



Evaluation of lot-to-lot consistency for BD GMP reagents on the BD FACSDuet™ Premium Sample Preparation System integrated with the BD FACSLyric™ Flow Cytometer

Poster #141

Farzad Oreizy, Angela Chen, Chih-Hung Lai, Rodrigo Pestana Lopes, Yang Zeng
BD Biosciences, 155 N. McCarthy Blvd, Milpitas, CA 95035

Abstract

We compared two lots of BD reagents manufactured under Good Manufacturing Practice (GMP) process in a 12-color panel on the BD FACSDuet™ Premium Sample Preparation System integrated with the BD FACSLyric™ Flow Cytometer. After sample processing using lyse/wash preparation method, each single-color reagent was acquired to generate Median Fluorescence Intensity (MFI) for a corresponding positive cell population. Percent difference of MFI between the two lots were averaged across three donors. Results: average %diff was 5.6 for CD8 FITC, 7.8 for Lambda PE, 10.5 for CD16 PerCP-Cy5.5, 3.4 for CD19 PE-Cy7, 7.2 for Kappa APC, 9.3 for CD20 APC-H7, 4.6 for CD5 APC-R700, 2.7 for CD3 BV421, 9.9 for CD45 V500-C, 0.7 for CD7 BV605, 9.4 for CD10 BV711, 3.0 for CD4 BV786. For the 12-color reagent cocktail, each lot was prepared and tested on the BD FACSDuet™ Premium Sample Preparation System integrated with the BD FACSLyric™ Flow Cytometer. The two lots of the 12-color panel showed comparable dot plot distribution. The percent difference of %Parent between the 2 lots were averaged across two donors. Results: average percent difference was 2.00 for Leucocytes, 0.98 for Lymphocytes, 0.11 for CD3+ T cells, 0.96 for CD19+ B cells, 1.66 for Anti-Kappa APC cells, 1.43 for anti-Lambda PE cells, 2.39 for CD8+ T cells, 0.69 for CD4+ T cells, 0.02 for CD5+ T cells, 0.28 for CD7+ T cells, 0.07 for CD20+ B cells, 0.10 for CD16+ NK cells. The 12-color antibody reagents demonstrated lot-to-lot consistency in dot plots and cell percentages.

Methods

Instrument setup:

- BD FACSLyric™ System was setup using BD™ CS&T Beads. Reference settings were setup using BD™ FC beads for the following channels: FITC, PE, PerCP-Cy5.5, APC and V500-C. The rest of the channels were setup using antibody reagent-stained cells.

Lot-to-Lot comparison of single-color reagents (Manual operation)

- Specimens: Three normal donor blood samples were used.
- Sample processing: Each single-color reagent was used to stain whole blood from three donors using the Lyse/Wash sample processing method with manual operation. Refer to Table 1.
- Acquisition: Samples were acquired manually on the BD FACSLyric™ Flow Cytometer.
- Gating and analysis: Granulocytes were gated for CD10 BV711. For the rest of the reagents, lymphocytes were gated to identify antibody specific cell populations.

Lot-to-Lot comparison of a 12-color reagent cocktail on the BD FACSDuet™ Premium Sample Preparation System integrated with the BD FACSLyric™ Flow Cytometer

- Specimens: Two normal donor whole blood specimens were used.
- Reagents: The 12 single-color reagents were mixed on the BD FACSDuet™ System to prepare a 12-color antibody cocktail containing BD Horizon™ Brilliant Stain Buffer.
- Sample processing: Whole blood samples were stained and processed automatically on the BD FACSDuet™ System using the Lyse/Wash sample processing method.
- Acquisition: Samples were automatically transfer to BD FACSLyric™ flow cytometer and acquired automatically.
- Gating and analysis: Granulocytes were gated for CD10 BV711. For the rest of the reagents, lymphocytes were gated to identify antibody specific cell populations.

Results (1): Lot-to-Lot comparison of single-color GMP reagents using three donors on the BD FACSLyric™ Flow Cytometer

Table 1. Liquid antibody reagents used in this study

Specificity	Clone	Fluorochrome	Volume per test (µL)
CD8	SK1	FITC	20
Lambda	1-155-2	PE	20
CD16	3G8	PerCP-Cy5.5	5
CD19	S125C1	PE-Cy7	5
Kappa	TB28-2	APC	5
CD5	L17F12	APC-R700	5
CD20	L27	APC-H7	5
CD3	SK7	BV421	5
CD45	2D1	V500-c	5
CD7	M-T701	BV605	5
CD10	H10a	BV711	2.5
CD4	SK3	BV786	5

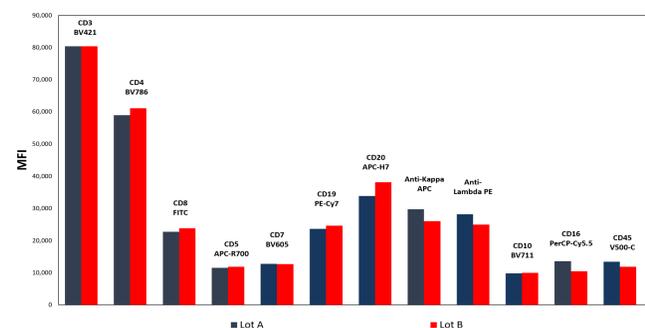


Figure 1: MFI comparison for the two reagent lots using antibody specific cell populations

Table 2 Lot-to-Lot Abs %difference in MFI

Single-color reagents	Donor 1	Donor 2	Donor 3	Average %diff
CD8 FITC	8.5	0.7	7.5	5.6
Lambda PE	8.4	6.5	8.4	7.8
CD16 PerCP-Cy5.5	13.6	9.1	8.7	10.5
CD19 PE-Cy7	3.0	4.5	2.8	3.4
Kappa APC	9.0	6.7	5.8	7.2
CD20 APC-H7	7.8	9.1	11	9.3
CD5 APC-R700	6.2	2.8	4.9	4.6
CD3 BV421	3.0	4.0	1.1	2.7
CD45 V500-C	8.6	10.6	10.6	9.9
CD7 BV605	0.4	0.8	0.8	0.7
CD10 BV711	9.6	8.8	9.9	9.4
CD4 BV786	2.5	3.5	2.9	3.0

- For each donor Lymphocytes, monocytes and granulocytes were gated/identified using forward and side scatter plot.
- To identify a positive cell population for a single-color reagent, a dot plot of SSC vs a fluorescence channel was used in data analysis. For example, CD3+ T cells were analyzed using a 2D plot of SSC vs CD3 BV421.
- For the CD10 BV711 stained cells, granulocytes were used to show MFI of the cell population.
- For cells stained with CD16 PerCP-Cy5.5, CD16+ lymphocytes were used to show MFI.
- For the rest of the single-color reagents, a lymphocyte subpopulation that was positive for each reagent was gated and used to generate MFI.
- Results of MFI were compared between Lot A and Lot B to generate % difference for each donor. The average %diff was generated across three donors. Refer to Table 2.

Results (2): Lot-to-Lot comparison of 12-color GMP reagent cocktail on the BD FACSDuet™ Premium Sample Preparation System integrated with the BD FACSLyric™ Flow Cytometer

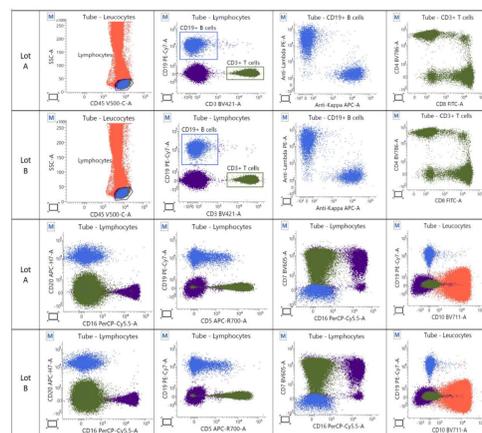


Figure 2: Dot plot comparison between the two lots of the 12-color antibody cocktail

Table 3 Lot-to-Lot Abs %difference in MFI

Cell population	Donor 1	Donor 2	Average %diff
Lymph.	2.01	0.06	1.04
Mon.	2.44	6.57	4.51
Gran.	2.39	0.17	1.28
CD3+	0.83	0.60	0.72
CD4+	0.70	0.67	0.69
CD8+	1.07	3.71	2.39
CD5+	0.07	0.03	0.05
CD7+	0.02	0.59	0.31
CD19+	6.38	4.47	5.43
CD20+	0.04	0.09	0.07
sigKappa	2.29	1.02	1.66
sigLambda	2.02	0.83	1.43
CD16+ (NK)	0.61	0.41	0.51

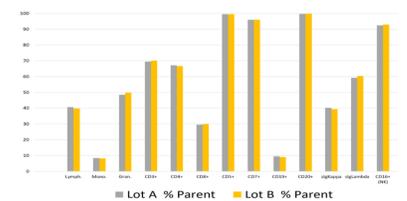


Figure 3: Percent Parent of each cell population for the two reagent lots

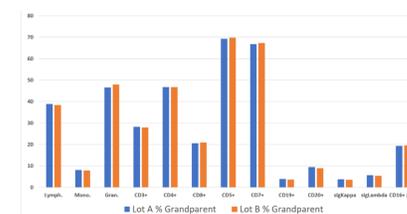


Figure 4: Percent Grandparent of each cell population for the two reagent lots

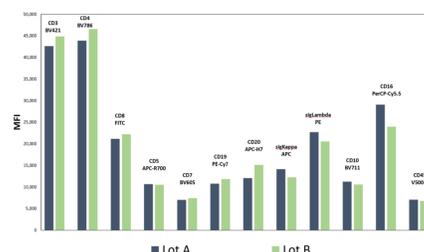


Figure 5: MFI comparison for the two reagent lots using antibody specific cell populations



Conclusions

- We evaluated BD reagents manufactured under GMP processes using different manufacturing lot numbers as specified on each reagent vial.
- The study design was based on customer's use perspective which was to stain a single tube per donor and test on multiple donors to compare lot-to-lot consistency.
- Study results showed that each of the single-color reagents demonstrated lot-to-lot consistency in MFI in the single-color reagent evaluation study.
- The 12-color panel antibody cocktail demonstrated lot-to-lot consistency in dot plots and cell percentages when the BD FACSDuet™ Premium Sample Preparation System integrated with the BD FACSLyric™ Flow Cytometer was used to process and acquire samples.

Class 1 Laser Product. This research is scientific in nature. Products NOT for diagnostic use.

BD, the BD logo, BD FACSLyric, BD FACSDuet and BD Horizon are the trademarks of Becton, Dickinson or its affiliates. © 2024 BD. All rights reserved. BD-120706 (v1.0) 0524