Reproducibility of a 10-color flow cytometry panel through transfer of application-specific target values from the BD FACSCanto™ Flow Cytometer to the BD FACSLyric™ Flow Cytometer

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Abstract

Despite rapid advances in flow cytometry, variability and inconsistency of data generated on separate cytometers remain a key challenge in the field. Cross-standardization of flow cytometers, achieved through transfer of application-specific target values, allows consistent and reproducible results across instruments irrespective of their institutional or geographical location.

In this study, we demonstrate the transfer of application settings from the BD FACSCanto™ Flow Cytometer to the BD FACSLyric™ Flow Cytometer, enabling convenient transfer of a 10-color panel between instruments. First, the BD FACSCanto™ Flow Cytometer was set up and characterized using the BD® Cytometer Setup and Tracking (CS&T) beads. Second, the desired application settings were applied and median values for each of the 10 fluorochromes were assessed using BD® FC Beads. Following setup and characterization of the BD FACSLyric™ Flow Cytometer, BD® FC Beads were sequentially acquired while adjusting the photomultiplier tube (PMT) voltages until each median value matched the corresponding values assessed on the BD FACSCanto™ Flow Cytometer.

Finally, 'Tube Settings' were created on the BD FACSLyric™ Flow Cytometer to save as a part of an assay. Importantly, the tube settings generated on the BD FACSLyric™ Flow Cytometer could be electronically imported to two additional BD FACSLyric™ Flow Cytometers, allowing transfer of settings between them regardless of BD® CS&T Bead lot number or location of the cytometer. A sample of blood stained with the 10-color panel and acquired on the BD FACSCanto™ Flow Cytometer and on three BD FACSLyric™ Flow Cytometers demonstrates consistency of results following standardization.

Materials & Methods

Materials

- BD FACSCanto™ Flow Cytometer
- BD FACSLyric™ Flow Cytometer
- BD FACSDiva™ CS&T Beads
- BD® CS&T IVD Beads or BD® CS&T RUO Beads, as appropriate for each instrument platform
- BD® FC Beads for each fluorescent channel on the flow cytometer
- FC Bead Dilution Buffer

Methods

- Apply Application settings on the BD FACSCanto ™ Flow Cytometer
- Acquire target Values on the BD FACSCanto™ Flow Cytometer
- Transfer Target Values to the BD FACSLyric™ Flow Cytometer
- Create Tube Settings on the BD FACSLyric™ Flow Cytometer
- Transfer settings between BD FACSLyric™ Flow Cytometers

Results

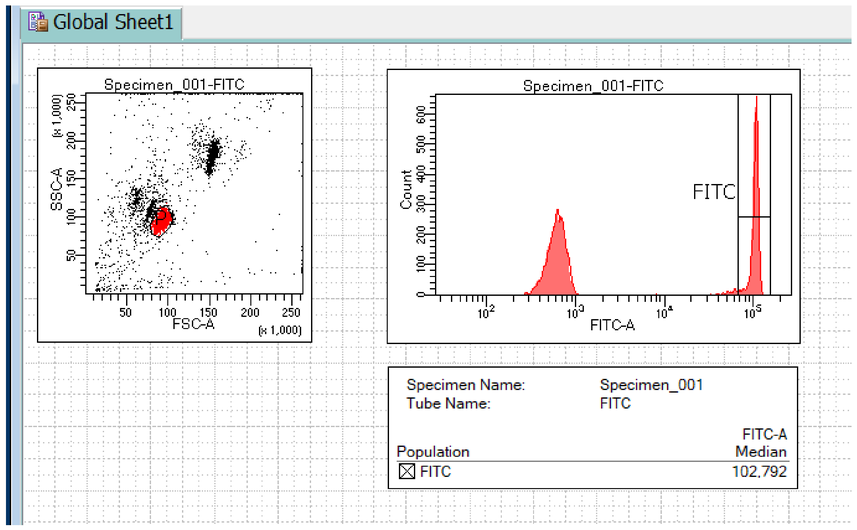

Figure 1. Target values acquired on the BD FACSCanto™ Flow Cytometer. First, a polygon gate was utilized to include bead population of interest. Second, a histogram for each fluorescence parameter to be measured was created. Prepared BD® FC Beads were recorded to include at least 10,000 gated events for each fluorochrome. Finally, an interval gate drawn on the bright bead population on each histogram was used to display the median value for each fluorescence parameter using statistics view. These median values were the target values for each fluorochrome for transferring to the BD FACSLyric™ Flow Cytometer.

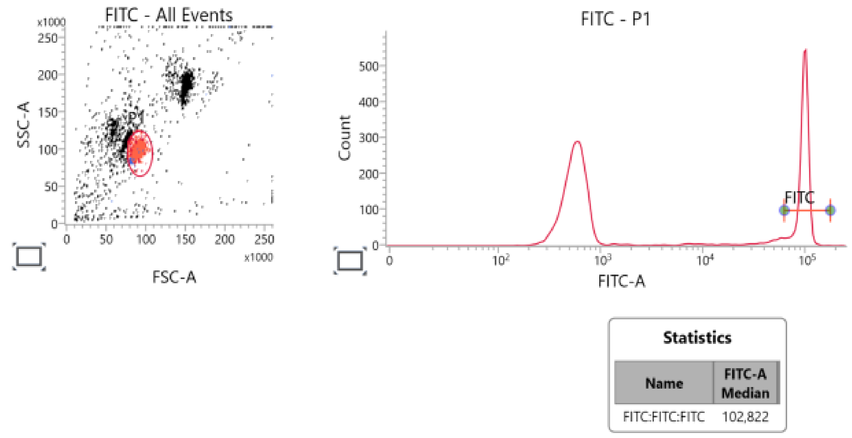

Figure 2. Target value transferred to the BD FACSLyric™ Flow Cytometer. Following instrument setup and daily QC, BD® FC Beads were previewed on a medium flow rate and PMT voltages (PMTV) were adjusted until the bright bead median value matched the corresponding bright bead median target value from the BD FACSCanto™ Flow Cytometer and 10,000 gated events were acquired. For each fluorochrome to be transferred, a new tube was labeled with the name of the fluorochrome, and the acquired tube was duplicated without data to carry over the adjusted PMTV. Tube settings were created once all tubes were acquired.

Results

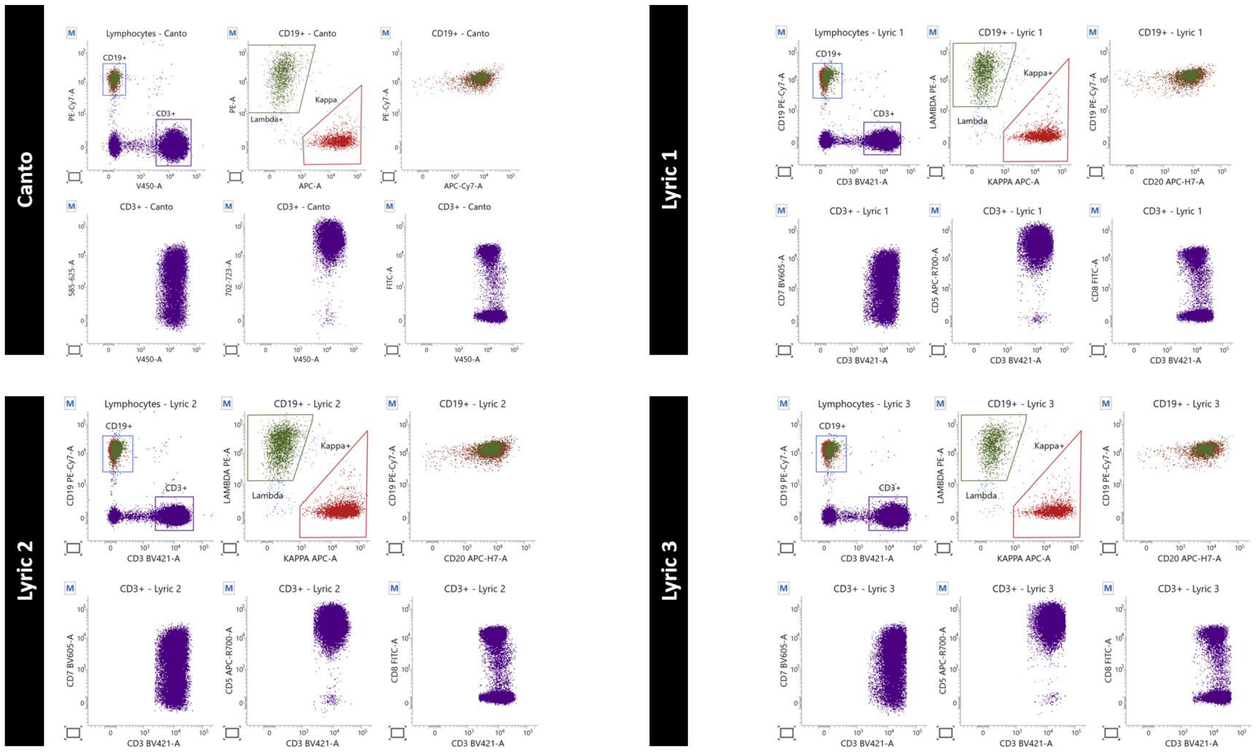

Figure 3. Representative data. Target values were created on BD FACSCanto™ II Flow Cytometer (Canto) and transferred to a BD FACSLyric™ Flow Cytometer (Lyric 1). Using the assay transfer feature in BD FACSuite™ Application, the settings were then transferred to Lyric 2 and Lyric 3 . Data from the BD FACSCanto™ II Flow Cytometer were analyzed in BD FACSuite™ Application for comparison.

Conclusions

- Transfer of application settings from the BD FACSCanto™ Flow Cytometer to the BD FACSLyric™ Flow Cytometer was accomplished.
- A 10-color panel was conveniently transferred from the BD FACSCanto™ Flow Cytometer to the BD FACSLyric™ Flow Cytometer.
- Tube settings were created on the BD FACSLyric™ Flow Cytometer to establish and maintain target values. These settings can be added to any assay.
- The generated tube settings were electronically imported to two additional BD FACSLyric™ Flow Cytometers.
- To view the accompanying application note, use this QR code.