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 (cellularity) 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 K562 long carcinoma cells were stained with DAPI (Attune Cytometer B) or propidium iodide (Cytometer C) and plated in 96-well plates at variable concentration (20 x 10^3 to 10 x 10^5 cells/well). Cells were acquired at a constant rate (300 µL/min) on all instruments. The observed number of events was plotted against the expected number of events.

Acquisition Speed Test. This test was designed to evaluate the effect of sample acquisition rate on performance as measured by efficiency and the coefficient of variation (CV). The Attune NxT sample slot was filled with mononuclear cells and K562 long carcinoma cells were stained with DAPI (Attune, Cytometer B) or propidium iodide (Cytometer C) and plated in 96-well plates at a constant concentration (2.5 x 10^5 and 2.5 x 10^6 cells/well). Cells were acquired throughout the entire range of rates available for each instrument. 30 to 1000 µL/min for the Attune NxT, 5 to 1000 µL/min for Cytometer B, and 5 to 1000 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 G2 peak was calculated as the standard deviation of the arithmetic mean DAPI (or PI) fluorescence/ the arithmetic mean DAPI (or PI) fluorescence.

Sample Carryover Test. This test was designed to evaluate sample carryover from one sample to the next. Calibrations (unstained, FITC, PE, APC) were concentrated to 30 x 375 µL and plated at constant fixed concentration, alternating between wells with PBS. Cells were acquired throughout the entire range of rates available for each instrument. 30 to 1000 µL/min for the Attune NxT, 5 to 1000 µL/min for Cytometer B, and 5 to 1000 µL/min for Cytometer C. Carryover was determined for each sample as the number of events in the M1 using well/well number of events in the proceeding load containing well.

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.

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

E. Michael Meyer, Albert D. Donnenberg