Variation and Statistics

NucleoCounter® NC-200™ - *Easiest Cell Count Ever!*

The precision of the determination of the cell concentration is dependent on the number of cells counted (n). It is normally assumed that the counting of random events follows the Poisson distribution, according to which the expected standard deviation is equivalent to the square root of the number of counted cells. The relative precision, expressed as Coefficient of Variation (CV) is therefore:

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

CV is often the preferred measure of variation from the 'true' count as it can be expressed as a percentage and allow quick comparisons between samples of different sizes.

Standard Deviation (SD) is calculated as the square root of the number of cells counted (√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 would have a SD of 20 cells (√400 =20), written 400 cells± 20 cells. For the same sample, the expected CV would be 5% (1/√400*100).

As both of these measures of variation are relative to the square root of the number of cells counted, the relationship between the measure of variation from 'true' count is not linear. In other words, the greater the number of cells counted, the smaller the SD and CV (Table 1).

Cell Conc. (cells/ml)	No. of cells counted[1]	Expected SD (cells)	Expected CV
5×10^4	70	8	12%
1×10^5	140	12	8.5%
5×10^5	700	26	3.8%
1×10^6	1400	37	2.7 %
5×10^6	7000	84	1.2%
1×10^7	14000	118	0.8 %

[1] 1.4 µl sample volume counted.

Table 1. Expected Standard Deviation and percent Coefficient of Variance expected solely from the Poisson/Normal distribution of a population of cells counted over the NucleoCounter® NC-200™ instrument's optimal range.

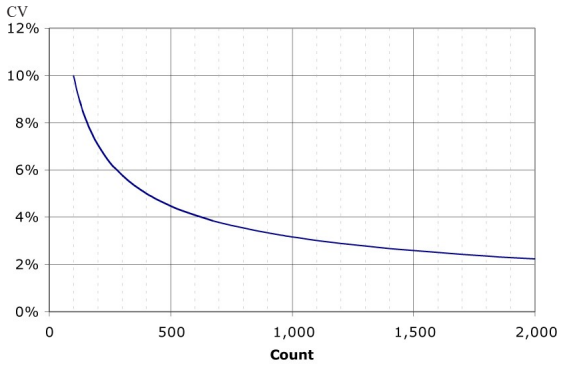

Figure 1. Relative standard deviation of the Poisson distribution ver- sus the number of counted objects in the range 100 to 2,000 counts.

Total standard deviation

The total standard deviation includes the contribution from Poisson together with a number of other sources of variation:

$$SD_{total} = \sqrt{(SD_{Poisson}^{\ 2} + SD_{cassette}^{\ 2} + SD_s^2 + SD....)}$$

Of these different sources of deviation the Poisson will be the greatest contributor. The $SD_{cassette}$ due to the cassette used in the NucleoCounter® NC-200™ is normally very low. All Cassettes are factory calibrated and the $SD_{cassette}$ is around 0.6%. SD_s is the deviation due to sample handling. Again this will vary depending on the skill of the operator but poor sample handling will lead to large deviations in both manual and automatic counts.

Manual counting of cells using a haemocytometer and Trypan blue staining has long been the universal standard for determining cell concentration and viability. When using this method considerably higher standard deviation of cell counts (and therefore also higher CV) are obtained compared with the NucleoCounter® NC-200™. First of all, typically fewer cells are counted. Unlike the Via1-Cassette™, which is user independent, the haemocytometer is highly user dependent in its reproducibility. The haemocytometer requires the user to be familiar with the correct use of the haemocytometer in order to avoid large variations. Deviation can also occur depending on the operator in manual haemocytometer counts since the manual count is highly subjective. One operator may include an object another may discard it. The NucleoCounter® NC-200™ is completely objective and will therefore not have this source for variation.