

TopCount *Topics*

TCA-008

Counting ^{51}Cr Released in Cytotoxicity Assays

Abstract

Microplate counting of supernatants from cell mediated lympholysis assays (CML) is easy and efficient using TopCount, a benchtop microplate scintillation counter. Samples may be counted with or without cocktail at efficiencies two to eight times greater than that obtained by conventional gamma counting. Data are presented from a typical cytotoxicity assay (CA) in which supernatants were split and counted on a gamma counter and on TopCount. Instrument correlation was excellent ($R^2 = 0.996$).

Introduction

Cytotoxicity assays are a fundamental tool for the study of cytotoxic T lymphocytes (CTL) and Natural Killer cell function. Typically, killing of ^{51}Cr -labeled target cells is determined by collecting supernatants and measuring ^{51}Cr release on a gamma counter. This method is encumbered by the time-consuming task of transferring supernatants individually to polystyrene tubes and by the low counting efficiency for ^{51}Cr of gamma counters.

A simplified and faster counting procedure is possible using TopCount, a benchtop microplate scintillation counter. With TopCount one transfers supernatants from the assay plate to either LumaPlates, PicoPlates, or white polystyrene microplates (*e.g.*, Packard OptiPlates), thereby retaining the original microplate format. LumaPlates and PicoPlates are 96-well microplates composed of solvent-resistant Borex plastic which contains white pigment to prevent optical crosstalk. White polystyrene microplates, such as OptiPlates, also prevent crosstalk. Supernatants may be counted in LumaPlates without cocktail or in PicoPlates and OptiPlates with MicroScint-20 (or -40) scintillation cocktail. LumaPlates, which contain a solid scintillator, yield the highest counting efficiency and lowest volume of waste.

Each microplate replaces 96 tubes, and the chance of accidentally rearranging tube or rack order is eliminated. TopCount's innovative technology enables counting of up to 12 samples simultaneously with an efficiency for ^{51}Cr which is two to eight times higher than gamma counting, depending on the type of microplate used and the degree of quench. TopCount has an absolute counting efficiency for ^{51}Cr of 21% compared to 2.7% by multidetector gamma counting [1.5" NaI(Tl) detectors].¹

Reported herein are the results from a typical CML. Supernatants were collected and then aliquoted in equal volumes for counting on a conventional gamma counter and on TopCount in LumaPlates and PicoPlates.

Methods

Cytotoxicity Assay:

P815 target cells (2×10^6) were infected with recombinant vaccinia virus (10^7 pfu) that express the influenza nucleoprotein gene. After infection, cells were resuspended for labeling in 50 ul of IMDM (7.5% FCS) plus 50 uCi of $\text{Na}_2^{51}\text{CrO}_4$ (Amersham; Arlington Heights, IL). One hour later cells were washed, resuspended in IMDM, and combined with effector cells at 10^4 cells/well in round-bottom 96-well plates with the following E:T ratios (27:1, 9:1, 3:1, and 1:1). The effector cells were CTL populations taken from BALB/cByJ mice immunized with the recombinant vaccinia viruses. Spontaneous release was determined by incubating target cells with an equal volume of media only (no effector cells), and maximum release was determined by incubating target cells in medium containing 1% SDS. Since the concentration of indicator dye was the same for all samples, including the detergent samples, the degree of color quench was the same for all samples. Cells were co-incubated for four hours at 37°C , and then 100 ul of supernatant was transferred to another microplate.² From

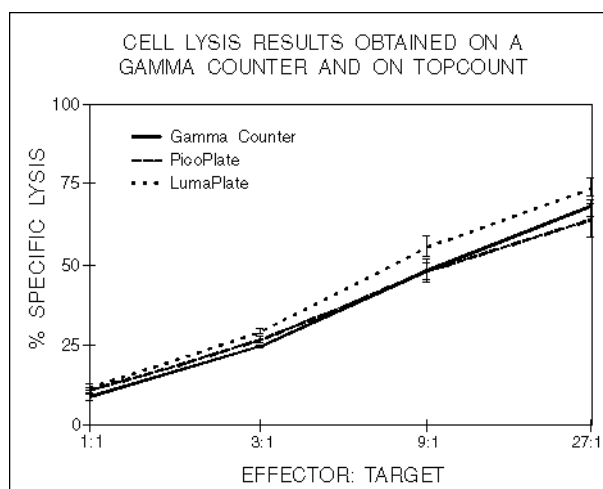


Figure 1.

the 100 μ l per replicate, 30 μ l was placed in a polystyrene tube for gamma counting with a Packard Cobra 5010, 30 μ l was placed in a PicoPlate well (plus 250 μ l of MicroScint-20), and 30 μ l was placed in a LumaPlate well (then air dried).

The percent specific lysis was calculated as follows: $[(E - M) / (T - M)]100$; T = maximal release, M = spontaneous release, E = experimental release.

Results

Figure 1 shows a comparison of the percent specific lysis between supernatants counted in tubes on a gamma counter and counted on TopCount in LumaPlates or PicoPlates. Killing measured via gamma counting had a range of 9.5% to 68.2%, while TopCount produced similar ranges using PicoPlates, 11.7% to 63.9%, and LumaPlates, 12.3% to 73.5%.

Instrument response correlations between gamma counting and LumaPlates and gamma counting and PicoPlates are presented in Figures 2 & 3. TopCount correlates very well with gamma counting, both $R^2 = 0.996$, respectively. LumaPlates had the highest count rates, $38,826 \pm 1,006$ CPM (maximal release), followed by PicoPlates, $18,724 \pm 394$, then gamma counting, $8,654 \pm 216$.

Conclusions

Cytotoxicity assays performed on TopCount have several advantages over similar assays quantified via gamma counting. TopCount delivers counting efficiencies for ^{51}Cr which are two to eight times that obtainable on a gamma counter. TopCount

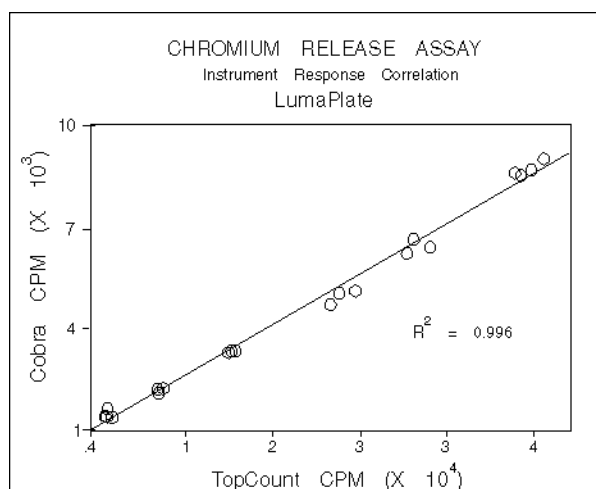


Figure 2.

reduces sample handling and sample mix-up by allowing direct microplate transfer and counting. Since a single 96-well microplate replaces 96 tubes, disposal costs are dramatically reduced, and only solid radioactive waste is produced with LumaPlates. Throughput is increased substantially; TopCount's twelve detectors can count 96 samples in a fraction of the time required by even a multidetector gamma counter. Samples can be counted with or without cocktail, and both methods correlate well with gamma counting, $R^2 = 0.996$.

1. Packard publication, TCA-005. TopCount Topics: Counting Aqueous Samples with the TopCount Microplate Scintillation Counter.
2. Packard would like to thank Dr. Laurence Eisenlohr at Thomas Jefferson University, Philadelphia, PA, for performing the cytotoxicity assays.

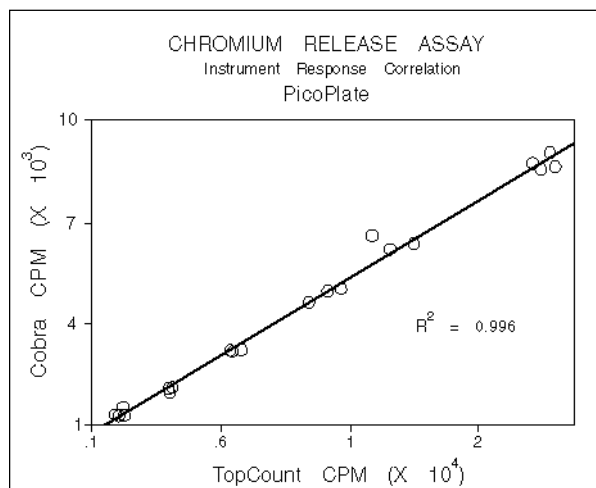


Figure 3.