkov counting and other counting methods have been used.² The plastic scintillation detector for determining soft beta nuclides, which is used without cocktail, has about a 30 year history.³⁻⁷ Internal sample counting with gas detectors has been used to measure soft beta emitting nuclides, but internal sample counting with plastic scintillation detectors has been less frequently reported in the literature. This paper describes a simple and convenient plastic scintillation method for routine internal sample counting.⁸⁻⁹ The application of this method, in measuring the amount of ^3H incorporated into cultured cells, was tested for its homogeneity, counting efficiency, reproducibility, and spectrum analysis.

MATERIALS AND INSTRUMENTS

The radioactive isotopes used as reference samples in this work were ^3H and ^{14}C labeled hexadecane. Also used were ^3H labeled lysine and thymidine and ^{35}S as Na_2SO_4 solution diluted by an emulsion cocktail. All radio isotopes were made by the Chinese Academy of Atomic Energy.

The plastic scintillator sheet (PSS) was made from styrene monomers purified by vacuum distillation. Fluors of PPO and POPOP at concentration levels of 10 g/L and 0.8 g/L, respectively were added to this monomer. The polymerization procedure was conducted in an oil bath at 110°C for 5 days. The PSS was pressure shaped into rectangular blocks 32 mm long, 15 mm

wide, and 0.25 mm thick. Two different detectors were made from the initial plastic sheet. One PSS detector was rectangular with base area dimensions of 24 mm \times 8 mm and a volume capacity of 0.996 mL. A second PSS detector was circular with a diameter of 12 mm. A glass filter paper disc used for cell harvesting measured 12 mm in diameter. Cocktails were made using a fluor concentration of 6 g/L of butyl-PBD in a solvent of pure toluene or 2:1 ratio of toluene/Triton X-100.

Radioactivity measurements were made using a Beckman Model LS-9800 liquid scintillation counter. The General Program and the Special Spectrum Analysis Program of the instrument were used in the counting procedures. A Hitachi Model S-520 scanning electron microscope was used for microscopic analyses.

METHODOLOGY AND RESULTS

The plastic detector used in this study sandwiches the radioactive sample between two PSS, and seals it with a gluing optical coupling solvent (GOCS). The radioactive sample may be in solution or on solid support.

The GOCS must have good qualities for both optical coupling and gluing the sample to the PSS. In general, aromatic solvents are superior for gluing. Solvents such as toluene, xylene, anisole, dioxane, ethyl acetate, chloroform, or dichloromethane can be used to soften or dissolve the PSS. Additional emulsifiers such as Triton X-100 in GOCS are necessary for aqueous sample counting. In fact, the toluene cocktail was found to be an excellent GOCS.

To prepare samples on filter paper support, they were dipped in cocktail. A radioactive sample, either dissolved or on support, is then fixed between two PSS by GOCS. It was found the GOCS should not be used in excess, otherwise the plastic sheets would become deformed. Finally, after gluing the two PSS together, they were placed in a 20 mL standard glass vial to be counted.

The sample prepared for counting is a composite of radioactive material, plastic scintillator, and GOCS. Figures 1 and 2 are scanning electron microscope photographs, shown amplified at 5000 and 2500 times. Figure 1 shows a cut section of fiberglass paper, and Figure 2 shows a torn section of the glass filter paper support sandwiched between the plastic scintillator. Figure 2 also shows some broken granules of wrinkled and cracked plastic scintillator folded around and among the glass fibers. Harvested cells in a ruptured broken state due to the treatment by the organic solvents contained in the GOCS are also shown. These photographs reveal that the distance between the glass fiber and plastic scintillator is 0.3 to 2.4 μm .

COUNTING EFFICIENCY AND REPRODUCIBILITY

As known radioactive reference standards, 10 μL of ^3H n-hexadecane and ^{14}C n-hexadecane were sealed between 2 plastic sheets and placed in an empty,

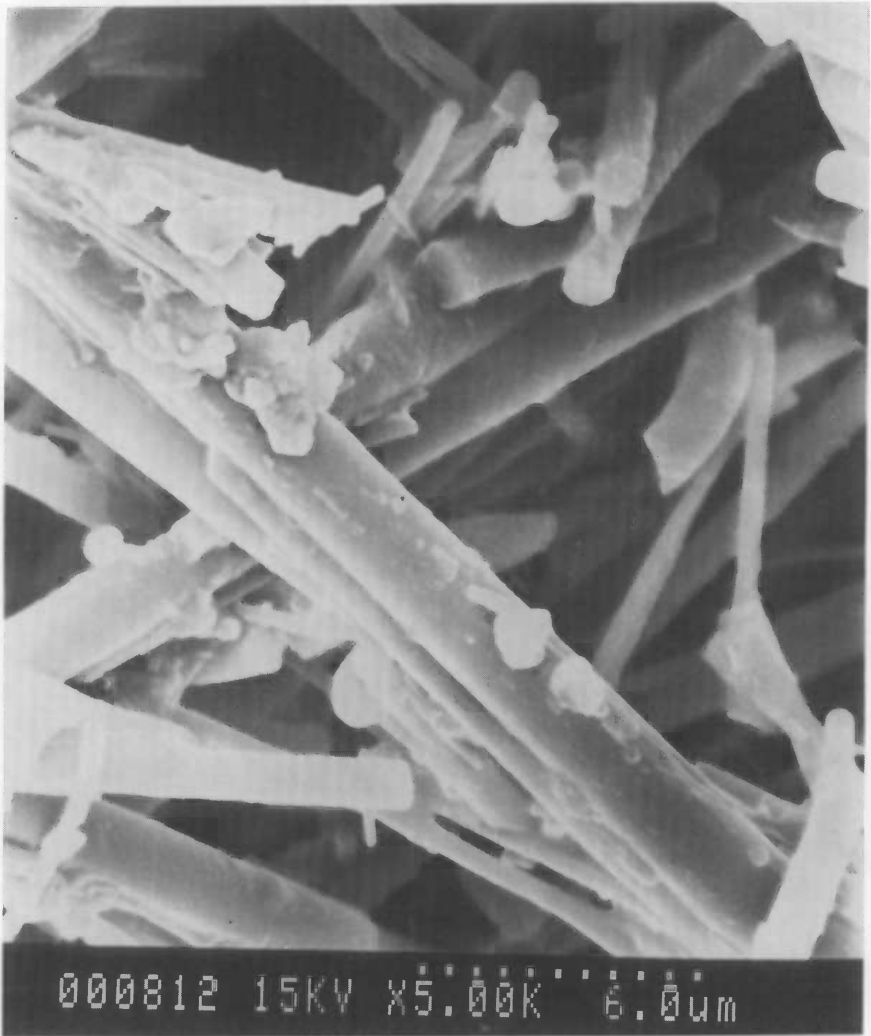


Figure 1. A cut section of filter glass paper.

standard 20 mL glass liquid scintillation vial. The counting efficiencies of ^3H and ^{14}C were $30.6 \pm 1.5\%$ (mean + S.D.) and $85.2 \pm 0.5\%$ using $100 \mu\text{L}$ of GOCS with a toluene b-PBD-6 g/L cocktail. The counting efficiencies, without the use of GOCS, were reduced to $5.9 \pm 1.3\%$ and $43.1 \pm 6.2\%$, respectively. These data show that GOCS not only increases ^3H and ^{14}C counting efficiencies but also decreases the relative standard deviation. For ^{35}S , as a Na_2SO_4 aqueous sample, a 2:1 toluene/Triton X-100 b-PBD-6 g/L cocktail was used as the GOCS. The relative counting efficiency of ^{35}S by PSS method is 82% compared to the emulsive cocktail counting method.



Figure 2. A torn section of the glass filter paper sandwiched between the plastic scintillator (broken granules of wrinkled and cracked plastic scintillator are folded among the glass fibers).

The shape of the PSS can be rectangular or circular. In the liquid scintillation counting procedure the rectangular PSS was leaned obliquely against the inner wall of the counting vial so that the radioactive sample was located in an elevated position relative to the counter phototubes. As a result, higher counting efficiencies could be obtained with the rectangular PSS than with the circular PSS, which was laid flat on the bottom of the vial during counting (Table 1).

The counting reproducibilities (coefficient of variation) of three measure-

Table 1. Comparison of the Spectral Parameters of the Plastic Scintillator Method with Homogeneous Counting

		H-3 Hexadecane			C-14 Hexadecane		
		Toluene Cocktail 10 mL	Plastic Scin. + GOCS	Plastic Scin.	Toluene Cocktail 10 mL	Plastic Scin. + GOCS	Plastic Scin.
Compton Electron Spetron	Main Peak Channel	790	640	550	780	640	550
	End Channel	850	760	750	840	760	750
Sample Spectrum	Main Peak Channel	270	240	180	510	510	510
	End Channel						
	1%	395	347	339	650	581	572
	5%	363	312	289	623	554	543
	10%	343	295	262	604	536	524

ments for the rectangular and circular PSS are $1.9 \pm 1.3\%$ and $2.3 \pm 1.2\%$, respectively. The ratio of cpm of circular to rectangular PSS is 0.862 ± 0.056 , which shows that the variation of height of the rectangular PSS position may cause larger counting error (coefficient of variation 6.7%). Because of less variation in the position of the circular PSS at the bottom of the vial, a smaller counting error (SD/mean) of $1.3 \pm 1.2\%$ was measured (Table 1). If GOCS is used in excess, the count of the rectangular PSS decreases progressively as its shape changes (Figure 3). The angle in the vial of the rectangular PSS to the PM tubes has little or no effect on counting (Figure 4). From these data, we can see that the rectangular PSS has higher counting efficiency than the circular PSS, but the circular PSS has better reproducibility than the rectangular PSS.

ENERGY SPECTRUM ANALYSIS

Table 2 shows the spectrum analysis from the external standard Compton electron spectrum of an empty 20 mL vial, a circular and rectangular PSS in a vial, and a 10 mL toluene cocktail in a vial. The data reveal that the main peak and end channel for the rectangular PSS, with and without GOCS, are significantly lower than the toluene cocktail sample. They also show the main peak channel for the plastic scintillator with GOCS is significantly higher than without GOCS, though the end channel is approximately the same.

Sample spectrum analyses of the main peak channels for ^{14}C homogeneous counting and rectangular PSS, with and without GOCS, had no obvious change. The ^3H main peak channels did decrease in the order mentioned previously. The end channels of the sample spectrum, which were eliminated at

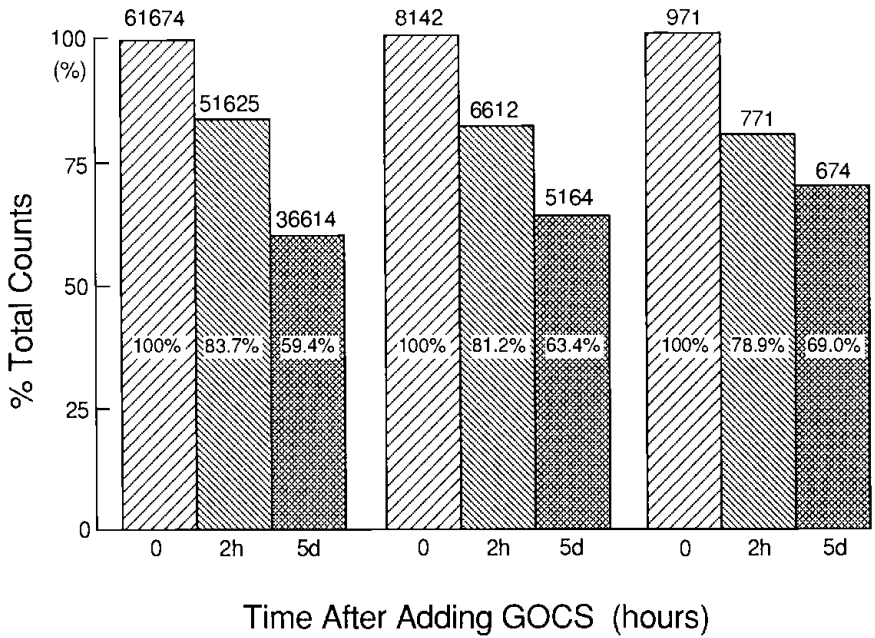


Figure 3. GOCS mount effect on counting efficiency.

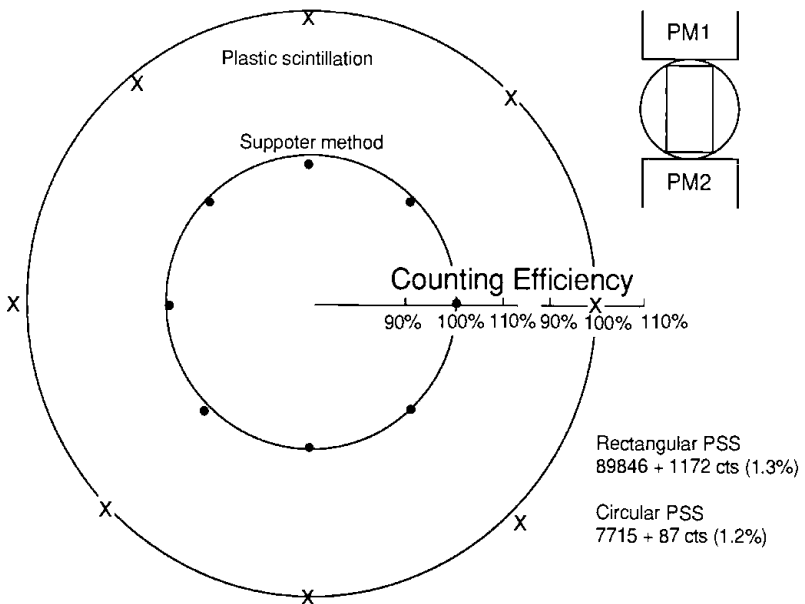


Figure 4. Angle effect on rectangular PSS with PM tube vs counting efficiency.

Table 2. Comparison of Background Spectral Parameters

		20 mL Empty Glass Vial ^b	Two Circle PSS ^a in a Vial ^b	Two Rec- tangle PSS ^a in a Vial ^b	10 mL Toluene Cocktail in a Vial ^b
	0	650	720	890	1000
End Channel	1%	588	611	803	983
Eliminated	5%	488	515	675	963
High energy	10%	451	472	618	905
Back- Ground (CPM)	Integral	28.5 ± 0.6	25.7 ± 2.1	28.8 ± 0.6	58.8 ± 2.8
	C-14 Channel (50—670)	28.5 ± 0.6	25.6 ± 0.5	28.7 ± 0.6	43.3 ± 0.7

^aPSS: Plastic Scintillator Sheet.^bVial Without Cap.

the 1%, 5%, and 10% high energy counts of the plastic scintillator with GOCS, increased slightly in comparison to that without GOCS. The end channels of homogeneous counting also increased compared to plastic scintillator with and without GOCS. The results for ³H are similar to that for ¹⁴C.

BACKGROUND SPECTRUM

The background spectrum was measured with a 20 mL glass standard vial (no cap), two rectangular PSS in a vial, two circular PSS in a vial, and 10 mL of a toluene-6 g/L of b-PBD cocktail in a vial. The main parameters of the background spectrum are listed in Table 3. The counting time was 90 minutes.

When high energy counts were eliminated at the 10% level, the end channel for the 20 mL empty glass vial decreased from 650 to 451. The rectangular and circular PSS in the glass vial decreased from 720 to 472 and from 890 to 618, respectively. The 10 mL cocktail in the vial decreased from 1000 to 905. These data indicate that the range of the background spectrum decreased about one

Table 3. Comparison of the Relative Counting Efficiency and Reproducibility of the Rectangular PSS with the Circular PSS

No. of Sample	Rectangle PSS		Circle PSS		Error (%) ^b	Effect of Circle Position (%)
	Mean ± SD (cpm) ^a	SD/Mean (%)	Mean ± SD (cpm)	SD/Mean (%)		
1	10495 ± 205	2.0	8335 ± 101	1.2	79.4	+2.9
2	11209 ± 26	0.02	10274 ± 134	1.3	91.7	+0.03
3	15736 ± 186	1.2	13060 ± 557	4.3	83.0	+0.3
4	17114 ± 395	2.3	15066 ± 356	2.4	88.0	-1.8
5	18033 ± 651	3.6	14187 ± 365	2.6	78.7	-1.5
Mean + SD	1.9 ± 1.3		2.3 ± 1.2		0.842 ± 1.056	1.3 ± 1.2

^an = 3.^bRelative Counting Efficiency of a Circle to Rectangle PSS.

third for the two kinds of PSS in their high energy background content. This decrease is greater than the homogeneous counting. This effect is clearly shown in Figure 5 and Table 3.

STABILITY

The radioactivities and backgrounds of PSS have been counted repeatedly over a period exceeding one year. The data show that the counts have not significantly changed. It is from these results that PSS are said to be stable and

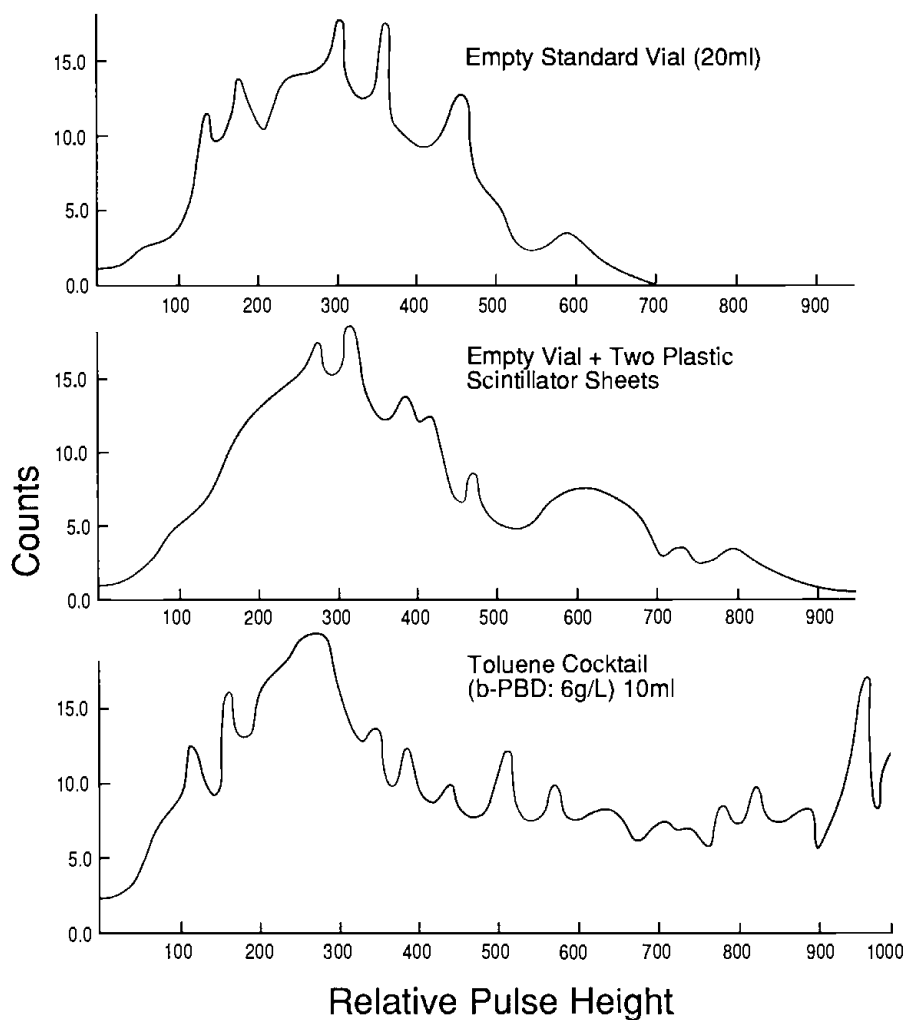


Figure 5. Background spectrum comparison of an empty 20 mL standard glass vial, two rectangular PSS in a vial, and 10 mL of toluene cocktail in a vial.

capable of being stored for long periods of time without undergoing any change.

APPLICATION TO MEASURING OF ^3H LABELED CULTURED CELLS

Screening of Chinese medicinal herbs for immunopotentiators was made with the aid of mitogen-induced lymphocyte transformation. Human peripheral blood lymphocytes were cultured in a flat-bottomed 96-well microliter plate at a concentration of 1×10^6 cells/well. A final 0.2 mL contained PRMI 1640 medium, supplemented with 10% fetal calf serum, and 100 units/mL of penicillin G, consisting of 100 $\mu\text{g}/\text{mL}$ of streptomycin sulphates. Mitogen and/or various concentrations of herbal extracts were added, and the cultures were kept under 37°C at a 5% CO_2 atmosphere. After 48 hours of incubation 0.5 or 1.0 μCi of ^3H -TdR was added to each well and incubation was continued for 6 to 12 hr. Cells were harvested with a Titertek multiharvester and collected on glass filter discs, after which the radioactivities were counted by the methods of supporter and rectangular PSS.

The stimulation index (S.I.) was calculated as follows:

$$\text{S.I.} = \frac{\text{cpm of test well}}{\text{cpm of control well}}$$

An S.I. value larger than 1.2 indicates a significant enhancement of lymphocyte transformation induced by the tested herbs.

The results shown on Table 4 indicated that herbs No. 162 and No. 520 exhibited significant enhancement of lymphocyte transformation. The S.I. values obtained in the supporter method were quite similar to that in PSS. Also the relative figure of merit of the PSS (290.0 ± 41.5) was higher than that in the supporter method (151.9 ± 8.1). The ratio of the PSS to supporter method was calculated at 1.91.

The macrophage active factor (MAF) was evaluated by means of macrophage tumor cell cytostasis (MTC). The self-prepared MAF was diluted by 1:25, 1:50, 1:100, and 1:200 and incubated with macrophages (Ms) at a 1×10^5 /well concentration for 12 hr at 37°C with a 5% CO_2 humidified air atmosphere. After two vigorous washings with RPMI 1640, p815 mouse macrophage-tumor cells were introduced at a concentration of 1×10^4 /well and incubated together with Ms under the same conditions for 30 hr. During the last 6 hr of incubation an aliquot of ^3H -TdR, at a 0.5 μCi concentration was added to each well. The degree of incorporation of ^3H -TdR by p815 cells was measured by both solid support and PSS counting methods.

The rate of cytostasis at different dilutions of MAF activated Ms were determined by the two counting methods listed in Table 5. The rate of cytostasis was calculated as follows:

Table 4. Plastic Scintillator Method Compared with LS Counting in Screening of Chinese Medicinal Herbs for Immunopotentiators

Group	Supporter Method				
	cpm (net) ± SD	S.I.	B (cpm)	E ² /B	
Cell ref.	1831 ± 346		65	153.8	
PHA: 2r	63213 ± 7465	34.5	66	151.5	
PHA: 1r	50032 ± 19042	27.3	68	147.1	
PHA: 0.05r	1954 ± 341		63	158.7	
No. 162A	4662 ± 222	2.54	66	151.5	
No. 520 ^a	6821 ± 1269	3.49	60	166.7	
No. 251 ^a	1671 ± 52	0.85	69	144.9	
No. 162A ^a	3527 ± 852	1.80	71	140.8	
Mean ± S.D.				151.9 ± 8.1	

Group	Plastic Scintillation Counting				
	cpm(net) ± SD	E (%) ^b	S.I.	B(cpm)	E ² /B
Cell ref.	1360 ± 79	74.3		19.5	282.8
PHA: 2r	48400 ± 2289	76.6	35.5	19.5	300.9
PHA: 1r	37285 ± 14607	74.5	27.4	19.5	284.8
PHA:0.05r	1406 ± 192	71.9		19.5	265.3
No. 162A	3300 ± 308	70.8	2.42	19.5	257.0
No. 520 ^a	5094 ± 535	74.7	3.62	19.5	286.0
No. 251 ^a	1185 ± 83	70.9	0.87	19.5	257.9
No. 162A ^a	3059 ± 937	86.7	2.17	19.5	385.6
Mean ± S.D.		75.1 ± 5.1			290 ± 41.4

^aPlus 0.05r PHA.^bRelative Counting Efficiency of Plastic Scintillation to Supporter Method.

$$\text{MTC rate} = \left(1 - \frac{(\text{p815} + \text{M}) \text{ cpm}}{\text{p815 cpm}} \right) \times 100\%$$

The result shows that even under a relatively high diluting situation (1:200), the MTC rate of MAF activated Ms was still as high as 72.1%, indicating the high activity of the self-prepared MAF. Also, the ratio of the relative figure of merit of the rectangular PSS to that of the supporter method was found to be 1.94.

Monitoring of self-immunity ability in humans was carried out through the assay for interleukin 2 (IL-2) activity. The peripheral blood lymphocytes of a renal transplant patient were stimulated by phytohemagglutinin (PHA) for 24

Table 5. Comparison of the Two Counting Methods in Evaluation of MAF by Means of Anti-Cancer Cell Proliferation

MAF Dilution	1:25	1:50	1:100	1:200	0	B ± S.D.	E ² /B
Supporter Method	1196	3037	10131	19601	70238	59 ± 17	169.9
Plastic Scint.	977	2392	8142	15322	57072	19.5 ± 7.8	329.0
Relative Eff. (%)	81.7	78.8	80.4	78.2	81.3	80.1 ± 1.4	

Table 6. Comparison of the Two Counting Methods in Monitoring of Self-immunity Ability in Renal Transplanting Patient

Titer %	50	25	12.5	6.25	3.125	0	B(cpm)	E ² /B
Supporter Method	219360	202110	18927	5609	2132	730	65 + 19	153.8
Plastic Scint.	161010	150460	14450	4237	1661	521	19 + 1.4	286.7
Relative Eff. (%)	81.7	72.2	74.3	73.1	76.9	74.5	73.8 + 3.0	

hr. The supernant, which was diluted by 1:2, 1:4, 1:8, 1:16, and 1:32, etc., was incubated with C57B1 mouse thymus cells which had been stimulated for 12 hr by Con A. Sixteen hours prior to harvesting ³H-TdR was added. The radioactivities in the cells collected on glass filter paper were compared by PSS counting and the supporter method.

Under proper dilution of the supernant, the thymus cells grew well where the activity of IL-2 was high, which demonstrates the renal transplant patientability for cell immunity. By this method, it is possible to predict the body rejection capability of a renal transplant patient. This procedure is at best a quasi-quantitative assay method, since it may be influenced by many factors. Its primary usefulness is its ability to compare the relative activity of different measurements for one person. As shown in Table 6, the two counting methods obtained comparable results. The ratio of E²/B of PSS (286.7) to the supporter counting method (153.8) is 1.86.

The PSS method applied in the above three experiments gives the same biological conclusions as the supporter method of counting. The PSS method has the advantage of a higher figure of merit, long storage, and no liquid waste. It is because of these attributes, we strongly recommend the PSS method, especially where small sample size or volume is to be prepared and counted and liquid scintillation waste disposal represents a problem.

Note: This manuscript has undergone extensive revision and condensation in order to meet the style requirements and space limitations of the proceedings. We sincerely hope that we have not changed the substance or connotation of any information intended to be conveyed by the authors. Eds.

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