Comparison of Sorting Capabilities of Beckman Coulter MoFlo™ XDP and Becton Dickinson FACSAria™ I and II

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Abstract

Advancement in the field of flow cytometry, especially with regard to analyzing and sorting rare populations (defined as <5% of sample population, 1:20 Poisson distribution) increases the requirement for high-throughput, accurate cell sorters. Therefore, Beckman Coulter, Inc. (BCI) developed three cell sorting assays to analyze the sorting capabilities of the Beckman Coulter MoFlo™ XDP as compared to the Becton Dickinson FACSAria™ I and II. **Methods:** In the first assay, to establish a baseline comparison of instrument performance, fluorescent beads (3% of sample population), in various concentrations, were sorted at different speeds. Statistics were collected on the events per second (EPS), hard abort rate, purity, and sort rate. In the second assay, lysed blood was sorted for CD19-PE positive events (2.8% of sample population) to differentiate biological versus bead sorting. Finally, whole blood was sorted for CD4-APC positive events (0.1% of sample population). The results were used to analyze the effects of sort population size on sort statistics. **Results:** At lower speeds, the XDP and the Aria demonstrated similar performance. However, at speeds above 20K EPS, the XDP produced fewer lost events and hard aborts, while achieving higher purity and faster sort rates than the Aria I and II.

Introduction

Researchers working in flow cytometry today increasingly require faster instruments to study rare populations and to increase laboratory productivity. Analysis and sorting of rare populations, such as stem cells, demands that the majority of the sample is analyzed, and that events are not lost due to instrument limitations. When a sample contains <5% population of interest, instrument performance is critical to producing confidence in the statistical distribution of data. Sorting rare populations is a lengthy process with samples that often degrade over time. Therefore, higher-speed instrumentation can help make the most of laboratory resources and help to preserve sample viability.

Cell sorters have evolved from the Los Alamos National Laboratory high-speed sorter (1978), developed for the Human Genome Project[1], to the advanced instrumentation on the market today. The Beckman Coulter, Inc. (BCI) MoFlo XDP is capable of sorting more than 70,000 events per second (EPS) with greater than 99% purity, and the Becton Dickinson (BD) FACSAria II sorter is capable of sorting 25,000 EPS with similar purity. New published research involving the sorting of rare populations was performed on high-throughput instruments, capable of producing exceptionally pure results. Researchers working with applications such as cervical cancer detection[2], CD34 positive enumeration[2], side population sorting[3], and others have used high-speed sorting to make great strides in scientific development. Instrument sorting capabilities are defined by how quickly events pass through interrogation, as well as hard abort rate, purity, and sort statistics. In the following section, sort factors are discussed in detail including performance comparisons between the XDP and the Aria.

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Sort Factors: An explanation

Both the XDP and the Aria II utilize stainless-steel-pressurized sheath tanks to deliver a stable fluidic stream to the laser interrogation point, but the sample interrogation methods differ. The XDP delivers sample to the interrogation point through jet-in-air, while the Aria delivers sample through a cuvette. Droplet deposition and deflection are created by different mechanisms for each instrument. However, both instruments divide the stream into droplets for sorting using a piezoelectric crystal that vibrates at a specific frequency for different nozzle tips and pressures. For general use, the XDP, using a 70 µm nozzle, is stable at 96 KHz frequency with pressure set at 60 psi. The Aria, using a 70 µm nozzle, is stable at 89 KHz with pressure set at 70 psi. Droplets contain particles that have already been interrogated by lasers upstream, and have also been analyzed by instrument electronics.

Sort decisions are applied to droplets depending on the operator settings, the location of the particle within the droplet, and the properties of the upstream and downstream particles. Depending on the sort mode, the electronics may abort or sort the particle.

The XDP divides sorting decisions into two parts, the Sort Mode and the Drop Envelope[5]. The user first selects the precision of the sort output: single, purify, or enrich. The user then selects the Drop Envelope: 0.5, 1, 1-2, 2 or 3 drops. The Drop Envelope is defined as the number of drops to which sort mode will be applied.

The Aria uses similar sort precision modes, where users define one of four sort modes: single cell, yield, purity, or 4-way purity. After choosing a preset sort mode, Aria users may adjust the Drop Envelope in 1/32nds for three masks: yield, purity and phase. The sort mode comparison is depicted in Figure 1, with the green drops representing the sorted drops and the red drops representing the aborted drops. The comparable sort modes between the XDP and the Aria are described at the bottom of Figure 1.

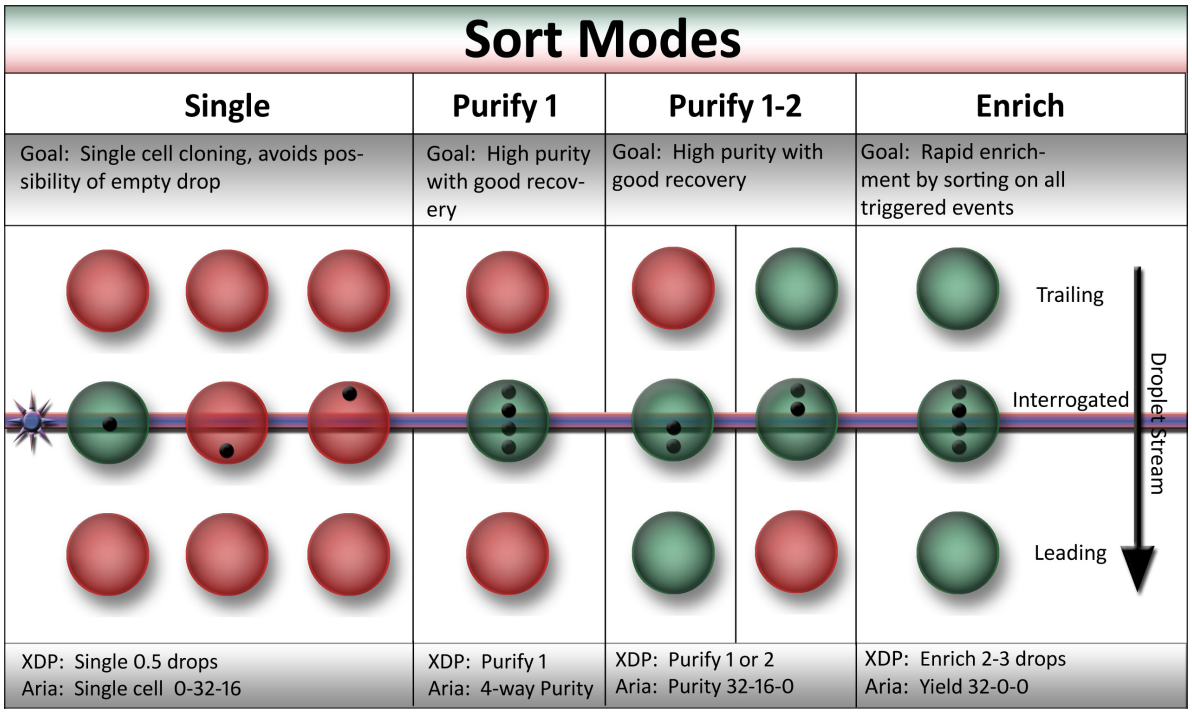

Figure 1. Sort modes for the XDP and the Aria. Green indicates sorted drops, red indicates aborted. Actual sort mode settings are described below the sort mode labels. Particles located within the green sorted drops can be in any one of the locations within the drop, but in only one. If there is more than one particle in a drop, the drop will be aborted.

Defining the same sort mode between instruments is critical when comparing the remaining sorting components: aborts, purity, and sort rate. Hard and soft aborts determine a portion of the sorted sample recovery and yield.

A **hard abort** occurs when the instrument cannot process information in time to make the sort decision[5]. The Aria defines the hard abort as an **electronic abort**[6]. Throughout this document, hard aborts will refer to the XDP's "hard aborts" and the Aria's "electronic aborts."

Coincidence is the primary source of hard aborts. Coincidence occurs when two events are too close together within the acquisition window and therefore both events are aborted. The XDP electronics use a narrow acquisition window (based on pulse width) to detect events. The Aria electronics use a wider acquisition window (based on pulse width) to detect events, thereby producing more coincident events that are aborted[7, 8]. An abort can also be caused by a **skipped sort**, where the complexity of the sort decision and the time available cause the sort to be aborted. Finally, a hard abort can be a **missed sort** where the electronics make a valid decision, but the sort card cannot process it and therefore the drop is aborted[9].

When a positive event defined by the sort gate fails to pass the criteria in the sort mode, drop envelope or stream precedence, it is defined as a **soft abort** or **coincidence**[5]. As the sample rate increases, conflicts increase in a linear manner. The soft abort rate is also seen in **sort efficiency** statistics defined as the sorted sample divided by sorted sample plus conflicts. When comparable sort modes are set, theoretically there should be no difference in the sort efficiency between the XDP and the Aria.

Sample purity depends largely on the operator-defined sort mode. Sample purity is defined as the percentage of desired cells in the sorted tube divided by the total cells sorted. If sort modes are similar, the purity should be similar in absence of differentiating factors such as droplet charging, accurate drop delay determination, and the stability of the droplet break-off. IntelliSort™ (XDP) and Sweet Spot™ (Aria) maintain the stability of the last-attached drop despite fluctuations in fluidics and temperature.

Drop delay is defined as the time it takes for a particle to travel from the interrogation point of the trigger laser to the last-attached drop in the stream[5]. The accuracy and stability of the drop delay is crucial to produce effective sorting. The XDP method, called Drop Delay Wizard, requires users to sort with fluorescent beads onto a microscope slide. The user visually confirms the location of the beads and then the drop delay is calculated to 1/100th of a drop. In contrast, the Aria uses an integrated Accudrop™ system using a diode laser and specialized drop delay beads. Users are required to run the beads and execute a sort, then use the fluorescence of the drop delay beads in a digitized image to determine the accurate drop delay. XDP users may have more confidence in their drop delay method since the output requires an actual count to determine a functioning sort. Aria users must infer from digital fluorescence images that the drop delay is accurate. In either case, if the drop delay is set properly, the sort purity between instruments should be comparable for a purity sort mode.

The rate at which particles are sorted per second is termed **sort rate**. The XDP and the Aria provide sort rate statistics by measuring the total number of particles sorted for a given period of time. If both instruments are set to similar EPS, sorting efficiency, and percent of population to be sorted, the sort rate should be similar at low speeds (less than 10K EPS). Theoretically, the XDP and the Aria should achieve the same sort rate for a given sorting assay. At higher speeds, however, lost events and hard aborts produced by the Aria will decrease the sort rate. Therefore, when using the Aria to isolate a specific number of events, the sort time will be longer as compared to the XDP.

Purpose of Study

To compare instrument performance, several sorting assays were developed at BCI to analyze the capabilities of the XDP and the Aria I and II. To determine instrument performance, two populations of beads (3 µm fluorescent and 7 µm blank) were mixed in various concentrations, and sorted at different speeds. Similar conditions were used to analyze biological samples. Lysed blood was sorted for CD19-PE positive events to differentiate biological versus synthetic sorting. A rare-event sort (0.1% of sample population) was performed on whole (unlysed) blood stained for CD4-APC to analyze the effects of a rare-event population on sort statistics. At lower speeds, the XDP and the Aria were similar, but above 20K EPS, the XDP produced fewer lost events and hard aborts, while achieving higher purity and faster sort rates than the Aria I and II.

Methods - Bead Assay

Sample preparation: Samples were prepared with 633 µL 7.06 µm Bangs Laboratory (Fishers, IN) carboxylated beads with a >95% singlet status, 10% w/v beads (4.90 x 10^8 beads per mL) and 1.9 mL Spherotech Ultra Rainbow Beads (Lakeview, IL). The primary sample was then mixed and split into two 1.2 mL micro-centrifuge tubes. The samples were spun at 14K rpm for 30 seconds. Then 933 µL of the supernatant was removed from one tube and the remaining sample was mixed. Dilutions were made by adding 500 µL of DI H_2O (+0.01% NP40) into fifteen 5 mL tubes. Serial dilutions were executed with 1100 µL of the bead mixture transferred to each tube in succession until tube 16 was reached. Samples were kept at 4°C (39°F) and used within one hour. Blank controls were made adding two drops of SpectrAlign Ultra Rainbow beads to 900 µL of DI H_2O in a 5 mL tube, and 10 µL of the Bangs beads to 990 µL of DI H_2O in another 5 mL tube.

Flow cytometry: The XDP and the Aria I and II were set up as described in their instrumentation manuals[4, 5] with calibration done according to manufacturer's specifications. The threshold settings were also kept constant, and the differential pressures set to 1100 EPS ± 100 for the lowest dilution sample. This ensured that both instruments had the same core stream width. Sorting was set up using IntelliSort™ (XDP) or Accudrop™ (Aria) with automatic drop delay.

Data acquisition – Gating and Histogram layout: The histograms were set up as follows: A. FSC-A vs. SSC-A, B. FITC Log-H vs. SSC-H, C. PE Log-H vs. SSC-H, D. FITC Log-H vs. PE Log-H. Gating structure followed with an elliptical gate on the SpectrAlign beads in histogram (A). The population in (A) was gated to (B) and (C). The positive populations in (B) and (C) were then gated to (D) and sorted. The gating strategy utilized XDP methods and was set identically between instruments.

Acquisition set-up: The threshold was set using an FSC trigger to eliminate debris and noise without compromising the singlet bead population. The voltages were set within the accepted PMT voltage range for maximum signal/minimum noise. All other parameters were removed from acquisition to reduce computer processor requirements. Sort decisions were set to default purity; purify with a drop envelope of 1 (XDP) and 4-Way Purity (0-32-0, Aria). The sort was set for continuous acquisition, sorting at least 15K events per sample. Each sort tube had 500 µL of DI H_2O (0.22 µm filtered). Samples were run for 10K events to capture an FCS file before sorting. If a clog occurred, the nozzle was sonicated and/or flushed for 10-20 seconds and the nozzle was replaced. Drop delay was recalculated and the lowest dilution was analyzed to ensure that the EPS were consistent between samples.

Data analysis: Each XDP file was exported as a 16 bit file (a reduction of 16 bits) and imported into FlowJo™ V. 7.2.5 (Treestar, Ashland, OR) for analysis. The Aria data files were exported as FCS 3.0 files and also imported into FlowJo.

Purity: The sorted samples were then removed and DI H_2O was flushed through the system until the FSC-A vs. SSC-A events totaled less than 1%. The scatter gate was enlarged to include all events except debris. The population in the scatter gate was then gated to FITC Log-H vs. PE Log-H. The purity of each sorted sample was determined to be the percentage of the population located in the second gate. Sorted samples were then reanalyzed, and 5K events were collected per sample. Samples were run on both instruments concurrently, using the same bead dilution tube consecutively to remove any variability due to sample preparation. Each experiment was done in triplicate on all instruments. Data was recorded for threshold count, events per second, hard aborts, sort time (seconds), sort rate, conflict count, efficiency and sort count.

Methods - Lysed Blood Assay

Sample preparation: Fresh blood was obtained from human volunteers, collected in EDTA-treated collection tubes, and stored at room temperature for no more than 24 hours. The blood was stained with mouse-anti-human antibodies (Beckman Coulter, Inc., Miami, FL) to CD3-Allophycocyanin (APC), CD4-Fluorescein Isothiocyanate (FITC) and CD19-Phycoerythrin (PE). Control samples were prepared by staining 100 µL of fresh blood with 5 µL of antibody, vortexed gently and incubated for 30 minutes in the dark at 22°C. To each sample, 1 mL of Fix-and-Lyse Versalyse™ (BCI) was added and samples were incubated for an additional 10 minutes in the dark at 22°C. Tubes were then centrifuged for 5 minutes at 300 g. The supernatant was removed and the remaining pellet was resuspended in 1 mL of 1% HAB (HBSS, 0.01% NaN3, 1 % BSA, 500 µmol/L EDTA) and centrifuged for an additional 5 minutes at 300 g. Finally, the supernatant was removed; the pellet was resuspended in 1 mL of 1% HAB, and placed at 4°C until analyzed. Multi-color samples were prepared in a similar manner in a much higher volume to allow for increased sample concentration. Four mL of fresh whole blood was added to four 50 mL conical tubes and incubated with 25 µL of each antibody, (CD3-APC, CD19-PE, and CD4-FITC). The samples were vortexed and incubated

at room temperature for 30 minutes in the dark at 22°C. Then 4 mL of Fix-and-Lyse Versalyse (BCI) was added to each tube, vortexed gently, and incubated for an additional 10 minutes. After centrifugation for 5 minutes at 300 g, the remaining pellets were washed by centrifugation with 4 mL of 1% HAB. Finally, the samples were pooled into 1 mL of 1% HAB and transferred to a 5 mL tube. If any red residue remained in the pellet, an additional 5 mL of Versalyse (BCI) was added; samples were incubated for 10 minutes, and were washed as previously mentioned. The sample was diluted in a 1:2 ratio for 5 samples by first adding 500 µL of 1% HAB to four 5 mL tubes. From the 1000 µL multi-color sample, 500 µL was used in serial dilution, mixing with 1% HAB before continuing to the next tube. All samples were stored at 4°C until run on the flow cytometer.

Flow cytometry: The XDP and the Aria I and II were set up as described in their instrumentation manuals[4, 5] with calibration done according to manufacturer's specifications.

Data acquisition – Gating and histogram layout: Histograms were set-up as follows (see Figures 4 and 5): A. FSC-A vs. SSC-A, B. FITC Log-H vs. SSC-H, C. PE Log-H vs. SSC-H, D. PE Log-H vs. PE Log-A. Gating structure followed with lymphocytes in (A) gated to (B). The CD4-FITC negative cells in (B) were gated to (C), the CD19-PE positives from histogram (C) were gated to (D), the sort gate. This structure removed any possible aggregates which would compromise purity. The gating strategy utilized XDP methods and was set identically between instruments.

Acquisition set-up: The threshold (3% on the XDP, 7,500 on the Aria) was set using a FSC trigger to eliminate debris and noise without compromising the sort population. The window extension on the Aria was set to 2 µs, which is the standard extension suggested by BD FACSAria II User's Guide[4]. Sorting was set up using IntelliSort (XDP) or Accudrop (Aria). Unstained and single control samples were run for 10K events to adjust the FSC and SSC so all lymphocyte populations were on scale. Automatic compensation was conducted with the control samples, CD3-APC, CD4-FITC, and CD19-PE.

Data analysis: Each XDP file was exported as a 16-bit file (a reduction of 16 bits) and imported into FlowJo V. 7.2.5 (Treestar, Ashland, OR) for analysis. The Aria data files were exported as FCS 3.0 files and also imported into FlowJo.

Sort: The lowest dilution sample was analyzed and the differential pressure adjusted so that the sample was running 7K EPS ± 500. The pressure was constant throughout the sort test. If a clog occurred, the drop delay was recalculated and the lowest dilution was analyzed to ensure that the EPS were consistent between samples. The CD19-PE positive population was between 2.8% and 3% (the XDP and the Aria used the same sample per trial). The sort settings were defined as purify 1 (XDP) and 4-way purity (Aria) to obtain the highest purity. Events were sorted continuously into 5 mL tubes with 500 µL 1% HAB into each sort tube. The samples were run for 30 seconds to stabilize EPS before sorting 75K cells per sample. After each sort, the tubes were vortexed and stored at 4°C until all samples were completed. After sorting, the instruments were rinsed with DI H_2O through the sample line for approximately 1.5 minutes until the events in the FSC vs. SSC were less than 1% of the total events. Sort statistics were recorded for data analysis.

Purity: To determine purity, the FSC-A vs. SSC-A gate was enlarged to include all events except debris. The (A) population was then gated to (D). PE Log-H vs. PE Log-A. Sorted samples were then reanalyzed for 5K events per sample and the sample purity was recorded using histogram (D).

Methods - Whole Blood Assay

Sample preparation: Fresh blood was obtained from human volunteers and collected in EDTA-treated tubes and stored at room temperature for no more than 24 hours. The blood was stained with mouse-anti-human antibodies (Beckman Coulter, Inc., Miami, FL) to CD3-APC, CD41-FITC for platelets, and CD135-PE for erythrocytes. Four samples were prepared for analysis: unstained, CD3-APC, CD41-FITC, CD135-PE and CD3/CD41/CD135. Each tube contained 1 mL of fresh blood and 20 µL of corresponding antibody was added. The samples were incubated for 30 minutes in the dark at 22°C. To fix the antibody to the cells, 1 mL of IOTest® 3 Fixative Solution (BCI) was added and the samples were incubated for 10 minutes in the dark at 22°C. Tubes were then centrifuged for 5 minutes at 300 g. The supernatant was removed and the remaining pellet resuspended in 1 mL of 4% HAB and centrifuged for an additional 5 minutes at 300 g. The supernatant was then aspirated and the pellet was resuspended in 1 mL of 4% HAB and placed at 4°C until analysis. Single color control samples were also prepared following the previous procedure except the antibody addition was reduced to 5 µL added to 100 µL of fresh blood and fixed with 100 µL of IOTest® 3 Fixative Solution. Dilutions of the whole blood sample were prepared by placing 600 µl into six 5 mL tubes. One mL of the multi-colored sample was added to 600 µL 4% HAB and used for serial dilution. For accurate flow rate determination, 200 µL Dako CytoCount™ beads (Dako, Glostrup, Denmark) were added to the lowest dilution. All samples were stored at 4°C until analyzed on the flow cytometer.

Flow Cytometry: The XDP and the Aria I and II were set up as described in their instrumentation manuals[4, 5] with calibration done according to manufacturer's specifications.

Data acquisition: Gating and histogram layout: Histograms were set-up as follows (see Figures 7 and 8): A. FSC-H vs. SSC-H, B. APC-Log vs. SSC-H, C. FITC-Log vs. SSC-H, D. PE-Log vs. SSC-H, E. PE-Log vs. APC Log, F. CytoCount beads (labeled PerCP or RPC-Cy7) vs. SSC-H. In log fluorescence gating, there are slight differences in Height and Area parameters, but it does not change sort population statistics significantly. Gating structure followed with leukocytes gated from (A) to (B). The CD3-APC positive population in (B) was gated to (C) while the dimmer CytoCount bead population was gated to (F). The CD41-FITC negative population in (C) was gated to (D). The CD135-PE negative population in (D) was gated to (E), the sort gate. In (F), the CytoCount beads were visible and a region was drawn to identify and quantify them. The gating strategy utilized XDP methods and was set identically between instruments.

Acquisition set-up: The threshold (1% on the XDP, 5,000 on the Aria) was set using an FSC trigger to eliminate debris and noise without compromising the sort population. The window extension on the Aria was set to 2 µs, which is the standard extension suggested by BD FACSAria II Users Guide[4]. Sorting was set up using IntelliSort (XDP) or Accudrop (Aria). Unstained and single control samples were run for 10K events to adjust the FSC and SSC so all populations were on scale. Automatic compensation was conducted with the control samples, CD3-APC, CD41-FITC, and CD135-PE.

Data analysis: Each XDP file was exported as a 16-bit file (a reduction of 16 bits) and imported into FlowJo V. 7.2.5 (Treestar, Ashland, OR) for analysis. The Aria data files were exported as FCS 3.0 files and also imported into FlowJo.

Sort: The lowest dilution samples were run and the differential pressures adjusted so that the number of CytoCount beads analyzed for 60 seconds was equivalent on each instrument (approximately 10K EPS). The differential pressure was constant throughout the sort tests. If a clog occurred, the drop delay was recalculated and the lowest dilution was analyzed to ensure that the EPS were consistent between samples. The CD3-APC positive population in histogram (E) was 0.1% of the total population. The XDP and the Aria used the same sample per trial. Sort settings were defined as purify 1 (XDP) and 4-way purity (Aria) to obtain the highest purity. Events were sorted continuously into 5 mL tubes with 500 µL 1% HAB in each sort tube. The sample was run for 30 seconds to stabilize EPS before sorting 5K cells per sample. After sorting, the tubes were vortexed and stored at 4°C. The instruments were then rinsed with DI H_2O through the sample line until the events in the FSC vs. SSC were less than 1% of the total events (approximately 10 minutes). Sort statistics were recorded for data analysis.

Purity: To determine purity, the FSC-H vs. SSC-H gate (G) was enlarged to include all events except debris. The (G) population was then gated to (H), PE-Log vs. APC-Log. Five thousand events from each sorted sample were reanalyzed, and the sample purity was recorded using histogram (H). (See Figures 7 and 8.)

Calculations

Expected events per second = (Starting EPS/dilution factor)
Calculated events per second = (Event count/analysis time)
Hard abort rate = (Hard aborts/acquisition time)
Sort rate = sort count/(sort count+conflict count)
Expected sort rate = % sorted region * expected EPS * efficiency

Results

Throughout the three sorting tests: bead, lysed blood, and whole blood; the XDP sorted up to three times faster than the Aria. Purity remained similar between instruments until speed 50K EPS. The XDP produced 5-10% higher purity at these higher speeds. The Aria aborted up to 18% of the sample throughout these sorting tests. The XDP had fewer aborts (1%) up to 100K EPS. Overall, samples were sorted much faster resulting in better purity on the XDP versus the Aria.

Results – Bead Assay

Lost events: The XDP has a significantly shorter analysis window (pulse width) than the Aria[7,8]. Therefore, the XDP is able to individually interrogate more events than the Aria and reduce lost events. During the bead sort, the XDP lost 9% of events at 30K EPS. The Aria lost the same percentage of events at 9K EPS. At 65K EPS, the XDP lost 18% of events, and the Aria lost 63%. (See Figure 2A.)

Hard Aborts: The XDP produced 0.54% hard aborts at 24K EPS and 0.7% hard aborts at 83K EPS. The Aria, however, produced 0.64% hard aborts at 5K EPS and increased to 18.6% hard aborts at 57K EPS. (See Figure 2B.)

Purity: The XDP achieved higher purity than the Aria on all sorted samples. The discrepancy was more evident as the speed increased. The XDP produced 98% ± 0.88% purity at 50K EPS. The Aria produced 94% ± 1.38% purity. (See Figure 2C.)

Sort Rate: The expected sort rate was calculated, and the actual sort rate for both the XDP and the Aria were compared against it. The sort region and pressure differential were kept constant between the instruments. The resulting efficiency for both instruments was equivalent for all samples. Therefore, the difference in sort rate between instruments defines the difference in sort speed. The XDP sorted up to 1K Sorts Per Second (SPS), and the Aria sorted 500 SPS. (See Figure 2D.) This indicates, for a specific sample, that the XDP can sort twice as fast as the Aria.

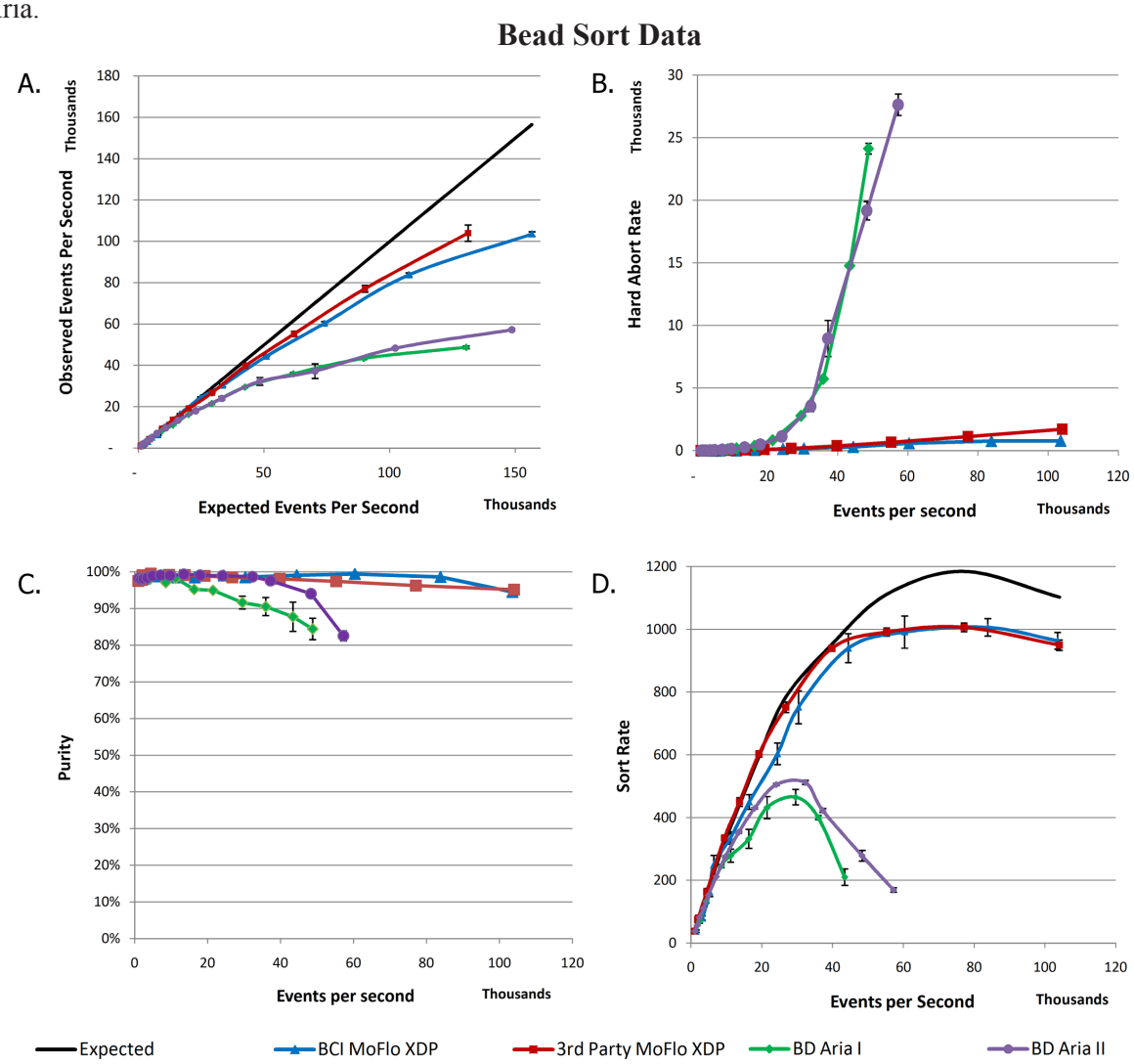

Figure 2. Bead Sort Results. A) The observed EPS graphed vs. the expected EPS. B) The hard abort rate vs. EPS. C) Purity of bead sorted sample vs. EPS. D) Sort rate vs. EPS.

Results – Lysed Blood Assay

Lysed blood was sorted for the CD19-PE positive population at five different speeds producing results similar to the bead sort. Sort protocols from both the XDP and the Aria are represented in Figures 4 (XDP) and 5 (Aria).

Lost events: Lost events were evident as the speed increased to an expected 47K EPS. The XDP lost 7% ± 3.2% and the Aria lost 20% ± 8% of the total events, measured in three separate trials. At the highest speed (85K EPS expected), the XDP lost 7% ± 2%, whereas the Aria lost 39% ± 1.6% of the total events. (See Figure 3A.) The standard error was higher for the lysed blood assay due to biological variation in blood samples.

Hard Aborts: The XDP produced fewer hard aborts than the Aria for the same samples. At 24K EPS, the XDP generated a hard abort rate of 0.28% ± 0.08%, whereas the Aria produced 6.1% ± 1.2%. (See Figure 3B.) When samples were run at higher speeds (85K EPS expected), the XDP produced 0.25% ± 0.08%, while the Aria was higher at 29% ± 0.6%. The Aria loses almost twenty times more events to hard aborts than does the XDP. When lost events as well as hard aborts are combined for total sample recovery, the

XDP has 60% higher recovery at an expected 85K EPS.

Purity: When the samples were run at 24K EPS, the XDP sort purity was 99% ± 0.7%, in contrast the Aria achieved 93% ± 0.5% sort purity. (See Figure 3C.) When the most concentrated sample was sorted, the XDP achieved 97% ± 1.4% purity at 80K EPS. The Aria's sort purity was 83% ± 7.3% at 52K EPS. The XDP produced higher purity than the Aria throughout the lysed blood sort.

Sort Rate: The XDP and the Aria produced similar sort rates for the same EPS, efficiency, and percent of sorted population, until speed reached 20K EPS. When speed exceeded 20K EPS, the sort rate for the XDP increased up to 2.5 times faster than the sort rate for the Aria. (See Figure 3D.) The XDP sorted for 50 minutes to recover the CD19-PE positive population from each sample in the lysed blood experiment, 1.3 times faster than the Aria that required 67 minutes.

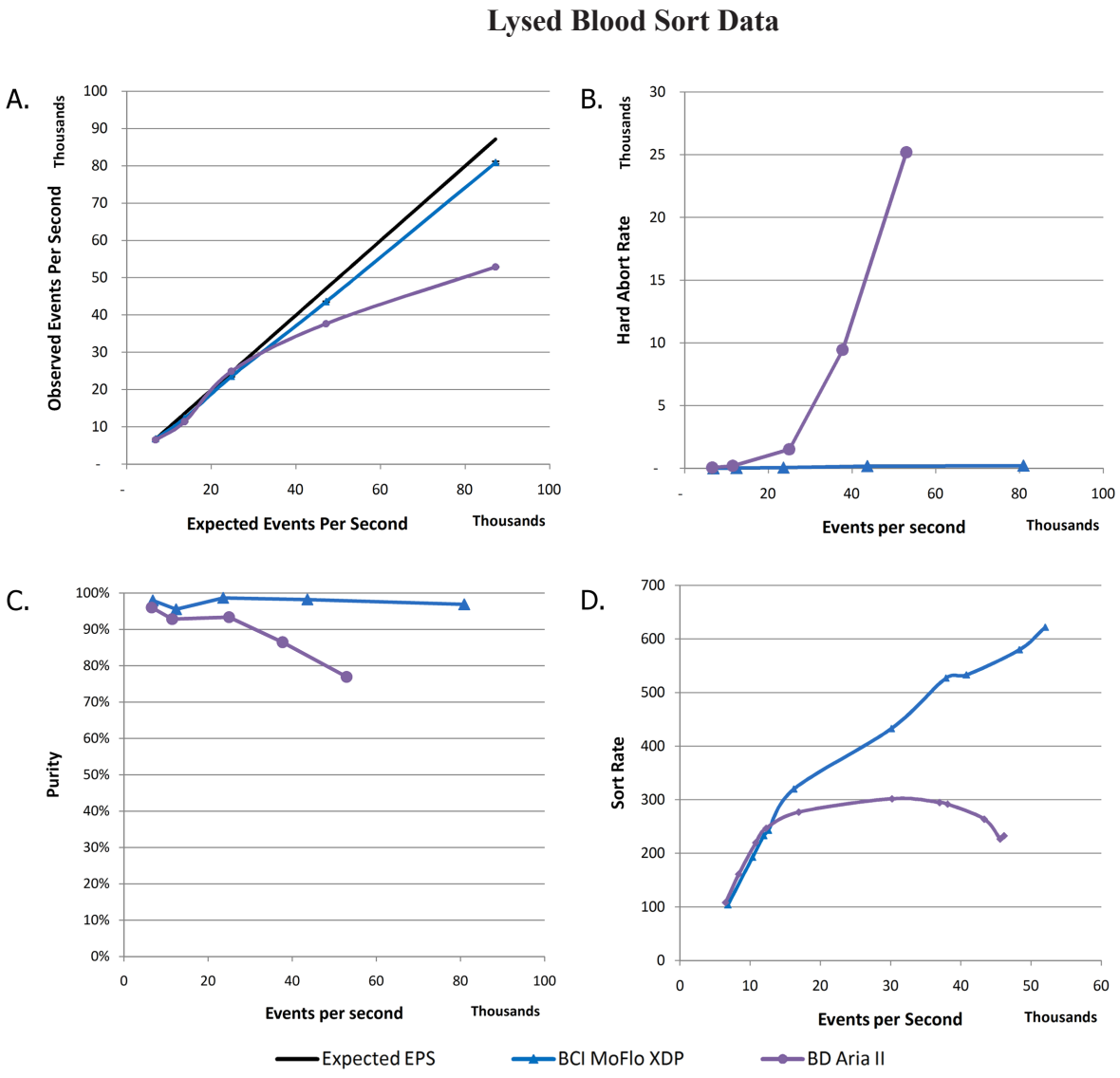

Figure 3. Lysed Blood Sort Results. (A) Expected EPS vs. observed EPS. (B) Observed EPS vs. hard abort rate. (C) EPS vs. purity of lysed blood sort. (D) EPS vs. sort rate with efficiency, EPS, and percent sorted compared between instruments.

Lysed Blood Sort Histograms - MoFlo XDP

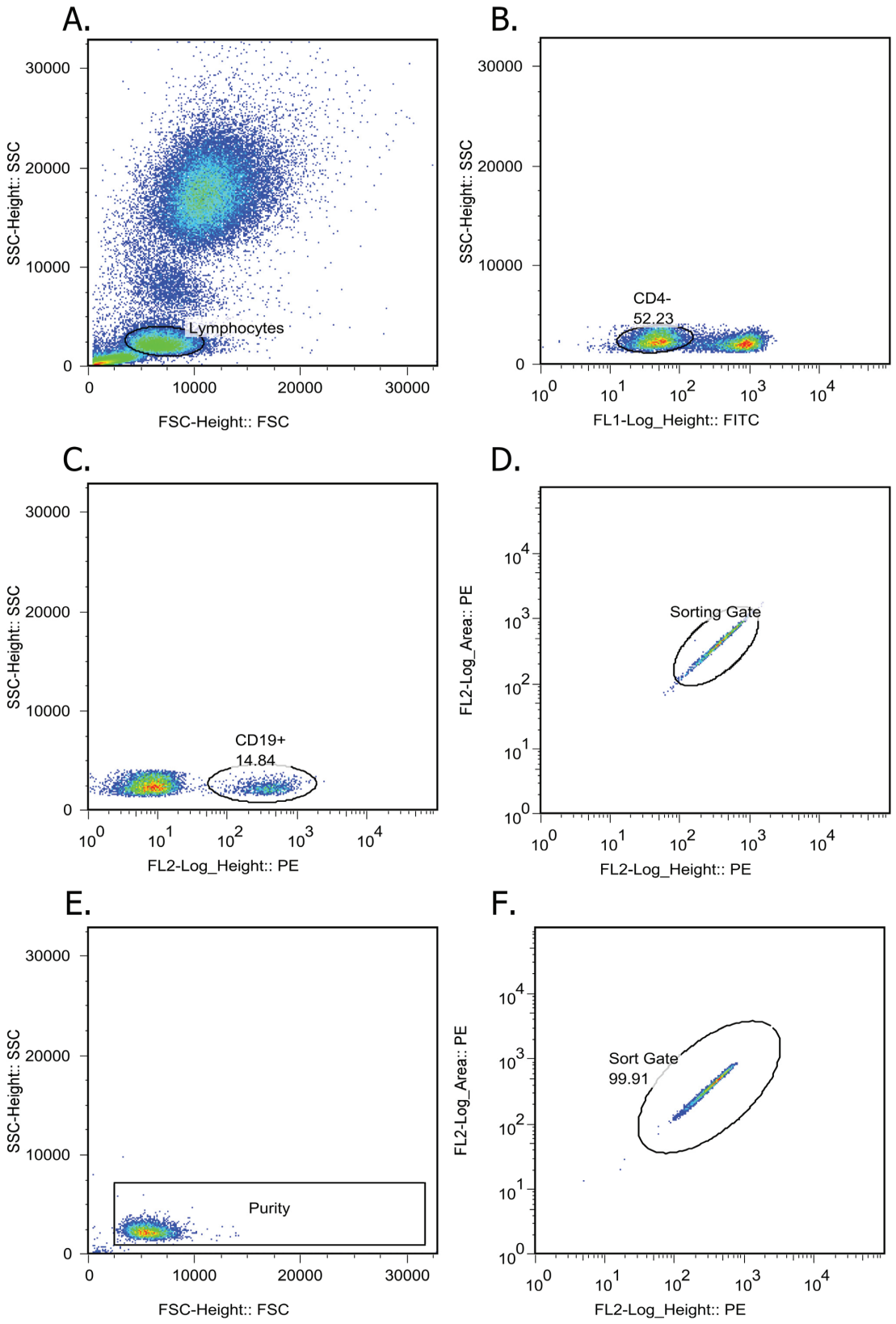

Figure 4. Lysed Blood Sort on the MoFlo XDP. Lysed blood sort gating scheme from (A) to (B), CD4-FITC negative cells were gated to (C) and then to (D). After sorting, the population was rerun with (E) to (F) with purity stated on the histogram of 99.91%. (The above example demonstrates the gating and reanalysis scheme using the 7K EPS sort.)

Lysed Blood Sort Histograms - Aria II

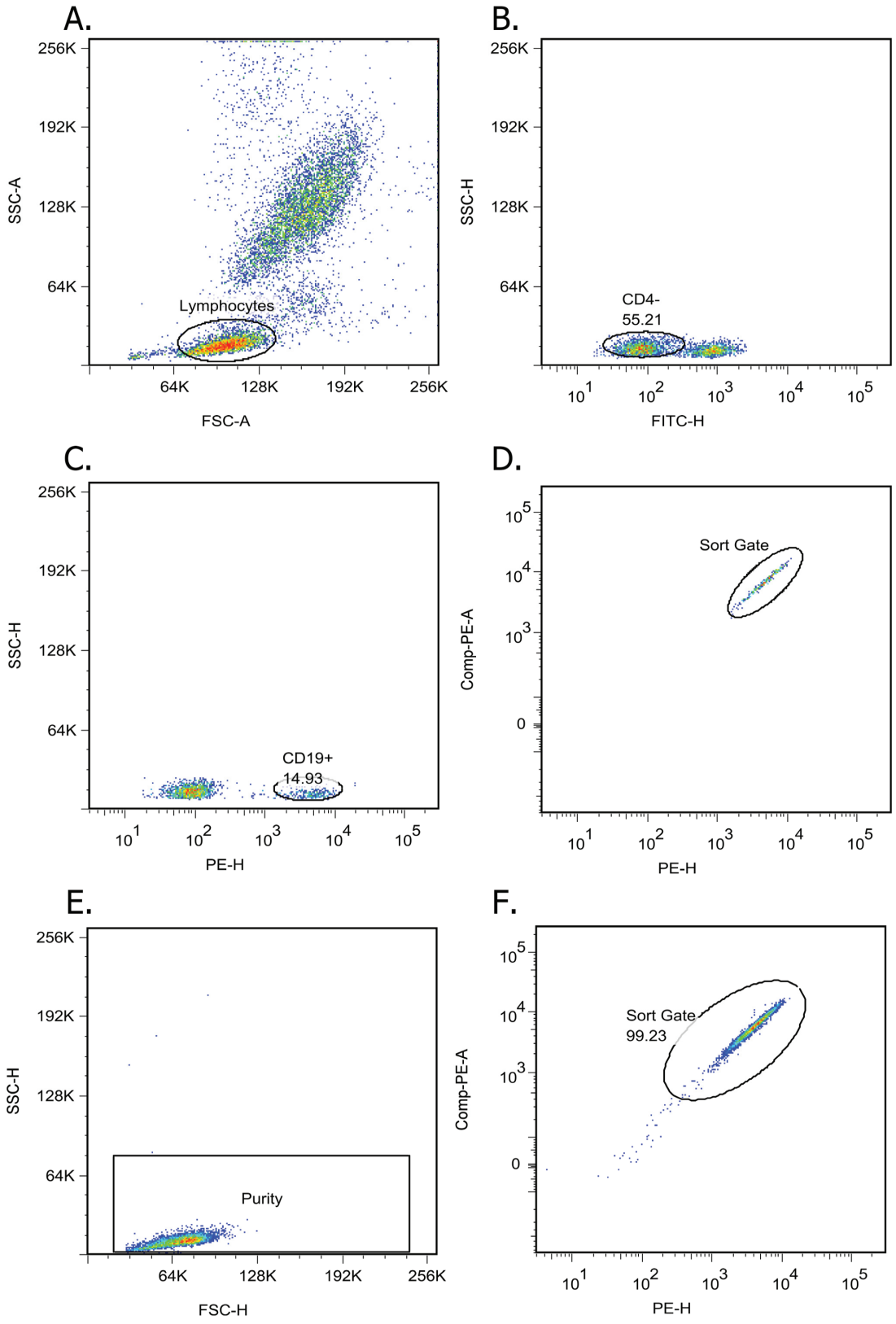

Figure 5. Lysed Blood Sort on the Aria II. Lysed blood sort gating scheme from (A) to (B), CD4-FITC negative cells were gated to (C) and then to (D). After sorting, the population was rerun with (E) to (F) with purity stated on the histogram of 99.40%. (The above example demonstrates the gating and reanalysis scheme using the 7K EPS sort.)

Results – Whole Blood Assay

The whole blood assay was designed to demonstrate a rare population sort that comprised 0.1% of the total sample. Sort protocols for the whole blood sort for 10K EPS are demonstrated in Figures 7 (XDP) and 8 (Aria).

Hard Aborts: As in the bead sort and lysed blood sort, the XDP produced a much lower hard abort rate than the Aria at all speeds. At 10K EPS, the lowest speed sort, the XDP measured 0.01% ± 0% hard aborts, while the Aria measured 1% ± 0%. (See Figure 6B.) At 62K EPS, the XDP measured 0.01% ± 0% hard aborts; in contrast to the Aria that measured 36% ± 4% hard aborts. The Aria loses incrementally more sample as the speed increases.

Purity: Although both instruments were set to identical sort modes, the XDP purity (99% ± 0.1%) was higher than the Aria purity (94% ± 0%) on the initial sample run at 10K EPS. This difference in purity increased to 17% ± 5% when the most concentrated sample was run on the Aria at 62K EPS. (See Figure 6C.)

Sort Rate: The sort rate is dependent on the variability of CD4 in the sample; therefore sort rates could not be pooled for an average. Because sort efficiency, EPS, and percent sorted were constant, the sort rate between the instruments could be compared. When sorting on 0.1% of the sample at 10K EPS, the XDP sort rate was 6.7 Sorts per Second (SPS). (See Figure 6D.) The Aria, running the identical sample, sorted 1.7 SPS. As the speed increased to 48K EPS, the XDP sorted at 10 SPS, whereas the Aria sorted at 2.5 SPS. Using this assay, the XDP collected 5K events in 53 minutes, 3.7 times faster than the Aria that required 3 hours and 20 minutes.

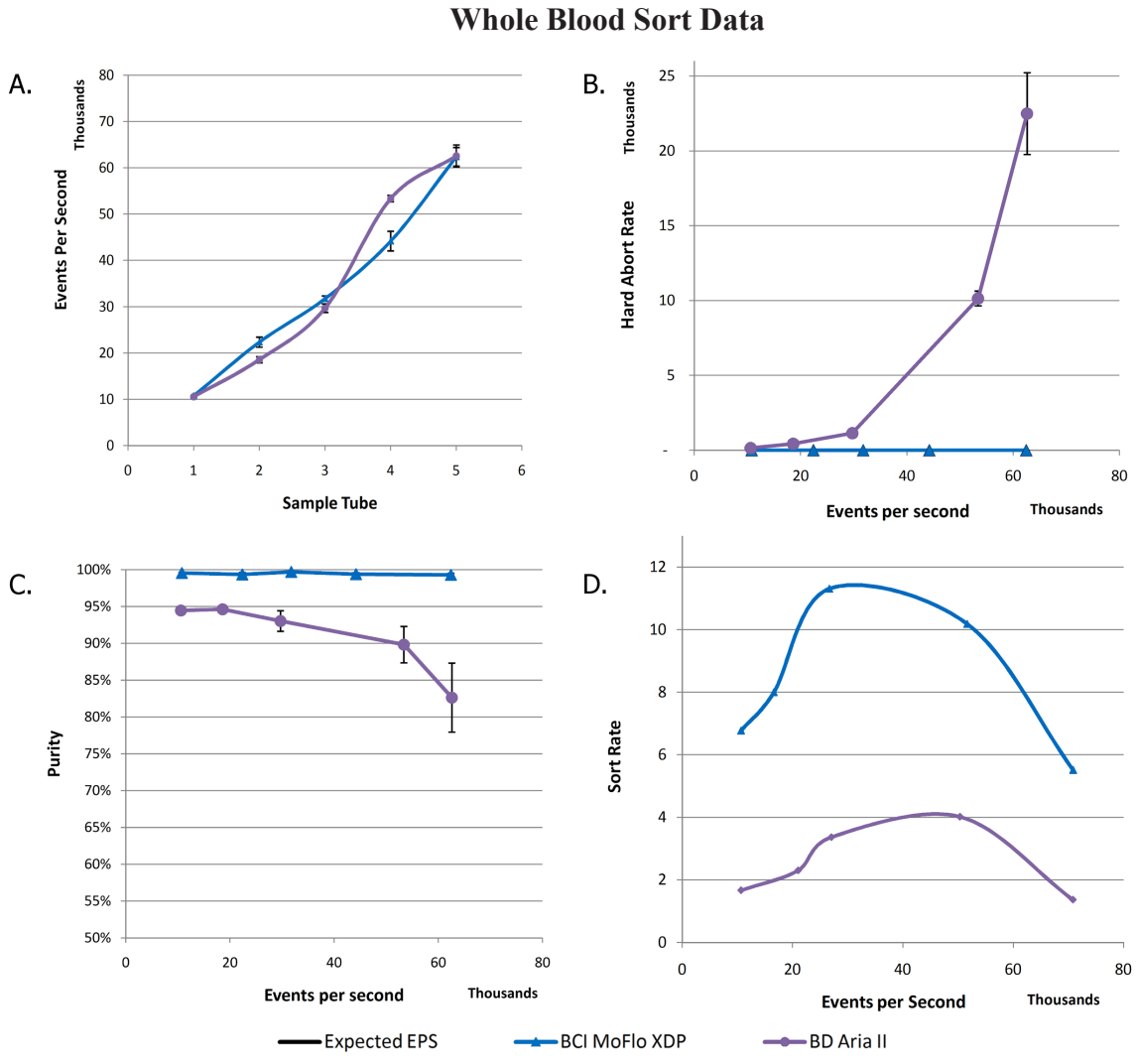

Figure 6. Whole Blood Sort Data. A) Sample tube (in increasing concentrations) vs. EPS. B) EPS vs. hard abort rate. C) EPS vs. purity. D) EPS vs. sort rate.

Whole Blood Sort Histograms - MoFlo XDP

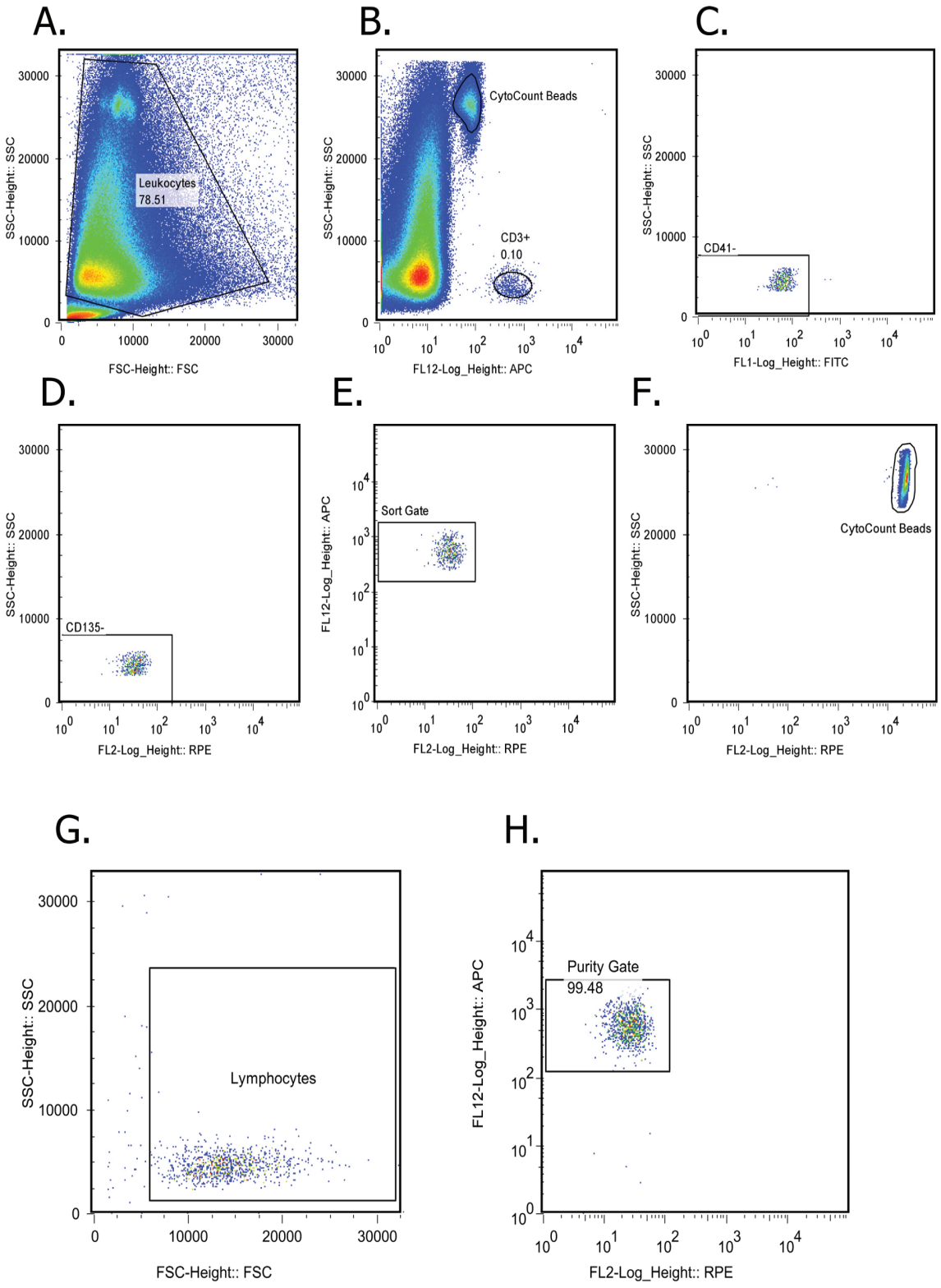

Figure 7. Whole Blood Sort on the MoFlo XDP. Cells not included in (A) were gated to (B), which included two separate populations of CD3-APC positive and CytoCount Beads. The CD3-APC positive population was then gated to (C) with the CD41-FITC negative population gated to (D) in which the CD135-PE negative population was gated to the sort gate (E). The CytoCount beads were analyzed on the RPE-Cy7 channel on the XDP (F). Purity was determined by gating from (G) to (H) with enlarged scatter gates and sort gates showing 99.48% purity.

Whole Blood Sort Histograms - Aria II

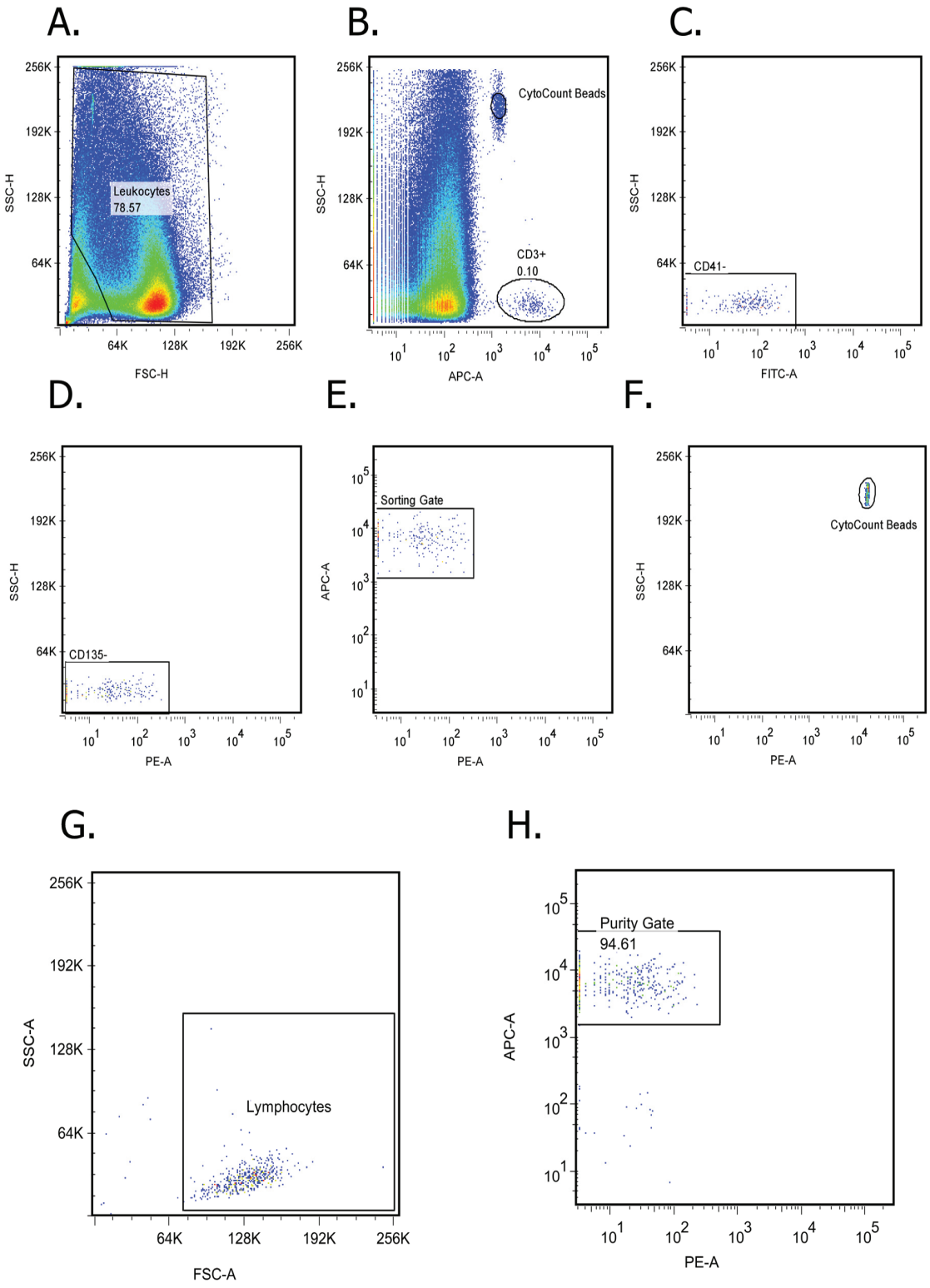

Figure 8. Whole Blood Sort on the Aria II. Cells not included in (A) were gated to (B), which included two separate populations of CD3-APC positive population and CytoCount Beads. The CD3-APC positive population was then gated to (C) with the CD41-FITC negative population gated to (D) in which the CD135-PE negative population was then gated to the sort gate (E). The CytoCount beads were analyzed on the Per-CP channel on the Aria (F). Purity was determined by gating from (G) to (H) with enlarged scatter gates and sort gates showing 94.61% purity.

Conclusions

The XDP and the Aria have different electronic acquisition structures and sample delivery methods. The XDP uses a narrow acquisition window (based on pulse width) to detect events and delivers sample through jet-in-air. The Aria uses a wider window as well as a pulse width extension and delivers sample through a cuvette[8].

Theoretical calculations[7] indicate that the pulse width of the Aria is almost five times longer (3.96 µs versus the XDP 0.82 µs) for a 13 µm cell, and is even wider when used with the window extension recommended for multi-laser analysis. An increased acquisition window is detrimental to doublet discrimination. A shorter window and pulse distinguishes each event separately giving a more accurate measurement of event count and speed.

Lost events

Pulse width becomes significant to instrument performance as speed and sample concentration increase. At speeds faster than 20K, the XDP measures more particles for the same core stream width and flow rate than the Aria. As speed increases, the Aria loses events possibly due to the wider acquisition window[8]. The XDP begins to lose events at higher speeds, but at a smaller percentage than the Aria. It is possible that a percentage of lost events is due to fluidic changes caused by more concentrated samples. During higher speed sorts, the XDP captures more distinct events than the Aria and, as a result, analyzes a greater percentage of sample.

Hard abort rate

During multi-laser analysis, adjustments to the pulse width must be made to account for changes in particle velocity. The XDP uses a "sliding-window" method to detect events across all three laser detection pinholes. This sliding window maintains pulse width integrity while permitting precise measurements through all laser lines[8]. In contrast, the BD FACSAria II User's Guide[4] recommends that users implement a fixed 2 µs window extension when performing multi-laser analysis. Time added to the acquisition window is intended to help the Aria detect the signal from all laser lines. In theoretical calculations, the resulting pulse width increases from 3.96 µs to 5.96 µs for a 13 µm cell, making the pulse width seven times longer than the XDP pulse width. When samples are run at higher EPS, the hard abort rate increases dramatically for the Aria. This may be because the number of particles within one analysis window increases, and therefore they are aborted. Aria users may adjust the window extension, but sample resolution may suffer.

Purity

The purity of the sorted sample is a result of several factors: sort set-up and drop delay, EPS, and sort decisions. The XDP and the Aria demonstrated comparable sort purity up to 20K EPS, but as the sort speed increased, the XDP achieved higher sample purity than the Aria in all assays. Because the sort modes for both instruments were set equivalently, the purity discrepancy may be explained by contaminants, or lost events, undetected in the sample. Lost events contaminate the sorted sample because they are undetected by acquisition electronics and can pass through to the sorted sample. As the speed increases, the effect of pulse width becomes more significant because more lost events are undetcted and contaminate the sorted drops. The XDP sort purity is higher at faster speeds due to narrower pulse width and no window extension. Therefore, XDP can deliver better sort purity than the Aria at event rates above 20K EPS and faster.

Efficiency

Sorting efficiency was equivalent between instruments. This indicates that the sort mode and drop envelope (or sort mode and masks for the Aria) were set in a comparable manner with the same sample flow rate throughout all sample concentrations. This reconfirms that the protocol development and testing process were performed accurately.

Sort Rate

The sort rate for the XDP was much higher than the Aria at speeds greater than 20K EPS. With EPS, sorting efficiency, and percent of sorted population similar between instruments, **the XDP sorts up to three times faster than the Aria**. The faster sort rate on the XDP is due to the instrument's lower number of lost events and hard aborts at higher speeds. Although the sort rate for the XDP and the Aria was similar at lower speeds, the XDP excels at sorting faster at event rates above 20K EPS and up to 80K EPS. For the whole blood sort (0.1% sorted population), the XDP was 3.7 times faster than the Aria. The XDP required 53 minutes to sort, whereas the Aria required 3 hours and 20 minutes. For the lysed blood sort (2.8% sorted population), the XDP was 1.3 times faster with a sort time of 50 minutes in contrast to the 67 minutes required on the Aria. In all sort assays, the XDP achieved a faster sort rate than the Aria at speeds above 20K EPS.

Summary

At lower speeds, the XDP and the Aria demonstrated similar performance. However, at speeds above 20K EPS, the XDP produced fewer lost events and hard aborts, while achieving higher purity and faster sort rates than the Aria I and II.

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