Automated Instrument Setup and Compensation on the BD FACSLyric™ Flow Cytometer

Casey J Fox,[1] Mamatha Bharadwaj,[1] Dina Huckaby,[1] Maclean Nyachienga,[1] Bao Nguyen,[1]
Rebecca Hershock-Quintana,[1] Darci Gorgone Chavez,[1] Nathan Whizin,[1] and Alan Stall[2]
[1] BD Life Sciences, San Jose, USA and [2] BD Life Sciences, San Diego, USA

Introduction

Multiparameter flow cytometry is used routinely to diagnose or track cell subsets in blood and tissue. As experiments increase in size and complexity, there is a need for instrument solutions that deliver both a streamlined, user-friendly workflow and exceptional performance. Here, we demonstrate the key usability and performance capabilities of the 10-color BD FACSLyric™ platform.

Results

In clinical and research labs, an optimized user workflow may improve efficiency. As shown in **FIGURE 1**, BD FACSLyric users can complete tasks at the click of a button for routine procedures. These include: automated instrument startup and shutdown; automated setup and quality control (QC) with BD™ CS&T beads, and highly precise compensation using BD™ FC beads. In addition, BD FACSuite™ Clinical software includes a menu of HIV IVD assays, automatic analysis capability, as well as preconfigured analysis reports.

To ensure that results are reproducible day to day, between instruments, or between sites, it is critical that cytometer setup is standardized and efficient. The BD FACSLyric system enables users to accurately adjust gains to achieve fluorescence (MFI) target values that are consistent across time. As shown in **TABLE 1**, BD FACSuite software sets BD CS&T beads to target MFI values with high accuracy (a percent difference of less than 0.4% for all parameters). Setup standardization between instruments or across sites also ensures that the same assays or experiments produce equivalent results. When assay settings were imported across six BD FACSLyric cytometers, target MFI values showed high reproducibility and a variance below 10% (**TABLE 2**).

Accurate compensation is essential for proper data analysis. As shown in **TABLE 3**, in a 20-minute procedure performed once every 2 months, BD FACSuite software and BD FC beads enable precise determination of spillover values (SOVs) with standard deviations typically less than 0.25. In addition, SOVs are valid for 60 days with no additional calibration, showing a variation of less than 0.5% over time (**TABLE 4**).

The capability of a flow cytometer to finely resolve dim or rare from bright populations improves data quality. As shown in **TABLE 5**, the BD FACSLyric system delivers improved resolution sensitivity in all detectors.

The standardized setup, accurate compensation, and improved resolution sensitivity of the BD FACSLyric system enables users to acquire high quality 6-color TBNK results using HIV patient and healthy control samples (**FIGURE 3**).

Conclusions

The results presented here demonstrate that the BD FACSLyric platform delivers user-friendly features, such as automated instrument setup and compensation, and high performance capability. These key features result in time savings every day while achieving reproducible and consistent results.

FIGURE 1. SIMPLIFIED USER WORKFLOW ON THE BD FACSLYRIC PLATFORM

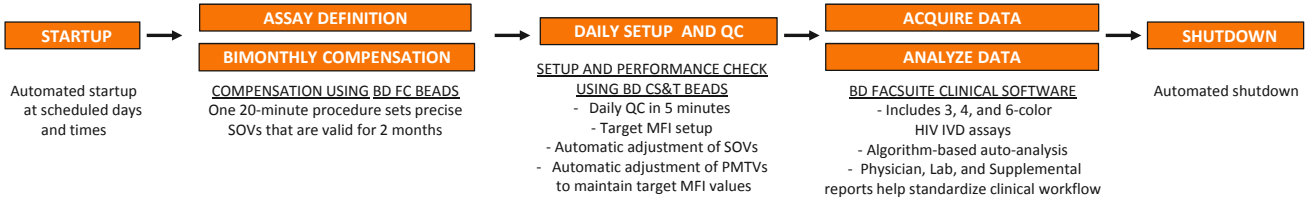

TABLE 1. ACCURACY OF TARGET MFI SETUP

Target MFI values were established and daily setup repeated three times using one lot of BD CS&T beads on one 10-color BD FACSLyric cytometer. For each parameter, the mean (n = 3) measured MFI value of CS&T bright beads was compared to the lot-specific target value. The percent difference = mean of (measured – target) / target * 100% is shown.

	CS&T BRIGHT BEAD		PERCENT
PARAMETER	TARGET	MEASURED	DIFFERENCE
FSC	17991	17992	0.01
SSC	126269	126009	-0.21
FITC	16369	16325	-0.27
PE	23693	23728	0.15
PerCP-Cy5.5	45375	45327	-0.11
PE-Cy7	28910	28806	-0.36
APC	40693	40718	0.06
APC-R700	42873	42869	-0.01
APC-Cy7	144141	144646	0.35
V450	9066	9060	-0.07
V500-C	32651	32663	0.04
BV605	6423	6440	0.26

TABLE 2. BETWEEN-INSTRUMENT REPRODUCIBILITY OF TARGET MFI VALUES

Daily QC with one lot of BD CS&T beads was run on six 10-color BD FACSLyric cytometers. Target MFI values were defined and peripheral blood samples acquired on BD FACSLyric Instrument 1 (INSTR 1). Settings from INSTR 1 were imported onto, and the same set of stained samples acquired on, INSTR 2 to 6. The MFI of positive populations was measured for all parameters across all instruments. The % CV is shown.

		MEDIAN FLUORESCENCE INTENSITY						%CV
PARAMETER		INSTR 1	INSTR 2	INSTR 3	INSTR 4	INSTR 5	INSTR 6	
CD4	FITC	1,891	1,699	1,816	1,865	1,661	1,691	5.6%
CD4	PE	16,781	16,781	17,393	18,363	16,993	17,482	3.5%
CD4	PerCP	1,287	1,332	1,242	1,367	1,287	1,439	5.3%
CD4	PerCP-	4,615	4,847	4,546	5,012	4,661	4,913	3.9%
CD4	PE-Cy7	25,402	27,600	26,066	29,213	30,510	26,393	7.2%
CD4	APC	20,064	21,163	20,768	20,600	20,990	18,848	4.2%
CD3	APC-R700	25,238	27,083	26,202	27,982	29,449	26,147	5.6%
CD8	APC-Cy7	27,942	29,440	29,174	30,553	31,283	29,738	3.9%
CD4	APC-H7	7,406	7,662	7,607	8,000	8,119	7,634	3.5%
CD4	V450	5,234	5,220	5,473	5,299	5,631	5,581	3.3%
CD4	V500-C	2,294	2,332	2,338	2,354	2,318	2,039	5.2%
CD7	BV605	6,760	5,805	5,925	5,733	6,047	5,637	6.8%

TABLE 3. PRECISION OF SPILLOVER VALUES

Daily setup and QC was done on three 10-color BD FACSLyric cytometers. Duplicate samples of one lot of BD FC beads were acquired on the three cytometers twice a day for eight days. For all experimental runs, SOVs were calculated for each sample. The standard deviation is shown.

		STANDARD DEVIATION OF SPILLOVER VALUES									
		DETECTOR									
		FITC	PE	PerCP	PE-Cy7	APC	APC-R700	APC-H7	V450	V500-C	BV605
PARAMETER	FITC		0.07	0.03	0.00	0.00	0.00	0.00	0.00	0.03	0.01
	PE	0.01		0.17	0.01	0.00	0.00	0.00	0.00	0.01	0.05
	PerCP	0.00	0.00		0.04	0.05	0.02	0.02	0.01	0.01	0.01
	PerCP-Cy5.5	0.00	0.00		0.07	0.01	0.04	0.04	0.00	0.00	0.00
	PE-Cy7	0.00	0.01	0.01		0.00	0.00	0.06	0.00	0.00	0.00
	APC	0.00	0.00	0.01	0.00		0.11	0.08	0.00	0.00	0.00
	APC-R700	0.00	0.00	0.06	0.02	0.07		0.38	0.00	0.00	0.00
	APC-H7	0.00	0.00	0.01	0.07	0.01	0.01		0.00	0.00	0.00
	APC-Cy7	0.00	0.00	0.01	0.13	0.08	0.02		0.00	0.00	0.00
	V450	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.11	0.01
	V500-C	0.03	0.01	0.01	0.00	0.00	0.00	0.00	0.05		0.05
	BV605	0.00	0.02	0.07	0.01	0.00	0.00	0.00	0.25	0.02	

TABLE 4. STABILITY OF SPILLOVER VALUES

On Day 1, one lot of BD CS&T or BD FC beads was acquired and SOVs captured. On Day 65, QC with the same lot of BD CS&T beads was repeated and the automatically updated SOVs recorded. The same lot of BD FC beads was newly acquired and SOVs captured. Newly acquired were compared to automatically updated SOVs. Delta spillover values (Day 65–Day 1) are shown.

		DELTA SPILLOVER VALUES (DAY 65 - DAY 1)									
		DETECTOR									
		FITC	PE	PerCP	PE-Cy7	APC	APC-R700	APC-H7	V450	V500-C	BV605
PARAMETER	FITC		0.03	0.00	0.02	0.00	0.00	0.01	-0.01	-0.10	-0.05
	PE	0.00		0.01	-0.01	0.00	0.00	0.00	0.00	0.00	-0.18
	PerCP	0.00	0.02		0.04	0.01	0.09	0.01	0.01	-0.01	0.04
	PerCP-Cy5.5	0.01	0.01		-0.16	0.04	0.03	-0.09	0.00	-0.01	0.00
	PE-Cy7	0.00	0.02	0.01		0.00	0.00	-0.16	0.00	0.01	0.00
	APC	0.00	0.00	-0.01	-0.01		-0.19	-0.19	0.00	0.01	0.00
	APC-R700	0.00	0.00	0.01	0.00	0.11		-0.49	0.00	0.00	0.00
	APC-H7	0.01	0.01	0.00	0.02	0.12	0.05		0.01	-0.01	0.01
	APC-Cy7	0.00	0.00	0.00	0.00	0.03	0.01		-0.01	0.01	0.00
	V450	0.00	0.00	0.00	0.01	0.00	0.00	0.00		0.00	-0.01
	V500-C	0.05	0.01	0.00	0.01	0.00	0.00	0.01	-0.13		0.02
	BV605	0.00	-0.01	-0.02	0.00	0.00	0.00	0.00	0.07	0.01	

FIGURE 2. IMPROVED RESOLUTION SENSITIVITY

One set of single-color stains of peripheral blood samples was acquired on a BD FACSCanto™ II and a BD FACSLyric cytometer. The stain index was calculated for all parameters on both machines. A table with the percent (%) increase in stain index (BD FACSLyric/BD FACSCanto II) is shown (leftmost panel). APC-Cy7 histograms from BD FACSCanto II (center panel) and BD FACSLyric (right panel) are also shown.

PARAMETER	% INCREASE IN STAIN INDEX
FITC	138
PE	160
PerCP	120
PerCP-Cy5.5	139
PE-Cy7	157
APC	166
APC-Cy7	290
BV421	251
BV510	108

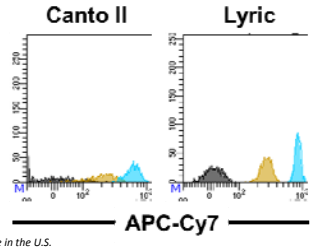

FIGURE 3. ACCURACY OF 6-COLOR TBNK ASSAY

Using three lots of the BD Multitest™ 6-color TBNK assay reagent, about 70 total healthy or HIV patient donor PBMC samples were acquired on BD FACSCanto II reference and BD FACSLyric test cytometers. Deming regression of the absolute counts on BD FACSLyric (see y-axis) and BD FACSCanto II (see x-axis) of $CD3^+CD4^+$ cells is shown.