

Counting clumpy primary cells: Comparison of the Countess® Automated Cell Counter with other methods of counting primary cells

Primary cells are cells isolated from living tissue that have undergone very few population doublings. Due to their propensity to clump, primary cells can sometimes be difficult to count. To evaluate the capability of the Countess® Automated Cell Counter of providing accurate counts of primary cells, we compared its performance with four other methods of cell counting. Because endothelial cells tend to clump during early passages, we limited our study to human umbilical vein endothelial cells (HUVECs) collected at the end of their first passaging.

Methods for counting primary cells

Early-passage human umbilical vein endothelial cells (HUVECs) (Figure 1) were counted using the following methods: a disposable hemocytometer, the Countess® Automated Cell Counter, a handheld impedance-based counter (“Counter I”), an autofocusing stand-alone counter (“Counter S”), and a computer-driven autofocusing counter (“Counter C”).

HUVECs were harvested using TrypLE™ Express dissociation reagent, spun down, and resuspended in EpiLife® medium.

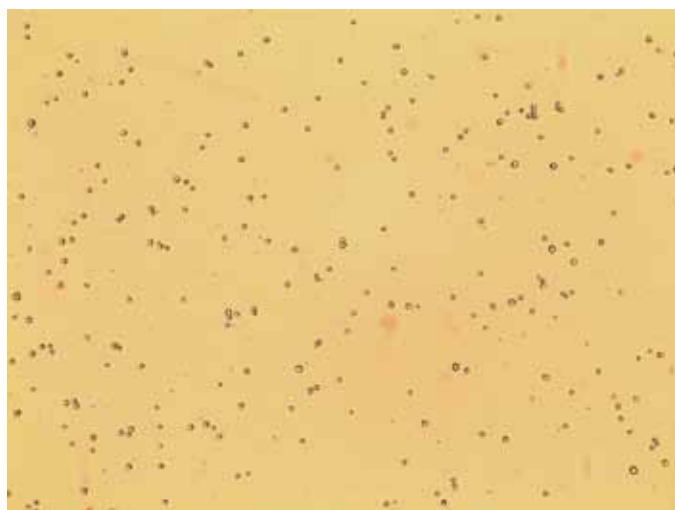


Figure 1. Human umbilical vein endothelial cells (HUVECs) in suspension after harvesting with TrypLE™ Express dissociation reagent.

Two additional serial 2-fold dilutions were made from the initial concentration of $\sim 1.25 \times 10^6$ cells/mL. A common pool of cells treated with trypan blue at a 1:1 ratio was used for cell counting, with the exception of Counter I, which uses impedance-based particle counting instead of trypan blue. Cells counted with this counter were diluted 1:1 with PBS. For each cell counting method, counts were obtained three times on three different samples.

The Countess® Automated Cell Counter, Counter S, and Counter C all use image-based counting techniques. For counts acquired using the Countess® Automated Cell Counter, the focus was manually checked and adjusted as necessary between samples. Due to drift, proper focus was difficult to obtain with Counter C. Once it was obtained, the focus knob was locked for the duration of the experiment; this approach is standard operating procedure for this instrument. Counter S employs an autofocus technology, and therefore no adjustments were made.

Comparison of cell counts between instruments

Similar cell counts were obtained with all methods for the initial concentration, with the exception of Counter I (Figure 2A). Because the initial concentration fell outside the accurate range for Counter I, its measurement was not comparable to those acquired by the four other methods.

For the second dilution (Figure 2B), the hemocytometer, the Countess® Automated Cell Counter, and Counter S gave comparable cell counts. The lower count obtained with Counter I may reflect the inability of this instrument to count anything other than single-cell suspensions. Counter I employs a 35- μ m filter to remove cell clumps, which may have resulted in slightly lower counts than those reported using the hemocytometer—the gold standard for cell counting. The Counter C measurement was similar to that of the hemocytometer, the Countess® Automated Cell Counter, and Counter S, but its error was relatively large.

This large error likely reflects focal drift, as well as debris that was noted in the optical path. The Countess® instrument gave cell counts closest to those acquired using the hemocytometer method, with a very small standard deviation. However, differences between cell counts were not statistically significant.

In the most dilute sample (Figure 2C), cell counts measured by all instruments were less accurate than and had higher variability compared to the hemocytometer. However, the results were comparable to each other, with the exception of Counter C.

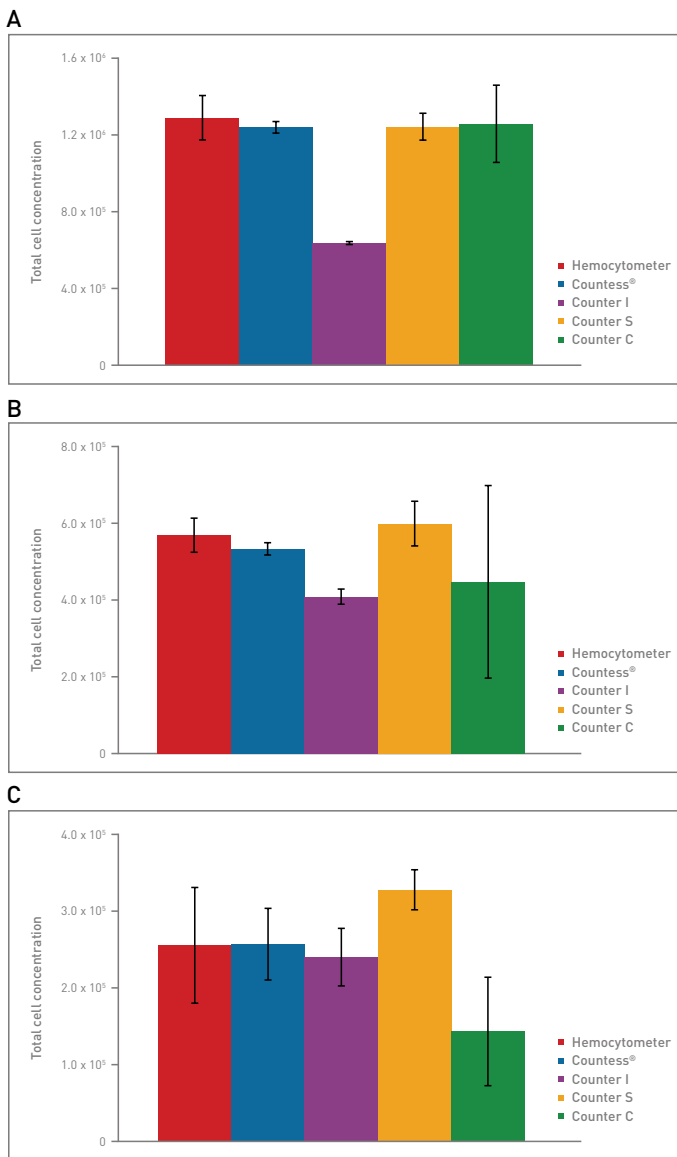


Figure 2. Comparison of human umbilical vein endothelial cell (HUVEC) concentration measurements using various methods of cell counting. Cells were counted with the hemocytometer, Countess® Automated Cell Counter, Counter I, Counter S, and Counter C. The averages of three measurements are shown; error bars represent standard deviations. **(A)** Initial concentration; **(B, C)** serial 2-fold dilutions.

Comparison of cell viability measurements between instruments

Viability was measured from the same samples that were used for cell counting, using the hemocytometer, Countess® Automated Cell Counter, Counter S, and Counter C. All four methods gave comparable results for the initial measurement, but as noted previously, Counter C has the highest error and lowest accuracy with less concentrated samples. The instrument is unable to provide an accurate viability reading at ~3 × 10⁵ cells/mL (Figure 3C).

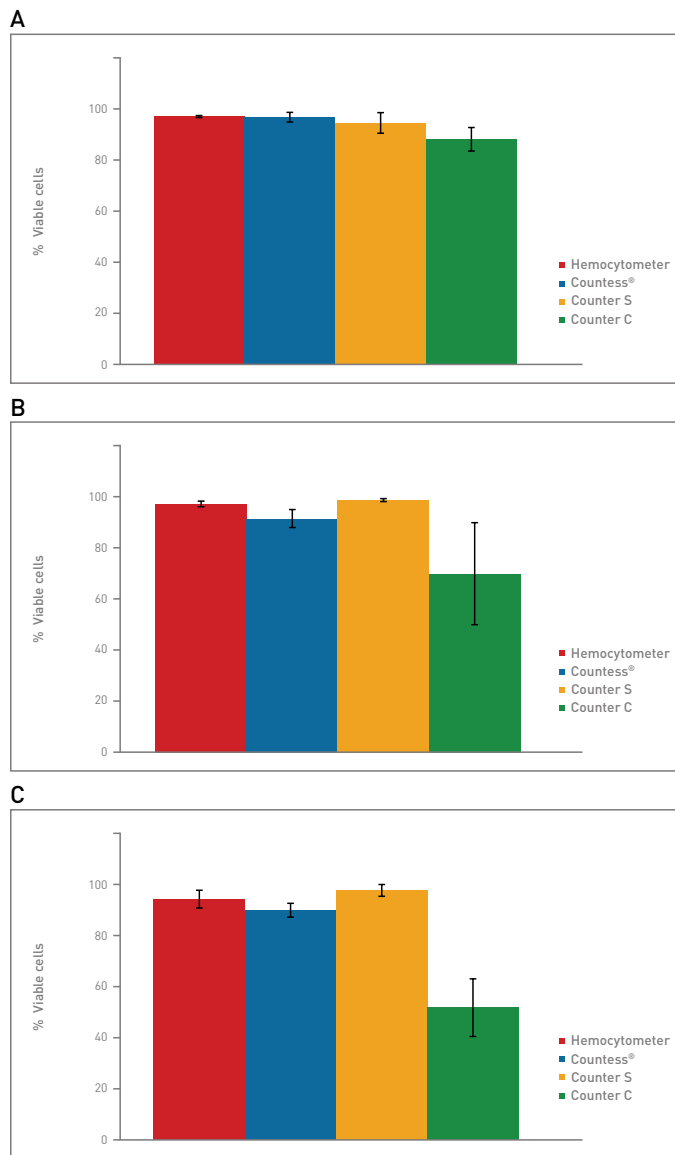


Figure 3. Comparison of human umbilical vein endothelial cell (HUVEC) viability measurements using various methods. Viability was determined using the hemocytometer, Countess® Automated Cell Counter, Counter S, and Counter C. The percentage of viable cells was determined using a trypan blue assay. The averages of three measurements are shown; error bars represent standard deviations. **(A)** Initial concentration; **(B, C)** serial 2-fold dilutions.

Summary

The Countess® Automated Cell Counter, Counter S, and Counter C all use trypan blue–based assays that provide cell counts and viability information. In this study, the Countess® Automated Cell Counter and Counter S provided comparable cell concentration measurements of clumpy, early-passage primary cells. Concentration measurements taken using Counter I were different at higher concentrations, but comparable to those of the Countess® instrument and Counter S at lower concentrations, where the measurements are much more variable. Counter C provided the poorest results, due to difficulty in maintaining proper focus of the image of the cells. All four counter methods gave viability results that agreed with that obtained by the hemocytometer for the highest concentration of HUVECs. Viabilities measured with the Countess® instrument and Counter S were comparable to the hemocytometer measurements at all cell concentrations. At lower cell concentrations, Counter C did not provide accurate viability data.

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