Technical Note 196

CellDrop Performance Data

Introduction

Accurate and reproducible cell quantification is a key control step in routine laboratory research, cell based manufacturing, and therapeutic processes.

Since the invention of the glass hemocytometer in the 1800s it has remained the accepted standard for cell quantification (1). Newer techniques for cell counting using flow-based and automated image-based systems have recently been introduced that offer significant advantages over manual counting using a hemocytometer. However due to the complex biological properties of cells and the number of preparation steps necessary before an actual count is taken, the creation of a universal cell counting standard to assess the accuracy of these new methods against the manual hemocytometer has been challenging (2).

The National Institute of Standards and Technology has proposed a robust method to assess cell counting linearity and reproducibility using serial dilutions to measure the proportional change of concentration on a cell counting device compared to the known proportion of the dilution series (3).

A version of this method was tested on three DeNovix CellDrop™ Automated Cell Counters to assess their linearity and reproducibility. For comparison, a manual hemocytometer was also tested using the same procedure.

Materials and Methods

Three CellDrop Automated Cell Counters were calibrated and certified using standard DeNovix procedures. The DeNovix Default Protocol in the Brightfield App (Min diameter 3, Max Diameter 30, Roundness 50) and DirectPipette™ at a chamber height of 100 µm was used for counting all samples.

A Brightline hemocytometer with 100 µm chamber depth (Hausser Scientific Horsham, PA) was used for comparison. Cells were counted manually under a microscope using standard protocols and cell concentration was calculated by applying the formula: Cells per mL = Total Cells Counted * 10,000

MRC5 and 293t cells were harvested from confluent cultures and centrifuged at 1000 RPM for 10 minutes. The pellets were resuspended in DMEM with 10% FBS and. Initial cell density was checked with one of the CellDrops.

A stock solution was prepared from this initial resuspension with a target concentration of 2.0×10^6 cells/mL. Three additional dilutions were prepared from this stock with target concentrations of 1.0×10^6, 5.0×10^5 , and 2.5×10^5 cells/mL. All four dilutions were checked with a Z2 Coulter Counter (Beckman Coulter, Brea, CA) as an independent reference.

Results

293t cells were prepared as described and ten replicates of each dilution were counted on three CellDrops and the manual Hemocytometer. Table 1 and Figure 1 present the linearity and reproducibility data for each CellDrop and the manual counting.

Figure 3 gives a detailed view of the 293t cells after counting using the CellDrop's default protocol. The live cells with bright centers and dark outlines can be seen circled in blue by the counting algorithm while smaller fragments of extracellular debris are excluded from the count.

MRC5 cell dilutions were prepared as described and also counted in replicates of ten on three CellDrop instruments and manually using the hemocytometer. As with the 293t cells, the CellDrop default protocol was used for counting. Table 2 and Figure 2 detail the reproducibility and linearity data obtained from the serial dilution replicates using both automated and manual methods

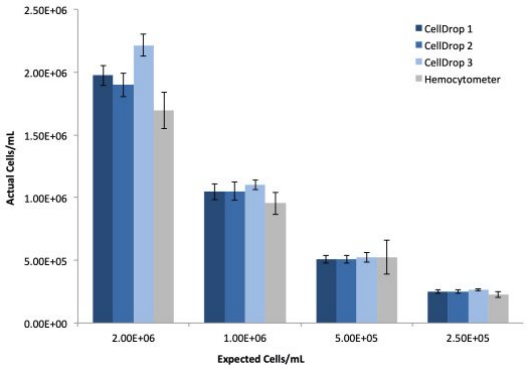

Figure 1: 293t Cell Count Linearity and Reproducibility .

Table 1: 293t Cell Count Linearity and Reproducibility.

Instrument	R^2	Average %CV
CellDrop 1	0.999	5.6
CellDrop 2	0.996	6.0
CellDrop 3	0.999	4.7
Hemocytometer	0.993	13.4

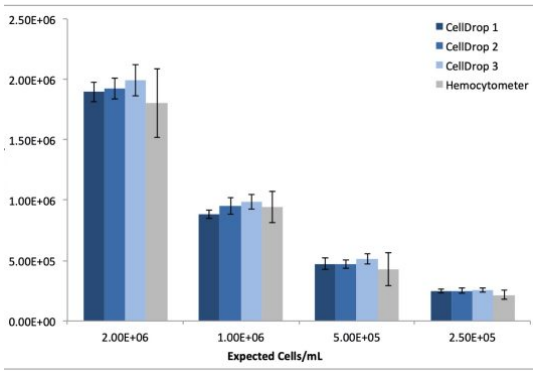

Figure 2: MRC5 Cell Count Linearity and Reproducibility

Table 2: MRC5 Cell Count Linearity and Reproducibility.

Instrument	R^2	Average %CV
CellDrop 1	0.999	6.3
CellDrop 2	0.999	6.9
CellDrop 3	0.998	6.9
Hemocytometer	0.998	14.5

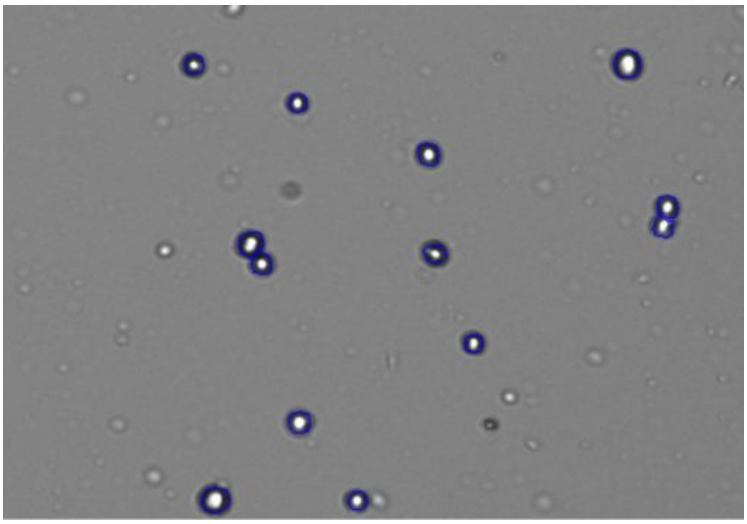

Figure 3: Brightfield counted 293t cells imaged on the CellDrop automated cell counter

Discussion

The data presented demonstrate that automated cell counting using CellDrop instruments delivers greater precision compared to the established manual methods. Superior precision is maintained across all dilutions tested for both cell lines and a more linear response was observed, demonstrated by superior R^2 values. The implementation of standard, automated cell counting procedures removes the subjectivity of manual cell counting allowing greater intra- and inter-user reproducibility and consistency of results over time. Automatic archiving of sample images and results also allows for quality control of culture health over time for traceability and auditing of data.

Additionally, laboratories are able to realize considerable time savings when moving to automated cell counting. During the present study, the time taken to perform manual cell counts using a hemocytometer ranged between two and five minutes from low to high concentration. This excludes time taken to transcribe results and apply calculations. Automated image capture, analysis, and reporting using the CellDrop was completed in less than 10 seconds for each sample.

In the absence of a universal cell counting standard it is not practical to attempt to assess the accuracy of one method vs another, however the time, linearity and reproducibility of the CellDrop Automated Cell Counters shows significant advantages over the manual hemocytometer.

References

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2. Tanavede V, Vaz C, Rao M, Vemuri M, Pochampally R. Research using Mesenchymal Stem/Stromal Cells: quality metric towards developing a reference material. Cytotherapy, 2015; 1169-1177
3. Sarkar S, Lund S, Vyzasatya R, Vanguri P, Elliot J, Plant A, Lin-Gibson S. Evaluating the quality of a cell counting measurement process via a dilution series experimental design. Cytotherapy 2017 1509-1521

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DeNovix Inc.
3411 Silverside Road
Wilmington, DE 19810, USA

Phone: +1.302-442-6911
Email: info@denovix.com
www.denovix.com