A comparison of the Countess® Automated Cell Counter to the Vi-CELL® XR Cell Viability Analyzer

Introduction

Many cell-based research studies require cells to be counted before an experiment is begun. This allows normalization of cell concentration between samples, minimizing error and variation in downstream results. Historically, the hemocytometer has been used in combination with light microscopy to quantify cells. Advancements in imaging technology have enabled the automation of cell counting, allowing for higher accuracy, decreased time-to-results, and increased ease of workflow.

This analysis compares the methodology and performance of two benchtop automated cell counting instruments, the Invitrogen™ Countess® Automated Cell Counter and the Beckman Coulter Vi-CELL® XR Cell Viability Analyzer. The Countess® cell counter provides data comparable to the Vi-CELL® XR Cell Viability Analyzer, but with the following benefits:

- Uses smaller sample and reagent volumes
- Provides faster time-to-results
- Involves significantly lower cost
- One-step installation with no external computer required
- Features an intuitive user interface
- Requires less bench space
- Does not require cleaning or maintenance

Countess® Automated Cell Counter

The Countess® Automated Cell Counter is a benchtop instrument that uses the standard trypan blue technique for determination of cell viability. Digital image capture and a sophisticated image analysis program determine the cell concentration, cell size, and percent viability of a cell population. The instrument contains a small microscope, a digital camera, and an internal computer, which together enable the user to view the sample, adjust the image for optical alignment, acquire an image, obtain cell count and viability, and calculate desired dilutions from total or viable cell concentrations in less than a minute.

Sample preparation for the Countess® cell counter is similar to that for a hemocytometer. Approximately 10 µL of a cell sample is mixed with an equal volume of 0.4% trypan blue, and 10 µL of this mixture is then transferred to a non-gridded disposable Countess® Chamber Slide. The slide is inserted into the Countess® instrument, and adjustments are made using the zoom function and fine-focusing knob to bring the image into focus. Specific image analysis parameters may be adjusted for unique cell types, but in general, this is not necessary to obtain an accurate count. A single press of a button on the touch screen begins the automatic image acquisition and analysis. After approximately 30 sec the results appear on the display, showing the image and readouts of total cells/mL, live cells/mL, dead cells/mL, percent viability, and average cell size. Cell counts can be effectively determined using concentrations between 1×10^4 and 1×10^7 cells/mL, with an optimum counting range of 1×10^5 to 4×10^6 cells/mL. Twelve samples can be counted in less than 10 min. A calculation screen can then be brought up to determine volumes of sample to media required for specific cell dilutions. Data may also be graphically analyzed and gated by cell diameter. The slide containing the counted sample is then removed and disposed of as hazardous waste. Because of the simple design of the instrument and the use of a disposable cell counting chamber, the instrument does not require any cleaning or maintenance.

Vi-CELL® XR Cell Viability Analyzer

The Vi-CELL® XR Cell Viability Analyzer is a benchtop video imaging system that also uses the trypan blue technique for determination of cell viability. An external PC is required to queue samples, run the complex fluidics system, and analyze the results. Final output includes cell concentration, cell size, and percent viability of a cell population. A reagent pack and hazardous waste collection bottle must be connected to the instrument with rubber tubing.

The Vi-CELL® instrument can automatically count up to 12 samples in a queue. Sample preparation by mixing with trypan blue is automated. A large volume (0.5 mL) of cell suspension is required, and it takes 2.5 min to count each sample. For counting fragile cells, settings must be adjusted to avoid applying excessive shear. Cells can be effectively counted over the range of 5×10^4 to 1×10^7 cells/mL. Data must be exported to an accessory computer for image storage and analysis. Because the Vi-CELL® instrument uses a fluidics system, each run generates 12 mL of hazardous waste.

Instrument comparison

For this analysis, both automated cell counting instruments were directly compared to the hemocytometer for technique, precision, and accuracy. The hemocytometer is considered to be the industry standard. In this experiment, a disposable hemocytometer was used to regulate sample volume. A standard bead solution (Beckman Coulter, Aperture Instrument Concentration Control, part number 177495) and cultured cells from the same sample were enumerated by all three methods. Estimated bead concentrations measured by both instruments were close to those measured with the hemocytometer; however, the Countess® cell counter showed the least variability of the three, and the Vi-CELL® analyzer the greatest variability (Figure 1). Cell concentrations obtained with the Countess® cell counter were comparable to those measured with the hemocytometer, while the Vi-CELL® instrument consistently gave higher concentrations than expected (Figure 2).

Summary

The Countess® Automated Cell Counter and the Vi-CELL® XR Cell Viability Analyzer are both trypan blue–based counting instruments. In this study, it was found that the Vi-CELL® instrument gave higher measurements of bead and cell concentrations than the Countess® cell counter and hemocytometer; in some cases the concentrations were >30% higher than those measured by the Countess® counter or hemocytometer (Figure 2).

While the Vi-CELL® analyzer is fully automated, our experiments have shown that it consistently produces higher counts than expected. The Vi-CELL® instrument is significantly more expensive, requires a dedicated computer, and occupies much more bench space than the Countess® Automated Cell Counter. In addition, the Vi-CELL® instrument requires extensive installation time and regular cleaning and maintenance. The Vi-CELL® instrument requires 0.5 mL sample volume, while the Countess® cell counter needs only 10 µL of potentially precious cells. Setup of reagent and waste collection bottles is a regular task when using the Vi-CELL® analyzer, whereas the Countess® instrument only requires simple mixing of trypan blue with the sample and generates no waste. Although trypan blue mixing is automated in the Vi-CELL® instrument, the time-to-results per sample is more than twice that of the Countess® cell counter. A summary of the features of both instruments is presented in Table 1. These comparisons demonstrate that the Countess® Automated Cell Counter is a reliable, more cost-effective, and less cumbersome alternative to the Vi-CELL® XR Cell Viability Analyzer.

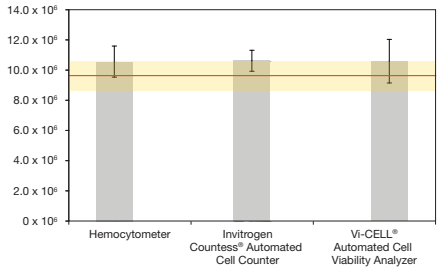

Figure 1. A comparison of the Countess® Automated Cell Counter and the Vi-CELL® XR Cell Viability Analyzer to the hemocytometer. A standard bead solution (Beckman Coulter, Aperture Instrument Concentration Control, part no. 177495) was counted with three instruments. The nominal concentration was 9.6 x 10^5 beads/mL ± 10% (indicated by the red line, with the yellow band representing percent variability). Each of the instruments determined the concentration of the beads to within the nominal range. However, the Vi-CELL® analyzer showed the greatest standard deviation, twice that of the Countess® cell counter.

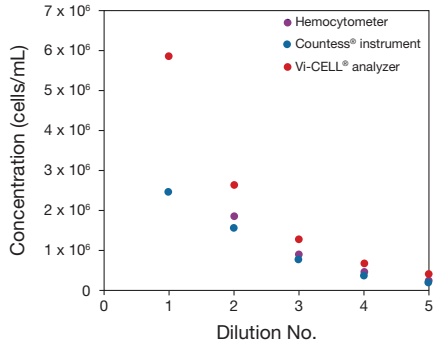

Figure 2. The Countess® Automated Cell Counter compares more favorably with manual cell counting. The cell concentrations of a dilution series of mouse embryonic stem cells were measured using a hemocytometer, the Countess® cell counter, and the Vi-CELL® XR Cell Viability Analyzer. There was a closer correlation between data from the Countess® cell counter and the hemocytometer than those from the Vi-CELL® instrument, which reported higher cell concentrations at all dilutions.

Table 1. Feature comparison of Countess® Automated Cell Counter to Vi-CELL® XR Cell Viability Analyzer.

Feature	Countess® cell counter	Vi-CELL® analyzer
Sample required	10 µL	500 µL
Count time	30–40 sec	2.5–5 min
Samples in automated queue	1	12
Method	Trypan blue and image analysis	Trypan blue and image analysis
Fluidics	Disposable chamber and capillary action	Small-bore tubing, pump, mixer, and built-in permanent flow cell
Size	Approximately 144 cm^2	1558 cm^2 plus computer
Setup	Plug-in	Installation by a field engineer
Maintenance	None	Regular cleaning and maintenance

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