Application Note

Precise and Accurate Counts and Viability Measurements Across Multiple Cell Lines Using the Muse™ Cell Count & Viability Assay

Introduction

The assessment of cell concentration in combination with viability is an important step in the characterization of cell health. Cell concentration and viability information can be used for monitoring proliferation rates, optimizing growth conditions and normalizing cell data for further studies, such as assessing the impacts of cytotoxic compounds. Current methods rely on multiple, sometimes complex, instrument platforms to provide these answers, reducing flexibility, limiting the ability to simply obtain comprehensive cell health information and adding increased costs to researchers. Other, simpler methods provide inconsistent results due to their dependence upon single-uptake dyes, which do not effectively discriminate between the various states of cellular demise. Therefore, there is a crucial need for analytical methods that provide rapid, robust and reproducible count and viability data to enable the efficient, daily execution of cellular research.

The Muse™ Cell Analyzer is a unique instrument that enables multidimensional cell health analysis on a single platform. The simplified format enables researchers of varying backgrounds and experience levels to obtain a comprehensive picture of cellular health. This small, robust benchtop cell analyzer effortlessly guides users through the acquisition and analysis of samples using mix-and-read assays with a highly simplified and intuitive touchscreen interface which delivers rapid measurements of cell concentration, viability, apoptotic status, and cell cycle distribution. Using multiparametric fluorescent detection of individual cells via microcapillary flow technology, the system enables highly sensitive and rapid detection of cellular samples using minimal cell numbers.

Figure 1.

Multidimensional cell health assessment on a single platform.

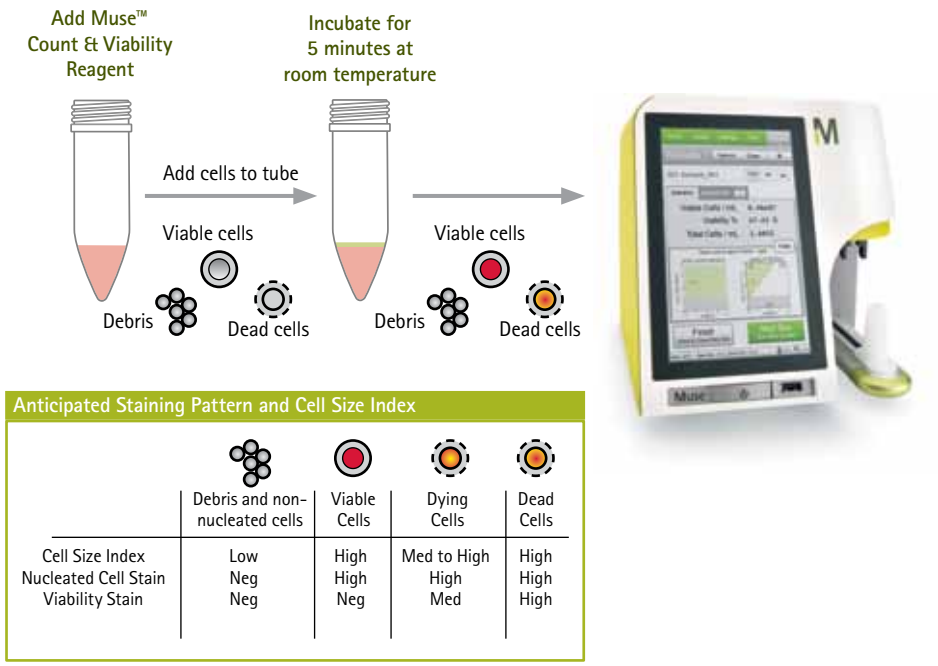
EMD Millipore is a division of Merck KGaA, Darmstadt, Germany

The Muse™ Count & Viability Assay is a simple, rapid, linear assay that provides cell concentration and viability information (Figure 2). In this application note, we show that the assay provides superior performance to conventional viability and count measurement by Trypan blue exclusion.

Key features of the assay include:

1. Accurate and precise data
2. Analyzer has small footprint and is portable
3. Mix-and-read protocols allow for rapid measurement and instantaneous results
4. Proprietary combination of two fluorescent dyes discriminate viable from dead, nucleated cells
5. Small cellular sample sizes
6. Validated on a variety of both suspension and adherent cell lines

The Assay utilizes a proprietary mix of two DNA intercalating fluorescent dyes in a single reagent (Figure 2). One of the dyes 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 resulting in both accurate cell concentration and viability results. Stained samples are then analyzed on the Muse™ Cell Analyzer using a guided touchscreen user interface. The Count & Viability Assay display results in an easy-to-read results page with an optional plot display. The use of dual fluorescent probes that clearly identify all nucleated cells, live and dead, allows for greater sensitivity and accuracy compared to colorimetric methods.



Anticipated Staining Pattern and Cell Size Index

	Debris and non-nucleated cells	Viable Cells	Dying Cells	Dead Cells
Cell Size Index	Low	High	Med to High	High
Nucleated Cell Stain	Neg	High	High	High
Viability Stain	Neg	Neg	Med	High

Figure 2.

Workflow (upper panel) and Principle (lower panel) for Muse™ Count & Viability Assay. The assay utilizes a proprietary mix of two fluorescent DNA intercalating dyes to provide information on total cell concentration and viability (lower panel). One membrane permeable dye stains all cells with nuclei, allowing for the distinction of cellular debris from cells without a nucleus. The second dye only stains cells whose membranes have been compromised. Dying and dead cells stain with both dyes, but dying cells have lower fluorescence intensity than do dead cells.

Materials and Methods

The Muse™ Count & Viability Assay uses a highly simplified workflow to provide count and viability results, as shown in Figure 2. Sample preparation is very simple with the one-step addition of the mix-and-read Muse™ Count & Viability reagent. Data from prepared samples are quickly acquired using the touchscreen Count & Viability Software Module.

The touchscreen interface workflow for the assay is shown in Figure 3. Briefly, a user enters the Count & Viability Module and hits "Run Assay". The touchscreen prompts the user to load a sample and, through simple on-screen instructions, guides the user through the optimization and verification of settings. The user then enters sample-specific information and then touches "Run Sample." The instrument displays the results screen with the calculated concentration values and provides the user the option to view the dotplot as well as adjust markers between samples (Figure 3, bottom).

Data can be stored on the device, exported in a report format and/or exported as a Microsoft Excel® file, thus enabling the production of a robust documentation trail with experimental details preserved. Result parameters include information on:

- Number of viable cells per mL
- Percent viability
- Total cells per mL
- Total viable cells in original sample
- Total cells in original sample
- Dilution factor (input value)
- Original volume (input value)
- Sample number
- Sample ID

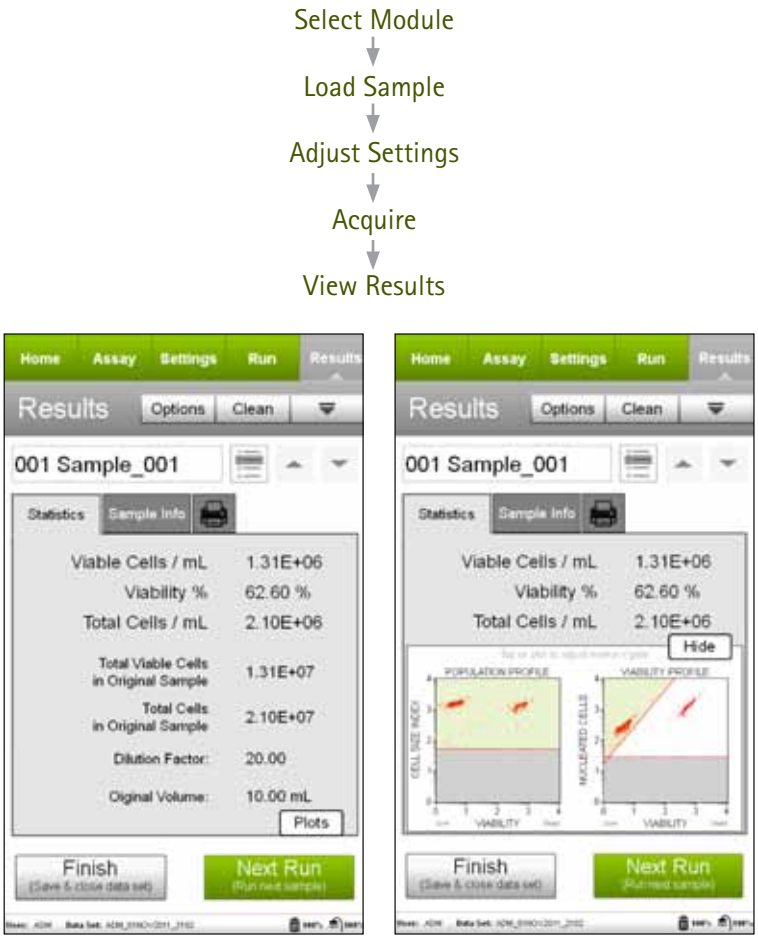


Figure 3.

The Muse™ Count & Viability Module requires just six steps to perform acquisition and analysis using a guided user interface. Concentration and viability results are displayed automatically at the completion of acquisition. Optional dotplots allow for visualization and further data manipulation.

Results

Counting Accuracy

The counting accuracy and linearity of the Muse™ Cell Analyzer was verified by measuring its ability to provide counts on multiple dilutions of reference counting beads. Figure 4 compares expected bead concentrations to bead concentrations measured using the Muse™ Cell Analyzer at multiple concentrations in the range of $1.0x10^4$ to $1.0x10^6$ beads/mL. The slopes and correlation coefficients of linear regression fit curves were both close to 1, demonstrating that excellent counting accuracy and linearity can be obtained using the Muse™ Cell Analyzer for the concentration range tested for reference counting beads.

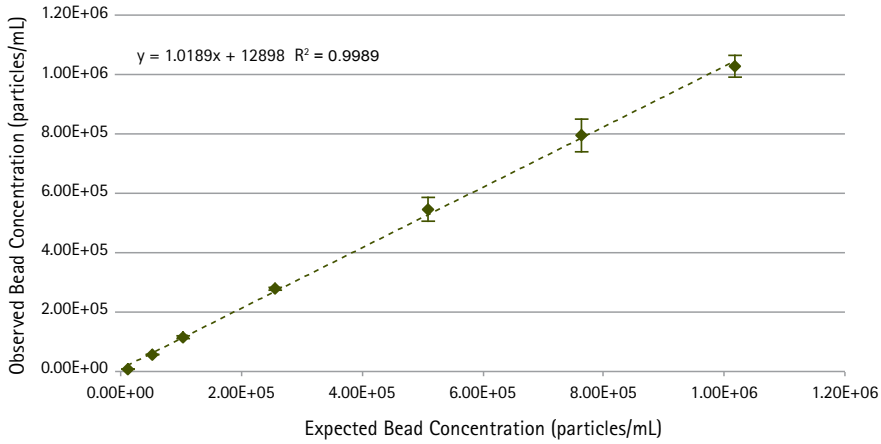


Figure 4.

The Muse™ Cell Analyzer provides accurate counting of reference counting beads. A standard bead solution (whose concentration had been determined using a Coulter Counter®) was obtained from an external vendor and diluted over a concentration range of $1.0x10^4$ to $1.0x10^6$ particles/mL. Expected bead concentrations were compared to observed bead concentrations. Each point represents the average of triplicate samplings and error bars represent corresponding standard deviations.

Versatility: Application to a variety of cell lines

The Muse™ Count & Viability Assay was used to determine cell concentration across several cell lines, including both suspension and adherent lines, at a variety of concentrations. Figure 5 shows the comparison of observed vs. expected cell concentrations for five of the cell lines tested. The theoretical concentrations were calculated based on the serial dilution of the original cell sample, whose concentration was established using the Muse™ Cell Analyzer. The slopes and R^2 values for all the cell lines tested closely approached 1, demonstrating that the assay can provide linear responses across a wide range of cell concentrations as well as diverse cell types.

Table 1 summarizes the list of cell lines validated to date for use with the Muse™ Count & Viability Assay. The data demonstrate accurate count and viability data for both suspension and adherent cell lines over a range of sample concentrations.

Cell Line Name	Adherent/Suspension	Origin	Source
Jurkat	suspension	Acute T Cell Leukemia – Human	ATCC TIB-152
HL-60	suspension	Promyelocytic Leukemia – Human	ATCC CCL-240
HB-8307	suspension	B Cell Myeloma – Human	ATCC HB-8307
CHO	adherent	Ovarian – Chinese Hamster	ATCC CCL-61
SF9	suspension	Insect Ovary *Spodoptera frugiperda*	Invitrogen 11496-015
K562	suspension	Bone Marrow Chronic Myelogenous Leukemia – Human	ATCC CCL-243
MCF-7	adherent	Breast Adenocarcinoma – Human	ATCC HTB-22
HeLa	adherent	Cervical Adenocarcinoma – Human	ATCC CCL-2
PC-3	adherent	Prostate Adenocarcinoma – Human	ATCC CRL-1435

Table 1.

Summary of cell lines tested with the Muse™ Cell Analyzer. The cell lines tested represent commonly used lines in research laboratories. They include adherent cells, suspension cells, mammalian cell lines and an insect cell line.

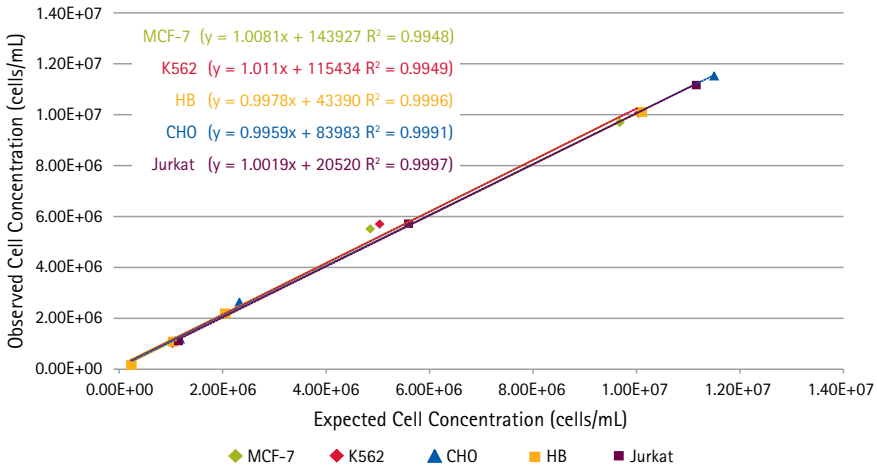


Figure 5.

The Muse™ Cell Analyzer performs with high linearity across multiple cell lines and a wide sample concentration range. The data show the comparison of observed vs. expected cell concentration results for serial dilutions of 5 representative cell lines shown, which include both adherent and suspension cells. Each point represents the average of three samplings.

Comparison of Muse™ counting compared to other counting systems

We compared the accuracy of the Muse™ Count & Viability Assay with other methods that provide count and viability information:

1. Traditional methods of cell counting that utilize Trypan blue staining such as manual hemocytometer counts
2. Automated image-based analysis of Trypan blue-stained samples.

Table 2 summarizes the features of each of the three methods for cell concentration and viability determination.

	Muse™ Cell Analyzer	Manual Hemocytometer	Automated Imaging-based Counting Device
Sample format required for acquisition	Tube-based	Slide-based	Slide-based
Staining type	Fluorescent dyes	Trypan blue	Trypan blue
Degree of operator bias	Minimal	Significant bias	None
Variability in number of cells counted	No variability	Number of cells counted is concentration-dependent and may vary between samples	Number of cells counted is not clear, concentration-dependent
Number of cells counted	More cells, increased statistical significance	Fewer cells	Fewer cells
Acquisition speed	1–2 minutes	Slower due to manual counting	~ 1 minute
Flexibility in sample reading/analysis	Greater flexibility in sample read time after staining	Samples must be analyzed soon after staining	Samples must be analyzed soon after staining
Data export features	Advanced export features, reanalysis of data, allows for documentation of report; Excel® file export option	Lost after read; manually written-down results	Exportable to .csv file – only counts exported

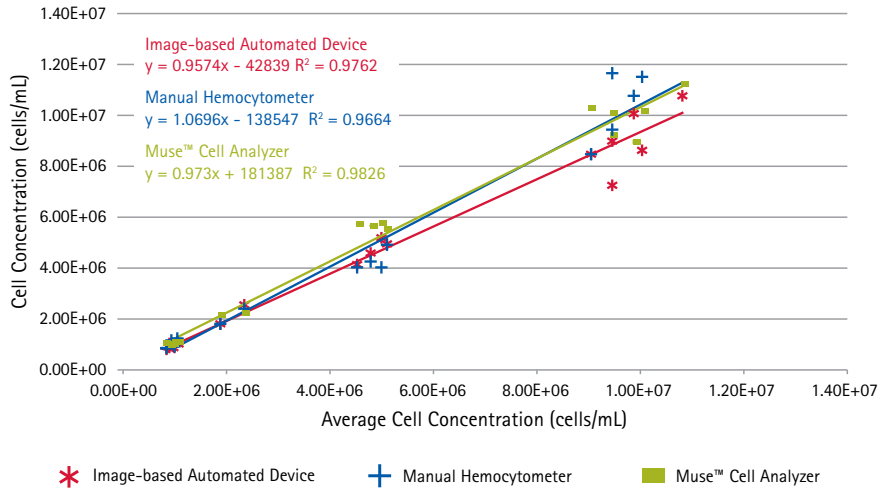
Table 2.

Comparison of features of Muse™ Cell Analyzer with features of other devices for measuring cell counts and viability.

Five different cell lines at multiple concentrations and viabilities were analyzed using the Muse™ Count & Viability protocol and manufacturer-recommended protocols for each of the other methods. Figure 6 depicts the comparison of the average of triplicate measurements for each individual cell counting method versus the average cell concentration calculated by taking the mean average cell concentration from all three methods together.

Regression statistics show that the Muse™ Cell Analyzer demonstrates excellent agreement and provides accurate and comparable results to a variety of viability methods and instruments.

A. Suspension Lines



B. Adherent Lines

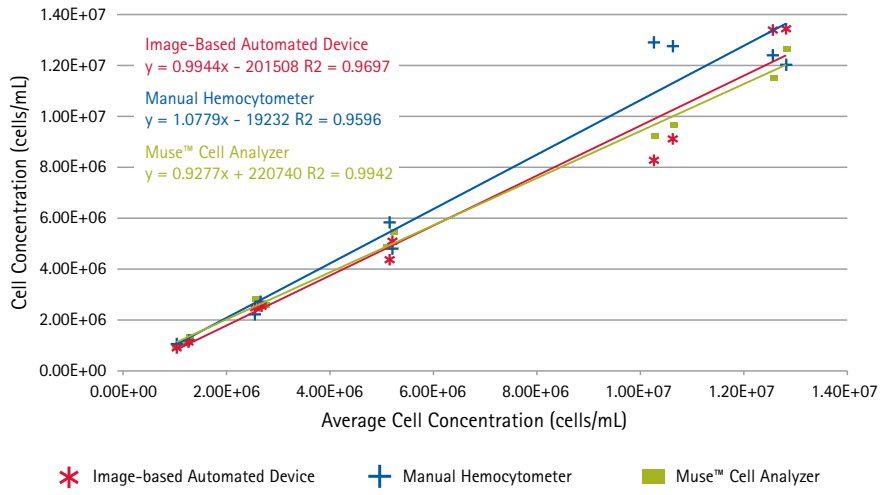


Figure 6.

The Muse™ Cell Analyzer provides accurate cell concentration measurements, comparable to results from other analysis methods, for both suspension (A) and adherent (B) cell lines. The plot represents comparison of Muse™ cell concentration data with combined average measured cell concentrations from all three counting methods (Image-Based Automated Device, Manual Hemocytometer and Muse™ Cell Analyzer). Each point represents the average of triplicate measurements.

Precision and Reproducibility

The precision of the Muse™ Count & Viability Assay was evaluated using the analysis methods and studies described above (Figures 5–6). Table 3 summarizes the average percent coefficient of variation (%CV) and %CV range obtained using the three methods to analyze 90 cellular samples from suspension and adherent cell lines at multiple concentrations.

The table demonstrates that the Muse™ Cell Analyzer provided average %CV of 4.0% for cellular concentration determination, which was lower than that observed for image-based automated counting (average %CV of 9.2%) and lower than that observed for manual hemocytometry (average %CV of 6.3%). While image-based automated counting methods and manual hemocytometry displayed broader ranges of %CVs, the Muse™ Cell Analyzer exhibited a narrow range of %CVs and consistently provided %CVs less than 10% over the entire range of samples tested. Higher %CVs were observed for the Trypan blue-based methods, particularly at lower cell concentrations. The data demonstrated that the Muse™ Cell Analyzer can provide superior precision for cell counting measurements for multiple cell lines across multiple concentrations.

Table 3 also demonstrates that the Muse™ Cell Analyzer has a lower average %CV (2.2%) for viability measurements compared to the other methods. The %CV for viability measurements on the Muse™ Cell Analyzer was < 7% for all samples tested.

	Cell Concentration		Viability	
Analysis Method	Average %CV	%CV Range	Average %CV	%CV Range
Muse™ Cell Analyzer	4.0%	0.3–8.8%	2.2%	0.4–5.6%
Image-based Automated Counter	9.2%	1.2–23.3%	3.7%	0.8–12.1%
Manual Hemocytometer	6.3%	0.5–15.3%	4.5%	0.5–9.2%

Table 3.

The Muse™ Cell Analyzer provides superior precision for cell concentration and viability measurements, compared to Trypan blue-based analyses. Data are based on triplicate measurements of 30 cellular samples from suspension and adherent cell lines at multiple concentrations and viabilities.

Figure 7 demonstrates viability results from multiple cell lines at multiple cell concentrations. Low variation between viabilities at each concentration was seen, as shown by the small standard deviation bars. The data support that the Muse™ Cell Analyzer provides reliable viability results across a wide concentration range, covering most cell concentrations encountered during standard culturing and cellular research.

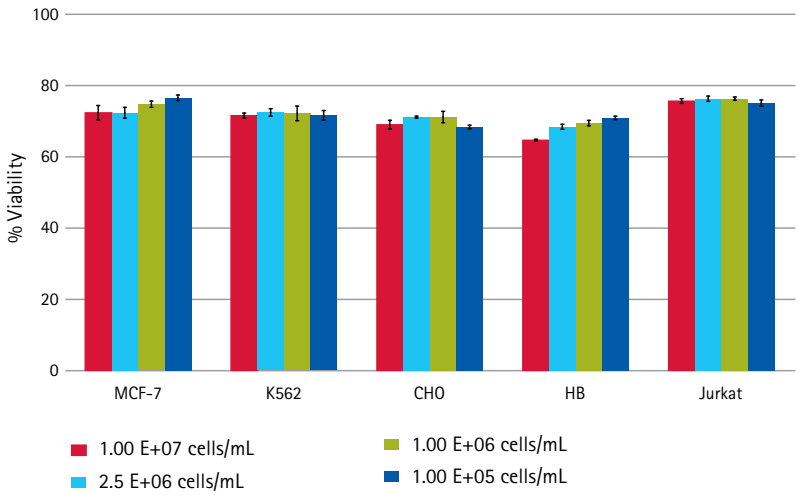


Figure 7.

Consistent viability results across various cell concentrations and cell types. Five cell types representing both adherent and suspension lines were harvested and viabilities determined across various cellular concentrations. Each bar represents the average of triplicate samplings and error bars represent their corresponding standard deviations.

Conclusions

The Muse™ Cell Analyzer is a multifaceted instrument that enables measurement of multiple cell health-related parameters on a single platform. Specific assay modules facilitate rapid, easy assessment of cell health using assays for counting and viability (shown in the present study), apoptosis detection and cell cycle distribution.

Performance data demonstrate excellent correlations with traditional, accepted analysis methods and confirm that this new platform yields accurate results for a variety of cell types and concentrations. Furthermore, the Muse™ platform yields superior precision compared to traditional methods of cell counting and viability measurement. By making cell health analysis simple, affordable and easily accessible, the Muse™ Cell Analyzer can help integrate cell health analysis into everyday cell culture workflows. As a result, cell-based experiments can be made more consistent and reproducible, enabling faster, more accurate decisions for more productive research.

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