Technical Note No. 2030

Effects of sample concentration on cell counting variation

NucleoCounter® NC-202™

This Tech Note explains the correlation between the number of cells counted and the precision of the cell count. The purpose is to explain the reproducibility of differing cell counting methods based on the number of cells counted.

Coefficient of Variation (CV) and Standard Deviation (SD)

Determining the precision of a cell culture's concentration depends on the number of cells counted (n). The counting of random events is normally assumed to follow a Poisson distribution (i.e. normal distribution), according to which the standard deviation is the square root of the number of events (in this case, cells counted). The relative precision, expressed as Coefficient of Variation (CV), is therefore:

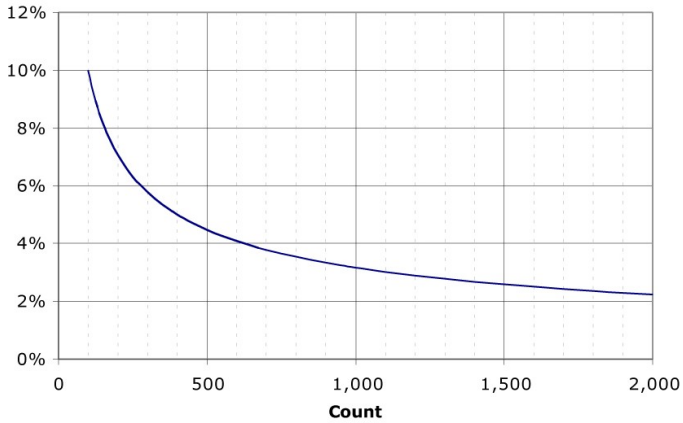
$$\text{CV } (\%) = \frac{\sqrt{n}}{n} * 100 = \frac{1}{\sqrt{n}} * 100$$

To measure the variation of the 'true' count, CV is often preferred, as it can be expressed as a percentage and allows for easy comparison of different sized samples.

The Standard Deviation (SD) is calculated as the square root of the number of cells counted. SD is expressed in the same unit as the objects counted, in this case cells. For example, a sample with 400 cells has an SD of 20 cells (√400 = 20), written as 400 cells ± 20 cells. For the same sample, the expected CV following the Poisson distribution is 5% (1/√400 × 100).

Cell conc. (cells/ml)	Counted cells (*n*)	Poisson SD (cells)	Poisson CV (%)
5×10^4	68	8	12.2%
1×10^5	135	12	8.6%
5×10^5	677	26	3.8%
1×10^6	1353	37	2.7%
5×10^6	6765	82	1.2%
1×10^7	13530	116	0.9%

The Standard Deviation and Coefficient of Variance (CV) expected solely from the Poisson (i.e. normal) distribution of a population of cells counted with the NucleoCounter® NC-202™.



Expected CV% derived from the Poisson distribution against the number of cells counted (n) in the range 100 - 2000 *n*.

As both measures of variation are relative to the square root of the number of cells counted (n), the relationship between the measures of variation from a 'true' count is not linear. In other words, the greater the number of cells counted, the smaller the SD and CV. The following table gives examples of expected SD and CV values at different cell counts and the graph explains the correlation between cell count and expected SD.

Total standard deviation

The total standard deviation (SD) includes the contribution from Poisson along with several other sources of variation:

$$SD_{total} = \sqrt{(SD_{Poisson}^2 + SD_{counting\ device}^2 + SD_{handling}^2 + SD_{...}^2)}$$

Between these different sources of variation, the Poisson will be the greatest contributor. The $SD_{counting\ device}$ is very low due to the use of Via2-Cassette™ with the NucleoCounter® NC-202™. Every Via2-Cassette™ is individually volume-calibrated at our factory and the volume SD is 0.6%.
$SD_{handling}$ measures the deviation from sample handling. This varies from lab to lab depending on the skill of the operator, but poor sample handling leads to large variations in both manual and automatic cell counting.
Other contributors (SD_{other}) also add to the total variation, and it is therefore important to know these contributors when wanting to decrease sources of error and optimize cell counting reliability. One contributor could be the precision of the pipetting volume, which is dependent on how well the pipettes have been calibrated.

Manual cell counting

Manual cell counting using a hemocytometer and trypan blue staining has been the universal standard for determining cell concentration and viability for decades. Using this method gives considerably higher SDs and CVs of cell counts compared to those when using the NucleoCounter® NC-202™[1]. Typically, fewer cells are counted in manual counting[2] and therefore the contribution from the Poisson distribution to the total variation will be greater. As such, the reproducibility of the hemocytometer is highly user-dependent, whereas the Via2-Cassette™ provides an option of sample processing and staining independent of the operator. The hemocytometer requires the operator to be familiar with its correct use in order to avoid often large variations.

[1] See *994-2029 Tech Note NucleoCounter® NC-202™ Performance* for full data (available online at www.chemometec.com/NC-202)
[2] Generally, it is recommended that you count at least 100 cells for hemocytometers, but users tend to count only 100-200 cells. This results in a Poisson contribution to the CV of 7-10% in addition to the other sources of variation. E.g. if 150 cells are counted in 5 big squares of a hemocytometer using trypan blue 1:1, the cell concentration will be (150 cells / 0.5 µl) × 2 = 6×10^5 cells/ml with an SD of 4.9×10^4 cells/ml (CV 8.1%). Analyzing the same sample on the NucleoCounter® NC-202™ will result in counting 812 cells with an SD of 2.1×10^4 cells/ml (CV 3.5%). These calculations only take the contribution from Poisson distribution into consideration.

User-dependent deviation also occurs in manual counting when recording an event, i.e. defining a cell to be counted, making manual counting highly subjective. One operator may include an object that another operator may discard during cell counting. For instance, different operators may have different perceptions of what exactly defines a cell, the specific borders to count within, or whether a cell stains positive for trypan blue and is thereby defined as dead. The NucleoCounter® NC-202™ is completely objective without these sources of variation, therefore providing much more reliable results.