

**Application Note:** *Enhanced Precision in the ELISPOT Assay with Guava ViaCount*

# Enhanced Precision in the ELISPOT Assay Using the Guava<sup>®</sup> ViaCount<sup>®</sup> Technique for Cell Counting

Dr. Guido Ferrari and Justin Pollara, Department of Surgery, Duke University, 27710 NC

## ABSTRACT

The ELISPOT assay for the evaluation of T cell response to vaccine candidates requires that the number of cells in the sample be accurately determined. This application note describes a study demonstrating the use of the Guava ViaCount cell counting technique provided significantly more precise cell counts over multiple samples than manual procedures such as Trypan Blue staining or the use of a fluorescent substrate. The accuracy of the Guava ViaCount procedure was found to be equivalent to the other techniques. An additional benefit of the Guava ViaCount technology is that an electronic record is generated that is easily transferred to other databases.

## INTRODUCTION

Evaluating the T cell response to a prospective vaccine is critical in measuring the effectiveness of the vaccine candidate (e.g. a prospective anti-cancer vaccine or anti-infectious disease vaccine). The T cell response is usually monitored by techniques such as the ELISPOT assay or with an intracellular cytokinine-staining assay.

The ELISPOT assay involves the enumeration of the spots on a membrane after incubation of PBMC with specific antigens in a 96-well plate. Each spot on the membrane represents a cell that is producing the cytokine of choice for the assay in response to the specific antigen stimulus. The responses to the antigen are typically reported as Spot Forming Cells per million PBMC (SFC/PBMC). The number of cells that are used as input for the assay is a vital parameter and the

accuracy and precision of the overall assay clearly is very dependent on the accuracy and precision of the measurement of the number of cells in the sample.

A number of different technologies are commonly used to count the number of viable cells, including staining with Trypan blue or with a fluorescence substrate. These techniques are then followed by visual counting methods. In our laboratories, we have found that these techniques lead to a high variation in the number of counts when the counting is performed at various times by the same operator or by different operators on the same sample. These issues will, of course, lead to uncertainty in the number of cells in the sample, and thereby lead to an uncertainty in the overall evaluation of the efficacy of the vaccine candidate.

Microcytometry provides an alternative approach for cell counting and we have evaluated the Guava Technologies ViaCount cell technology to determine if it is possible to obtain more reliable and accurate count. This note describes our evaluation of the Guava system to count cells.

## EXPERIMENTAL

Eight samples were collected from normal individuals, separated after overnight resting by standard Ficoll procedure. Remaining cells were cryopreserved and seven of these samples were used for counting cryopreserved samples after thawing (day 1) and after overnight resting (day 2). Three additional samples were used as source of cryopreserved peripheral blood mononucleated cells (PBMC). Cells were counted using the Guava PCA instruments in parallel with Trypan blue exclusion dye, Erythrosin B exclusion dye, AOEB

(Acridine Orange/Eosin B) fluorescent methods in all the samples for accuracy, precision, and linearity. Flow cytometric method TruCOUNT by BD was also used for the accuracy counts.

For the determination of the accuracy of the various methods, eight different samples were run in triplicate. For the determination of linearity of results, five different samples were run in a serial two-fold dilution. For the determination of precision, three different samples were each split into 10 aliquots. All aliquots were counted once by each procedure.

## RESULTS

The accuracy of the counts that were obtained with the Guava ViaCount were as accurate as those taken with the other procedures and no significant differences were observed (always  $p > 0.1$ ).

When serial two-fold dilutions were done on both fresh and cryopreserved samples with initial counts  $> 2 \times 10^6$  PBMC/mL, we determined that linearity was lost at the 1:16 dilution. For the Guava PCA method, the R2 value was  $> 0.89$  and the range for the other methods (Trypan blue, Erythrosin B, AOEB) was  $> 0.86$ .

The precision for the number of counts measurement using the three methods for cell counting described above is as follows:

Method	CV Average
Guava	$3.15 \pm 0.57$
Trypan Blue	$6.91 \pm 0.63$
Fluorescent	$9.35 \pm 0.38$

The ability of the Guava ViaCount system to provide more precise values for the cell count led to the ability to obtain more precise data from the ELISPOT assay. As an example of this point, we present the trend analysis of SFC/ $10^6$  PBMC data obtained using the AMJ2L cell sample in the ELISPOT assay. This data shown in Figure 1 was collected over a period of 10 days; the yellow line represents results for each assay using the Guava ViaCount method for cell counting, while the red line represents the results of the assay using the Trypan Blue method for determining the number of cells.

Figure 1A presents the measurements for each of the eight samples when plotted against the mean of all the Guava measurements. The data point for each assay represents the average of triplicate measurements for each sample. The Guava samples do not vary much above one to two standard deviations away from the mean for all 8 samples compared to the manual counts. This trend is even more clear when the average Trypan blue value is used as the baseline mean (Figure 1B).

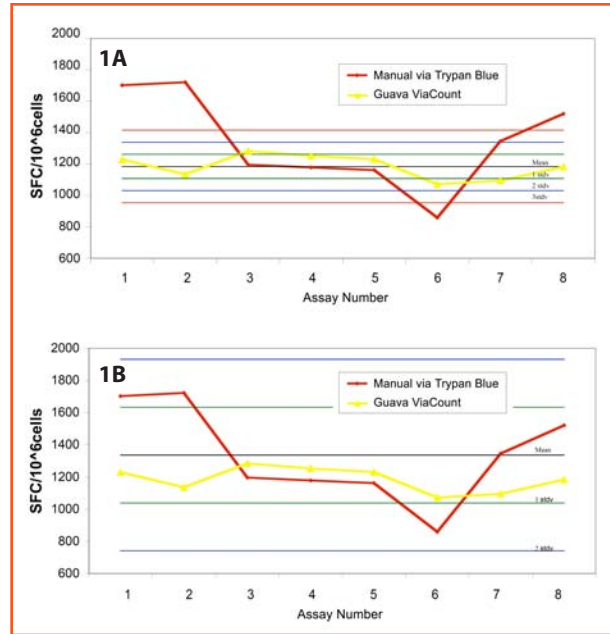


FIGURE 1: Plot of SFC/ $10^6$  using the AMJ2L Cell Sample in an ELISPOT assay. Data from one sample per day (measured in triplicates) over eight days was plotted against the mean of all Guava measurements (A) or the mean of all manual Trypan Blue measurements (B).

## CONCLUSIONS

Cell counting using the Guava ViaCount technique clearly led to more precise counts than was obtained by use of methods based on Trypan Blue or using a fluorescence substrate. The greater level of precision leads to a significant improvement in the SFC/ $10^6$  PBMC data that we collected for evaluation of vaccine candidates.



### USA & World

**Guava Technologies, Inc.**  
25801 Industrial Blvd.  
Hayward, CA 94545 USA  
Tel: 510.576.1400  
Tel: 866.448.2827 (US only)  
www.guavatechnologies.com  
info@guavatechnologies.com

### European Office

**Guava Technologies**  
Guava House  
Drope Rd.  
St. Georges Super Ely  
Cardiff CF5 6EP UK  
Tel: +44 1446 760112  
Fax: +44 1446 761015  
info@guavatechnologies.com

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