Variation and Statistics

NucleoCounter® NC-3000™ - Fast and Precise!

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:

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

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).

A) Via-1

Cell Conc. (cells/mL)	No. of cells counted in Via-1	Theoretical SD (cells)	Theoretical CV %
5×10^4	160	13	7,9
1×10^5	320	18	5,6
5×10^5	1600	40	2,5
1×10^6	3200	57	1,8
5×10^6	16000	126	0,8
1×10^7	32000	179	0,6

B) A2

Cell Conc. (cells/mL)	No. of cells counted in A2	Theoretical SD (cells)	Theoretical CV %
5×10^4	560	24	4,2
1×10^5	1120	33	3,0
5×10^5	5600	75	1,3
1×10^6	11200	106	0,9
5×10^6	56000	237	0,4
1×10^7	112000	335	0,3

C) A8

Cell Conc. (cells/mL)	No. of cells counted in A8	Theoretical SD (cells)	Theoretical CV %
5×10^4	120	11	9,1
1×10^5	240	15	6,5
5×10^5	1200	35	2,9
1×10^6	2400	49	2,0
5×10^6	12000	110	0,9
1×10^7	24000	155	0,6

Table 1.
Expected Standard Deviation and percent Coefficient of Variance expected solely from the Pois- son/Normal distribution of a population of cells counted over the NucleoCounter® NC-3000™ instrument's optimal range. a) Cells counted in Via-1 cassettes b) Cells counted in NC-Slides A2™ and c) Cells counted in NC-Slide A8™.

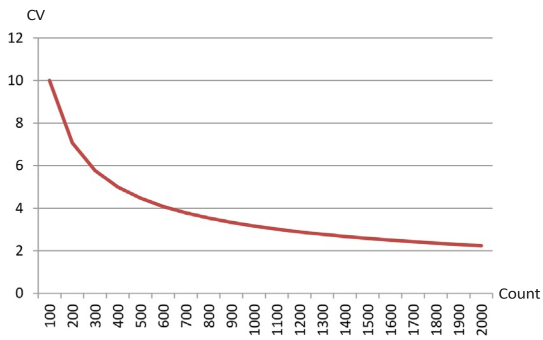

Figure 1.
Relative standard deviation of the Poisson distribution versus the number of counted objects in the range 100 to 2,000 counts.

Total standard deviation

The total standard deviation observed when measuring cell concentrations is the sum of all the individual factors causing variation. These include normal statistical variation as well as variation introduced by the user and the consumables.

$$SD_{total} = \sqrt{(SD_{Poisson} + SD_{sample\ Media} + SD_{s} + SD....)}$$

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-3000™. First of all, typically fewer cells are counted by manual methods. Unlike the NucleoCounter® instruments, which are 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® range of instruments is completely objective and will therefore not have this source for variation.

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