

Accurate Absolute Counting and Viability Measurements for Cell and Gene Therapy Workflows, Using Cytel[®] Microcapillary Flow Cytometry Technologies

Tate Sessler, Julie Clor, Katie Gillis, and Kamala Tyagarajan, Cytel[®] Biosciences, Fremont, CA

Background

The development of cell and gene-based therapies has augmented the need to determine accurate counts and phenotypic characterization of cells in every step of the process—particularly in the isolation, modification, manipulation, manufacture, and administration of the cellular product.¹ While cell counting is a fundamental measure needed for the cellular product, dosage, characteristics of viability, and purity are also extremely important in understanding and ensuring that the desired cellular effects will be obtained. These parameters are also needed to understand stability, shipping/handling, and storage conditions for cell therapy products.

Cell counting and viability are crucial in cell therapy processes as the cells need to be isolated, differentiated or transformed, cultured and expanded, and then relevant doses need to be formulated. Analytical methods that provide simple yet rapid, robust, and reproducible cell counting and viability data are especially important in the workflow.² Cell enumeration for cell therapy can often be challenging due to small amounts of precious sample available at different steps, and can often involve heterogeneous sample mixes. A wide variety of cell types are used in cell and gene therapy workflows, including whole blood, PBMCs, T cells and engineered T cells, NK cells, dendritic cells, bone marrow cells, stem cells, or pluripotent stem cells.

Figure 1.



Figure 1. Example workflow for cell and gene therapy research and cell manufacturing. The Cytel[®] Guava[®] Muse[®] and Cytel[®] Guava[®] easyCyte[™] counting assays offer accurate and precise quality control measurements for cellular count and viability at any step in the process.

The Cytel[®] Guava[®] Muse[®] cell analyzer and Cytel[®] Guava[®] easyCyte[™] systems are based on microcapillary flow cytometry and are useful instruments for cell and gene therapy labs due to their easy capability for multi-dimensional cell health and characterization analysis. These systems have easy-to-use interfaces and can provide measurements through their ability to count many hundreds or thousands of cells automatically when providing statistically relevant, accurate, and precise results on a variety of samples. These systems leverage the power of microcapillary flow using low sample volume and quick turnaround of results, and can perform absolute counting without external beads, as well as offer complete solutions with optimized kits and software. In addition, the same systems have the capability to perform extensive cell health analysis or characterize and count specific immune cell populations determining purity or identity. Superior reproducibility and repeatability are observed and have been demonstrated over other cell counting methods.

Users can use single tube measurements on the Guava Muse cell analyzer or Cytex® Guava® easyCyte™ SL systems, or perform plate-based measurements on the Cytex® Guava® easyCyte™ HT systems. The assays and systems have been applied to a variety of cell lines as evidenced by numerous publications and several cell therapy-based applications.^{3, 4, 5} The application of the Muse® Cell Count & Viability Assay to a variety of cell lines, and its advantage when compared with other methods of cell counting, have been previously shown.⁶ The Guava Muse cell analyzer and Guava easyCyte systems have broad capability to provide not only cell counts and viability results but can also be utilized for immunophenotyping, apoptosis, or cell signaling studies. This gives users the opportunity to address a range of cell and gene questions in one platform.

Assay Principle

The Muse® Count & Viability Assay and Guava® ViaCount™ Reagent use a combination of fluorescent nuclear staining dyes to provide dead and live cell information based on fluorescence and scatter parameters using simplified flow cytometry. The reagents include a premixed formulation of two DNA intercalating dyes as a single reagent for a quick mix and read assay. One dye is membrane permeant and will stain all cells with a nucleus. The second dye only stains cells whose membranes have been compromised and are dying or dead. This combination allows for the discrimination of nucleated cells from those without a nucleus or debris, and live cells from dead or dying cells (**Figure 2**) resulting in both accurate cell concentration and viability results. In only a few minutes, samples may be acquired on the Muse cell analyzer or on the Guava easyCyte platforms. The assay specific module provides total cell concentration, percent viability, and corresponding live or dead populations.

The use of a dual fluorescent assay allows for the clear distinction of all nucleated cells from debris, distinguishes live from dead cells, and enables obtaining data with greater sensitivity and accuracy when compared to colorimetric approaches often used by vision-based methods. Detection of T cells can be achieved even in the presence of erythrocytes or formulation reagents. Settings can be adjusted depending on sample and cell type and nucleated cells can be distinguished from non-nucleated populations or debris, thus even heterogeneous samples can be easily analyzed. The method is morphology independent in providing results.

The assay specific software modules for count and viability simplify sample set up and acquisition with pre-configured plot set-ups and assay results that include total cell concentration, percent viability, and concentrations for corresponding live or dead populations. Data can be stored on the device, exported in a PDF report format and/or exported as a CSV file, enabling the production of a robust documentation trail with experimental details preserved.

Figure 2.



Figure 2. Guava® ViaCount™ Reagent and Muse® Count & Viability Reagent workflow on the Cytex® Guava® easyCyte™ or Cytex® Guava® Muse® systems.

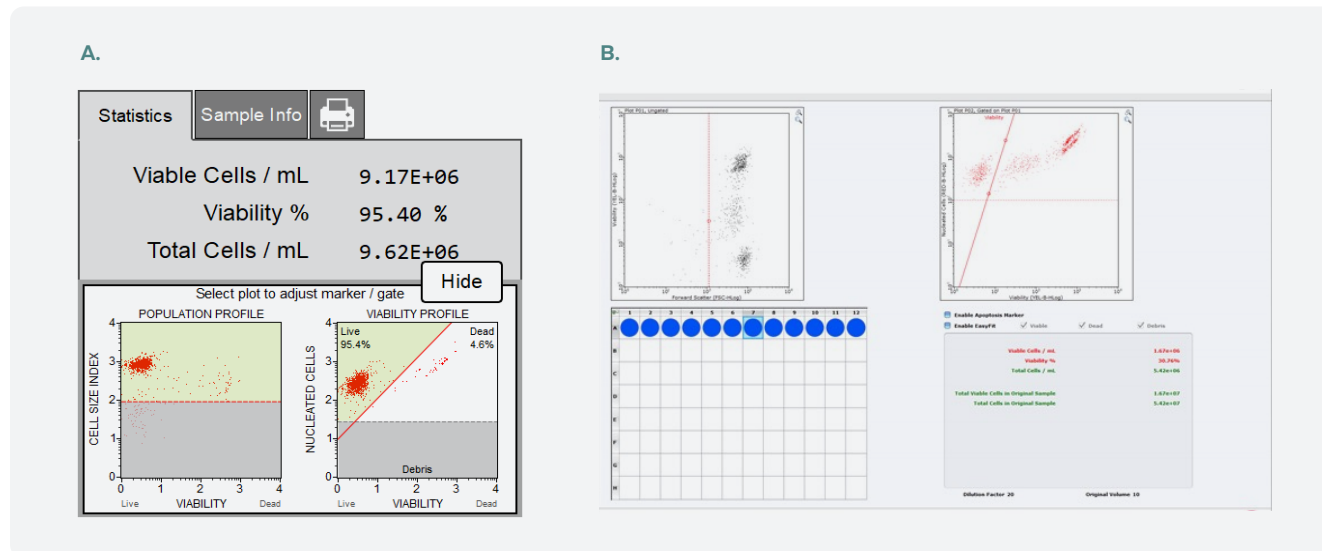
Figure 3.

Figure 3. Dedicated software outputs allow for easy interpretation of results on the Cytek® Guava® Muse® cell analyzer (A) or the Cytek® Guava® easyCyte™ system (B). Results can be exported as CSF files or PDF files for further analysis.

In this application note, we show performance data regarding precision, inter-instrument comparison, and linearity in the use of Guava ViaCount and Muse Count & Viability assays for evaluating peripheral blood mononuclear cell (PBMC) samples, isolated T cells, and Jurkat cells to obtain cell concentration and viability data in plates or tubes.

Results

High Reproducibility of Measurements

The reproducibility of measurements was evaluated by performing precision evaluation on PBMC and isolated CD3 T cell samples from the same adult human sample. Samples were prepared using either the Guava ViaCount or Muse Count & Viability kits, as described in the package inserts. Ten replicates of samples at three concentrations labeled A, B, and C, in the range of 1-10 million cells/mL were prepared and acquired on the Guava Muse cell analyzer in tubes and on the Guava easyCyte HT system in plates. Cell concentration and viability data was mined from these studies and %CV calculated. The results in **Table 1** and **Table 2** indicate %CVs of <10% for all measurements for cell concentrations on both platforms. Viability data also demonstrated %CVs of < 10% and indicated good reproducibility in measurements at all concentrations tested for both sample types.

Table 1. Data from analysis of PBMCs and CD3 T cells using Guava® ViaCount™ Reagent on Cytek® Guava® easyCyte™ HT system

Sample	Avg Conc (cells/mL)	%CV	Viability	%CV
PBMCs				
A	9.78E+06	5.3	87.4	1.5
B	5.11E+06	4.8	87.1	1.3
C	1.02E+06	3.2	86.3	1.7
CD3 T Cells				
A	1.01E+07	3.0	90	1.3
B	5.14E+06	3.3	88.7	1.1
C	1.11E+06	4.6	87.7	1.3

Table 2. Data from analysis of PBMCs and CD3 T cells using Muse® Count & Viability reagent on Cytex® Guava® Muse® cell analyzer

Sample	Avg Conc (cells/mL)	%CV	Viability	%CV
PBMCs				
A	1.07E+07	5.0	89.7	1.1
B	5.31E+06	5.4	88.8	1.1
C	1.07E+06	4.5	88	1.1
CD3 T Cells				
A	1.11E+07	5.4	92.8	1.1
B	5.62E+06	4.2	90.2	1.2
C	1.14E+06	2.9	89.3	1.3

Instrument Repeatability (Intermediate Precision)

Evaluation of inter-instrument variability for cell count and viability measurements on Guava easyCyte HT systems was evaluated. In these studies, PBMC samples at multiple concentrations were prepared with Guava ViaCount Reagent and run on multiple instruments. Samples were acquired in plates on four Guava easyCyte systems using the ViaCount software module. Concentrations were determined for six replicates at multiple concentrations of samples. %CV, average intra-assay %CV, and inter-assay %CV were calculated for each instrument.

Results demonstrate low inter-instrument %CV (typically <10%) at multiple concentrations tested.

Table 3. Average cell concentration and viability for Guava® ViaCount™ Reagent on the Cytex® Guava® easyCyte™ HT systems

	Cell Concentration (cells/mL)			Viability (%)		
	Sample A	Sample B	Sample C	Sample A	Sample B	Sample C
Inst 1	9.14E+05	5.43E+06	1.02E+07	87.9	86.5	86.3
Inst 2	9.86E+05	5.45E+06	1.10E+07	86.2	84.9	85.3
Inst 3	9.53E+05	5.34E+06	1.04E+07	86.3	86.7	86.2
Inst 4	9.33E+05	5.68E+06	1.14E+07	88.5	86.9	85.6
Avg Conc	9.57E+05	5.49E+06	1.09E+07	87.2	86.3	85.9
SD	2.68E+04	1.73E+05	5.26E+05	1.2	1.12	0.4
%CV	2.8%	3.1%	4.8%	1.4%	1.3%	0.5%

Linearity of Samples

Linearity of measurements was evaluated for both PBMCs and Jurkat cells using the Guava ViaCount assay on the Guava easyCyte HT system. Samples were diluted in a measurement range of 10^6 to 10^7 million cells/mL, stained with the Guava ViaCount Reagent, and acquired on the Guava easyCyte HT system. Cell concentrations of expected values versus observed were graphed for both cellular populations with regression and R^2 values calculated. As **Figure 4** and **Figure 5** show, R^2 values above 0.9 were seen for both populations and linear correlations were also seen.

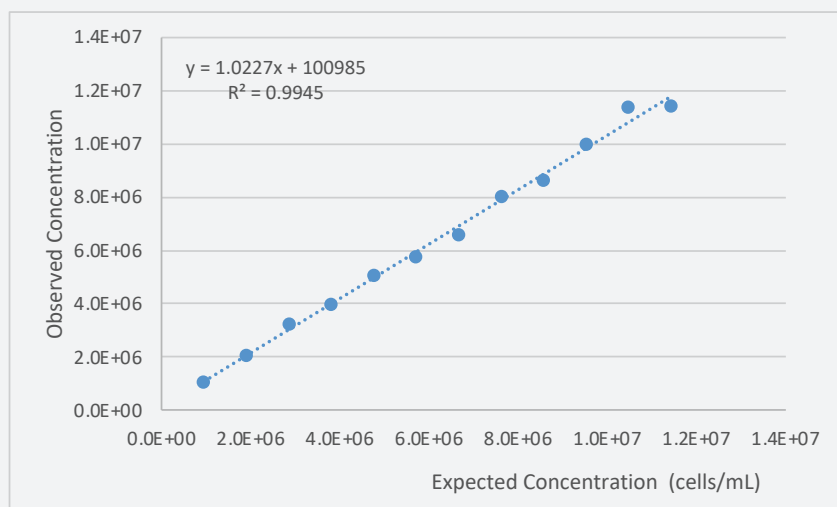
Figure 4.

Figure 4. Expected versus observed concentrations for PMBCs for the Guava[®] ViaCount[™] Reagent on the Cytex[®] Guava[®] easyCyte[™] HT system.

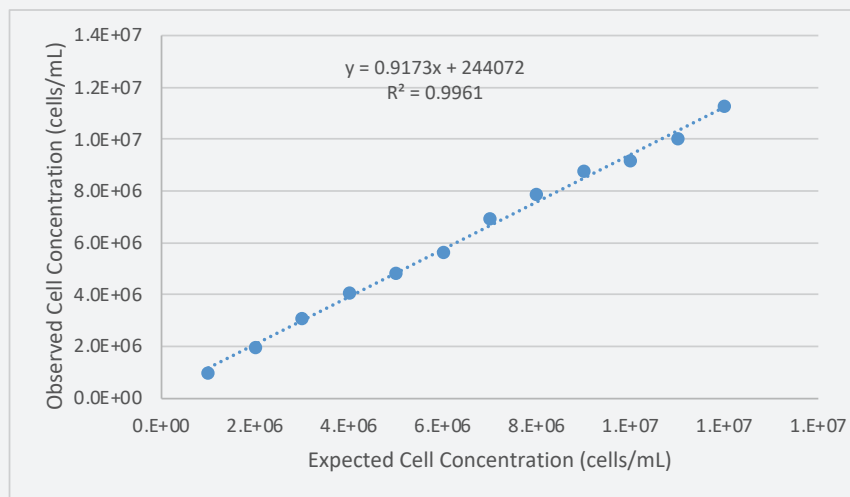
Figure 5.

Figure 5. Expected versus observed concentrations for Jurkat cells for the Guava[®] ViaCount[™] Reagent on the Cytex[®] Guava[®] easyCyte[™] HT system.

Response to Viability Changes

The response to changes in viability was studied with samples of live and dead Jurkat cells mixed in various ratios. These samples were prepared with the Guava ViaCount assay and acquired on the Guava easyCyte HT system. The results show the expected viability values corresponding to the ratio of live and dead cells (**Figure 6**).

Additionally, viability measurements at multiple concentrations of the same sample were evaluated with the Guava ViaCount Reagent. Samples at approximately 20% and 75% viability were diluted to multiple cell concentrations and analyzed with the assay. The results (**Figure 7**) demonstrate that similar viability percentages were obtained for the sample irrespective of concentration. The ability of the assay to assess different viability measurements both across a constant concentration as well as at variable concentrations highlights the accuracy of viability measurements seen with the assay.

Figure 6.

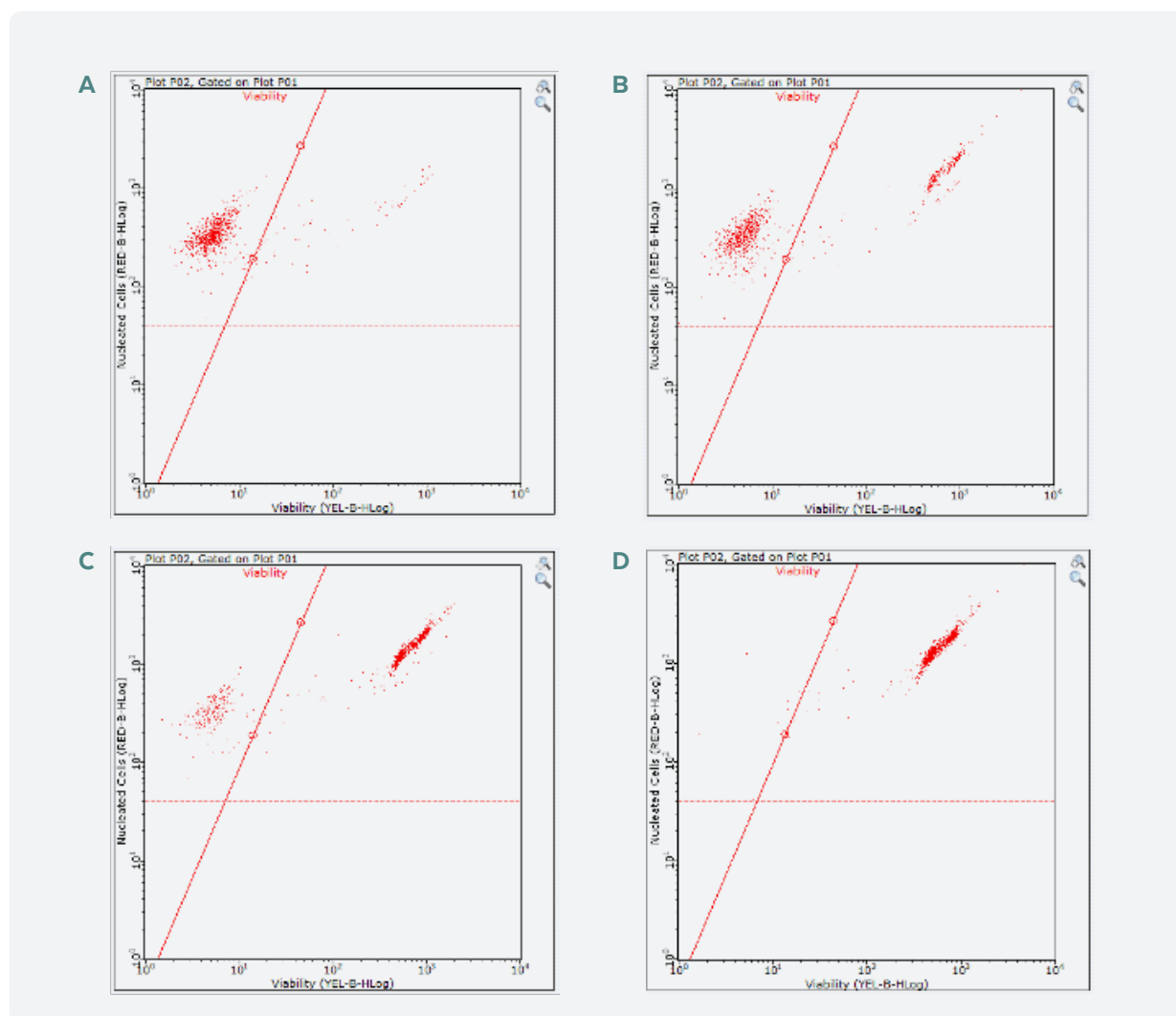
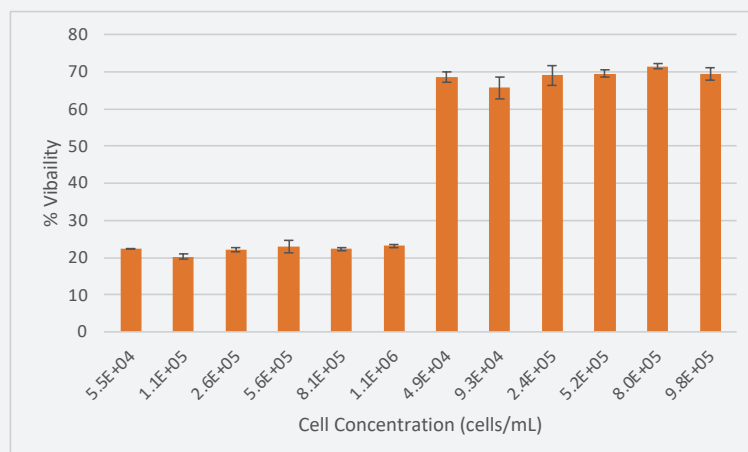


Figure 6. Response of assay to changes in viability. Sample A (Live), Sample B (3:1 Live:Dead), Sample C (1:3 Live:Dead), and Sample D, (Dead Jurkat cells).

Figure 7.**Figure 7.** Viability percentages across a variety of cell concentrations using the Guava[®] ViaCount[™] Reagent.

Detection of Apoptotic Cells

The Guava ViaCount and Muse Count & Viability assays can discriminate between apoptotic, live, and dead cells on the Cytex[®] microcapillary systems. To evaluate apoptotic cells, live, dead, and overgrown Jurkat cells were assayed with either the Guava ViaCount Reagent or the Guava Nexin[®] Reagent, which allows for the detection of cellular apoptosis as shown by phosphatidyl serine exposure. Samples were stained with either kit following the manufacturing instructions and acquired on the Guava easyCyte HT system. **Figure 8A** shows the analysis of the ViaCount data with the optional apoptotic marker, allowing for identification of a population of cells that are intermediate between live and dead. The percentage of this population corresponds well with the percentage of apoptotic cells determined by the Nexin assay (**Figure 8B**). This additional capability of the Guava ViaCount assay, along with the apoptotic marker, can be valuable for identifying apoptotic and dying cells.

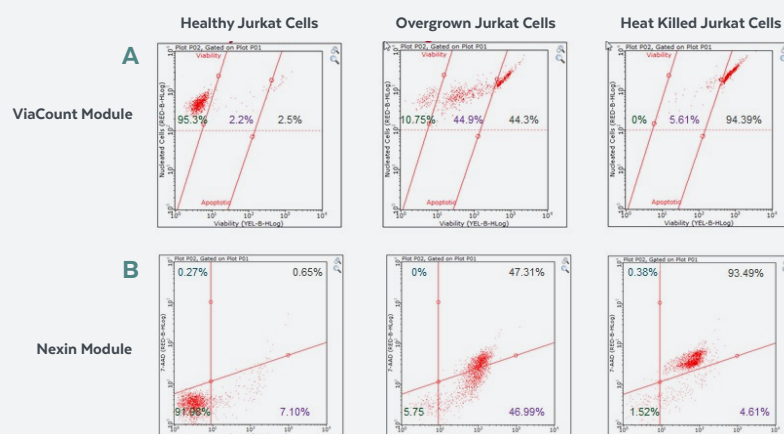
Figure 8.

Figure 8. Application of apoptotic marker in Guava[®] ViaCount[™] Reagent. Data was analyzed with the Guava[®] ViaCount[™] Reagent module with the application of the apoptotic marker (**A**). The lower panel represents the same samples analyzed with the Guava[®] Nexin Reagent (**B**) assay which helps identify apoptotic populations.

Comparison to other methods

To assess accuracy of cellular concentrations using the Guava ViaCount and Muse Count & Viability assays, three different suspension cell lines were used. Jurkat, HB, and K562 cells were prepared at multiple concentrations and viabilities and analyzed with the Muse Count and Viability protocol, or the manufacturer recommended protocol for each of the other methods. Regression statistics show that the Guava Muse cell analyzer demonstrates excellent agreement and provides accurate and comparable results to a variety of viability methods and instruments. This study demonstrates that the cell count results from the Muse cell analyzer correspond well with other cell counting methods across a wide range of concentrations.

Figure 9.

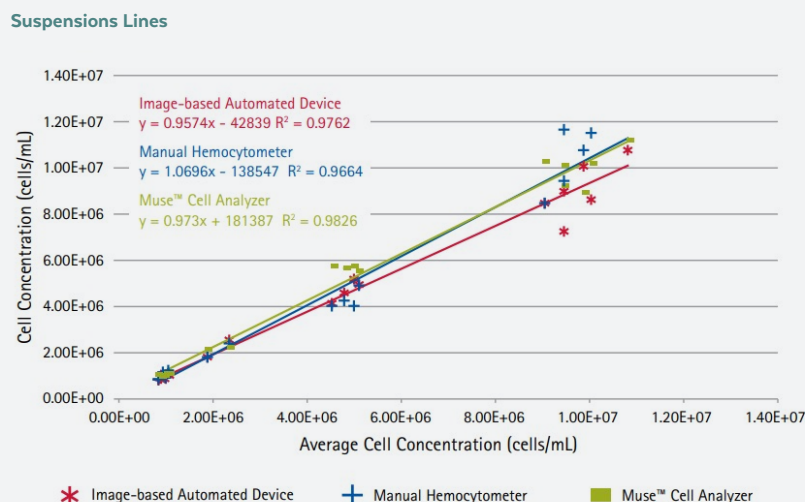


Figure 9. The plot compares Cytex® Guava® Muse® cell analyzer cell concentration data with the combined average of measured cell concentrations from all three counting methods (the image-based automated device, manual hemocytometer, and the Cytex® Guava® Muse® cell analyzer). Each point represents the average of triplicate measurements.

Conclusions

Determination of cell concentration and viability information is crucial in cell and gene therapy research, production, and manufacturing steps. Analytical methods that provide simple yet rapid, robust, and reproducible cell counting, and viability data are especially important in the workflow. The Cytex Guava easyCyte systems and Cytex Guava Muse cell analyzer utilize microcapillary cytometry along with dedicated kits (Guava ViaCount and Muse Count & Viability) for cell counting and viability along with dedicated software to provide statistically robust, fluorescence based staining and measurements. In these studies, we demonstrate the application of Guava ViaCount on PBMCs, T cells, and Jurkat cells and demonstrate that precise and accurate results can be obtained for a variety of sample types. The Guava microcapillary flow based cell counting and viability kits can provide easy-to-use but robust and powerful solutions to cell therapy researchers.

References

1. Weber EW, Maus MV, Mackall CL. The Emerging Landscape of Immune Cell Therapies. *Cell*. 2020 Apr 2;181(1):46-62.
2. Abou-El-Enein M, Elsallab M, Feldman SA, et al. Scalable Manufacturing of CAR T cells for Cancer Immunotherapy. *Blood Cancer Discov*. 2021 Sep;2(5):408-422.
3. Panebianco C, Trivieri N, Villani A, et al. Improving Gemcitabine Sensitivity in Pancreatic Cancer Cells by Restoring miRNA-217 Levels. *Biomolecules*. 2021 Apr 26;11(5):639.
4. Feng X, Han H, Zou D, et al. Suberoylanilide hydroxamic acid-induced specific epigenetic regulation controls Leptin-induced proliferation of breast cancer cell lines. *Oncotarget*. 2017 Jan 10;8(2):3364-3379.
5. Nakashima H, Ivanov P, Anderson PJ, et al. Tumor Interferon Signaling Is Regulated by a lncRNA INCR1 Transcribed from the PD-L1 Locus. *Mol Cell*. 2020 Jun 18;78(6):1207-122.
6. The Muse® Cell Count & Viability Assay Provides Improved Accuracy and Precision for Cell Counting and Viability Measurements Across Multiple Cell Lines. Luminex Corporation. <https://www.luminexcorp.com/muse-cell-analyzer/#documentation>.

For Research Use Only. Not for use in diagnostic procedures.

©2023 Cytex Biosciences, Inc. Cytex, Guava, Muse, EasyCyte, ViaCount and Nexin are trademarks of Cytex Biosciences, Inc. All other trademarks are the property of their respective owners.

APPNOTE526100
July 2023

