Comparison of BD FACSLyric™ Instrument Performance in a Global Setting

Bieke Soen [1], Jarne Schelpe [2], Miet De Baere [2], Asha Soosapilla [3], Eggy Yeh [4], George Wei [4], Veronica Nash [1], Christopher Rota [1], Ans De Beuckelaer [1] and Nithianandan Selliah [1]

[1]Cerba Research, Ghent, Belgium and New York, USA; [2]Cerba HealthCare Belgium, Division CRI, Ghent, Belgium; [3]Laverty Pathology, Sydney, Australia; [4]CerbACT Asia, Taipei, Taiwan.

Is the Cytometer Setup & QC software module of the BD FACSLyric™ instrument sufficient to monitor instrument performance?

Background

As flow cytometry is a powerful tool to characterize cellular populations, it is critical to have standardized instruments within and across different labs and/or regions for global clinical trials. The Cytometer Setup & QC software in the BD FACSLyric™ instrument should correct for daily fluctuations within one instrument and across instruments using Bright Bead Median Target Values (BBMV). To assess the capability of the software module to standardize flow cytometry assays, we evaluated the Median Fluorescence Intensity (MFI) between instruments and within instruments over time, using both BD® Cytometer Setup and Tracking (CS&T) beads (BD Biosciences) and SPHERO™ Ultra Rainbow calibration particles (Spherotech).

Method

To monitor instrument performance and reproducibility of MFI values, experiments were performed across a total of 15 instruments located in four different countries; Belgium (6), USA (4), Taiwan (2) and Australia (3). A specific lot of two types of calibration beads, CS&T beads (LOT 2091889) and Ultra Rainbow calibration particles (LOT AP03), were chosen to monitor all 12 channels of the BD FACSLyric™ instrument. The experiments using CS&T beads were not performed in Taiwan due to lack of same lot of the reagent.

During the experiments, both types of calibration beads were prepared according to the manufacturer's recommendation and acquired daily for five consecutive days on a total of 15 BD FACSLyric™ instruments. In order to perform the experiments on optimally functioning flow cytometers, acquisition of the beads was always done after a successful performance QC (pQC). To ensure that the resulting MFI values were obtained independently from the built in Setup & QC software module, beads were acquired in experiment mode on the Lyse/Wash (LW) setting, without compensation. Next, data were analyzed using FACSuite™ software for all 12 channels, as shown in Figure 1. For CS&T beads, the MFI value of the positive peak was determined, and for the Ultra Rainbow calibration particles, the MFI of the 5th peak was obtained. Statistical analysis was performed on the resulting MFI values for all 12 channels to evaluate stability of MFI values over time and alignment of MFI values across instruments, using the formulas below in MS Excel:

$$\%CV = \frac{SD}{Mean} \times 100$$

$$\%\ difference = \frac{MFI_{\ reference\ instrument} - MFI_{\ value\ instrument\ of\ choice}}{MFI_{\ reference\ instrument}} \times 100$$

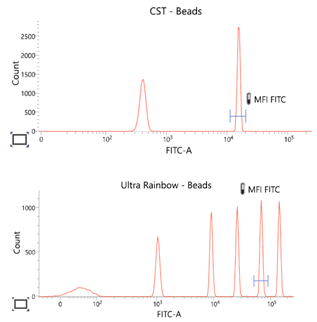
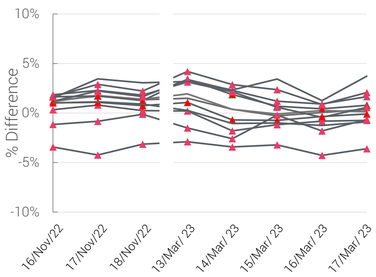


Figure 1: Representative histograms for CS&T beads (top) and Ultra Rainbow beads (bottom). The positive peak for CS&T and 5th peak for Ultra Rainbow beads are chosen for MFI value.

Results

MFI values are stable over time



B

	#264	#293
FITC	0.4%	1.7%
PE	0.2%	1.9%
PerCP-Cy5.5	1.3%	0.9%
PE-Cy7	1.0%	2.0%
APC	2.0%	2.7%
APC-R700	1.8%	0.7%
APC-Cy7	1.4%	2.0%
V450	0.4%	1.2%
V500-C	0.5%	2.1%
BV605	0.2%	2.1%
BV711	1.5%	0.2%
BV786	1.2%	2.8%

Figure 2: MFI values from CS&T beads were collected for two non-consecutive weeks, with a time period of five months in between. Data is displayed as % difference between MFI on the first day (15/Nov/2022) of acquisition and the MFI on the days shown on the graph. Representative data from one instrument is shown (**A**). The influence of a cQC was evaluated in two instruments. The table shows %Difference in MFI values from CS&T beads before and after the execution of a cQC (**B**).

MFI values of 12 channels were evaluated daily for two weeks with a gap of five months in between both weeks. Analysis was performed on all instruments using the same CS&T bead lot. Figure 2A shows representative data for one instrument. All 15 Flow cytometers demonstrated an identical trend, in which % difference is <5% when compared to the MFI on the first day of acquisition. These data show that the CS&T software module of the BD FACSLyric™ corrects for daily fluctuations.

As the execution of a characterization QC (cQC) changes the Bright Bead Median Target values (BBMTV) of LW settings, MFI values were assessed by measuring CS&T beads before and after cQC on two instruments (Figure 2B). %Difference is <5% (maximum is 2.8%), showing the limited influence of cQC on MFI values. Further investigation is needed to assess effects of a Bead Lot Transfer (new CS&T lot), another event that may influence BBMTV. These data will provide further information on the MFI stability across various bead lots, which is necessary for testing samples in long term clinical trials.

Comparison of MFI values between instruments

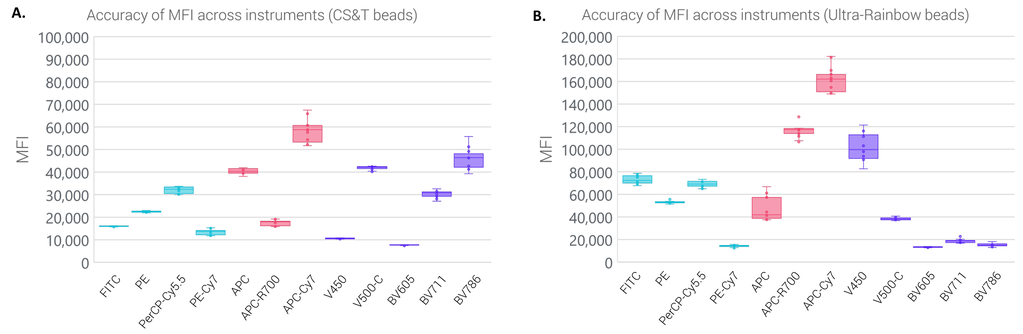


Figure 3: CS&T beads (**A**) and Ultra Rainbow beads (**B**) were acquired on all 15 instruments across the globe. Box and Whisker chart of MFI values show median, 25th percentile, 75th percentile, minimal value and maximal value. Data from all instruments are shown for each of the 12 channels, with channels of the 488 nm laser shown in blue, channels of the 640 nm laser shown in pink, and channels of the 405 nm laser shown in purple.

			% Difference														
	#292 (MFI)	%CV of MFI	US #058	US #061	US #158	US #161	EU #114	EU #052	EU #265	EU #264	EU #292	EU #293	TW #246	TW #248	AUS #020	AUS #099	AUS #353
FITC	78,659	5.14%	14.0%	9.2%	4.0%	1.7%	12.1%	9.7%	10.7%	1.1%	0.0%	2.7%	3.3%	8.8%	10.7%	14.1%	5.5%
PE	53,350	2.13%	1.2%	2.3%	0.5%	0.5%	0.6%	-0.9%	1.4%	1.9%	0.0%	3.6%	1.3%	1.6%	-5.5%	1.9%	3.2%
PerCP-Cy5.5	68,861	3.45%	0.9%	3.1%	-4.8%	-3.1%	2.3%	-4.0%	5.5%	5.6%	0.0%	1.3%	-4.0%	0.1%	-2.4%	3.8%	-0.9%
PE-Cy7	13,838	6.44%	2.2%	1.8%	0.8%	0.5%	-10.1%	-13.2%	5.9%	10.5%	0.0%	-6.7%	-6.4%	-1.8%	-4.7%	7.8%	-1.4%
APC	45,340	21.88%	8.9%	5.0%	13.3%	8.6%	-47.3%	-38.0%	8.3%	8.8%	0.0%	2.3%	17.3%	14.7%	-22.8%	-46.4%	3.3%
APC-R700	116,892	5.90%	6.8%	6.2%	5.1%	3.0%	-10.4%	-10.1%	8.0%	8.8%	0.0%	-1.1%	-0.5%	2.5%	-1.9%	4.3%	0.6%
APC-Cy7	160,621	6.50%	3.1%	4.9%	1.4%	3.1%	-13.5%	-13.0%	7.2%	6.0%	0.0%	-5.8%	-1.9%	3.6%	-0.5%	6.7%	4.7%
V450	97,941	10.84%	-12.8%	5.7%	-16.6%	-4.2%	-13.9%	4.3%	-15.1%	7.3%	0.0%	-23.9%	-1.7%	15.5%	-1.3%	-19.4%	5.8%
V500-C	37,857	2.70%	-1.5%	-1.5%	-4.2%	-1.1%	-5.0%	3.0%	1.3%	-1.0%	0.0%	-1.2%	1.1%	0.4%	-1.6%	-3.6%	-7.8%
BV605	13,016	2.82%	-0.9%	1.9%	-5.7%	-2.1%	-3.9%	2.8%	-3.4%	-1.9%	0.0%	-2.2%	1.5%	-0.5%	-1.1%	-3.8%	-7.8%
BV711	17,780	9.25%	1.7%	6.2%	-7.2%	1.6%	-13.8%	-6.7%	3.1%	3.3%	0.0%	-7.2%	0.0%	3.1%	-29.5%	5.9%	-9.3%
BV786	15,133	9.32%	6.3%	9.1%	-1.9%	4.1%	-19.8%	-6.6%	8.6%	11.5%	0.0%	-8.5%	2.6%	4.6%	-11.2%	13.9%	-3.4%

Table 1: %Difference of all instruments is calculated to reference instrument #292. US = United States of America, EU = Europe (Belgium), TW = Taiwan, AUS = Australia. %CV (Coefficient of Variation) of MFI over all instruments is shown. %Difference/%CV <10%: black; 10%-20%: purple; >20%: pink. Data are from acquisition of Ultra-Rainbow beads.

Next, MFI values were compared between all the instruments. In order to cover different ranges across the MFI spectrum of the cytometers, data from both types of calibration beads were evaluated (Figure 3). Box and Whisker chart analysis reveals a higher variation for MFI values of the APC, APC-Cy7, V450 and BV786 channels compared to other channels. As shown in Table 1 for Ultra Rainbow beads, the variation is further confirmed when reviewing APC and V450, which has a %CV of 21.88% and 10.84%, respectively.

To further investigate which of the instruments are deviating, the %difference was calculated for all instruments using instrument #292 as reference. #292 was chosen as reference because its MFI values were closest to the average of all instruments. For several instruments %difference of more than 20 was observed, most notably on the APC channel. Variation of MFI values across different BD FACSLyric™ instruments were more significant than anticipated. This highlights the importance of selecting instruments with similar MFI values during assay validation and, when possible, incorporating quantification beads for normalization of MFI values.

Conclusion

Evaluation of MFI values across all 12 channels for an extended period shows that the BD FACSLyric™ instrument is capable of generating reproducible results over time. However, the data from calibration beads show that the Setup & QC software module is not able to ensure optimal alignment of MFI values across multiple instruments for all channels. The most significant differences were observed on the APC, APC-Cy7, V450 and BV786 channels. Extensive troubleshooting and discussions with the manufacturer did not reveal any lab-related causes for these deviations. Inherent differences in lasers and detectors, or setup during the installation could be the reason for the seen differences in certain instruments.

These observations underline the importance of including an independent QC step with calibration beads to monitor MFI values across multiple instruments, and to select instruments with similar MFI values during assay validation for global clinical trials.

Cerba Research
www.cerbaresearch.com
Flow Cytometry Science Team
flowcytometry@cerbaresearch.com

Cerba Research can develop and validate customized flow cytometry panels for global clinical trials. Connect with our scientific team to learn how we can enhance your research and develop specific flow cytometry panels.