

## Technical Note No. 2029 Rev. 1.2

# NucleoCounter® NC-202™ Performance data

**The NucleoCounter® NC-202™ is a high precision cell counter using the Via2-Cassette™ for sample loading and staining. The data in this document demonstrates the performance of the NucleoCounter® NC-202™ in comparison with manual cell counting.**

### Introduction

Cell density greatly impacts cell behavior in a broad range of cell-based applications such as research experiments, bioassays, and bioprocessing.

Precise and robust cell counting is critical to achieving reproducibility in such applications. The following document summarizes the performance of the NucleoCounter® NC-202™ in comparison with manual cell counting using a generic counting chamber (the Bürker-Türk) and trypan blue.

### Background

The NucleoCounter® NC-202™ is a high precision cell counter using low magnification fluorescence microscopy and automated image analysis to identify live and dead cells.

The Via2-Cassette™ combines cell sampling, staining, and loading of the counting chamber into a single workflow. Together, the NucleoCounter® NC-202™ and

Via2-Cassette™ generate data with low inter- and intra-operator variation. The NucleoCounter® NC-202™ can count all mammalian cell types, including primary cells and aggregated cells.

NC-View™, the accompanying NucleoCounter® NC-202™ software, provides operational control and easy validation of cell counts by displaying images and results in an intuitive user interface. 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™ instruments in parallel with manual counting.

### Conclusion

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

---

## Experimental setup

A panel of cell types (Appendix I) were counted using three different NucleoCounter® NC-202™ instruments. 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. 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 counting variation.

## Cell concentration range

To confirm the accuracy of the NucleoCounter® NC-202™ instrument in the entire counting range ( $5 \times 10^4$  to  $1 \times 10^7$  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 clear linear correlation with an  $R^2$  of 0.956.

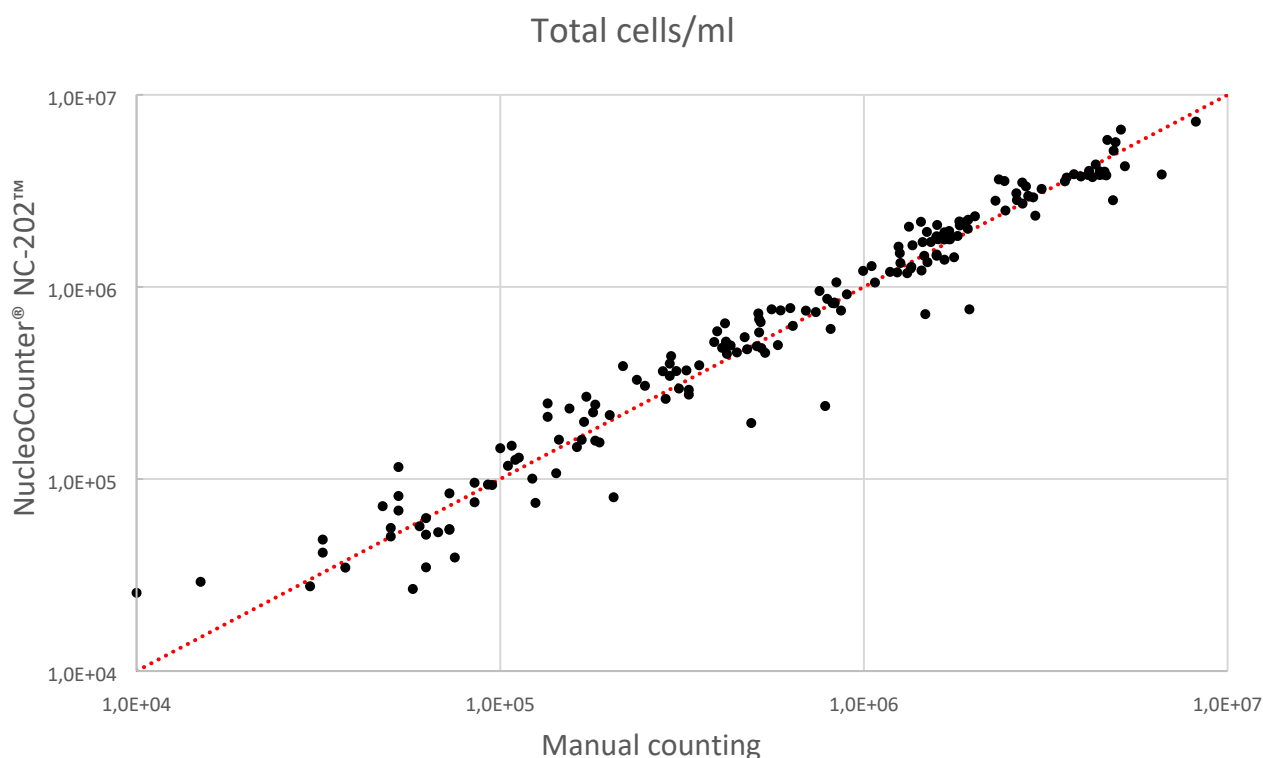


Figure 1. NucleoCounter® NC-202™ total cell count correlates with manual counting. The graph presents data from 15 different cell types with 166 measurements using three NucleoCounter® NC-202™ instruments and manual counting. A line of unity, where  $X = Y$ , is indicated by a dotted line.

## Viability range

The NucleoCounter® NC-202™ provides viability measurements from 0 – 100% using the widely used stain DAPI to quantify the number of non-viable cells. There is a clear linear correlation between viabilities determined by the NucleoCounter® NC-202™ and by trypan blue exclusion in manual counting (Figure 2).

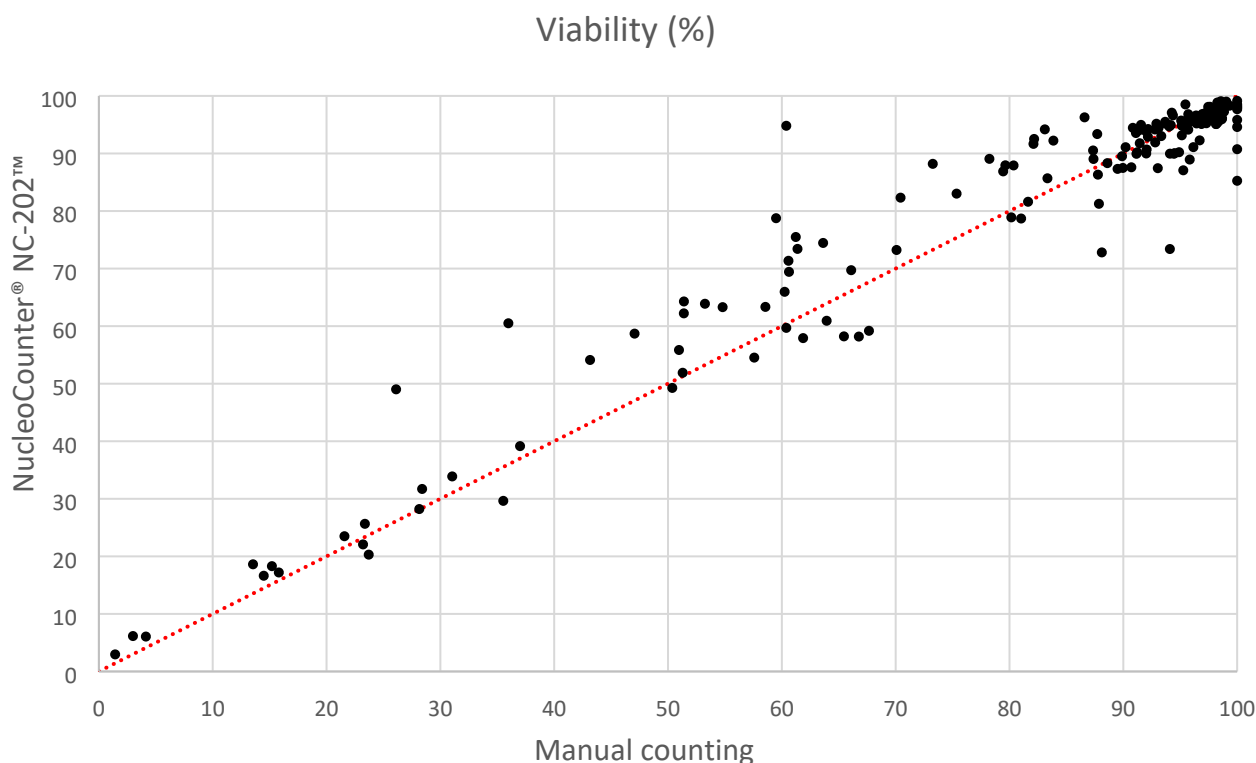


Figure 2. NucleoCounter® NC-202™ viability correlates with manual assessment by trypan blue. The graph presents data from 15 different cell types with 166 measurements using three NucleoCounter® NC-202™ instruments and manual counting. A line of unity, where  $X = Y$ , is indicated by a dotted line.

### Cell counting precision

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

To demonstrate counting precision, the coefficient of variation (CV) was calculated from replica NucleoCounter® NC-202™ or manual counts and plotted against the cell concentration (Figure 3). The NucleoCounter NC-202™ showed significantly lower variation; on average 4.1% (Figure 3A) as compared to an average of 8.2% for manual counting (Figure 3B).

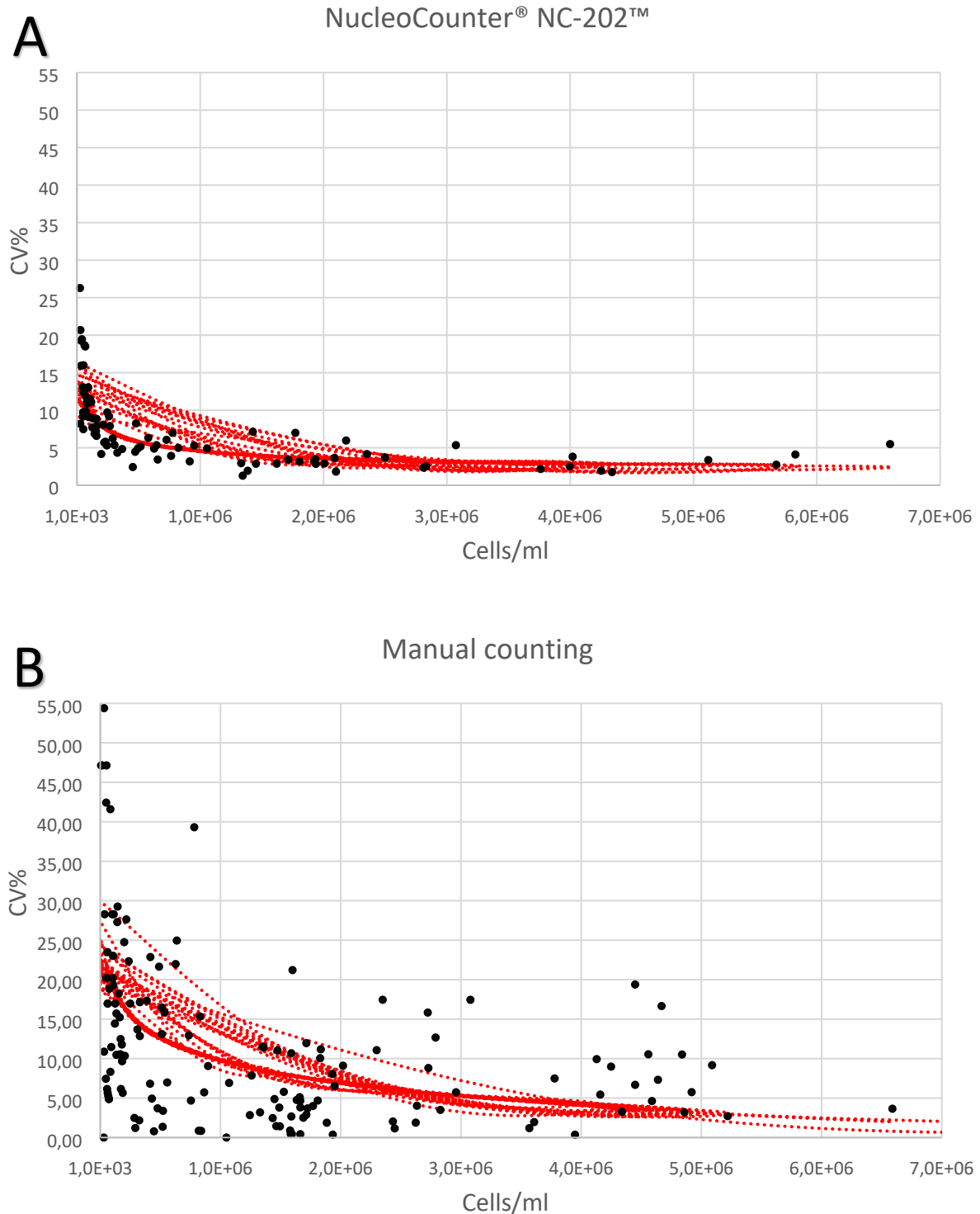


Figure 3. NucleoCounter® NC-202™ cell counting is more precise than manual counting. The graph presents data from 15 different cell types with 166 measurements using (A) three NucleoCounter® NC-202™ instruments and (B) manual counting. CV indicates coefficient of variation. Curve fitting is indicated by a dotted line.

### Instrument-to-instrument repeatability

All NucleoCounter® NC-202™ instruments are calibrated to a reference instrument at time of manufacture to ensure that all instruments acquire consistent data and perform to the same high standard. The LED light sources are constant over time, which ensure stable image acquisition regardless of the age of the instrument. The optics are mechanically adjusted during production and cannot be changed, and do not require any adjustment by the user. Consequently, all NucleoCounter® NC-202™ instruments are inter-comparable, regardless of production year.

When normalized total cell counts are compared between three NucleoCounter® NC-202™ instruments, no significant difference is observed, evident by a P-value of 0.998 in a one-way ANOVA test: n =228, 15 different cell lines (Figure 4).

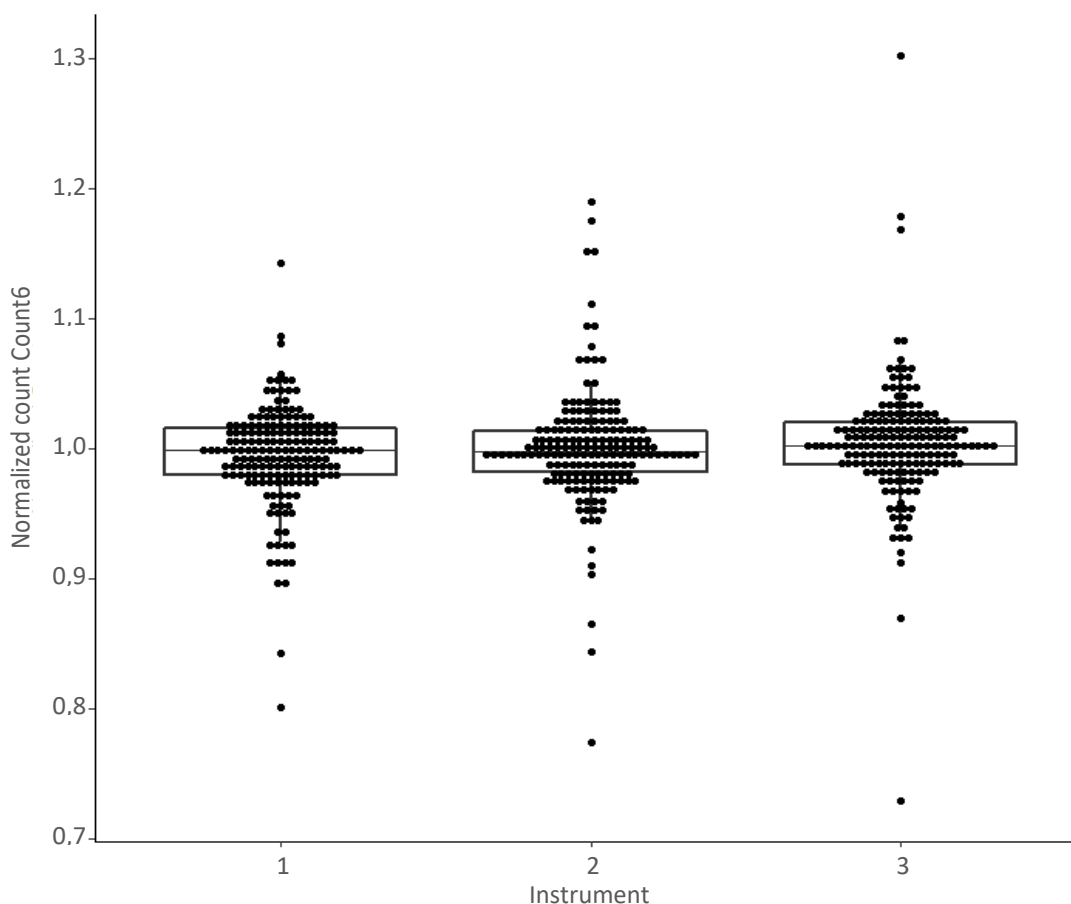


Figure 4. Instrument-to-instrument variation. The graph presents normalized cell count data from 15 different cell types at different concentrations (a total of 166 individual measurements) using three different NucleoCounter® NC-202™ instruments. One-way ANOVA test shows no significant difference between 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
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
MSC*	Human	Adipose tissue	Stem cells
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

#### Handling and storage

For handling and storage of ChemoMetec® instruments, reagents, cassettes and NC-Slides refer to the corresponding product documentation. For other reagents refer to the material data sheet from the manufacturer of the reagents and chemicals.

#### Warnings and precautions

For safe handling and disposal of the ChemoMetec® reagents, cassettes and NC-slides refer to the corresponding product documentation and the NucleoCounter® NC-202™ user guide. For other reagents refer to the safety data sheet from the manufacturer of the reagents and chemicals required for this protocol. Wear suitable eye protection and protective clothes and gloves when handling biologically active materials.

#### Limitations

The NucleoCounter® NC-202™ system is FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE. The results presented by the NucleoCounter® NC-202™ system depend on correct use of the reagents, Cassettes and the NucleoCounter® NC-202™ instrument and might depend on the type of cells being analyzed. Refer to the NucleoCounter® NC-202™ user's guide for instructions and limitations.

#### Liability disclaimer

This application note is for RESEARCH PURPOSES ONLY. It is not intended for food, drug, household, or cosmetic use. Its use must be supervised by a technically qualified individual experienced in handling potentially hazardous chemicals. The above information is correct to the best of our knowledge. Users should make independent decisions regarding completeness of the information based on all sources available. ChemoMetec A/S shall not be held liable for any damage resulting from handling or contact with the above product.

#### Product disclaimer

ChemoMetec A/S reserves the right to introduce changes in the product to incorporate new technology. This application note is subject to change without notice.

#### Copyright

Copyright © ChemoMetec A/S 2020. All rights reserved. No part of this publication and referred documents may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, without the prior written consent of ChemoMetec A/S, Gydevang 43, DK-3450 Allerød, Denmark.

ChemoMetec, NucleoCounter and Xcyto are registered trademarks owned by ChemoMetec A/S. Via2-Cassette and NC-View are trademarks of ChemoMetec A/S. All other trademarks are the property of their respective owners.