

>>> Guava[®] ViaCount[®]

The new standard for cell counting and viability assessment

The Guava ViaCount Assay Accurately Determines Total Cell Counts

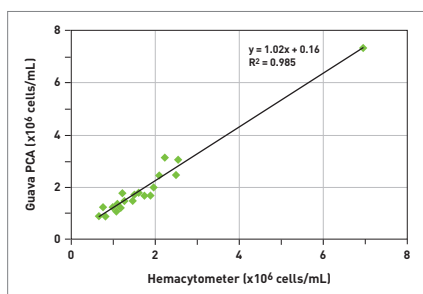


FIGURE 1 Total cell counts determined with the Guava ViaCount assay matched counts obtained with the trypan blue-based assay¹

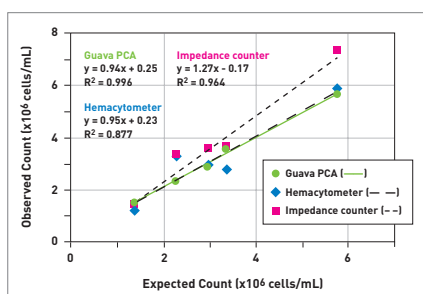


FIGURE 2 High levels of accuracy and linearity were observed with the Guava ViaCount assay when compared to other cell counting methods²

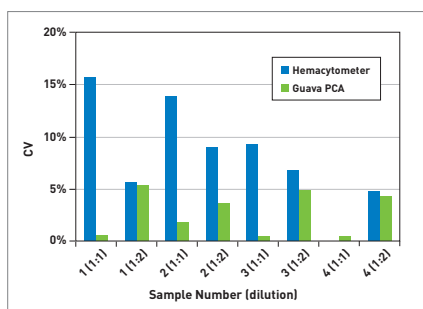


FIGURE 3 Technician-dependent variability was reduced by 68% with the Guava ViaCount assay, from an average CV of 8.1% for the hemocytometer to 2.6% for the Guava PCA¹

➤ In order to assess counting performance of the Guava ViaCount assay with mesenchymal stem cells, twelve cell samples were obtained and counted using both the ViaCount assay performed on the Guava PCA™ system and a trypan blue-based assay on a hemocytometer¹. In addition, four of the samples were assayed after dilution to 50% of their initial concentration. Data for total cell counts are shown in Figure 1 along with linear regression statistics. In this study performed by Osiris Therapeutics, Inc., a strong correlation between the two assays was observed, as indicated by a slope of 1.02 and an R² value of 0.985.

➤ In a separate study performed by Raven Biotechnologies, Inc., various methods for cell counting were evaluated, including the Guava ViaCount assay on the Guava PCA system, trypan blue dye exclusion on a hemocytometer and an impedance-based assay on an automated counting instrument. All of these methods were compared to a predicate method for enumerating cells utilizing an FDA-cleared reference counting bead (TruCOUNT™), using samples covering a range of cell concentrations. As shown in Figure 2, the Guava ViaCount assay demonstrated excellent correlation and linearity (R² = 0.996) versus the predicate method. The trypan blue-based assay also correlated well with the predicate method, with only fair linearity (R² = 0.877). The impedance-based assay exhibited a significant bias (slope = 1.27), consistently overestimating cell counts.

The Guava ViaCount Assay Reduces Technician-Dependent Variability

➤ In order to evaluate technician-dependent variability, two technicians counted a total of 8 cell samples using both the Guava ViaCount assay on a Guava PCA system, and trypan blue dye exclusion on a hemocytometer¹. Cell concentrations ranged from approximately 7x10⁵ to 1.8x10⁶. In this study, also performed at Osiris Therapeutics, the Guava ViaCount assay clearly showed improved precision for assessing total cell counts, with an average CV of 2.6%, in contrast to a CV of 8.1% for the trypan blue-based assay.

The Guava ViaCount Assay Provides an Improved Assessment of Cell Viability

➤ Samples of 4 different mammalian cell cultures were tested to evaluate the accuracy of viability assessments obtained with a manual hemacytometer using trypan blue and a Guava PCA system using the Guava ViaCount assay². Results obtained by these assays were compared with results from a propidium iodide-based assay on a flow cytometer.

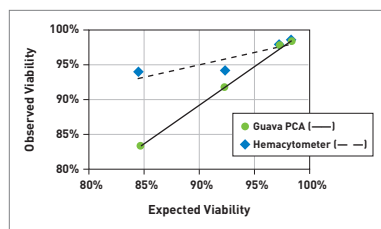


FIGURE 4 More accurate viability assessments were observed with the Guava ViaCount assay when compared with the trypan blue dye exclusion assay on a hemacytometer²

As shown in Figure 4, the trypan blue-based assay overestimated viability of the samples. Potential reasons for the discrepancies observed include the inability of the trypan blue-based assay to identify apoptotic cells, differential uptake of the trypan blue dye by dying cells, operator subjectivity associated with interpreting trypan blue-stained samples³ and the greater sensitivity of fluorescence-based methods.

➤ A study was performed to compare the accuracy of two methods for determining human progenitor cell viability and to correlate the results with biological function⁴. Methods used were trypan blue exclusion and a nucleic acid-binding, fluorometric assay utilizing a combination of the dyes acridine orange and propidium iodide. This fluorometric assay is analogous to the Guava ViaCount assay, with a different set of dyes being used. In this study, live cells from bone marrow were mixed with heat-killed cells from the same bone marrow source, in varying proportions to create a set of standards used to evaluate the accuracy of the viability assays. For comparison to a measure of biological function, the viability standards were plated then evaluated for the number of colonies forming on each plate.

Figure 5 depicts accuracy of the trypan blue exclusion and nucleic acid binding assays evaluated with viability standards, as well as correlation with CFU-GM colonies formed when the viability standards were plated and cultured. The nucleic acid binding assay showed a higher degree of linearity and accuracy than the trypan

blue-based assay, particularly at lower viability levels, where the trypan blue-based assay significantly overestimated viability. In addition, the nucleic acid binding assay correlated better with the functional CFU-GM frequency. It was also reported that this assay was much more stable over time than the trypan blue-based assay.

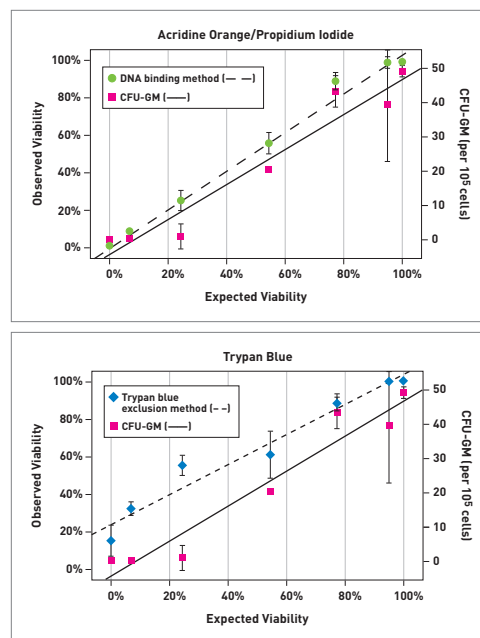


FIGURE 5 Nucleic acid binding method for viability assessment is more accurate and correlates better with cell function when compared with trypan blue dye exclusion⁴

References

- ¹ Data provided courtesy of Don Simonetti, Osiris Therapeutics, Inc.
- ² Data provided courtesy of Amy Hirst, Raven Biotechnologies, Inc.
- ³ Altman SA, et al. *Biotechnology Progress* 9: 671-674, 1993
- ⁴ Mascotti K, et al. *Transfusion* 40: 693-696, 2000



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