Recommendations for accurate carryover measurements on the Attune NxT Flow Cytometer

Introduction

The Invitrogen™ Attune™ NxT Flow Cytometer has less than 1.0% carryover when the standard rinse cycle is used. Carryover is measured by acquiring a fixed volume of sample, followed by acquiring a fixed volume of a particle-free, buffer-only solution such as phosphate-buffered saline (PBS). The concentration of particles detected in the PBS/buffer blank divided by the concentration of particles in the sample, multiplied by 100, yields the percent carryover (Figure 1).

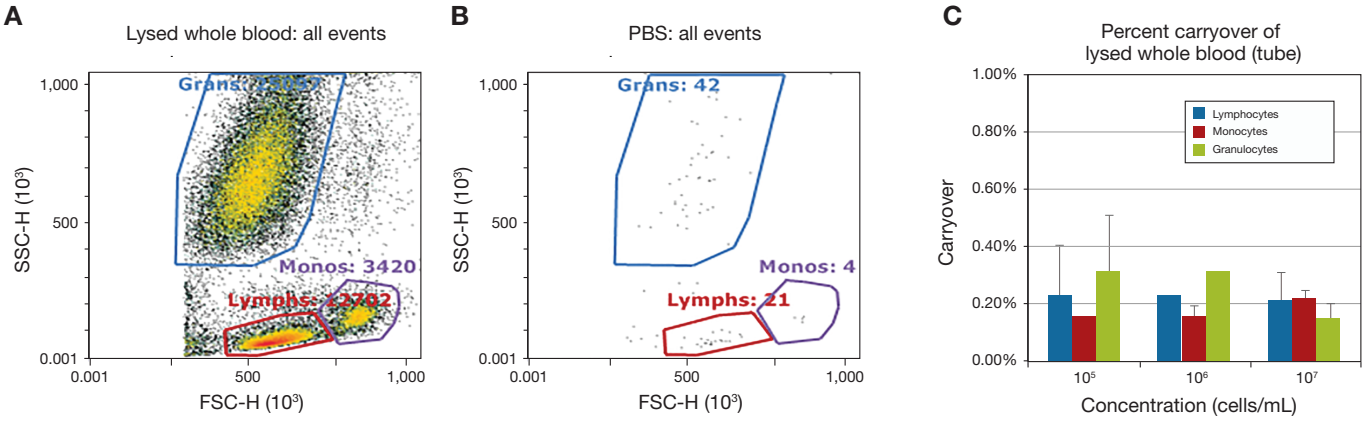

Figure 1. Analysis of carryover from a tube. (A) A forward scatter (FSC) vs. side scatter (SSC) plot of human lysed whole blood at 10^6 cells/mL, and **(B)** analysis of PBS blank with the same gates. The numbers displayed for each population of lymphocytes, monocytes, and granulocytes are the concentrations in cells/µL. **(C)** Percent carryover of lysed whole blood (LWB) as a function of cell concentration for the three main cell population gates; 50 µL of lysed whole blood was acquired, followed by 50 µL of PBS. Percent carryover = (concentration of PBS subset/concentration of LWB subset) x 100%. In this example, the lymphocyte carryover is (21/12,702) x 100% = 0.17%. The error bars show the standard deviation (n = 3) of the calculated percent carryover at each concentration.

The percent carryover of each of individual subset is calculated as:
Percent carryover = PBS subset/LWB subset x 100
In example shown in figures 1A and 1B, the lymphocyte carryover is calculated at 21/12,702 x 100 = 0.17%

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Key considerations for accurate carryover measurements:

- Remove any excess sample droplet that may remain on the sample injection port (SIP) when acquiring from a tube. Such a droplet can be either wiped away or removed by touching the sample vessel to the SIP while removing the vessel to wick away this excess droplet.
- It is good practice to acquire samples of lower particle or reagent concentrations before acquiring samples with higher concentrations of particles or reagents.
- When setting up plates with the Invitrogen™ Attune™ Autosampler (Figure 2), running blank buffer wells between samples will help reduce carryover.
- The "SIP sanitize" function with Attune™ wash or debubble solution instead of bleach can be used to reduce carryover, as these solutions contain detergents that assist in the removal of stubborn particles.
- Collect enough events to achieve statistically significant detection (e.g., >400 events for cells or particles of interest)—see "How many events must be acquired?" (pg. 14) in *BioProbes* 71 (p.14), available at thermofisher.com/bioprobes. For further discussion on statistical considerations of rare-event detection, see Allan AL, Keeney M (2010) *J Oncol* 426218.
- When analyzing rare events:
  - It is important to make sure the instrument and fluids used are clean and free of particles that could contribute falsely to the measurements of the rare population. Sample buffers should be filtered to remove any background particulates that could be measured along with carryover.
  - It can be helpful to include time as a parameter and look for any bursts or breaks of data during acquisition that may indicate a problem with the sample (such as clumping) or with the fluidics of the instrument.

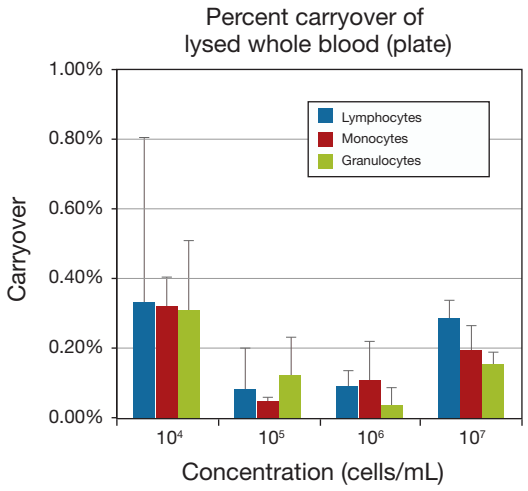

Figure 2. Analysis of carryover using the Attune NxT Autosampler with one rinse and one mix. Carryover is less than 0.40% using only one rinse cycle for each of the three main cell populations of lymphocytes, monocytes, and granulocytes at four different cell concentrations. The error bars show the standard deviation (n = 3) of the calculated percent carryover at each concentration. At 10^4 cells/mL concentration, the measurement error is higher due to the poor counting statistics (<400 events in each subpopulation).

Find out more at **thermofisher.com/attune**

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