TECHNICAL NOTE

How Does Red Blood Cell Contamination Impact Cell Counting? (NC-202™ vs Trypan Blue-Based Counter)

Abstract

Distinguishing between nucleated and non-nucleated cells in peripheral blood mononuclear cells (PBMCs) can be difficult. As a result, accurate cell counting of PBMCs in the presence of residual red blood cells can be challenging. This technical note compares the cell counting accuracy of the NucleoCounter® NC-202™ and a trypan blue-based automated cell counter in the presence of red blood cell contamination, demonstrating the superior performance of the NC-202™.

Introduction

Red blood cell contamination can interfere with accurate cell counting, particularly when using trypan blue-based techniques. Trypan blue stains red blood cells, so they are mistaken for non-viable cells and included in the total cell count.

The NucleoCounter® NC-202™ is an automated cell counter that overcomes the problems caused by red blood cell contamination by specifically counting only nucleated cells. The NC-202™ uses two dyes contained within the Via2-Cassette™: acridine orange (AO) and 4',6-diamidino-2-phenylindole (DAPI). AO binds to negatively charged molecules such as DNA in cell nuclei, staining live cells. DAPI also binds with DNA, but it cannot permeate the cell membrane, so it only stains dead cells. The NC-202™ detects the two fluorescence signals from these dyes to determine the total cell count and viability.

Red blood cells are non-nucleated cells, and their hemoglobin quenches fluorescence, so they are excluded from the NC-202™ cell count. As a result, the NC-202™ provides accurate PBMC counting and cell viability data, even in the presence of contaminating red blood cells.

Here, we compare the effects of red blood cell contamination on the cell counting accuracy and precision of the NC-202™ and a trypan blue-based automated cell counter.

Methods

We prepared six PBMC samples with increasing red blood cell contamination levels ranging from no contamination to around 2 x 10^7 red blood cells/ml (see Figure 1). Then, we counted the cells in each sample using a trypan blue-based automated counter and the NC-202™. All counts were completed in triplicate and averaged to calculate each sample's total cell count and viability.

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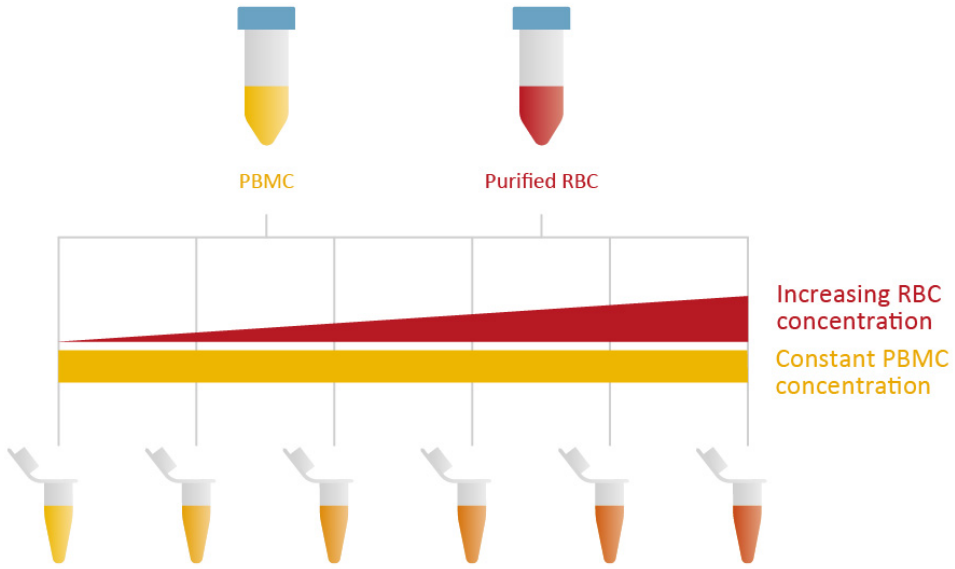

Figure 1: Schematic diagram showing how we prepared PBMC samples with increasing red blood cell contamination. RBC = red blood cells; PBMC = peripheral blood mononuclear cells.

Results

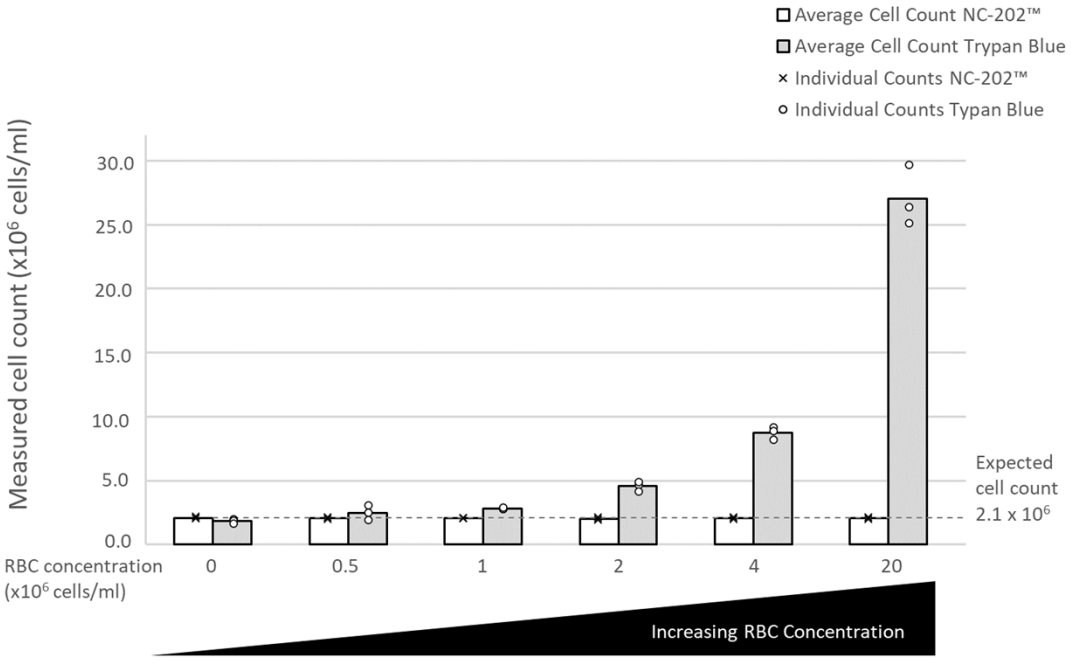

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Figure 2: Graph showing the measured cell count of PBMCs with increasing levels of red blood cell contamination using a trypan blue-based automated cell counter and the NucleoCounter® NC-202™. RBC = red blood cells; PBMC = peripheral blood mononuclear cells.

Figures 2 and 3 show the total cell count and viability for each sample and counting technique. The NC-202™ provided consistent results and low %CVs throughout the experiment, indicating high accuracy and precision.

As shown in Figure 2, the total cell counts obtained using the trypan blue-based automated cell counter were affected by the presence of red blood cells, even at low contamination levels. What's more, the trypan blue-based counter hugely overestimated the PBMC counts at higher red blood cell concentrations.

Viability measurements using the trypan blue-based counter were also impacted by the presence of red blood cell contamination (see Figure 3), with viability dropping as contamination increased, confirming that the trypan blue-based cell counter assumes that the red blood cells are dead cells. The %CV of the trypan blue-based counter varied from 3.3-22.6% throughout the experiment, reflecting its reduced precision in the presence of red blood cell contamination.

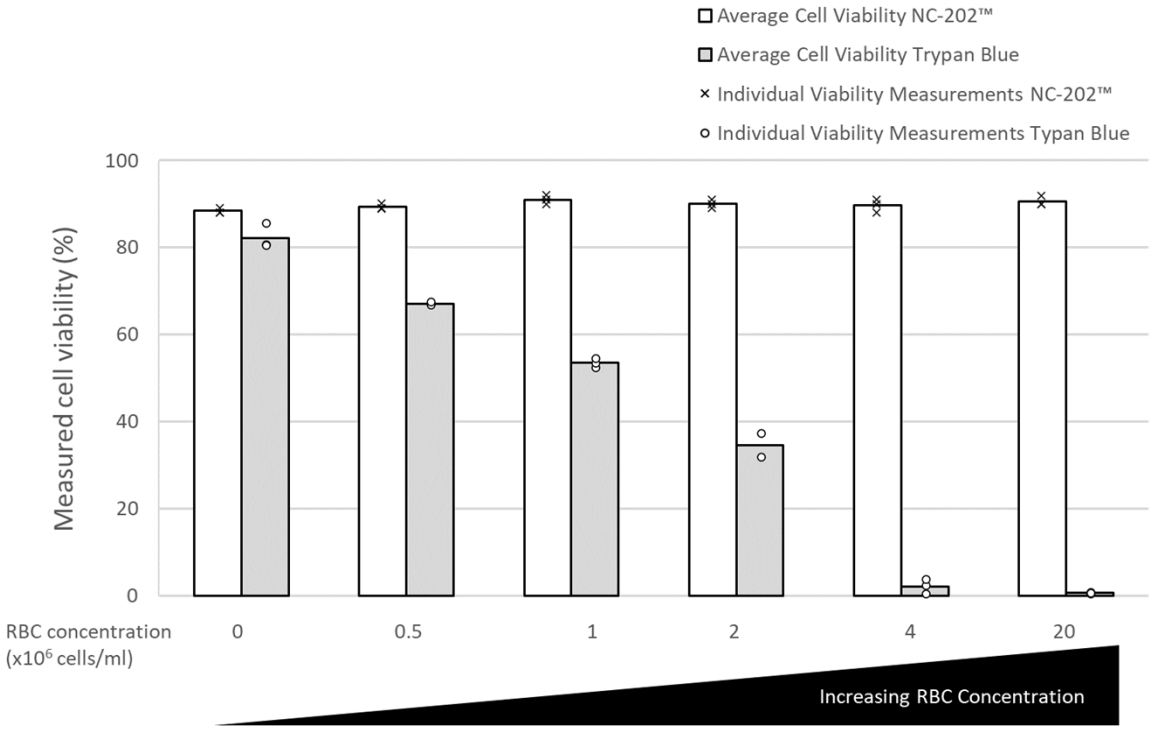

Figure 3: Graph showing the measured cell viability of PBMCs with increasing levels of red blood cell contamination using a trypan blue-based automated cell counter and the NucleoCounter® NC-202™. RBC = red blood cells; PBMC = peripheral blood mononuclear cells.

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Conclusion

The results presented here demonstrate that the NucleoCounter® NC-202™ provides superior accuracy and precision for PBMC counting in the presence of red blood cell contamination than trypan blue-based automated counters.

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.

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