A new technique for enhanced cell counting precision with the ELISPOT assay

by Dr. Guido Ferrari, Justin Pollara and Louise Saufley

The ELISPOT assay for the evaluation of the T-Cell response to vaccine candidates requires that the number of cells in the sample be accurately determined. This article describes a study which demonstrates that the use of the ViaCount cell counting technique from Guava Technologies provides significantly more precise results than manual procedures such as Trypan blue staining or the use of a fluorescent substrate prior to counting. The accuracy of the procedure was found to be equivalent to the other techniques. An additional benefit of this new technology is that an electronic record is generated that is easily transferable to other databases.

The evaluation of the response of T-Cells to a prospective vaccine is a critical aspect of any study of the effectiveness of vaccine candidates, such as prospective anti-cancer vaccines or vaccines against infectious diseases. The T-Cell response is usually monitored by techniques such as the ELISPOT assay, or an intracellular cytokine-staining assay.

The ELISPOT assay involves the enumeration of the spots on a membrane after incubating peripheral blood mononucleated cells (PBMCs) with specific antigens in a 96-well plate. Each spot on the membrane represents a cell that can produce an appropriate cytokine in response to the specific antigen stimulus. The response to the antigen is typically reported as Spot Forming Cells per million peripheral blood mononucleated cells (SFC/PBMC). The number of cells that are input for the assay is a vital parameter and the accuracy and precision of the overall assay is clearly very dependent on the accuracy and precision of the counting of the number of cells in the sample.

Several different technologies are commonly used to count the number of viable cells, including staining with Trypan blue or with a fluorescent substrate, followed by visual counting methods. In our laboratories, it has been found that these techniques lead to a high variation when the counting is performed at different times by the same operator, as well as when the counting is performed by different operators on the same sample. This does, of course, lead to uncertainty in the number of cells in the sample, and thereby leads to an uncertainty in the overall evaluation of the efficacy of the vaccine candidate.

Microcytometry provides an alternative approach for cell counting and this article describes an evaluation of the ViaCount cell counting technology to determine if it is possible to obtain more reliable and accurate cell counts.

Methods

Ten samples were collected from normal individuals and separated after standing overnight by a standard Ficoll procedure. The remaining cells were cryopreserved and seven of these cell samples were used for counting after thawing (day 1) and after standing overnight (day 2). Three additional samples were used as sources of PBMCs. Cells in all the samples were counted using a Guava Personal Cell Analysis (PCA) system (Figure 1) in parallel with Trypan blue exclusion dye, Erythrosin B exclusion dye, and AOB fluorescent methods of staining and counting in order to compare the accuracy, precision and linearity. A flow cytometric method (TruCOUNT, BD Biosciences) was also used for comparing the accuracy of the counts. In order to compare the accuracy of the various methods, ten different sample were run in triplicate. To determine the linearity of the results, five different samples were run in a serial two fold dilution. In order to compare the precision, three different samples were run ten times with each procedure except for the TruCOUNT method.

Results

Counts that were obtained with the ViaCount were as accurate as those obtained with the other procedures; no significant differences were observed (p>0.1). It was found that linearity was lost from the 1:16 dilution onwards for both fresh and cryopreserved samples with initial count >2x10^6 PBMC/mL. For the Guava PCA method, the R^2 value was >0.89; the range for the other methods was >0.86. The precision using the three methods for cell counting described above is shown in Table 1. The ability of the ViaCount system to provide more precise values for cell counts resulted in more precise data with the ELISPOT assay. The trend analysis of SFC/10^6 PBMC data obtained with the AMJ2L cell sample in the ELISPOT assay was used to illustrate this; the data shown in Figure 2 were collected over a period of 10 days. The yellow line represents results for each assay using the 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 3 represents an analysis of the data obtained with ViaCount, with the Trypan blue method shown as a reference, to determine the mean +/- SD. It is clear that the ViaCount assay technique generates results with considerably less variation over time.

Conclusions

![Figure 1. The Guava personal cell analysis (PCA) system.](image)
![Figure 2. Plot of SFC/10^6 using the AMJ2L Cell Sample in an ELISPOT assay. Data averaged over 10 days (one sample/day). ViaCount method for cell counting = Yellow Line, Trypan blue method = Red Line.](image)

<table>
<thead>
<tr>
<th>Method</th>
<th>CV Average</th>
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<tbody>
<tr>
<td>Guava</td>
<td>3.15 +/- 0.57</td>
</tr>
<tr>
<td>Trypan Blue</td>
<td>6.91 +/- 0.63</td>
</tr>
<tr>
<td>Fluorescent</td>
<td>9.35 +/- 0.38</td>
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Table 1. Data to show precision of the three methods of cell counting.

![Image](image)
Cell counting using the Guava ViaCount technique clearly led to more precise counts than were obtained using methods based on staining with Trypan blue or using a fluorescence substrate prior to cell counting. The greater level of precision lead to a significant improvement in the SFC/10^6 PBMC data for evaluation of vaccine candidates.

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