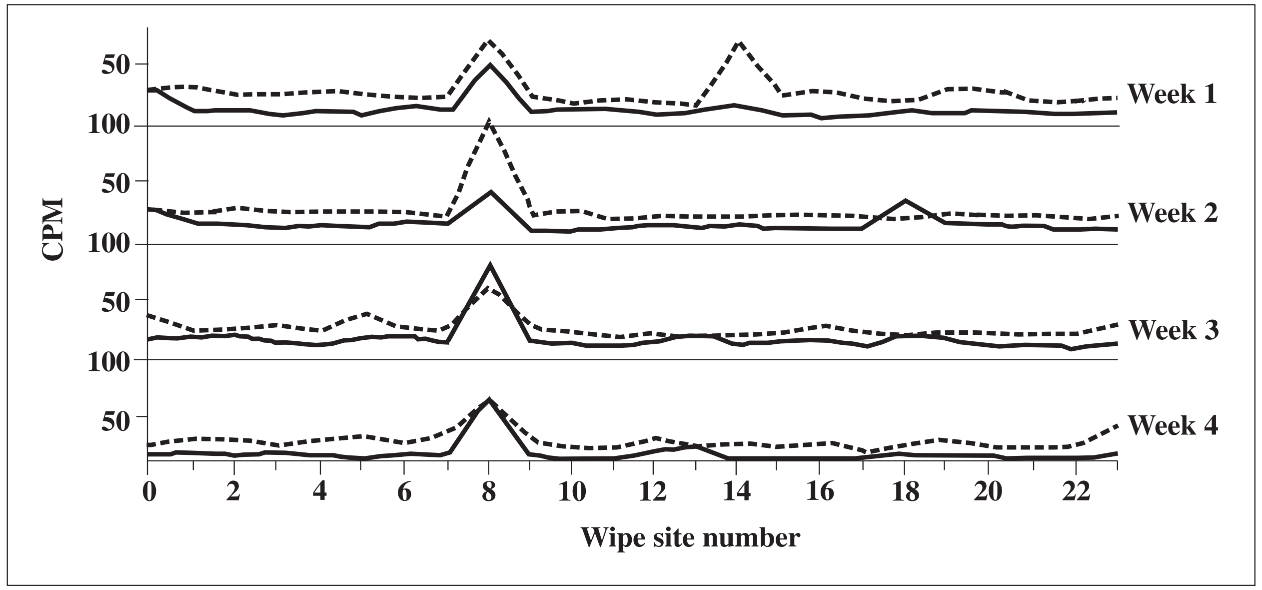

Figure 3.

Wipe tests for four sequential weeks counted on a Packard TopCount (——) and a conventional LSC (------). Wipe site number eight served as a positive control.

appear to be generated by static or luminescence from dust or other small particles interacting with the cocktail. Increasing the lower energy discriminator for both the TopCount and the LSC reduced the incidence of false positive readings. However, any apparently contaminated area should be subjected to a follow-up wipe test and decontaminated as necessary until shown to be uncontaminated.

Discussion

There are several caveats to be borne in mind with the procedure as outlined above. The use of small pieces of filter paper does expose the person doing the wipe to possible exposure. However, the use of forceps, gloves, and standard good laboratory practice should minimize this. The second problem of false positives, using either TopCount or LSC, must be considered. Setting the TopCount discriminator at 2-256 channels rather than 0-256, greatly reduces the incidence of false positives while the sensitivity of the system is only marginally effected. This is a common technique in scintillation counting which eliminates single photon events that result from luminescence (exposure to bright light or from dust) or static electricity discharge.

The counting efficiency and detection limits of the TopCount and LSC seem to be comparable in our study. Both systems reliably detected contaminated areas. The use of a single instrument for all our radioactive detection has greatly simplified operations in the laboratory. The advantages in speed, low waste production, and cost certainly indicate that TopCount is an excellent choice for analyzing complex biological assays as well as routine laboratory wipe tests.

Furthermore, the TopCount sample screening view mode makes it easy to simultaneously view all samples in a microplate, and immediately detect hot spots. Storing the resulting data on the internal hard disk of the TopCount meets the university requirement for a permanent record.

TopCount *Topics*

TCA-027

Use of the TopCount™ for Radioactivity Determinations in Wipe Test

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Introduction

The University of Connecticut School of Pharmacy recently initiated a three credit course in high throughput screening (HTS). In addition to classroom lectures, a laboratory was set up to give hands-on experience in HTS. The laboratory is funded, in part, by a state sponsored initiative bringing academia and industry together in drug design and development, and is used as a core facility to assist drug discovery. Packard Instrument Company has been a strong supporter of the HTS program at the University of Connecticut since its inception. A TopCount microplate scintillation and luminescence counter and a FilterMate™ harvester from Packard are used to assist our teaching efforts and also to enhance our drug discovery program. The TopCount and FilterMate are used primarily to run radioligand binding assays for lead screening using combinatorial and natural product libraries, and for lead evaluation where we obtain full dose-response curves using a variety of radioligands and protocols.

One of the consequences of our increased productivity using HTS methods has been an increase in the amount of radioactivity handled in the laboratory. This has necessitated an increase in the frequency of wipe tests to monitor work areas. Since the TopCount has become our primary scintillation counter, we were interested to see if it could be used in place of a conventional liquid scintillation counter (LSC) to analyze these wipes, simplifying the needs of the laboratory to one instrument. Additionally, we would increase the speed and efficiency of our wipe test, while also reducing costs and radioactive waste.

A series of experiments were conducted to verify the ability of the TopCount to replace our current method of assaying for low levels of radioactivity.

Methods and Results

The current protocol uses 60 cm^2 Kimwipe® strips, wetted with isopropanol (iPA). An area of approximately 100 cm^2 is wiped. The tissue is then placed in a 20 mL glass scintillation vial and 10 mL of Opti-Fluor™ (Packard) scintillant is added. The vial is vortexed, left for two hours in the dark and then counted in a conventional LSC for five minutes with a window set to include ^{3}H through ^{32}P energies. This protocol is in line with the University of Connecticut radiation safety policy and is conducted in 22 locations of two laboratories that are currently used for radiosynthesis and binding experiments. Data from the LSC are then manually transcribed into an Excel® spreadsheet where they are converted to DPM for analysis by the user for any possible areas of contamination.

In order to adapt this protocol to the TopCount, there were a variety of factors to consider. The material for the wipe test has to fit into the 24-well microplate, the material has to efficiently release radioactivity into the limited quantity of cocktail, the speed and efficiency of the system needs to be comparable to the current protocol, and the protocol needs to be cost effective.

In the first series of experiments, we examined the type and size of material used in the wipe assay. For these experiments Kimwipes (60 cm^2 and 25 cm^2), cotton buds, and 4.25 cm diameter Whatman No. 1 filter paper (whole and half) were chosen as possible wipe material.

An initial consideration was the amount of scintillation fluid required for efficient counting. After placing the materials in the wells of a PicoPlate™-24 (Packard), MicroScint™-20 (Packard) scintillation

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fluid was added in 250 µL aliquots. The volume required to saturate and cover the material was recorded. PicoPlates were selected over polystyrene plates because they are resistant to scintillation fluid for an extended period of time. These results are summarized in Table 1.

Material	Saturate (mL)	Cover (mL)
60 cm^2 Kimwipe	1.25	1.75
25 cm^2 Kimwipe	1.00	1.40
Whole filter	0.50	1.00
Half filter	0.25	1.00
Cotton bud	0.25	1.75

Table 1.
The amount of scintillation cocktail required to saturate and cover the five different types of materials tested for use in the wipe assay.

For the large Kimwipes, approximately 1.25 mL of fluid was required before free liquid could be observed. The 25 cm^2 Kimwipe required a little more than 1 mL. For the whole filter paper, 0.5 mL caused oversaturation while 0.25 mL was adequate to oversaturate the half filter paper and the cotton buds. While 1 mL was adequate to cover the filter papers completely, the 25 cm^2 Kimwipes required approximately 1.4 mL (which was dependent on the degree of compaction). The cotton buds and 60 cm^2 Kimwipe extended to the top of the wells and therefore required 1.75 mL of scintillant to completely immerse them.

A second series of experiments, involving all the test materials shown in Table 1, examined the relative counting efficiency of the various materials. 10 µL of a solution of ^{3}H flunitrazapam was added to each test material which was placed in a well of a 24-well plate and 0.5, 1, or 1.75 mL of scintillant was added. The samples were left in the dark for two hours before being counted. Four wells were used for each experimental variable and the CPM were averaged.

The results shown in Figure 1 for 1.75 mL indicate that the half filter method was the most efficient compared to the control (radioactivity counted in cocktail without filter material). The results for 0.5 mL and 1 mL of scintillant were essentially the same, however, the CPM variations for these lower volumes were much greater, particularly with the Kimwipes. These results clearly demonstrate that the smaller the amount of wipe material filling the well, the more efficient the counting.

Initial results showed operating difficulties with the 60 cm^2 Kimwipes and the cotton buds. Both the cotton buds and the large Kimwipes on occasion extended beyond the top of the well of the microplate. In the case of the cotton buds, this presented a solid surface above the well of the microplate which interfered with sealing and the proper positioning in the stackers and in the TopCount. When using the 60 cm^2 Kimwipes, the scintillant on occasion overflowed the well. Either the total amount of tissue filling the well made adding scintillant difficult or capillary action from the tops of the Kimwipes caused overflowing when in contact with the protective plate cover during sealing. While neither of these actions appeared to contaminate neighboring wells (due to the lip surrounding each well), it is uncertain what effect this would have on overall efficiency and possible contamination of the instrument. Therefore, the use of this material was discontinued.

With economy and efficiency as two main goals, we decided to concentrate on the half filter papers. They were the easiest of all the materials to fit into the 24-well plate and gave the most efficient counting with the least amount of cocktail. Experiments with these papers were conducted to determine the overall efficiency and reproducibility of the test.

Wipe tests were conducted with both iPA moistened and dry filter paper over pre-contaminated surfaces to assess the effects of using iPA as a wipe agent. The results from areas contaminated with three concentrations of radioactivity showed that moistening the filter paper before the wipe resulted in a three to six fold increase in the measured counts over the dry filter paper. This was consistent for both the TopCount and the LSC and clearly indicated the value of using moistened filter paper. Isopropanol had no significant effect on the counting efficiency up to and beyond the volume required to saturate a half filter paper (80 µL, data not shown).

Since we were interested in the overall efficiency of the system, a series of serial dilutions of radioactivity were set up to determine the detection limits of the TopCount and the LSC. The results shown in Figure 2 indicate that while the LSC reported higher CPM's, the detection threshold (in terms of quantity of radioactivity above base line) for both systems was essentially identical.

Using the data from the above experiments, we produced a new protocol for wipe tests based on the TopCount. Using half a 4.25 cm Whatman No. 1 filter paper, wetted with isopropanol and held with tweezers, an area of approximately 100 cm^2 is wiped. The paper is then placed in a well of a PicoPlate-24. 1 mL of MicroScint-20 scintillant is added, the plate is left for two hours in the dark, and then counted in a Packard TopCount for five minutes with a wide window of 2-256 channels. The TopCount screening function was used to alert the user to any possible areas of contamination.

The results from four consecutive weeks of wipe tests are shown in Figure 3. Both the TopCount and the LSC faithfully detected wipe site number eight, which served as the positive control. The variation in the peaks at wipe site number eight is a combination of differences in preparing the contaminated area, wiping technique, and efficiency of detection.

One of the problems we have occasionally encountered with our current wipe test assay is false positive readings. These false peaks are shown for the LSC in week one, wipe site number 14 and for TopCount in week two, wipe site number 18. These

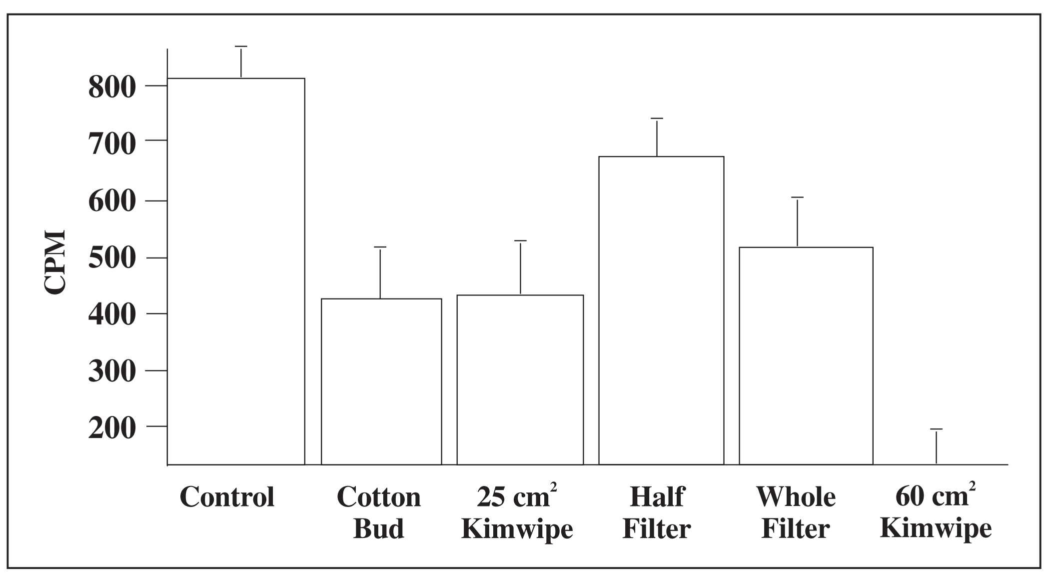

Figure 1.
The effects of various wipe agents on counting efficiency of the Packard TopCount (using 1.75 mL scintillation liquid). The control is in the absence of wipe material.

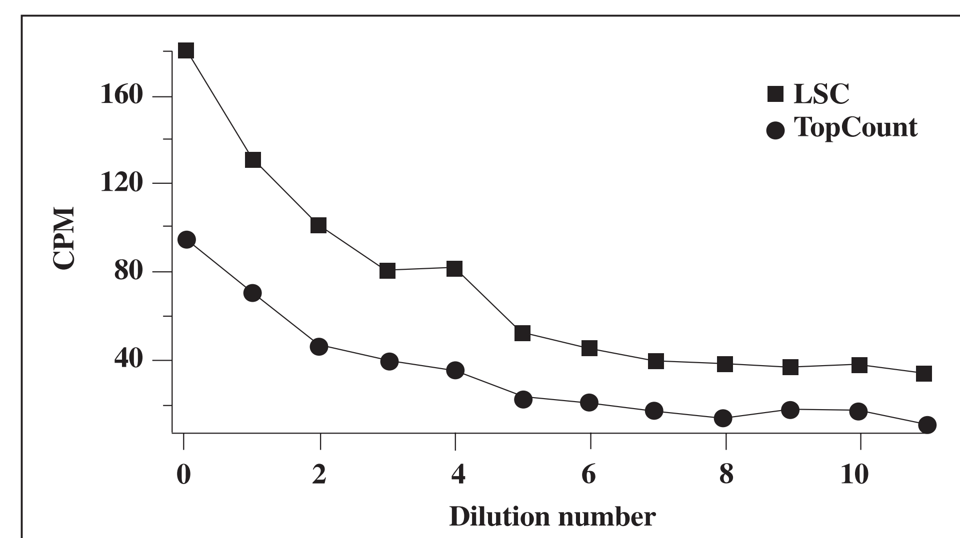

Figure 2.
Serial dilutions of radioactivity counted in an LSC and a Packard TopCount demonstrating the relative sensitivity of the two systems.