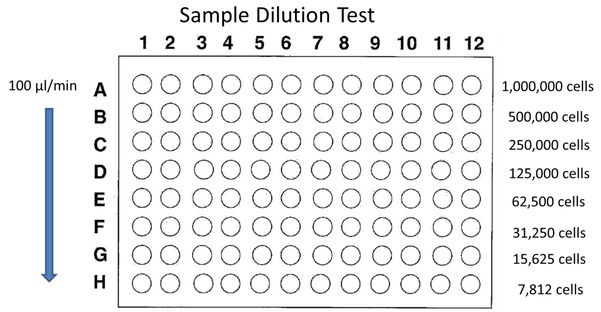
An Objective Test to Qualify Plate Samplers for Complex Flow Cytometric Assays

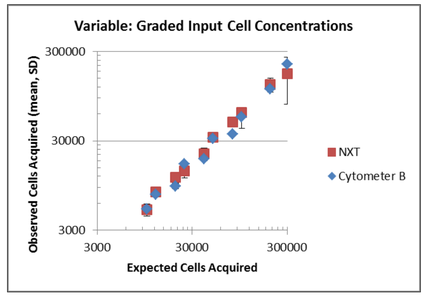
E. Michael Meyer, Albert D. Donnenberg

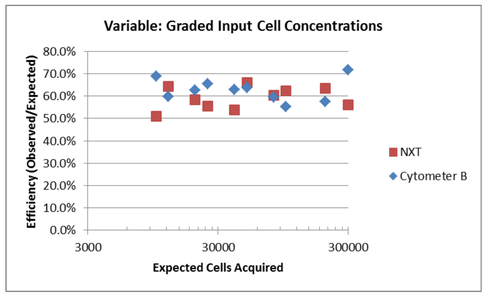
INTRODUCTION. Plate samplers are available for a variety of analytical flow cytometers. Originally, the intended use was for high throughput screening during which small sample volumes (<50 µL) are rapidly acquired with a simple readout such as viability. The requirements for more demanding applications such as multi-color cytometry and rare event detection are more stringent. Important considerations include fluidic stability, event recovery and sample carryover. In order to compare available systems, we propose an objective set of tests using cells and beads to quantify these parameters.

METHODS. We performed three tests comparing the performance of the Invitrogen Attune NxT plate sampler with that of 2 other current cytometers with manufacturer-supplied plate samplers (B and C). Sample resuspension (2 mixes on the Attune NXT and Cytometer B) and washing between wells (2 wash cycles) was standardized between instruments. Mixing on Cytometer C was by plate agitation and was not practical with fully loaded wells. The Attune NxT, equipped with an auto-sampler, has novel fluidics which include acoustic focusing and a syringe driven sample stream. It was provided to the University of Pittsburgh Cancer Center's Cytometry Facility by Invitrogen for this evaluation.

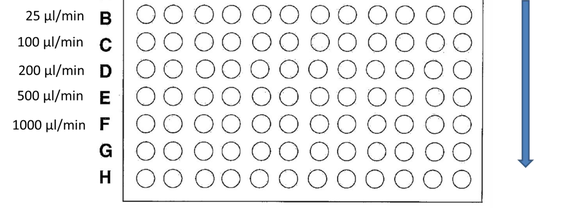


Sample Dilution Test. This test was designed to evaluate the effect of sample density (cells/mL) on performance as measured by efficiency, where efficiency was calculated as the observed number of cells acquired/expected number of cells acquired. This test depends on the performance of both cytometer and plate sampler. Fixed and permeabilized human peripheral blood mononuclear cells (PBMC) and A549 lung carcinoma cells were stained with DAPI (Attune, Cytometer B) or propidium iodide (Cytometer C) and plated in 96-well plates at variable concentrations (10×10^3 to 10×10^6 cells/mL in half $\log_{10}$ steps). Cells were acquired at a constant rate (100 µL/min) on all instruments. The observed number of events was plotted against the expected number of events.

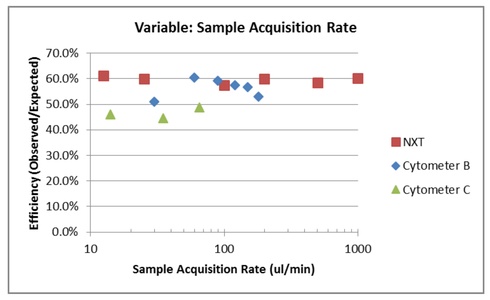


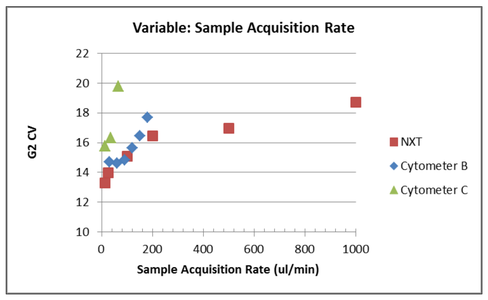


In this data set , cell density does not have a major effect on acquisition efficiency. Both the Attune NxT and Cytometer B had an average efficiency of around 60%. Higher cell densities did result in greater variability in efficiency as reflected in larger standard deviations. Technical difficulties prohibited including Cytometer C in this data set.

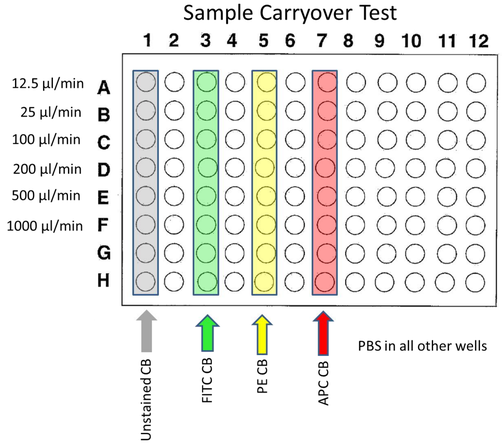


Acquisition Speed Test. This test was designed to evaluate the effect of sample acquisition rate (µL/min) on performance as determined by efficiency and the coefficient of variation (CV) of the 2N DNA peak in PBMC. Fixed and permeabilized human peripheral blood mononuclear cells and A549 lung carcinoma cells were stained with DAPI (Attune, Cytometer B) or propidium iodide (Cytometer C) and plated in 96-well plates at a constant concentrations (1.25×10^5 and 1.25×10^6 cells/mL). Cells were acquired thought the entire range of rates available for each instrument: 12.5 to 1000 µL/min for the Attune NXT; 30 to 180 µL/min for Cytometer B; and 14 to 65 µL/min for Cytometer C. The observed number of events was plotted against the expected number of events and efficiency was calculated as observed/expected. The CV of the 2N DNA peak was calculated as the standard deviation of the arithmetic mean DAPI (or PI) fluorescence/ the arithmetic mean DAPI (or PI) fluorescence.

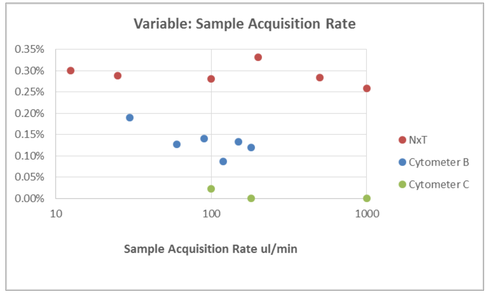




When the sample density is held constant and the sample rates are varied, both Cytometer B and the Attune NxT have constant efficiency (approximately 60%.) throughout their ranges of operation. Data show that Cytometer C had lower efficiency at lower sample rates (45-50%). The data suggest that the Attune NxT's performance is constant at all sample rates, but perhaps Cytometer B has a "sweet spot" at approximately 90 µL/min. As expected, the calculated CV of the G2 peak increases on all cytometers as a function of sample rate, due to greater uncertainty of the cell position within the sample stream. On cytometers B and C, performance deteriorated exponentially at relatively low rates (180 µL/min for Cytometer B and 65 µL/min for Cytometer C). In contrast, the CV of the Attune NxT increased only gradually through 1000 µL/min, a sample rate 5 times greater than the maximum on Cytometer B, and 15 times greater than that of Cytometer C.



Sample Carryover Test. This test was designed to evaluate sample carryover from one sample to the next. Calibrite beads (unstained, FITC, PE, APC) were concentrated to 10×10^6/mL and plated at constant bead concentration, alternating beads with wells containing only PBS. Cells were acquired thought the entire range of rates available for each instrument: 12.5 to 1000 µL/min for the Attune NXT; 30 to 180 µL/min for Cytometer B; and 14 to 65 µL/min for Cytometer C. Carryover was determined for each sample as the number of events in the PBS only well/the number of events in the proceeding bead-containing well.



Sample carryover after 2 washes was independent of sample acquisition rate and acceptable on all instruments (<0.35%), but was lowest on Cytometer C, which has the simplest and most direct fluidic system.

CONCLUSIONS. The tests described here permit the objective comparison of plate samplers from different vendors. In addition, the data can be used as a benchmark to optimize plate-based sample acquisition, in order to maximize event yield and acquisition efficiency and minimize sample-to-sample carryover.