TECHNICAL NOTE

How Does Red Blood Cell Contamination Impact Cell Counting with the NC-202™?

Abstract

In cell counting, distinguishing between nucleated and non-nucleated cells in peripheral blood mononuclear cells (PBMCs) can be difficult. As a result, it can be challenging to obtain accurate PBMC cell counts in the presence of residual red blood cells. The data in this technical note demonstrates the cell counting accuracy of the NucleoCounter® NC-202™, even in the presence of contaminating red blood cells.

Introduction

Accurate cell counting of peripheral blood mononuclear cells (PBMCs) is essential in a range of research applications. However, obtaining reliable PBMC counts can be tricky. Even if you carefully isolate your PBMCs, some red blood cell contamination can remain.

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. However, at very high concentrations, red blood cells can interfere with the fluoresence signals and impact cell counting. That's why we recommend that you use our 'Blood' protocol, which includes a red blood cell lysis step, if you are counting cells in whole blood.

So how much red blood cell contamination can the NC-202™ tolerate without it impacting its cell counting accuracy and requiring red blood cell lysis prior to counting?

The following technical note outlines how we investigated the effects of red blood cell contamination on the NC-202™. We also provide data demonstrating the performance of the NC-202™ in the presence of red blood cell contamination and determine how much contamination it can tolerate before impacting accuracy.

Methods

We prepared seven PBMC samples with increasing red blood cell contamination levels ranging from no contamination to around 4 x 10^7 red blood cells/ml (see Figure 1). Then, we counted the cells in each sample using 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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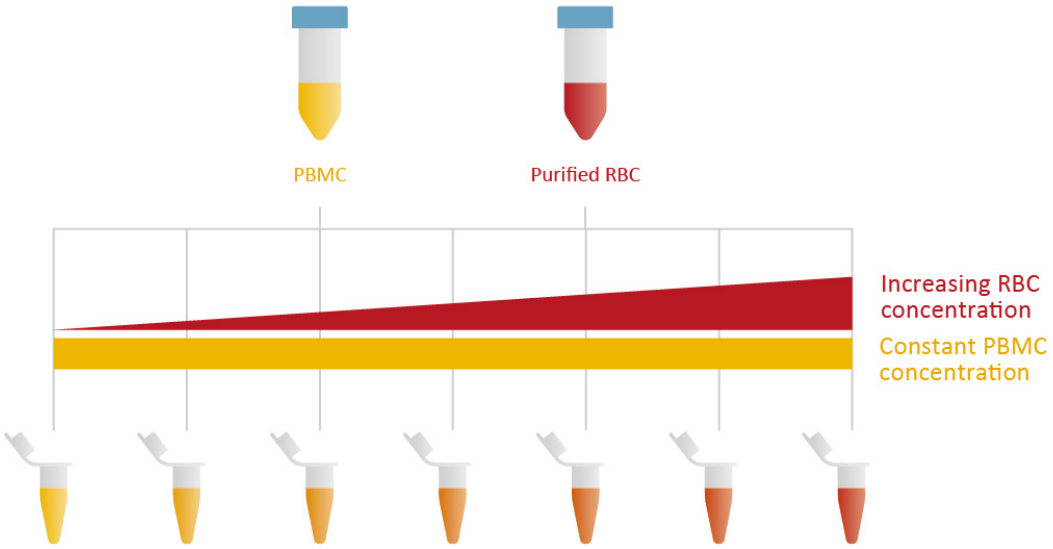

Figure 1: Schematic diagram showing how we prepared PBMC samples with increasing red blood cell contamination. RBC = red blood cells; PBMC = peripheral blood mononuclear cells.

Results

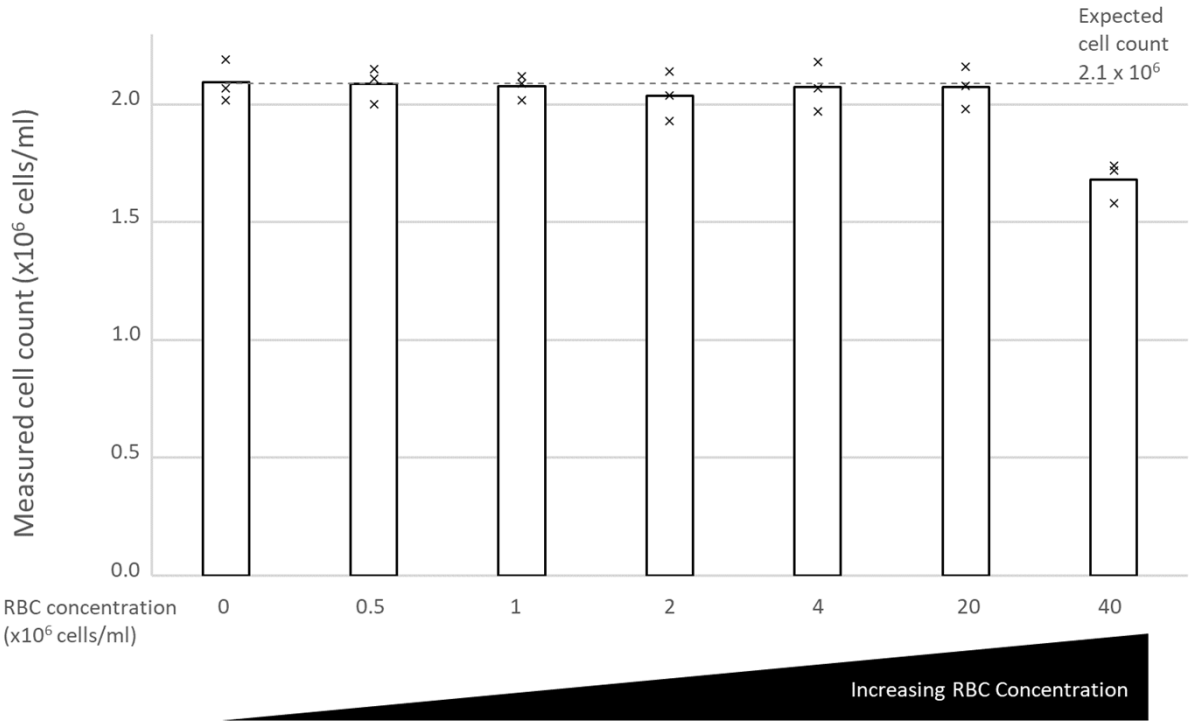

Figure 2: Graph showing the measured cell count of PBMCs with increasing levels of red blood cell contamination using the NucleoCounter® NC-202™. The bar chart shows the average of three measurements, the data points show the three individual measurements. RBC = red blood cells; PBMC = peripheral blood mononuclear cells.

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Figures 2 and 3 show the total cell count and viability for each sample, respectively. As expected, the NC-202™ continues to produce consistent cell counting data, despite the presence of contaminating red blood cells. What's more, the %CV for the total cell count (3.7-5.2%) and viability (0.6-1.7%) remained similar throughout the experiment, indicating consistently high precision.

However, at high red blood cell concentrations (above 2 x 10^7 cells/ml), the measured total cell count decreased, suggesting that contamination at this level impacts the counting accuracy of the NC-202™ (see Figure 2).

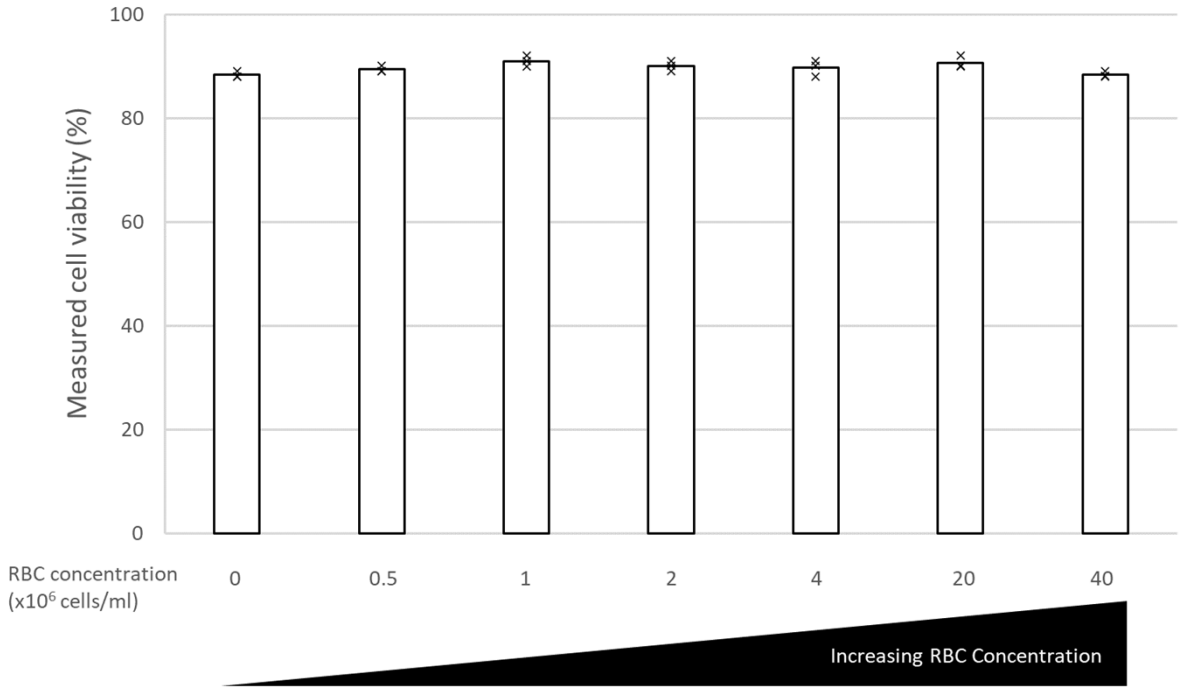

Figure 3: Graph showing the measured cell viability of PBMCs with increasing levels of red blood cell contamination using the NucleoCounter® NC-202™. The bar chart shows the average of three measurements, the data points show the three individual measurements. RBC = red blood cells; PBMC = peripheral blood mononuclear cells.

Conclusion

The results presented here demonstrate that the NucleoCounter® NC-202™ provides high accuracy and precision for PBMC counting, even in the presence of red blood cell contamination. What's more, our data confirms that the NC-202™ Count & Viability protocol can tolerate red blood cell concentrations up to around 2 x 10^7 cells/ml without influencing the cell counting results.

If you have higher levels of red blood cell contamination, we recommend using the 'Blood' protocol, which we have designed for reliable cell counting in samples with high concentrations of red blood cells, including whole blood.

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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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