Technical Note No. 2029 Rev. 1.0

NucleoCounter® NC-202™ Performance data

The NucleoCounter® NC-202™ is a high precision cell counter that uses Via2-Cassettes™ for sample loading and staining. This document presents data demonstrating the performance of the NucleoCounter® NC-202™ in relation to manual counting.

Introduction

Cell density greatly impact cell behavior and a broad range of cell-based applications such as research experiments, bioassays, and bioprocessing.
Precise and robust cell counting is critical to achieve reproducibility in such applications. The following document summarizes the performance of the NucleoCounter® NC-202™ in comparison with manual cell counting using Bürker-Türk counting chambers and trypan blue.

Background

The NucleoCounter® NC-202™ is a high precision cell counter that uses low magnification fluorescence microscopy and automated image analysis to identify live and dead cells. The Via2-Cassette™ combines cell sampling, staining, and counting chamber into a single workflow. Together the NucleoCounter® NC-202™ and Via2-Cassette™ generate data with low inter- and intra-operator variation.

The NC-View™ software provides operational control and easily validates the cell count, by displaying images and results in an intuitive user interface.
The NucleoCounter® NC-202™ can count all mammalian cell types, including primary cells and aggregated cells.
NC-View™ is designed to maintain data integrity and is compatible with the 21 CFR part 11 guidelines.

This document summarizes a complete dataset where a large panel of cell lines were counted with three NucleoCounter® NC-202™, in parallel with manual counting.

Conclusion

The NucleoCounter® NC-202™ displays superior performance in terms of linearity, precision and instrument-instrument variation.

Experimental setup

A panel of cell types (Appendix I) was counted using three different NucleoCounter® NC-202™ to quantify the performance. For the NucleoCounter® NC-202™ cell counts were performed using the standard ‘Count & Viability’ protocol with Via2-Cassettes™. Manual cell counting was done in parallel, to serve as a counting reference. The manual counts were carried out in duplicates using 0.4% trypan blue and a Bürker-Türk counting chamber. The same operator performed all the manual cell counts to minimize sample variation.

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Cell Concentration Range

To confirm the accuracy of the NucleoCounter® NC-202™ in the entire counting range ($5x10^4$ to $1x10^7$ cells/ml), cell counts were performed on cell samples with a wide range of concentrations. The average cell count value was plotted for the NucleoCounter® NC-202™ and manual counts (Figure 1). These counts showed a good linear correlation with an R^2 of 0.918.

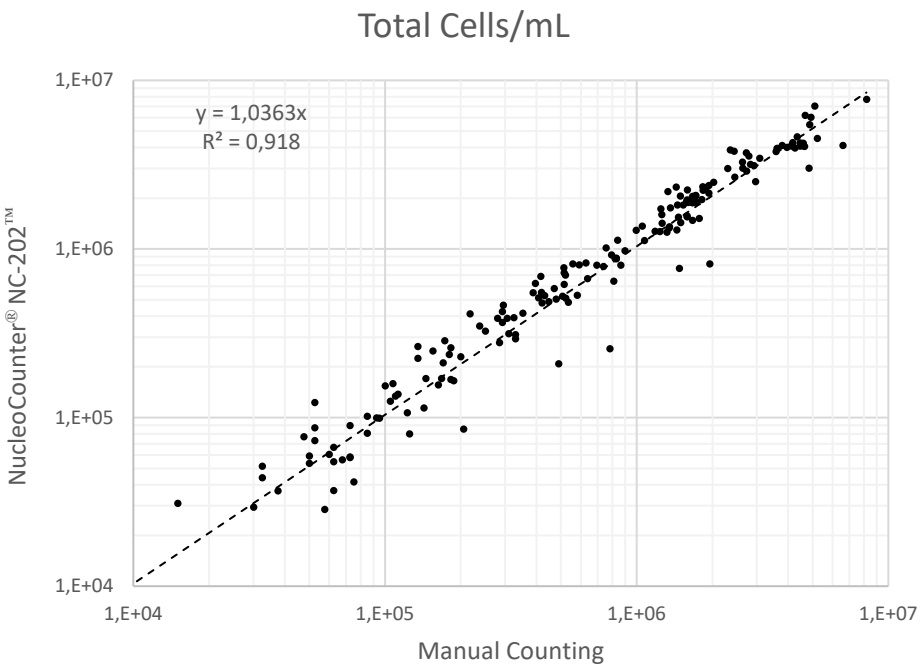

Figure 1. NucleoCounter® NC-202™ total cell count correlates with manual counting. The graph presents data from 15 different cell types with 166 measurements using three NucleoCounter® NC-202™ instruments and manual counting.

Cell Counting Precision.

The precision of a cell count depends on the number of counted cells. Normally the variation of a cell count is assumed to follow the Poisson probability distribution, where measurements of discrete events will deviate with the root of the number of counted events. In addition, variation in sample collection and processing will also contribute to the overall deviation.

To demonstrate the counting precision, the coefficient of variation (CV) was calculated from replicated NucleoCounter® NC-202™ and manual counts and plotted against the cell concentration (Figure 2). The NucleoCounter NC-202™ gave significantly lower variation on average 4.1% (A) compared to manual counting 8.2% (B).

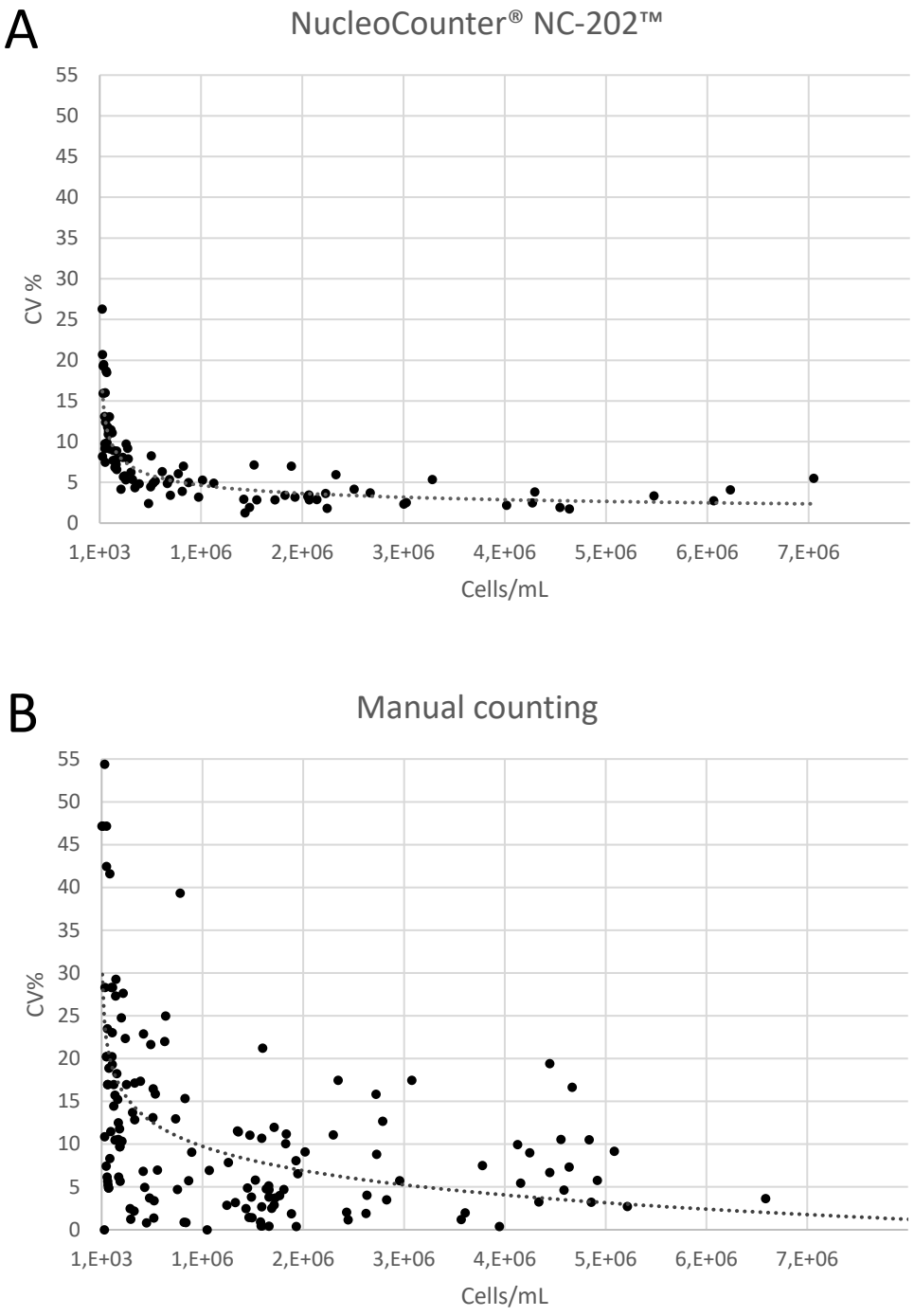

Figure 2. NucleoCounter® NC-202™ cell counting is more precise than manual counting. The graph presents data from 15 different cell types with 166 measurements using three NucleoCounter® NC-202™ instruments (**A**) and manual counting (**B**). CV indicates coefficient of variation.

Viability Range

The NucleoCounter® NC-202™ provides viability measurements from 0 to 100% using the well-recognized dead stain DAPI to quantify the number of non-viable cells. There is clear correlation between viabilities determined by the NucleoCounter® NC-202™ and trypan blue exclusion in manual counting (Figure 3).

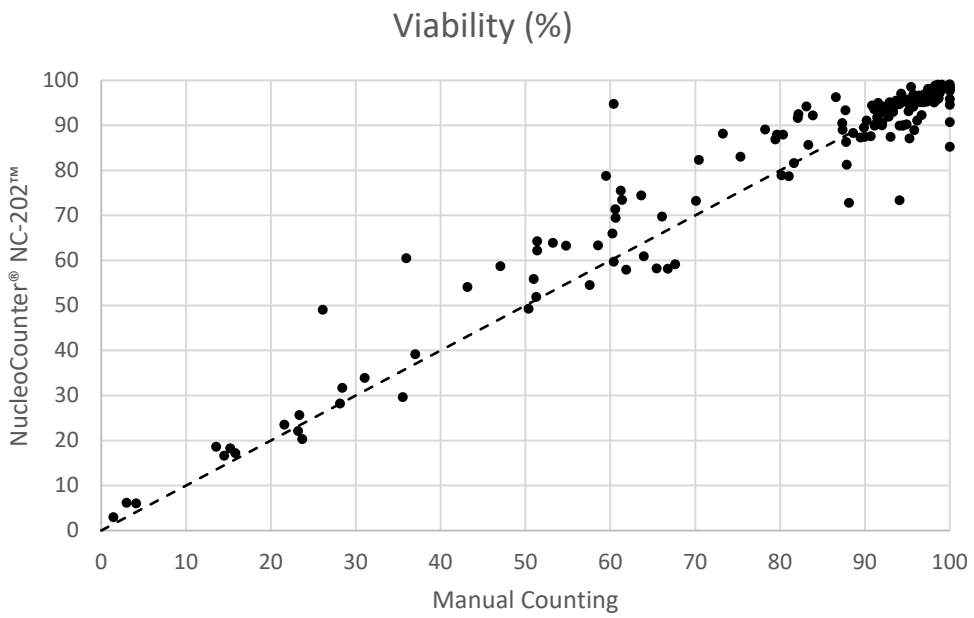

Figure 3. NucleoCounter® NC-202™ viability correlates with manual assessment by Trypan Blue. The graph presents data from 15 different cell types with 166 measurements using three NucleoCounter® NC-202™ instruments and manual counting.

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Instrument-Instrument repeatability

All NucleoCounter® NC-202™ instruments are calibrated to a reference instrument to ensure that all instruments acquire data under the same conditions. The LED light sources are constant over time, which ensure stable image acquisition over time. The optics are mechanically adjusted during production and cannot be changed. Consequently, all NucleoCounter® NC-202™ instruments are inter-comparable, regardless of production year.

When normalized total cells counts are compared between three NucleoCounter® NC-202™ instruments, no significant difference is observed, P = 0.998 (one-way ANOVA n =228, 15 different cell lines).

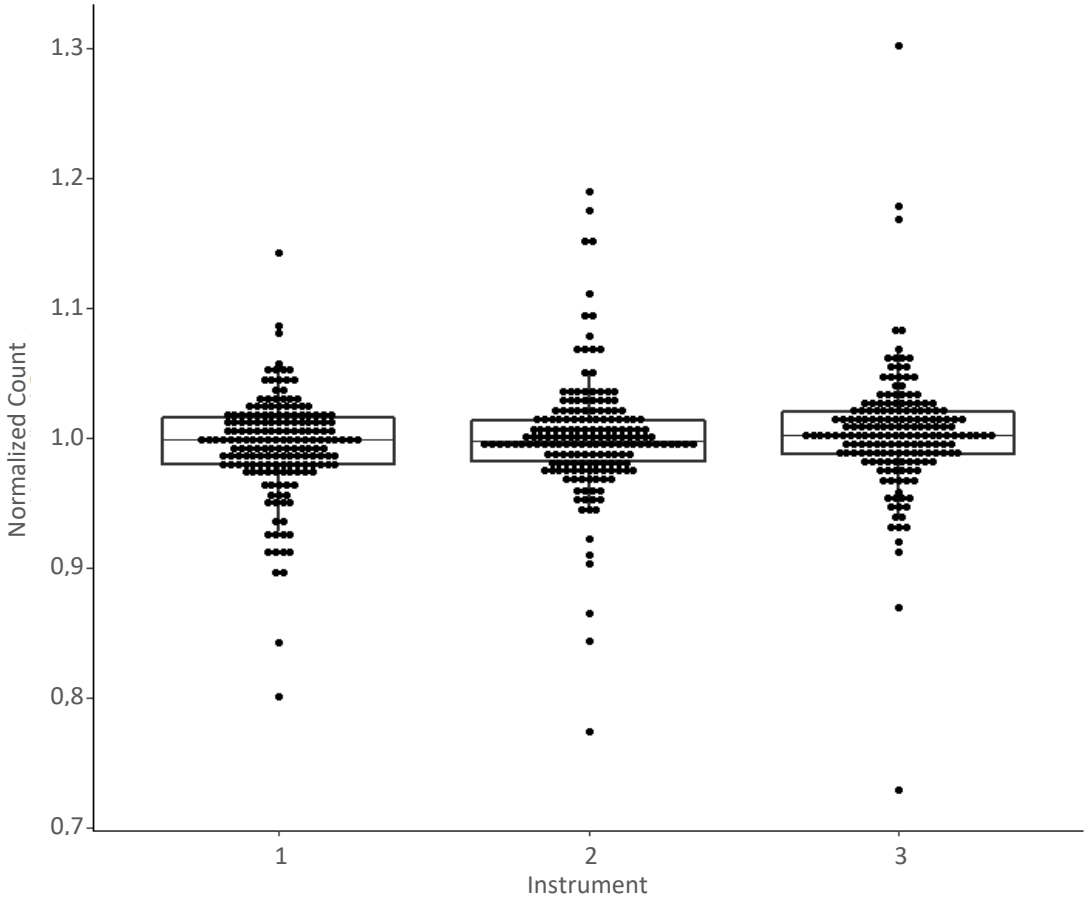

Figure 4. Instrument to instrument variation. The graph presents normalized count data from 15 different cell types with 166 measurements using three NucleoCounter® NC-202™ instruments. One-way ANOVA test show no difference between the three NucleoCounter® NC-202™ instruments, P = 0.998.

Appendix I:

List of cell types used for this technical note.

Cell type	Species	Tissue	Remarks
3G5	Mouse	Blood	B lymphocyte, suspension
BSC-1	African Green Monkey	Kidney	Epithelial, adherent
BHK-21	Hamster	Kidney	Fibroblast, adherent
CHO	Chinese Hamster	Ovary	Epithelial-like, adherent
COS-7	African Green Monkey	Kidney	Fibroblast, adherent
ES-E14*	Mouse	Embryo	Embryonic stem cells, spherical, adherent
FreeStyle™ CHO-S	Chinese Hamster	Ovary	Epithelial-like, suspension
FreeStyle™ 293-F	Human	Embryonic Kidney	Epithelial, suspension
HEK293T	Human	Embryonic Kidney	Epithelial, adherent
HeLa	Human	Cervix	Epithelial, adherent
HMEC*	Human	Breast	Epithelial, adherent
MSC*	Human	Adipose tissue	Stem cells
JM1	Human	Blood	pre-B lymphoblast, suspension
Jurkat A3	Human	Blood	T lymphocyte, suspension
MCF7	Human	Mammary Gland	Epithelial, adherent
MDA-MB-231	Human	Mammary Gland	Epithelial, adherent
MR1	Mouse/Hamster	Blood	B lymphocyte, suspension
NIH/3T3	Mouse	Fibroblasts	Fibroblast, adherent
PBMC*	Human	Blood	Lymphocytes, suspension
PC-3	Human	Prostate	Epithelial, adherent
U-2 OS	Human	Bone	Epithelial, adherent
WEHI-S	Mouse	Fibrosarcoma	Epithelial, adherent
YAC-1	Mouse	Blood	Lymphoblast, suspension

*Primary cells

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