

## MICROVOLUME LIQUID SCINTILLATION COUNTING WITH PLATE COUNTERS

TUULA STARK and TAPIO YRJÖNEN

Wallac Oy, P. O. Box 10, SF-20101 Turku, Finland

**ABSTRACT.** We review the Wallac Betaplate™, and Microbeta™ multidetector liquid scintillation counters designed for counting samples on filters and microtitration plates. For Microbeta™, the new microtitration plate design requires special counting and calculation methods, which include normalization, crosstalk elimination and DPM counting. Normalization compares efficiencies and background of all the detectors, whereas crosstalk counting eliminates increased background from other samples on the sample plate. Samples for Microbeta™ are deposited on a 96- or 24-well sample plate. The total sample volumes are up to 400  $\mu$ l for 96-well plates, and up to 1.3 ml for 24-well plates.

### INTRODUCTION

Liquid scintillation (LS) counters are traditionally designed for counting separate samples deposited in vials, with sample volumes of 1–20 ml. More sensitive detection systems have enabled the use of smaller amounts of radioactivity in samples while maintaining the same counting precision. Microvolume samples are popular because they not only use less reagents but also produce less radioactive waste. However, counting geometry is usually less than optimal with traditional counters for microvolume samples or for samples deposited on a flat matrix. The need for an instrument optimized for these purposes has become widely recognized. Thus, LS counter manufacturers have modified their instrument designs, and introduced plate counters for microvolume liquid scintillation counting (LSC). We describe here this development at Wallac Oy. The development of plate counting and the Betaplate™ counter have been presented (Warner *et al.* 1985; Potter *et al.* 1986; Warner & Potter 1986; Potter & Warner 1989). Yrjönen *et al.* (1993) also described the Microbeta™ and the plate-counting concept. The new plate counter concepts offer the benefits of better counting geometry and the use of a more appropriate sample vessel for microvolume samples, *i.e.*, microtitration plates, which enable handling of up to 96 samples.

In 1987, Wallac introduced Betaplate™, the first LS counter to count samples on plates. It was designed for counting sample preparations on filters, as proposed by Warner *et al.* (1985), Potter *et al.* (1986), Warner and Potter (1986), Potter and Warner (1989). The Microbeta™, a further development of the plate-counting concept, was introduced in 1990. A microtitration plate counter, the Microbeta™, counts samples directly from 96- or 24-well sample plates (Lehtinen, Yrjönen & Sonne 1991).

### PLATE COUNTING

LSC on plates differs only in a few aspects from LSC in vials. The main similarity is that the radioactive sample is mixed with a scintillator, and the light signal that is produced is measured with two opposing photomultiplier tubes (PMT) operating in coincidence. On the other hand, the plate counting concept has added new features to LSC. First, plate counters use several detectors in the same instrument. Because the samples are arranged in a linear, two-dimensional array, several detectors can lie parallel and close together. Space is also saved because the sample volume is so small that no lead shielding is needed to reduce background (Potter & Warner 1989).

The use of several detectors in one instrument requires a system ensuring that the results obtained from each detector are equivalent, *i.e.*, independent of variations in counting efficiencies, crosstalk

between and background counts of individual detectors. Crosstalk occurs when the activity of samples is being measured on microtitration plates made of translucent plastic material, and whose sample wells are not optically isolated from each other. This normally results in an increased count rate of a low-activity sample if adjacent to a high-activity sample.

To compare the different detectors with each other, and to eliminate possible crosstalk, their efficiencies and backgrounds are equalized by using standard samples. In Wallac plate counters, the efficiency equalization and background subtraction is done by predetermining relative efficiencies and background counts of each detector. Crosstalk normalization is needed so that the Microbeta™ can accept “all” commercially available microtitration plates. Figure 1 exemplifies the positions of a standard sample for crosstalk normalization. Crosstalk subtraction is done by normalizing the magnitude of the crosstalk effect on other samples (Yrjönen, Oikari & Lehtinen 1991). In a normalization run, a background and an active sample are measured sequentially in every detector. The increase in background is subtracted from the pulses of individual adjacent samples. Intercomparisons are made, and relative individual counting efficiencies are calculated automatically (Microbeta™ Instrument Manual 1992).

	1	2	3	4	5	6	7	8	9	10	11	12
A	Bg											
B												
C												
D												
E												
F											Bg	
G											A	
H												

Fig. 1. A standard sample for crosstalk normalization in Microbeta™. Bg is a background sample and A is the standard active sample.

When determining DPM, *e.g.*, absolute disintegrations rather than observed count rates, of individual samples in a multidetector counter, the relation between the counting efficiency and the quench level of each sample must also be known. Because, even with matched detectors, some differences are exhibited in energy and efficiency responses (an energy-scale difference will affect the quench-level measurement), standard samples representing different quench levels should be measured in each detector.

To avoid this time-consuming individual detector standardization, we have incorporated a procedure into the software (Yrjönen & Kouru 1991) in which all the standard quench samples (Fig. 2) are measured with only one detector. The conventional quench curve (Fig. 3) defines the relation between the counting efficiency and the quench level of one sample in one detector. With the new software, only two of the standard samples are measured in every detector to define the relation between the energy and efficiency responses of the detectors (Figs. 4, 5) (Microbeta™ Instrument Manual 1992).

#### SAMPLE PREPARATION

The sample preparation in microvolume counting on plates differs somewhat from microvolume counting in vials: the total sample volume, including scintillant, is limited on a plate, and all the samples on plates need to be microvolume samples. The maximum sample volume is ~1 ml for the Wallac Microbeta™ and ~400  $\mu$ l for the Betaplate™.

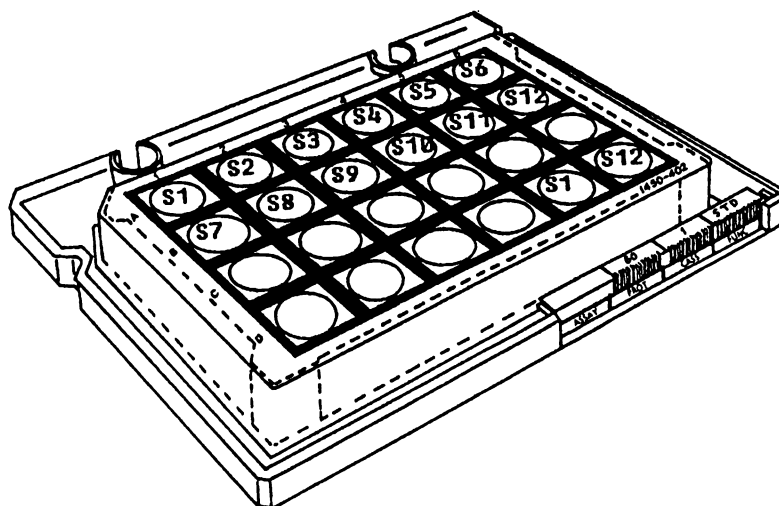


Fig. 2. The positions of DPM standardization samples on a 24-well plate for the Microbeta™ counter. S1 to S12 are quench calibration standards. Replicates of S1 and S12 in positions D5 and D6 are used as standards in energy and efficiency calibrations (Microbeta™ Instrument Manual 1992).

Other types of microvolume samples are deposited on a filter mat. The plate counter is ideal for these samples, because the sample preparation is significantly simpler than using vials. Sample preparation for plate counters includes processing the filter mat in one piece, so that up to 96 samples are prepared at the same time. This contrasts with placing the filter mat into vials individually, which is both time-consuming and laborious (Potter *et al.* 1986; Potter & Warner 1989; Potter *et al.* 1987). Counting efficiency is also better in plate counters because of better counting geometry. Counting geometry places special demands on microvolume sample placement in vials; different adapters may be needed. In plate counters, the counting geometry is ideal for microvolume and filter samples, because the instrument is designed specifically for the plates, and the PMTs are at the correct angle, close to the sample.

Sample preparation with plates is also easier to automate than with vials. Commercial pipetting stations are designed for use with plates of standard microtitration plate format. Sample identification is also easier with the plate format. In the sample preparation for plate counters, when total volume is limited, the loading capacity of the scintillation liquid becomes the determinant. The problem of scintillation liquid loading capacity is being overcome with new and less toxic cocktails, which provide another advantage in that they do not dissolve the different plastics used as plate material.

Applications for plate counters range from harvested cells (Potter *et al.* 1987) to DNA samples (Hyypiä *et al.* 1990; Potter, Tan & Ratcliffe 1991; Matzinger 1991; Potter & Le Jeune 1991). The only requirements for samples are that they be microvolume samples and that they use appropriate cocktails. The different sample types can be divided into groups by physical and chemical appearance. One classification of different sample types is shown in Table 1, which also shows recommended scintillants. We recommend the use of safe cocktails for liquid samples and the new melttable solid scintillator, Meltilex™, for solid samples and filters.

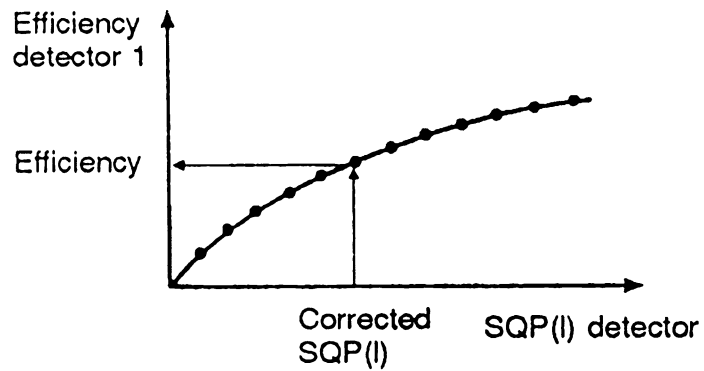


Fig. 3. The quench correction curve, counting efficiency vs. quench parameter. The curve is made with up to 12 points in the Wallac Microbeta™ (Microbeta™ Instrument Manual 1992).

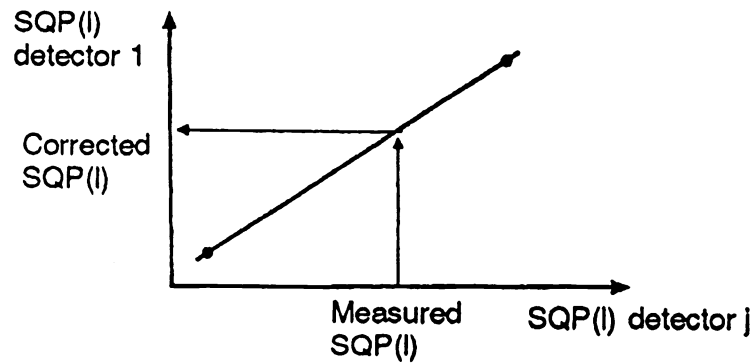


Fig. 4. The energy calibration curve, made with two points. The SQP(I) value is corrected from the energy calibration curve. It is now the value it would have been if the sample had been counted in detector 1 (Microbeta™ Instrument Manual 1992).

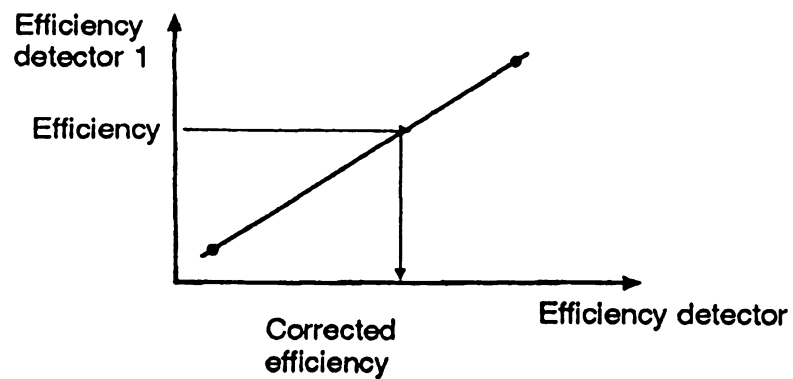


Fig. 5. The efficiency calibration curve, made with two points. The efficiency calibration curve is used to obtain the actual detector efficiency corresponding to the detector 1 efficiency obtained from the quench correction curve (Microbeta™ Instrument Manual 1992).

TABLE 1. Sample Preparation in the Microbeta™ and Betaplate™\*

	<b>Sample type</b>		
	<b>Non-aqueous</b>	<b>Aqueous</b>	<b>Cells, cell organelles, receptors</b>
<b>Liquid</b>	Organic chromatographic eluates	Water, buffer, serum, plasma, urine, gradients	Blood, tissue homogenates
	MicroBeta: <i>96-well plate</i> 5-100µl sample + 150µl OSHS <i>24-well plate</i> 5-250µl sample + 500µl OSHS  Betaplate T-Tray: 5-100µl sample + 200µl OSHS	MicroBeta: <i>96-well plate</i> 5-100µl sample + 150µl OPHS3 or 5-125µl sample + 125µl HL <i>24-well plate</i> 5-300µl sample + 500µl OPHS3 or 5-400µl sample + 400µl HL  Betaplate T-Tray: 5-100µl sample + 200µl OPHS3 or 5-150µl sample + 150µl HL	MicroBeta: After cell lysis <i>96-well plate</i> 5-100µl sample + 150µl OPHS3 or 5-125µl sample + 125µl HL <i>24-well plate</i> 5-300µl sample + 500µl OPHS3 or 5-400µl sample + 400µl HL  Betaplate T-Tray: After cell lysis 5-100µl sample + 200µl OPHS3 or 5-150µl sample + 150µl HL
<b>Solid</b>	Lipids, drugs, steroids, pesticides	RIA-precipitates, amino acids, saccharides	Cells, organelles immobilised into wells
	MicroBeta: <i>96-well plate</i> sample + 250µl OSHS <i>24-well plate</i> sample + 750µl OSHS  or sample + MeltiLex  Betaplate T-Tray: sample + 300µl OSHS  or sample + MeltiLex	MicroBeta: Sample is dissolved into water or scint. liquid <i>96-well plate</i> 5-100µl sample + 150µl OPHS3 or 5-125µl sample + 125µl HL <i>24-well plate</i> 5-250µl sample + 500µl OPHS3 or 5-400µl sample + 400µl HL  Betaplate T-Tray: Sample is dissolved into water or scint. liquid 5-100µl sample + 200µl OPHS3 or 5-150µl sample + 150µl HL	MicroBeta: <i>96-well plate</i> sample + 250 µl OPHS3 or sample + 250µl HL <i>24-well plate</i> sample + 750µl OPHS3  or sample + MeltiLex  Betaplate T-Tray: sample + 300µl OPHS3 or sample + 300µl HL  or sample + MeltiLex
<b>On support</b>	Receptor-ligand-complexes, drugs, metabolites, lipids	Protein-hydrolysates, nucleotides on filter	Proteins, organelles, cells on filter, receptor-ligand-complexes
	Betaplate, MicroBeta: Filter + MeltiLex	Betaplate, MicroBeta: Filter + Betaplate Scint or filter + MeltiLex	Betaplate, MicroBeta: Filter + Betaplate Scint or filter + MeltiLex

\*Abbreviations: OSHS = Optiscint HiSafe™; OPHS3 = Optiphase HiSafe™ 3; HL = HiLoad

P  
h  
y  
s  
i  
c  
a  
l  
  
A  
p  
p  
e  
a  
r  
a  
n  
c  
e

## CONCLUSION

The plate-counting concept enables high-throughput counting with a better counting geometry for microvolume samples and filter mats. Software control enables efficiency calibration, quench evaluation and crosstalk suppression of individual detectors. Sample preparation for plate counting is easy to automate. New scintillators optimize the sample volume and facilitate the sample preparation.

## REFERENCES

- Hyypiä, T., Auvinen, E., Kovanen, S. and Ståhlberg, T. H. 1990 Rapid quantification of DNA spot hybridization by flatbed scintillation counting. *Journal of Clinical Microbiology* 28(1): 159–162.
- Lehtinen, K., Yrjönen, T. and Sonne, V. 1991 Sample plate liquid scintillation counter. U. S. Patent No. 5,061,853. Washington, U. S. Patent Office 1991.
- Matzinger, P. 1991 The JAM test. A simple assay for DNA fragmentation and cell death. *Journal of Immunological Methods* 145: 185–192.
- Microbeta™ Instrument Manual 1992 Wallac Oy, Turku Finland.
- Potter, C. G., Gotch, F., Warner, G. T. and Østrup, J. 1987 Lymphocyte proliferation and cytotoxic assays using flat-bed scintillation counting. *Journal of Immunological Methods* 105: 171–177.
- Potter, C. G. and Le Jeune, S. 1991 Quantitative analysis of nucleic acid dot blots using the Betaplate™ flat-bed scintillation counter. *Technique - A Journal of Methods in Cell and Molecular Biology* 3(3): 117–121.
- Potter, C. G., Tan, C. C and Ratcliffe, P. J. 1991 Quantification of <sup>32</sup>P-labeled samples in gel fragments using the flat-bed liquid scintillation counter. *Analytical Biochemistry* 197: 121–124.
- Potter, C. G and Warner, G. T. 1989 Flat-bed liquid scintillation counting of whole cells and hybridization samples. In Balows, A., Tilton, R. C. and Turano, A., eds., *Proceedings of the Fifth International Symposium on Rapid Methods and Automation in Microbiology and Immunology*. Brescia, Italy, Brixia Academic Press: 9–18.
- Potter, C. G., Warner, G. T., Yrjönen, T. and Soini, E. 1986 A liquid scintillation counter specifically designed for samples deposited on a flat matrix. *Physics in Medicine and Biology* 31(4): 361–369.
- Warner, G. T. and Potter, C. G. 1986 New liquid scintillation counter design eases vial disposal problems. *Health Physics* 51(3): 385.
- Warner, G. T., Potter, C. G., Yrjönen, T. and Soini, E. 1985 A new design for a liquid scintillation counter for micro samples using a flat-bed geometry. *International Journal of Applied Radiation and Isotopes* 36(10): 819–821.
- Yrjönen, T. and Kouru, H. 1991 A method for evaluating sample activities in a multidetector liquid scintillation counter. European Patent Publication No. WO 91/19999. München, European Patent Office 1991.
- Yrjönen, T., Oikari, T. and Lehtinen, K. 1991 A method for correcting measuring values when measuring liquid scintillation samples deposited on sample plates. U. S. Patent Application S.N. 07/756,684. Washington, U. S. Patent Office 1991.