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Variability Analysis of the Vi-CELL BLU Cell Viability Analyzer against 3 Automated Cell Counting Devices and the Manual Method

Andrew Wu [AWU01@BECKMAN.COM]
Applications Scientist, Beckman Coulter Life Sciences

Introduction

The manual cell counting method using a hemocytometer is often regarded as a trusted and reliable method. However, there are multiple key differences between manual and automated counting which can lead to unreliable results. The manual method involves several error-prone steps such as taking a precise sample, mixing with trypan blue and delivering it to the counting chamber, as well as subjective counting using a microscope and incremental counting device. Precise counts are often limited by time constraints of counting a limited number of grids before viability begins to drop off, which can lead to increased variability and reduced statistical confidence in the results. Live and dead cells must be differentiated subjectively by the operator, which can lead to inconsistent results even within the same laboratory (Salinas et al. 1997). With automated counters, the sampling and mixing steps are performed in a highly repeatable and consistent manner, and image-based counting algorithms streamline the enumeration process. The Vi-CELL BLU cell viability analyzer offers an automated solution to manual counting. The device prepares the sample by mixing with trypan blue and automatically delivers it to the flow cell to take up to 100 images in as little as 90 seconds. It can be used with both individual tubes and 96-well plates. The device has been shown to have low instrument-to-instrument variability as described in the, “Evaluation of Instrument-to-Instrument Performance of the Vi-CELL BLU Cell Viability Analyzer” application note.

In the following application note, a comparison of cell counts and viability was performed for 3 suspension cell lines on 4 different automated counting instruments from different manufacturers against the manual method using a hemocytometer. The comparison was performed by a third party and the provided raw data was analyzed with JMP 16.

The three different cell lines and medium types are listed below

Cell line	Medium	Days of Culture
CHO K1	CDCHO + Pen-Strep	9
HEK Exip293	Expi293 + Pen-Strep	7
Sf9 ExpiSf9	Sf-900 + Pen-Strep	8

Table 1. Cell lines and medium types.

Measuring devices and specifications

Method	Cell Diameter Range	Density Range	Sample volume	Analysis time	Automated Prep	Automated Counting
XR	3-70µM	5E+04 - 1E+07	500µL	<180 sec	Yes	Yes
BLU	2-60µM	5E+04 - 1.5E+07	200µL	<130 sec	Yes	Yes
Device 2	4-70µM	1E+05 - 8E+07	400µL	228 sec	Yes	Yes
Device 1	NA	1E+04 - 1E+07	10µL	10 sec	No	Yes
Manual	NA	NA	10µL	Variable	No	No

Table 2. Instrument devices and specifications. Instrument settings optimizations were performed by representatives of each manufacturer.

Culturing method

Cells were taken from LN2 storage and were allowed to recover in 15mL of media over 4-6 days. Cultures were then expanded in 50mL flasks for 4-6 days. Next, 4 flasks were seeded at 0.5e6 cells/mL in 75mL of media.

Sampling method

5mLs from each flask was taken and diluted at 10, 30, 50, 70 and 90%. For each cell type, 3-4 replicates per dilution were tested on each day for up to 9 days.

Statistical methods

Values were divided by the dilution factor to get a standardized estimated undiluted value. Viability was calculated by dividing the viable cell count by the total cell count.

Contaminated samples and samples with instrument failures during analysis were not included in statistical analyses. Clear outliers were manually removed when values were visibly off from the trend of the entire data set. For example, if a replicate was over or under ~1.5 times the mean of the replicates of the same sample. No outliers were manually removed from the manual counts due to the high spread. Values with viability readings less than 70% for Days 1-6 were removed. A total of 42 outliers were removed from the data set of 2240 data points.

Variability analysis results

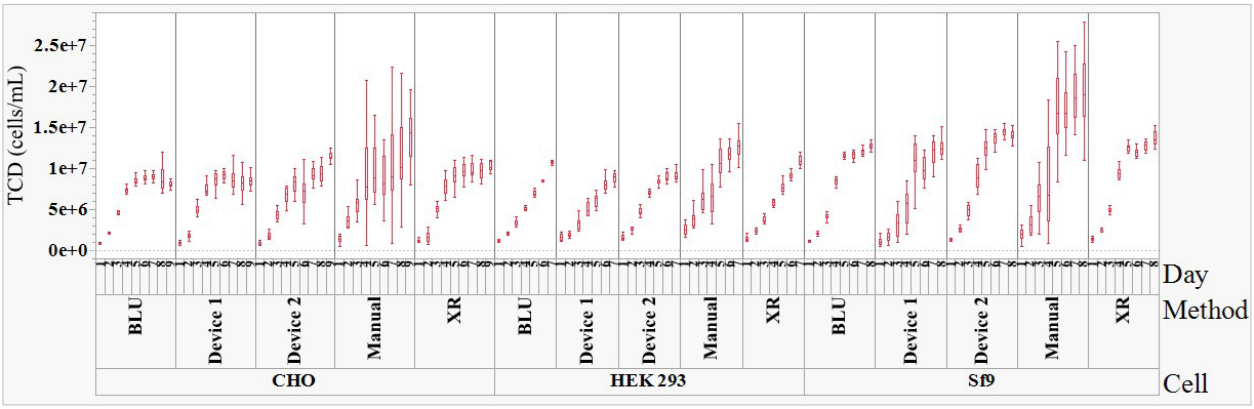

Figure 1. Total Cell Count (TCD).

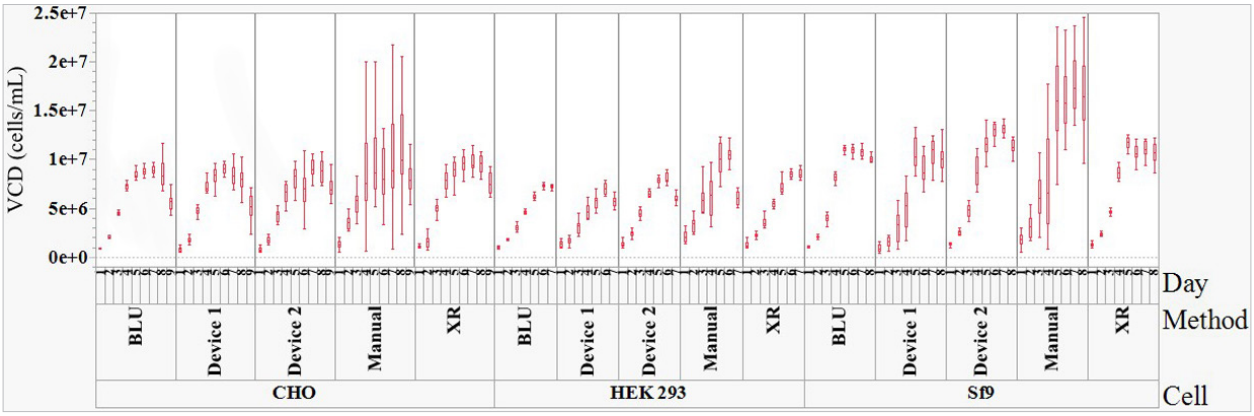

Figure 2. Viable Cell Count (VCD).

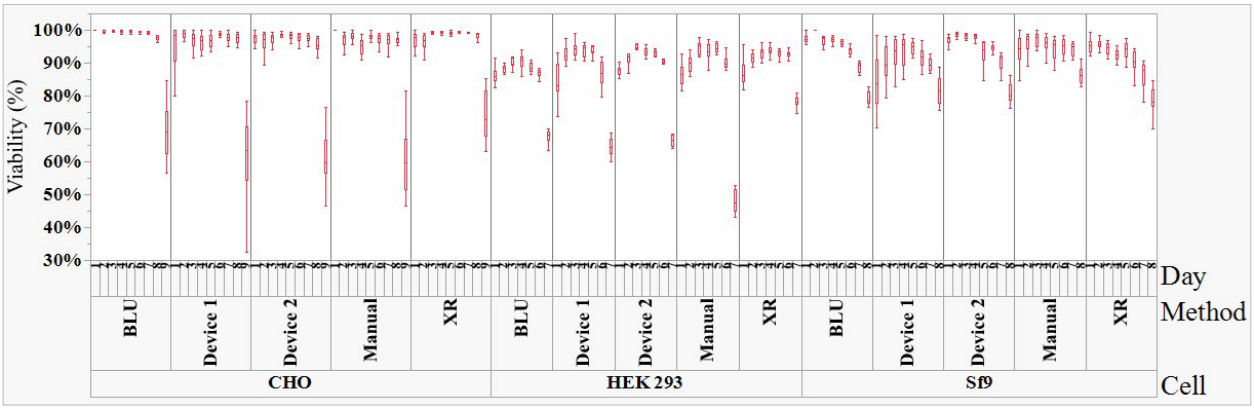

Figure 3. Viability %

Figures 1-3. Variability of results (up to 4 replicates) by days (7-9) and methods (5). Data was normalized to the estimated initial sample density by dividing results by the dilution factor.

Coefficient of variation for replicates expressed as a percentage (%CV): Mean results were averaged across days by cell line

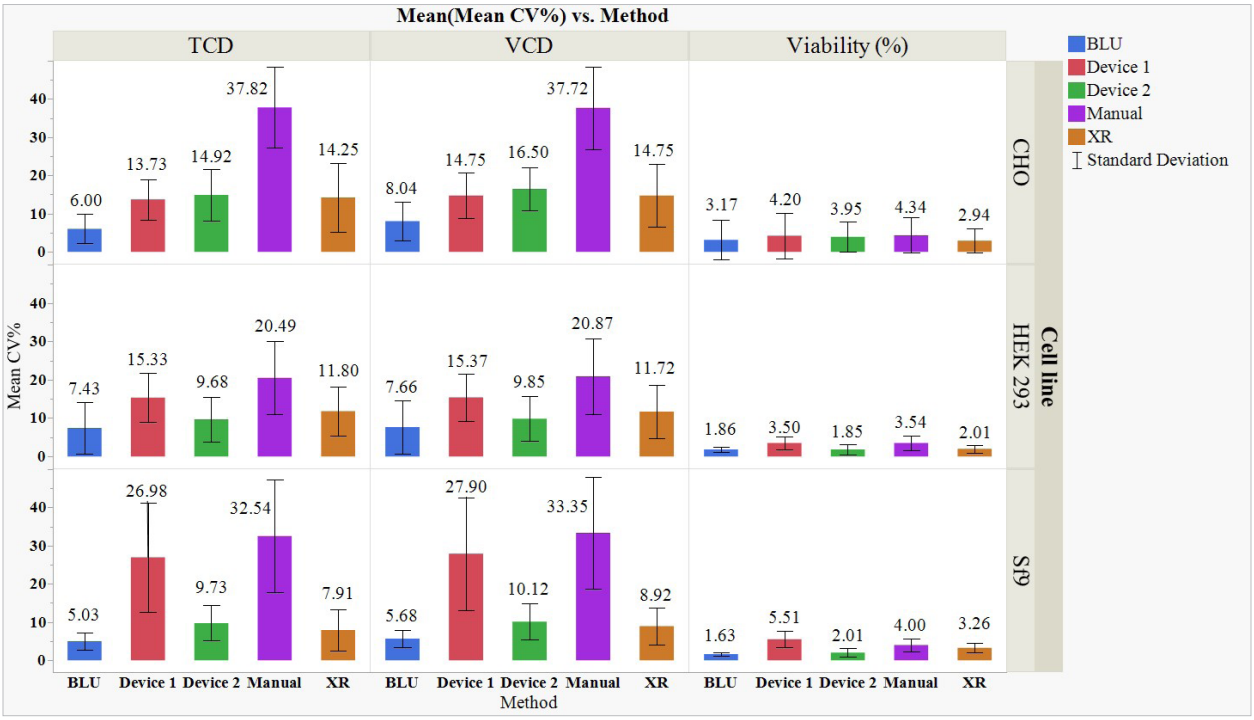

Figure 4. Mean %CV results for TCD, VCD and Viability % by Method and Cell Type. CV (%) is the mean of %CV values averaged across all days for each cell type and method.

Conclusion

Based on the results presented, the Vi-CELL BLU cell viability analyzer emerges as the optimal choice for cell counting when considering variability between measurements. Its low variability among replicates across days and cell lines, coupled with its sample run time savings, makes the Vi-CELL BLU analyzer an invaluable tool for monitoring cell health in bioprocessing applications such as virus and antibody production.

A low replicate %CV can be beneficial to consistently determine target values without the need to run excessive replicates. For high- and medium-throughput applications, the time savings and freedom to run alternative samples in place of excessive replicates can reduce costs associated with inefficiencies. Furthermore, its low instrument-to-instrument variability solidifies its position as the go-to instrument for cell-based process monitoring when instruments are required across multiple sites. With the Vi-CELL BLU cell viability analyzer, researchers can confidently make critical decisions based on consistent results when monitoring cell health.

References

1. Salinas, M et al. Ann Rheum Dis. Oct 1997; 56(10): 622-626. Comparison of manual and automated cell counts in EDTA preserved synovial fluids. Storage has little influence on the results.

2. Beckman Coulter. 2019. Application Note. Evaluation of Instrument to Instrument Performance of the Vi-CELL BLU Cell Viability Analyzer.

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