Measurements Across Multiple Cell Lines

Cell Count & Viability Assay

The assessment of cell concentration in combination with viability is an important step in the characterization of cell health. 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.

Fig. 1: Multidimensional cell health assessment on a single platform.

Introduction

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.

The Muse Cell Analyzer enables multidimensional cell health analysis on a single platform. This benchtop cell analyzer guides users through the acquisition and analysis of samples using mix-and-read assays with an 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.

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

The assay utilizes a proprietary mix of two DNA intercalating fluorescent dyes in a single reagent. 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 using a guided touch screen user interface. The assay display results in a 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.

Materials and Methods

The assay sample preparation is simple with the one-step addition of the mix-and-read reagent.

The touchscreen interface workflow for the assay is simple. Briefly, a user enters the Count & Viability Module and hits "Run Assay". The touchscreen prompts the user to load a sample and 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.

Result parameters include information on the 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 and sample ID.

Results

Counting Accuracy

The counting accuracy and linearity was verified by measuring its ability to provide counts on multiple dilutions of reference counting beads. The comparison of expected bead concentrations to bead concentrations measured using the Muse Cell Analyzer at multiple concentrations in the range of 1.0 x 10^4 to 1.0 x 10^6 beads/ml. The slopes and correlation coefficients of linear regression fit curves were both close to 1, demonstrating that counting accuracy and linearity can be obtained for the concentration range tested for reference counting beads.

Application to a Variety of Cell Lines

Figure 2 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 analyzer. The slopes and R2 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. The data demonstrate accurate count and viability data for both suspension and adherent cell lines over a range of sample concentrations.

Comparison to other Counting Systems

Table 1 summarizes the features of each of the three methods for cell concentration and viability determination. Five different cell lines at multiple concentrations and viabilities were analyzed using the protocol and manufacturer-recommended protocols for each of the other methods. Regression statistics showed that the Muse Cell Analyzer demonstrates agreement and provides accurate and comparable results to a variety of viability methods and instruments.

Precision and Reproducibility

Table 2 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 shows provided average %CV of 4.0 % for cellular concentration determination, 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 Instrument 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.

Table 2 also demonstrates a lower average %CV (2.2 %) for viability measurements compared to the other methods. The %CV for viability measurements was < 7 % for all samples tested.

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.

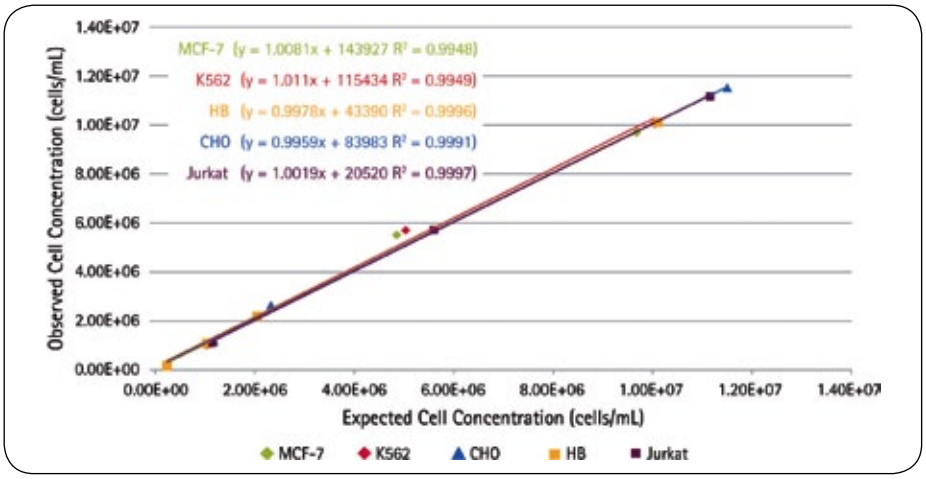

Fig. 2: Comparison of observed vs. expected cell concentration results for serial dilutions of 5 representative cell lines (adherent and suspension cells). Each point represents the average of three samplings.

	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 1: Comparison of devices for measuring cell counts and viability.

	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: Data are based on triplicate measurements of 30 cellular samples from suspension and adherent cell lines at multiple concentrations and viabilities.

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