NucleoCounter® NC-100™
A Note on Analytical Performance

Introduction

This document is an analysis of the analytical performance of the NucleoCounter® instrument for the counting of mammalian cells (NC-100™). The results present here are based on tests conducted at ChemoMetec.

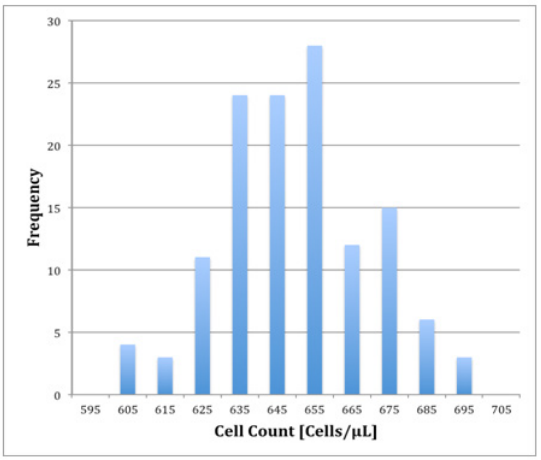
The analytical performance is firstly the precision of the NucleoCounter® method, secondly its reproducibility, and thirdly a comparison to the manual Hemacytometer method based on staining cells with Trypan Blue (TB).

Precision

In order to estimate the precision of the NC-100™ a suspension of CHO cells was lysed using Reagent A100 and B. The cell lysate was then measured repeatedly under stable condition in order to evaluate NC-100™ ability to repeat the measurement. Cassettes from two lots, produced with more than 3 months interval were used. Care was taken to mix the lysate before each NucleoCassette™ was loaded and the measurements were carried out without unnecessary delay between each determination.

A total of 130 measurements were performed and the results are summarised in the following table and histogram. In the table the *Mean* and *StDev* are the observed sample mean and standard deviation in Cells/µl. *CV%* is the Coefficient of Variation determined as the ratio between the standard deviation and mean.

n	130
Mean	639.3
StDev	19.7
CV%	3.1%
$SD_{Poisson}$	17.9
SD_{Method}	19.5



The parameter $SD_{Poisson}$ is calculated from the results and is the expected standard deviation according to the Poisson distribution of random events. The NC-100™ counts cells in appr. 2 µl of cell lysate and thus on average the number of counted cells (random events) is twice the average concentration. According to the Poisson distribution we get the expected standard deviation as follows:

$$SD_{Poisson} = \sqrt{2 * Mean_{Cells/\mu L}}$$

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The parameter SD_{Method} is the expected standard deviation of the method, assuming only significant effect being that of the Poisson distribution and that of the size interval of the sample compartment thickness of the NucleoCassette™, which is 4µm. The sample thickness of each NucleoCassette™ is determined during production and this thickness is coded on the NucleoCassette™ where it is read by the instrument in order to calculate the volume analysed. An interval size of 4µm implies a random thickness error of about 1.2µm or about 1.2% as the average sample thickness is about 100µm. The ideal method precision is therefore as follows:

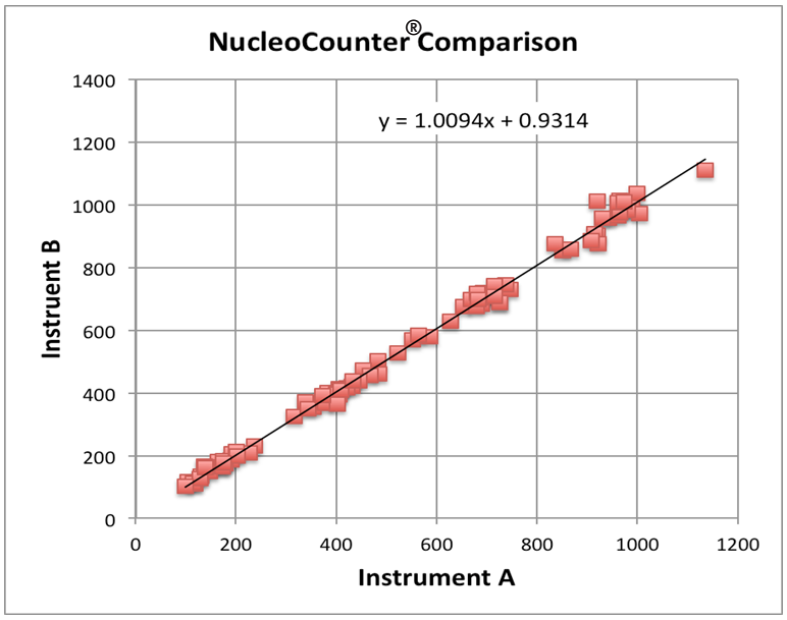
$$SD_{Method} = \sqrt{SD_{Poison}^2 + (0.012 * Mean_{Cells/\mu L})^2}$$

The results of the precision testing show that the observed standard deviation of 19.7 Cells/µl (3.1% relative to the mean) are not significantly different from the theoretical precision of the method, which is 19.5 Cells/µl under the conditions of the test.

Reproducibility

In order to evaluate the reproducibility of NucleoCounter® instruments two instrument from different production runs were used to produce results from the same sample material. The instruments were individually calibrated according to the standard procedure at ChemoMetec and no additional adjustment of instruments was carried out.

A total of 84 different samples were prepared and two Cassettes loaded from each cell lysate. The correlation between the average of the two measurements obtained by each instrument in Cells/µl is shown in the following graph.



The results from the test suggest that the correlation between the two instruments was linear, with observed slope of 1.009 (sd=0.008) and intercept of 0.9 (sd=4.9). The calcluated slope is not significantly different from unity and the calculated intercept is not significantly different from zero.

Further the difference between the duplicate measurements of each sample was not significantly different from the difference between measurements on the two instruments suggesting that the difference between the two instruments is largely dominated by the reproducibility of the method (SD_{Method}).

Manual Cell Counting Method

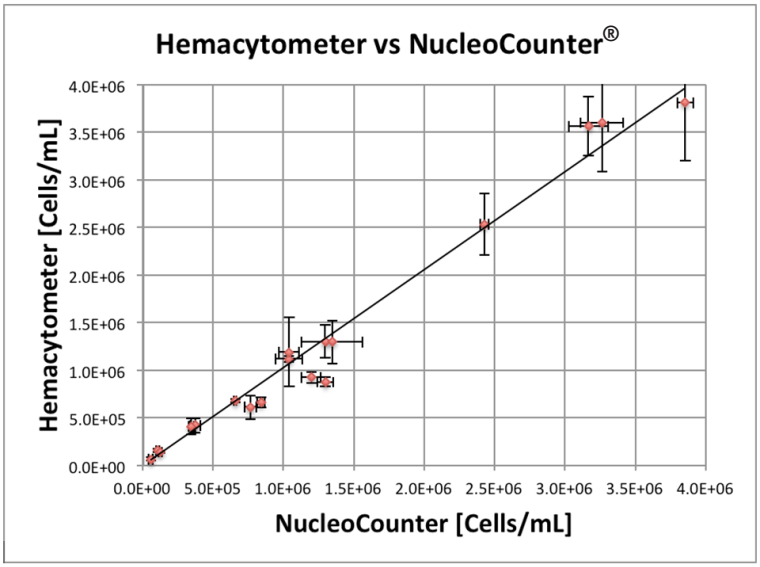
The task of comparing two different methods for cell counting is made difficult in view of the fact that there does not exist a reference method, against which a method can be calibrated. The response of each method is therefore dependent on several factors, many of which are physical properties, such as volume ratio of dilution and determination of sample chamber volume, which can be compensated for if the level is know.

Unfortunately there are also other factors which are difficult to quantify, but nevertheless have significant influence on the determination of cell concentration. One of the most important of these factors is the objective distinction or identification of an object. Factors such as the presence of cell fragments, and variation in the size of cells, can make the identification of a cell an objective task, resulting in potential difference between different methods. Here it is important to emphasise that in this respect, cell counting according to the manual method, but carried out by two individual operators should be regarded as two separate cell count methods, unless careful training and coordination between the operation has been carried out. In any case, frequent validation between results obtained by different operators must be carried out.

The NucleoCounter® is of course also affected by objective parameters in its determination of cell count, which makes the result obtained by a NucleoCounter® potentially different from any other cell counter method. But the nature of the method and the process of production assure that this objectivity is virtually identical among all NucleoCounter® instruments making results of the NucleoCounter® virtually independent on the instrument used, or even which person operates the instrument.

At ChemoMetec a test was conducted where 9 different cell lines were analysed with the manual Trypan Blue methods as well as the NucleoCounter® method. The cell lines were: A3, CHO, H1-L7, H1L7, HEK293, Hela, MCF-7 (high density), MCF-7 (low density), U2OS. A total of 18 samples were analysed and each sample was measured in triplicate on both methods. The total number of cells that were counted with the manual method was about 40 in each Hemacytometer determination.

The findings from the test are shown in the following graph, which shows the average of the results of the Hemacytometer plotted against the average of results of the results of the NucleoCounter®.



The horizontal and vertical lines at each data point in the graph represent the observed standard deviation of the triplicate measurements for the respective method. The position of the points relative to the best-fit line shown in the graph shows that many of the measurements fall within one standard deviation from the line, which is in accordance with the expected appr. 67% of the Normal distribution and it also shows that virtually all observations are within 2 standard deviations of the best-fit line.

The results demonstrate that there is generally good agreement between the manual Hemacytometer method and the NucleoCounter® method. Further it also shows that for the 9 different cell lines tested no significant difference was shown between the results of the two cell counting methods.