5 Tips for Better Cell Counting

Counting cells is a tedious job that needs to be done properly, be it in industry or academia, be it manually or automated, you want trustworthy results to continue your research. To help you with this, we have tips to get more out of your cell counting. These tips might surprise you, even if you have years of experience in manual counting. After reading this, you should be able to count your cells quicker and obtain better results from your experiments. And maybe you will enjoy counting cells even a bit more with the Corning® Cell Counter (Cat. No. 6749).

Tip #1: Sample Preparation: Improving the First Step that Counts

However you decide to determine your cell concentration, be it manually or automated, you need a proper sample for an accurate value. An incorrect sample is one of the main causes for invalid counts. What can be done to prevent incorrect samples from being analyzed? By following these 3 steps, you can gain more accurate results, as well as less variation between operators.

Standardize Your Protocols

Each operator has their own way to handle cell counting, starting with trypsinizing cells to the way pipets are handled. By standardizing protocols across your whole facility and making sure these are properly followed (via routine assessments), you should increase the accuracy of the counts from sample to sample.

It may take some time and effort to get everybody in line to follow the protocol, but in the long run, this will result in more accurate counts, leading to better results from experiments and saving you money.

Maintain a Homogenous Suspension

When analyzing your sample, it is assumed that the cell distribution within the sample is representative for the whole suspension. Yet, it is well known that when a cell suspension is allowed to rest for a short period of time, cells will gravitate towards to the bottom of the tube. This results in a concentration gradient within the suspension, so any sample taken will be unrepresentative.

By using a vortex before sampling or by using the classic "finger-flick," you regain a homogenous distribution. Some operators prefer to resuspend the cell suspension when taking a sample with the pipet. Whatever procedure is performed, be sure to make it the standard protocol.

Eliminate Causes of Debris Inclusion

With most counting protocols, chances are there will be some level of debris present. This can be a significant cause for misclassification when not properly adjusted for, e.g., when debris is included as a cell (false positive) or a cell is excluded as debris (false negative).

Accuracy of the results will improve by minimizing the amount of debris included in the sample. Otherwise, decreasing misclassification by training for manual counting or improved detection parameters for automated systems will also give more accurate results.

Conclusion

To improve your sample and thus your results, focus on standardizing protocols and follow up on these as best as you can. This will minimize errors caused by sample preparation but also inter-operator variation. Also, by keeping the sample as homogenous as possible and by excluding debris, the accuracy of the count can increase greatly.

Tip #2: A Better Understanding of the Total Cost of Cell Counting

The procedure for manual cell counting is simple: harvest the cells, dilute them, put them on a hemocytometer slide, observe through the microscope, and start clicking. Anybody with basic cell culture experience can do it, and it is an inexpensive way to get accurate counts and thus significant results. But is it the least expensive way?

A certain level of expertise is needed to perform manual cell counting accurately. The ability to distinguish between live cells, dead cells, cell clumps, and debris takes time and training. To improve productivity in the laboratory, automated cell counters were introduced. Improved automated results come with a financial cost. This raises the financial question, is manual counting less expensive than automated counting?

Which Counting Setup is Financially Better?

Whatever your counting setup is, there are always 3 cost factors to account for: initial purchase cost, operating cost (e.g., consumables), and operator cost. Manual counting is by far the least expensive option regarding purchasing. You have your setup consisting of a glass counting chamber and a clicker for around $185. This does not include a common bright-field microscope which is needed but seen as common lab equipment. The operating cost is one cover slip per measurement, which comes down to about $0.025. Simply stated, it is a very inexpensive setup. So why are automated cell counters financially interesting? When taking operator cost into account for both manual and automated setup something interesting occurs.

The Times are Changing

A seasoned operator will spend at least four minutes to analyze the cells compared to an automated setup that will only take several seconds. This reduces the operator cost dramatically, especially as the number of counts increases.

Even with a modest purchasing cost of an automated setup starting around $3,700, an automated setup can be a return on investment with increasing amounts of cell counts. This even surpasses the increase in operating costs, as automated setups often require more expensive consumables. These custom disposable cell counting consumables are approximately a 15X increase compared to the cover slip required by manual counting.

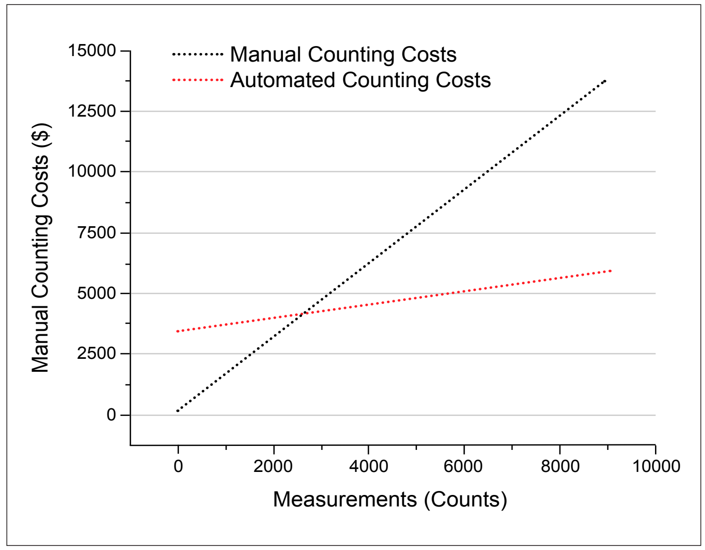

Figure 1. Representation of total cost of manual and automated cell counting. Both included their respective purchase cost, operating cost and operator cost. A break-even point is reached at approximately 2,600 counts.

Now, what can be done with this information? If we calculate the total costs for both manual and automated setups, including initial purchase cost, operating, and operator cost, we see a break-even point where manual cell counting will become more expensive due to its high operating costs (Figure 1).

Conclusion

The initial cost of a cell counting setup is an important factor when comparing cell counters. But only looking at the initial purchase price may cost you more in the long run. We showed that by incorporating all costs of a setup, a better financial comparison between different systems can be made.

It is advised to make your own comparison as purchase cost, operating cost, and operator cost can differ greatly based upon your situation and location. From this, the break-even points can differ greatly when comparing manual and automated systems, or in some cases, may not occur at all.

Tip #3: A Little Something to Assist You

The procedure for manual cell counting is simple: harvest the cells and then count the cells. However, this procedure is prone to errors. Perhaps the first time you misused the pipet and added too much trypsin. Or maybe an incorrect calculation left you with hardly any cells in your flask. Not all knowledge was yet ingrained in your mind.

With time, you got more experienced and more proficient in cell counting. Still, sometimes you needed to improvise. Perhaps the T-175 flasks ran out, and you needed to switch to T-25 flasks to maintain your cells. But how many mL goes into a T-25 again?

To aid you when you need a little assistance with such minor trivia, we have provided you with this cell concentration cheat sheet. This template includes general information about flasks and a useful calculator. To download the template, click the button below.

DOWNLOAD THE TEMPLATE

Tip #4: Accuracy of Cell Counters Explained

One of the most important aspects when researching laboratory equipment for purchase is its accuracy. An important question is, how do you determine the accuracy of the system? And, what is the difference between "precision," "trueness," and "accuracy?" These three terms are used interchangeably, but have very different meanings.

To begin, let us properly look at the definition that should be used. ISO 5725 uses two terms "trueness" and "precision" to describe the accuracy of a measurement method. "Trueness" refers to the closeness of agreement between the arithmetic mean of a large number of test results and the true or accepted reference value. "Precision" refers to the closeness of agreement between test results. The general term accuracy is used in ISO 5725 to refer to both trueness and precision.*

The term accuracy was at one time used to cover only the one component now named trueness, but it became clear that to many persons it should imply the total displacement of a result from a reference value due to random as well as systematic effects.*

The term bias has been in use for statistical matters for a very long time, but because it caused certain philosophical objections among members of some professions (such as medical and legal practitioners), the positive aspect has been emphasized by the invention of the term trueness.*

The general term for variability between repeated measurements is precision. Two conditions of precision, termed repeatability and reproducibility conditions, have been found necessary and, for many practical cases, useful for describing the variability of a measurement method. Precision is normally expressed in terms of standard deviations.*

To summarize, only a device that measures with both a high degree of trueness and a high degree of precision will produce accurate results.

How to Measure the Trueness of a Cell Counter

The trueness of a cell counter is described as how close the calculated average value of the measurements of the device are to the actual concentration. To determine this, you need to analyze a sample with a known concentration using the device.

You also must be aware that cell counters can perform differently at the extremes of their operating concentration range. Knowing this, a simple test for trueness assessment is performed by diluting a sample with a known concentration several times. This will create a calibration curve.

This curve will reveal the trueness of the cell counter within the whole operating range, and not only at one specific concentration.

If you produce such a calibration curve, a cell counter with a high trueness will show two things:

- A linear relationship between measured concentration and theoretical concentration
- An incline close to one

*Literature source: www.iso.org

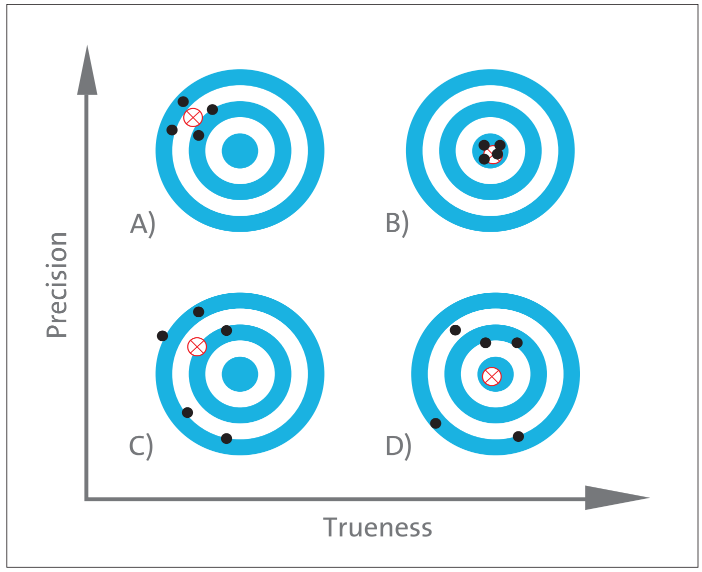

Figure 2. Various kinds of accuracy presented with increasing trueness and precision. (A) With only high precision, a system will underscore or overscore the actual sample. (B) Only with high trueness and precision is a system accurate. (C) With low trueness and low precision, very low accuracy is achieved. (D) With only high trueness, the average value is correct, but many samples are needed.

How to Measure the Precision of a Cell Counter

Now that you know how to determine the trueness, we will describe the precision of a cell counter. As stated previously, the precision of a cell counter defines how robust the system is. Therefore, if you count the same sample multiple times you should get similar results.

Statistically this is represented by the coefficient of variation (CV value) and can be calculated by using Equation 1. With σ being the standard deviation and $\bar{x}$ being the mean of your dataset.

$$CV(\%) = \frac{\sigma}{\bar{x}} * 100\% \quad \text{(Equation 1)}$$

This calculated CV value should always be compared to its theoretical CV value, which can be calculated using Equation 2. The theoretical CV value is a reference value for the measured CV value to determine the precision.

The measured CV value should be close to the theoretical value but should not be lower than the theoretical CV value. The measured CV value will be higher because an increased standard deviation due to error caused by the system and the operator on top of the theoretical CV value.

$$CV_{Th}(\%) = \frac{\sqrt{n}}{n} * 100\% = \frac{1}{\sqrt{n}} * 100\% \quad \text{(Equation 2)}$$

Summary

We've shown how to determine the accuracy of a cell counter. It showed that accuracy is made up of two components, trueness and precision. It is shown how these two are related to the performance of a cell counting system. With this information, you can evaluate information provided by manufacturers and put a system to the test.

To investigate the trueness of a device, you should perform a calibration curve. A cell counter with a high trueness will show a linear relationship between measured concentrations and the theoretical concentrations.

For precision, you should look for the CV value given by manufacturers of a system and compare it to the theoretical value. The CV value should always be lower than the theoretical value.

Tip #5: Switch from Manual to Automated

It seems there are some who have a clear prejudice regarding an automated cell counter or manual cell counting. To investigate this properly there is one question you must answer: What do you want to count?

The answer to this question will influence what kind of counting is best suited for you. Perhaps you want to measure only one cell type multiple times per day or investigate a wide array of cells and bacteria. When making your decision between manual and automated setups, whatever you want to count, the following 4 aspects need to be considered.

Costs

Manual cell counting has the lowest initial purchase and operating cost due to its reusability. Especially at small number of counts it is the best choice regarding budget (see Tip #2).

Nevertheless, that does not include the cost of the time the operator needs to perform the count and the time required to gain experience in manual counting. Accordingly, cost can greatly increase in total when the amount of counts increases.

For automated cell counting, the initial purchase cost can be a large hurdle to overcome as they are often 1,000X more expensive. Their advantage is that they greatly decrease processing time per sample. An additional cost is the necessity of consumables.

These consumables can surpass the initial purchase cost greatly when the device is used for a large amount of cell counts. With increasing numbers of counts, automated systems may be lower in costs than manual counting due to its reduction of operator costs.

Accuracy

For both manual and automated setups, the results obtained must be accurate. With manual cell counting its strengths are in its versatility, accurate classification, and early problem detection. Because the operator directly observes the cells, any errors can quickly be assessed.

But the operators can also be a disadvantage, as their results are subjective and inter-operator variation can be high. The counting protocol from one person may differ greatly from another, even if they count the same grid from the same hemocytometer.

Automated cell counting has the advantage that it has a lower error rate per sample and does not suffer from the subjectivity present with manual cell counting. Also, being automated, it has a high reproducibility rate compared to manual cell counting.

However, since there is no human element classifying cells, the possibility of misclassification is higher. This can allow for automated systems to be precise but have a low level of trueness, thus underestimating the actual concentration.

Versatility

Manual cell counting is easily adaptable to many situations, making it highly versatile. At the same time, it is limited by its operator. These operator limitations are time needed to perform a count and proper counting through experience.

Automated cell counting allows for a higher throughput of samples; thus, more counts can be performed. This results in higher productivity and less misclassification between samples but limits its versatility available with a specific system.

Reporting

For manual cell counting, reporting has not changed since the first hemocytometer was used in the 19th century. The operator simply writes results in their lab journal.

On the automated side, a lot has changed, allowing for more in-depth and faster reporting of the cell counts. With current setups, there are more detailed analysis reports available, including graphical display of the cells counted. Furthermore, with cloud data storage, the operator can reanalyze data later. This may provide new results otherwise overlooked.

Conclusion

Before deciding between automated or manual cell counting, start with defining what cells are to be counted. Only when it is clear what you want to count and in what quantity, can you properly evaluate setups which are available to you. Focus on details of both manual and automated systems regarding their costs, accuracy, versatility, reporting, and how these are related to your requirements.

Once you can relate this information to your situation, you can determine which system suits your needs, allowing you to make the best decision to either continue counting manually or perhaps switch to automated counting.

Tip

When trying a new setup, always try to arrange a trial so you can see if it fits your needs. For a demonstration of the Corning® Cell Counter, contact your local Corning Account Manager.

For more specific information on claims, visit the Certificates page at **www.corning.com/lifesciences**.

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