Enhanced Reproducibility of Multicolor B-Cell Assays Using the Automated Universal Assay Setup Features of the BD FACSLyric™ System and Dry-Format Reagent Panels

Marybeth Sharkey[1], Alan Stall[1], Dina Huckaby[1], Marsha Griffin[1]; [1]BD Life Sciences, San Jose, CA
Jeffrey Jorgensen[2], Sa Wang[2], Russell Higgins[3], Kenneth Holder[3], Chandra Krishnan[4], Cathy Spadaccini[5], Jean Coviello[6], Franklin Fuda[7], Weina Chen[7], Andrea Sheehan[8], Martha Luna[9], April Ewton[10], Kimberly Monnin[11], Laura Sulak[12], Christina Montalvo[12], Nawir Hakim[13], Alireza Torabi[13], Harry Wilson[13]
[2]University of Texas MD Anderson Cancer Center, Houston; [3]UT Health Science Center, San Antonio, [4]Dell Children's, Austin, [5]Christus Santa Rosa, San Antonio, [6]Brooke Army Medical Center, San Antonio, [7]University of Texas Southwestern, Dallas, [8]Texas Children's Hospital, Houston, [9]Renaissance Hospital, Edinburgh, [10]The Methodist Hospital, Houston, [11]Clinical Pathology Laboratories, Austin, [12]St. Luke's Episcopal Hospital, Houston, [13]University Medical Center, El Paso

Introduction

Accurate and reproducible assays are the cornerstone of meaningful results from multi-site clinical trials. Two of the major sources of variability in such studies are 1) setup and cross-site standardization of instruments and 2) reagent consistency. Data presented here show that the Automated Universal Assay Setup functionality of the BD FACSLyric™ cytometer with BD FACSuite™ software, which leverages BD™ CS&T and BD™ FC Beads, provides the necessary instrument setup as well as enhanced assay portability. However, even with standardized instrumentation, variations in reagent panels over time is the most common source of variability in results from assays. BD Life Sciences has developed multicolor dry format cocktails which minimize assay variations and provide long-term reagent stability to further enhance assay reproducibility. Together these features provide consistency in assay performance across multiple instruments

Methods

- Assay:

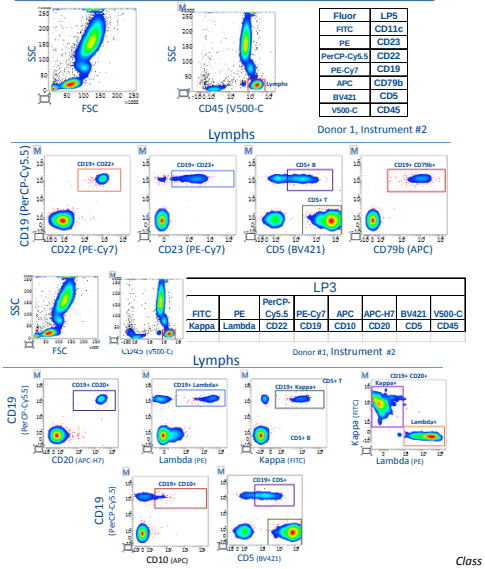
Two B-cell panels (LP3 and LP5) were designed by the TexFlow Consortium. BD prepared the panel reagents as multicolor dried format cocktails to maximize staining reproducibility.

- Two donors were used for the course of the study.
- At each time point, stained samples were run on three BD FACSLyric instruments set up for lyse/wash samples using Universal Setup.

-Day 1: Instruments were set up with CQC spillover values established using the BD™ FC Beads 7-Color Kit.

-Days 14, 28, 33, 77: instruments were setup using PQC. The saved Universal compensation was applied.

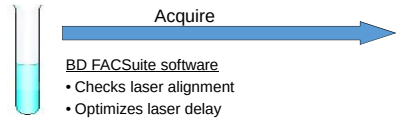
Assay Results



Fluor	LP5
FITC	CD11c
PE	CD23
PerCP-Cy5.5	CD22
PE-Cy7	CD19
APC	CD79b
BV421	CD5
V500-C	CD45

Fluor	LP3
FITC	Kappa
PE	Lambda
PerCP-Cy5.5	CD22
PE-Cy7	CD19
APC	CD10
APC-H7	CD20
BV421	CD5
V500-C	CD45

ONE step (Performance QC with CS&T beads) required to run standard lyse/wash assays



BD FACSuite software
- Checks laser alignment
- Optimizes laser delay
- Performs instrument QC on all channels
- Adjusts PMTVs to match assay MFI target values
- Assigns spillover values (SOVs) for compensation

- Instrument performance update
- Performance measurements tracked in Levey-Jennings graphs
- The instrument is fully set up to run all assays using lyse/wash or lyse/no-wash sample prep

The BD FACSLyric™ cytometer offers:

- Ease of use
- Reproducible, accurate data
- Flexibility
- Portability

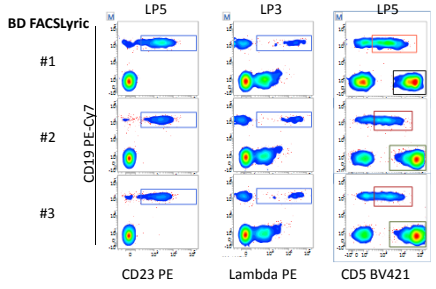
Reproducibility of BD FACSLyric cytometers

Assay reproducibility across instruments

Data consistency across multiple BD FACSLyric systems

LP5		Median Fluorescence Intensity						
Donor	Instrument	BV421 CD5	V500-C CD45	FITC CD11c	PE CD23	PerCP-Cy5.5 CD22	PE-Cy7 CD19	APC CD79b
#1	#1	83611	22626	2112	2462	10013	12913	10947
	#2	73390	23714	2002	2488	9847	12853	10637
	#3	71466	27404	2047	2304	9868	12516	10808
	AVG	**76156**	**24581**	**2054**	**2418**	**9909**	**12761**	**10797**
	StdDev	**6528**	**2504**	**55**	**100**	**90**	**214**	**155**
	% CV	8.6%	10.2%	2.7%	4.1%	0.9%	1.7%	1.4%
#2	#1	94910	22612	3330	3137	11856	12695	12169
	#2	82306	27914	3286	3547	11377	12781	10487
	#3	80372	27486	3282	2960	11093	12893	10624
	AVG	**85863**	**26004**	**3299**	**3215**	**11442**	**12790**	**11093**
	StdDev	**7895**	**2945**	**27**	**301**	**386**	**99**	**934**
	% CV	9.2%	11.3%	0.8%	9.4%	3.4%	0.8%	8.4%

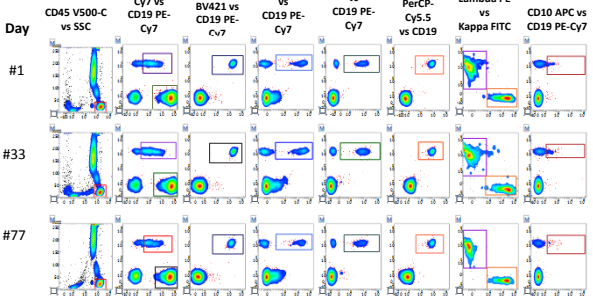
LP3		Median Fluorescence Intensity							
Donor	Instrument	BV421 CD5	V500-C CD45	FITC Kappa	PE Lambda	PerCP-Cy5.5 CD22	PE-Cy7 CD19	APC CD10	APC-H7 CD20
#1	#1	78936	23062	8693	28419	9842	11843	4106	26749
	#2	69867	27222	8739	23674	9249	12202	4205	26186
	#3	70056	24012	9094	25790	9896	12649	4001	25861
	AVG	**72953**	**24765**	**8842**	**25961**	**9662**	**12231**	**4104**	**26265**
	StdDev	**5182**	**2180**	**219**	**2377**	**359**	**404**	**102**	**449**
	% CV	**7.1%**	**8.8%**	**2.5%**	**9.2%**	**3.7%**	**3.3%**	**2.5%**	**1.7%**
#2	#1	94113	22413	8847	23691	10410	12268	8042	22981
	#2	80368	27572	9102	22656	10835	12158	8166	21883
	#3	77657	23158	8447	25853	10874	12555	7885	21527
	AVG	**84046**	**24381**	**8799**	**24067**	**10706**	**12327**	**8031**	**22130**
	StdDev	**8823**	**2788**	**330**	**1631**	**257**	**205**	**141**	**758**
	% CV	**10.5%**	**11.4%**	**3.8%**	**6.8%**	**2.4%**	**1.7%**	**1.8%**	**3.4%**



Assay reproducibility across time

Assay reproducibility- fluorescence

		CD5+	CD19+ CD23+	CD11c Grans	CD19+ CD23+	CD19+ CD22+	CD19+ CD22+	CD19+ CD79b+
		MFI						
Inst	Day	BV421 CD5	V500 CD45	FITC CD11c	PE CD23	PerCP-Cy5.5 CD22	PE-Cy7 CD19	APC CD79b
#1	1	83611	19371	1330	2462	10013	12913	10947
	14	70695	17981	1145	2697	9772	12400	9205
	33	84289	18793	1249	2699	10185	12559	10309
	43	75356	17774	1382	2355	9539	13121	11183
	77	66007	18696	1677	2814	13189	11631	10559
	AVG	75992	18523	1357	2605	10540	12525	10441
	Std Dev	7474	462	183	195	1605	616	387
	%CV	**10%**	**2%**	**14%**	**7%**	**15%**	**5%**	**4%**
#2	1	71466	19710	1323	2304	9868	12516	10808
	14	64285	18668	1130	2233	9450	12450	8546
	33	81762	19843	1192	2538	10817	12886	11346
	43	71918	19404	1281	2498	9691	12897	12715
	77	57968	18359	1599	2749	12893	10709	10211
	AVG	69480	19197	1305	2464	10544	12292	10725
	StdDev	9777	622	177	128	1359	1031	1082
	%CV	**14%**	**3%**	**14%**	**5%**	**13%**	**8%**	**10%**
#1 & 2	AVG	72440	18891	1328	2528	10542	12398	10596
	Std Dev	8701	708	182	200	1374	727	1151
	%CV	**12%**	**4%**	**14%**	**8%**	**13%**	**6%**	**11%**



Results were acquired across three instruments over a two-month timeframe. The assays delivered excellent resolution for all parameters with accurate compensation. Data was collected in the absence of daily compensation controls or any manual adjustment. Spillover values typically showed less than 0.5% difference over time. Analysis of the median fluorescence intensity (MFI) reproducibility of individual populations across instruments, a key requirement for cross-site assay portability and reproducibility, showed variances between 2% and 15% depending upon the detector. When looking over time, similar MFI variances were seen, albeit slightly larger due to donor differences.

Conclusions

Dried reagents stabilize assay performance over time, while the enhanced features of the BD FACSLyric system enable the use of Universal Setup to deliver equivalent results when tested using three platforms. These features enable standardization of assay results, simplifying data comparison of the assays to deliver equivalent performance over time and across instruments.