

Performance of NucleoCounter® NC-200™

The NucleoCounter® NC-200™ offer precise measurement of cell concentrations and viability

Introduction

The most commonly used method to determine cell concentration today, although highly imprecise, is still manual counting of cells in hemocytometers. Advancements in microscopy and software image analysis have made it possible to develop automated image based cell counting, providing easy, objective and accurate results.

The NucleoCounter® NC-200™ and its performance are described in this technical note in terms of accuracy, precision and measurement range.

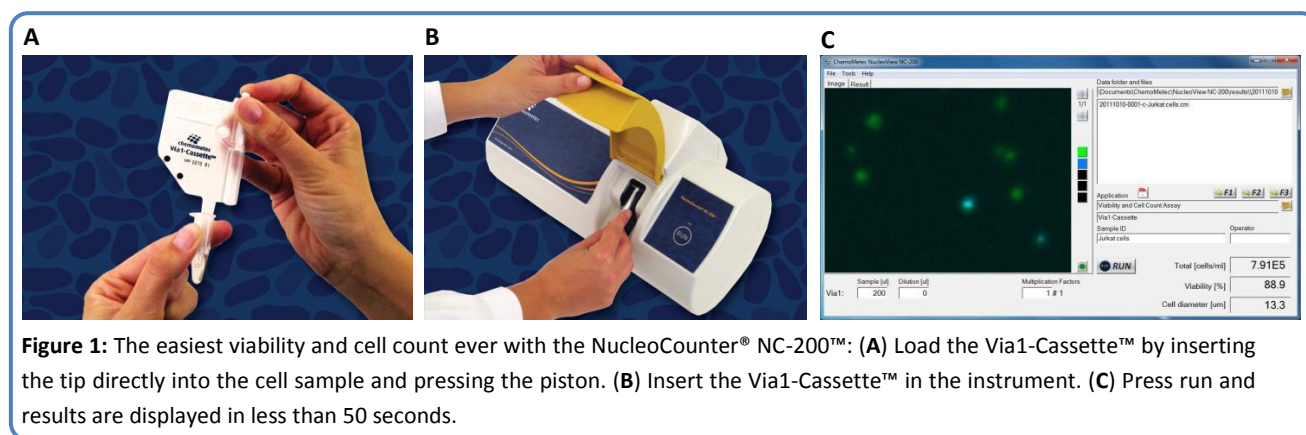
The NucleoCounter® NC-200™ system

If high quality in cell counting is important for you, the NucleoCounter® NC-200™ is the right instrument for you. The NucleoCounter® NC-200™ provides precise and objective cell counts, in a fast and easy manner. To perform a viability and cell count assay, you only have to load a Via1-Cassette™ with a representative cell sample, insert the cassette into the NucleoCounter® NC-200™ and

press 'Run' (Figure 1). In less than 50 seconds the image and results are displayed showing the total cell concentration (in cells/ml), the viability (in %) and an estimated cell diameter (in μm). Furthermore, extended results are available giving the cell concentrations of viable and non-viable cells, the standard deviation on the determination of the cell diameter, together with information about the degree of cell aggregation ("clumpiness").

Key benefits of the NucleoCounter® NC-200™

- **Ease of use:** Insert a Via1-Cassette™ loaded with your cell sample and press 'Run' to get viability and cell concentration results.
- **No pre-treatment:** The inside of a Via1-Cassette™ is coated with two different fluorescent dyes that immediately stain the different cell populations allowing determination of cell concentration and



viability. The first dye, Acridine Orange (AO) stains the total cell population, whereas the second dye, DAPI, only stains the non-viable cells. As the cells are stained inside the Via1-Cassette™ no pretreatment is required for this type of analysis.

- **High precision:** The volume of the measurement chamber of each Via1-Cassette™ has been measured and is calibrated for the determination of the cell concentration with high precision. The volume counted is much higher than the volume normally counted in hemocytometers, hence the statistical variation is lower in the NucleoCounter® NC-200™
- **Objective cell count:** The automation of cell counting eliminates the individual subjective judgment of users, providing a standardized method that enables comparison of data between different laboratories.
- **Calibration free:** The instrument is calibrated by ChemoMetec and no subsequent calibration is needed.
- **User safety:** Sample handling is safe since the dyes are enclosed inside the Via1-Cassette™ at all times.
- **Maintenance and service free:** The construction of the NucleoCounter® NC-200™ contains no internal flow system to become contaminated or blocked.
- **Flexible software package:** Protocols can be adapted by the user to fit specific needs. The software is 21 CFR part 11 ready for regulated environments.

Performance

The NucleoCounter® NC-200™ produces very precise results with small average coefficients of variation (CV), indicating a high level of reproducibility when counting cells. The average percent CV, calculated by measuring cell concentrations of approximately 1×10^6 cells/ml of 8 different cell lines (n=3), is 3.4 % (standard deviation ± 0.9). The precision of the determination of the cell count is dependent on the number of cells counted. It is normally assumed that counting random events follows the Poisson distribution, according to which the expected standard deviation is equivalent to the square root of the number of counted events. Therefore, the theoretical CV of the NucleoCounter® NC-200™ instrument at the cell concentration of 1×10^6 cells/ml can be calculated

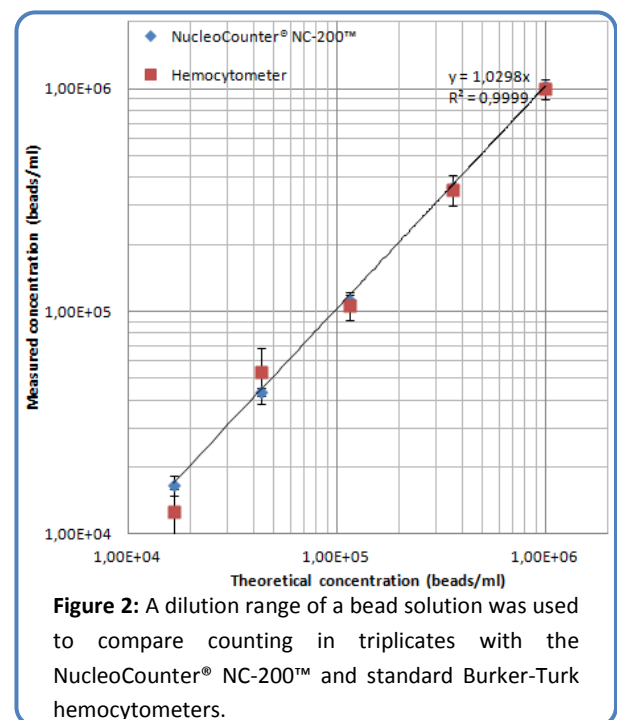


Table 1: List of cell types verified on the NucleoCounter® NC-200™.

Cell type	Species	Tissue	Remarks
BSC-1	African green monkey	Kidney	
CEF*	Chicken	Embryo	
CHO	Chinese hamster	Ovary	
COS-7	African green monkey	Kidney	
Embryonic stem cells*	Human	Embryo	
HEK-293	Human	Kidney	
HeLa	Human	Cervix	
Hematopoietic cells*	Mouse	Bone marrow	
HepG2	Human	Liver	
Jurkat	Human	Blood	
K562	Human	Bone marrow	
MCF7	Human	Breast	
NHBE*	Human	Bronchi	Assay requires lysis
NHDF*	Human	Skin	Assay requires lysis
NHEK*	Human	Skin	Assay requires lysis
NIH/3T3	Mouse	Embryo	
SF9	Fall army worm	Ovaries	
SVF cells	Human	Adipose tissue	
T-cells (purified)*	Human	Blood	
U2OS	Human	Bone	
Vero cells (grown on micro carriers)	African green monkey	Kidney	Assay requires lysis
WEHI-164	Mouse	Fibrosarcoma	
Whole blood*	Human	Blood	Assay require RBC lysis and incubation

* Primary cells.

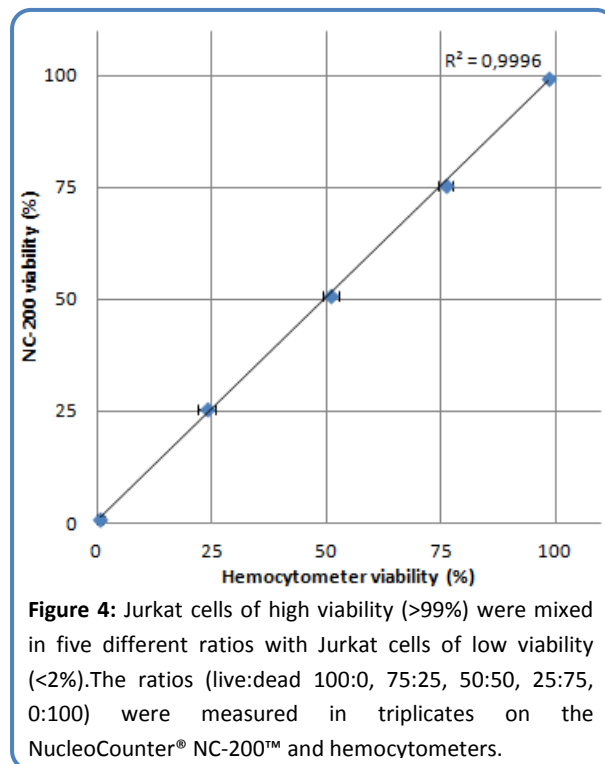
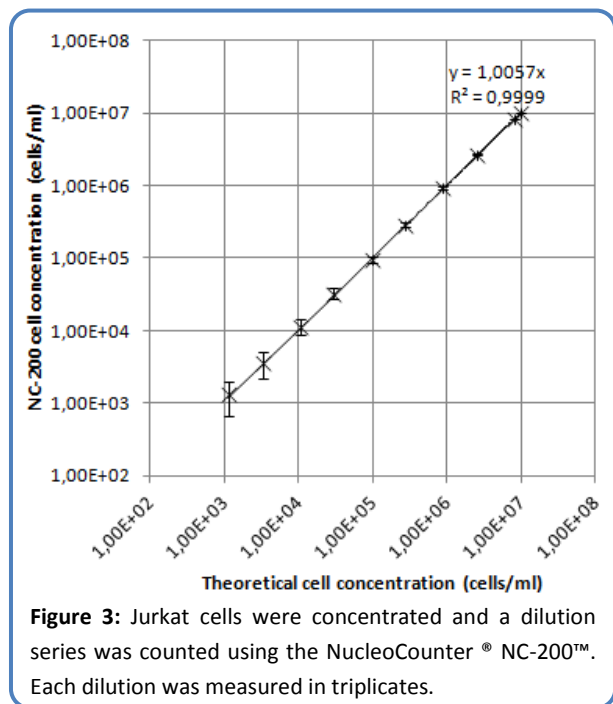
In the assays that require lysis, a solution is added to the sample for the total cell count. An additional cassette is required in this assay to measure the viability. In the whole blood assay, lysis of red blood cells (RBCs) is obtained by addition of a solution and an incubation step before analysis. This assay only requires one cassette for a viability and cell count analysis of leukocytes.

to be 2.7%. Cell concentration measurements performed by manual counting in hemocytometers commonly gives CVs above 10% when counts are performed by several individuals in blinded experiments. Comparing NucleoCounter® NC-200™

counts on a dilution range of a bead solution with manual counts performed with Burker-Turk hemocytometers shows a very good correlation between the results obtained with the two counting methods.(Figure 2).

Cell concentration range

The working range of the NucleoCounter[®] NC-200™ is 5×10^3 to 1×10^7 cells/ml in the tested solution, and the optimal range is 5×10^4 to 5×10^6 cells/ml. A warning will be given when analysis results are outside the optimal range. The working range is determined by the linear range where measurements can be performed (Figure 3). However, the majority of mammalian cell lines display non-linear counting tendencies above 5×10^6 cells/ml and, on the basis of this, the upper limit of the optimal range was set. The lower limit of the optimal range is set at 5×10^4 cells/ml, due to that determinations below this cell concentration becomes increasingly uncertain, again due to low number of cells counted as described above. The theoretical CV at the cell concentration of 5×10^4 cells/ml is 12%. It is recommended that the linear



range is determined for the employed cell lines to assure reliable results. Counting of a variety of different cell types has been verified at the NucleoCounter[®] NC-200™ (Table 1).

Viability range

The NucleoCounter[®] NC-200™ provides viability measurements in the full range from 0% to a 100%. DAPI is as known stain used to quantify the number of non-viable cells. There is good correlation between viabilities determined by the NucleoCounter[®] NC-200™ and the hemocytometers (Figure 4).

Handling and storage

For handling and storage of ChemoMetec instruments and 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-200™ user's 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-200™ system is FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE. The results presented by the NucleoCounter® NC-200™ system depend on correct use of the reagents, NC-slide, cassettes and the NucleoCounter® NC-200™ instrument and might depend on the type of cells being analyzed. Refer to the NucleoCounter® NC-200™ user's guide for instructions and limitations.

Liability disclaimer

This technical 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 technical note is subject to change without notice.

Copyright

Copyright © ChemoMetec A/S 2003. All rights reserved. No part of this publication 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.