

# Accuracy and Precision Comparison of the Hemocytometer and Automated Cell Counting Methods

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## Abstract

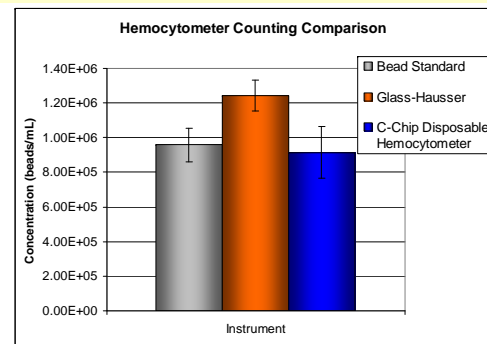
The hemocytometer is the most widely used device for determining cell concentrations, requiring consistent criteria and tenacity to obtain measurements correctly and reproducibly. As an alternative, the Countess™ Automated Cell Counter employs digital imaging and an image analysis algorithm to identify and enumerate cells in a sample. To compare the performance of these two methods, the accuracy, precision, and effective ranges were determined for total cell count and percent viability. First, using a standardized bead solution, the accuracy and precision of glass and disposable hemocytometers were measured and found to be 30% and 5% different from the anticipated value, respectively. Subsequently, disposable hemocytometers and the automated counter were used to determine total cell numbers and percent viability for multiple cell types over a range of cell concentrations. Using a Student's t-test, the results showed the Countess™ instrument measured cell concentrations and viability as accurately and precisely as the disposable hemocytometer. Additionally, the effective concentration range for the Countess™ instrument was two times greater than the hemocytometer, and the viability range matched the hemocytometer. The Countess™ Automated Cell Counter overall produces results much more rapidly without the problem of operator tedium and fatigue or compromised accuracy and precision.



The Countess™ Automated Cell Counter uses the trypan blue dye exclusion method to calculate cell concentration and provide viability information.

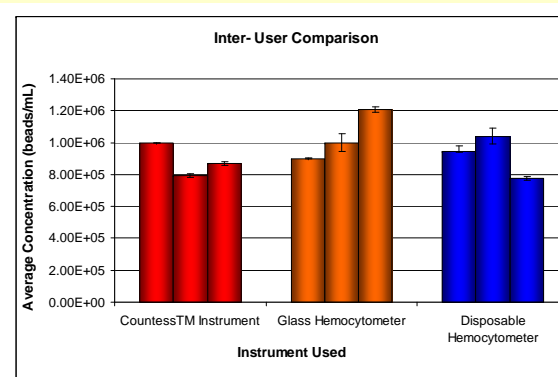
[www.invitrogen.com/countess](http://www.invitrogen.com/countess)

**Figure 1 – Counting variation between traditional glass and disposable plastic hemocytometers**



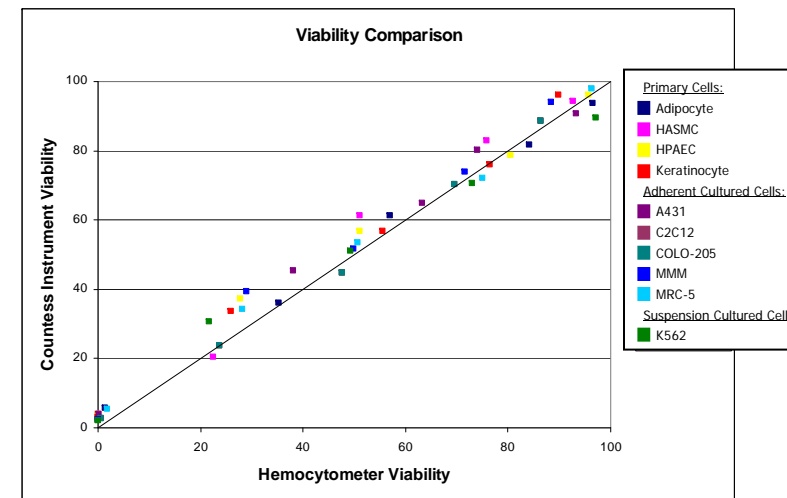
A C-Chip disposable hemocytometer (Manufacturer Digital Bio Technology) contains two separate, fixed-volume chambers. This is much more consistent than the glass hemocytometer which relies upon the surface tension of the sample to hold the loose cover slip at the correct depth. Three preparations and counts were performed for each type using a standard latex bead sample (Coulter bead standard,  $9.57 \times 10^5 \pm 10\%$ ) following the manufacturer's suggested protocol. The results show a difference of 30% and 5% from the expected value for the glass and disposable hemocytometers, respectively. Error bars show one standard deviation above and below the mean concentration for each hemocytometer type. These data, combined with ease of use comparisons, resulted in the use of only the disposable hemocytometer in subsequent hemocytometer comparison experiments.

**Figure 2 – Comparison of inter- and intra-user error for both manual and automated cell counting methods**



To test the inter-user variation for manual and automated cell counting methods for multiple users, three separate Countess™ slide chambers were loaded with a standard bead sample by three different, trained users. Each chamber was counted by each of the users. The same work flow was followed for the hemocytometer counts. The error bars show differences in user count results, while the column heights show differences in sample preparation for each instrument. As shown in the chart above, the variation in total bead counts between users is as much as 5.5% when using the hemocytometer, and less than 1% when using the Countess™ Automated Cell Counter. Sample preparation difference was approximately 12% for the Countess™ instrument, and approximately 15% for each of the hemocytometers.

**Figure 3 – Automated cell counting viability determination across multiple cell types**



This experiment was used to judge the accuracy of the Countess™ instrument viability readings. Ten cell lines were counted on the Countess™ Automated Cell Counter – 4 primary lines (keratinocyte, HPAEC, HASMC, and adipocyte), 5 adherent cell lines (C2C12, A431, MRC-5, MMM, and COLO-205), and 1 cultured suspension line (K562). For each cell line five samples were prepared using different ratios of live cells and heat killed cells, to represent theoretical viability points ranging from 0% to 100% live cells. One chamber slide was prepared for each sample and counted three times each using the Countess™ instrument. Counts of ten squares using disposable hemocytometers were performed for each sample.

## Results

The experiments shown were used to compare the performance of manual cell counting using a hemocytometer to the Countess™ Automated Cell Counter.

- Accuracy of the instruments was determined by performing several counts of the same sample. The Countess™ instrument had a smaller standard deviation for both count and viability data.

- The Countess™ instrument has an accurate range to  $4.0 \times 10^6$  cells/mL and can count to  $1.0 \times 10^7$  cells/mL, while manual counting methods are limited to approximately  $1.0 \times 10^6$  cells/mL.

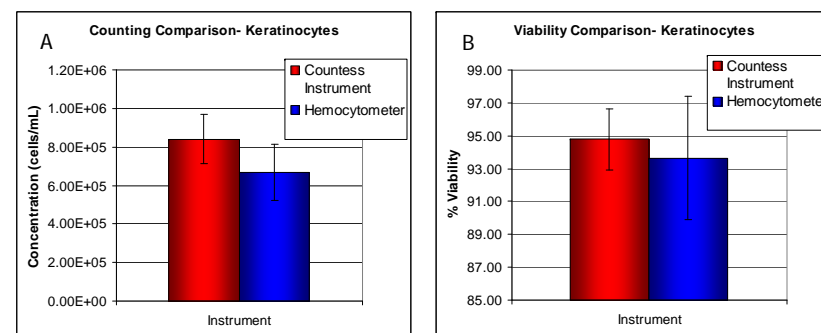
- Viability is easily measured by the Countess™ instrument by using trypan blue dye exclusion. Viability measurements for both manual and automated cell counting agree.

- Inter-user variation of total cell count was shown to be less than 1% using the Countess™ instrument and as much as 5.5% for trained users counting manually.

## Conclusions

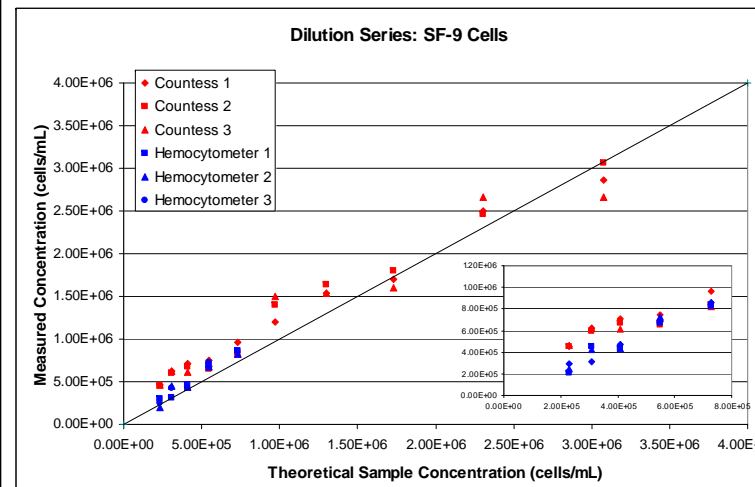
Due to the critical nature of accurate cell counts in biochemical and cellular assays, it is important that the cell counts be easy to perform, be accurate, and give reproducible results. We have shown that when compared to manual counting using a disposable hemocytometer, the Countess™ instrument gives more precise and as accurate results. The concentration range of the Countess™ instrument extends much higher than what a hemocytometer user could count by eye. This removes the need for dilutions prior to counting, thereby improving work flow. Cell viability readings were shown to be equivalent to those determined visually and quantified using a hemocytometer. Inter-user variation is also markedly decreased by using the Countess™ instrument. These experiments were performed using over 25 different cell types, including primary cell lines, with similar results. The Countess™ automated cell counter streamlines the enumeration of cells to obtain quicker and more reliable results which reduces user fatigue and tedium. As is evident in these experiments, automated cell counting improves results by providing consistent and accurate data which is important for reproducibility and statistical confidence.

**Figure 4- Accuracy and Precision- Automated cell counting compared to a traditional hemocytometer**



Three separate Countess™ counting chambers were loaded with primary keratinocyte cell samples and counted three times each for a total of nine measurements. These results were compared to three counts of ten squares each in a disposable hemocytometer. Using the standard trypan blue exclusion method and following the manufacturer's protocol, count and viability were determined for both instruments. Student's t-test was used to show that the mean of the two groups are equal for a 95% confidence interval. Error bars show one standard deviation above and below the calculated mean total cell concentration based on the results obtained from each instrument.

**Figure 5-Effective concentration range-Automated cell counting compared to a traditional hemocytometer**



A serial dilution series of SF-9 cells was made starting with a fresh highly concentrated cell sample. Three Countess™ slide chambers were counted three times each for all of the samples in the dilution series. A manual count of ten squares was performed using a disposable hemocytometer three times for each sample below  $1 \times 10^6$  cells/mL. Due to the density of the cells, it was difficult to manually count the samples using the hemocytometer for cell concentrations above  $1 \times 10^6$  cells/mL. The Countess™ instrument was capable of accurately counting cells at a much higher concentration than was possible using a hemocytometer, which reduced the need for dilution of the sample before counting and improved the workflow.