

Abstract

Flow cytometry plays a critical role in modern medicine and biological research. Multiparameter flow cytometry assays rely on high-quality reagents and instrument systems for reliable and reproducible data. BD manufactures flow cytometry reagents under current Good Manufacturing Practices (cGMP) for a variety of fluorescence conjugates. BD GMP reagents are produced with qualified equipment, QC testing and documentation in compliance with ISO 13485:2016 Quality Management Standard and in line with many principles defined in U.S. 21 CFR 820 "Quality System Regulation" – Medical Devices. In this study we compared two lots of BD GMP reagents individually and in a 12-color panel using the BD FACSDuet™ Premium Sample Preparation System integrated with the BD FACSLyric™ Flow Cytometer.

The chosen antibodies were CD8 FITC/anti-Lambda PE/CD16 PerCP-Cy5.5/CD19 PE-Cy7/anti-Kappa APC/CD5 APC-R700/CD20 APC-H7/CD3 BV421/CD45 V500-C/CD7 BV605/CD10 BV711/CD4 BV786. Two lots of every single-color reagent were processed individually using stain/lyse/wash method and acquired on the BD FACSLyric™ Flow Cytometer using whole blood from three healthy donors. The Median Fluorescence Intensity (MFI) of each single-color reagent was generated for the corresponding positive cell population and compared across both lots for each donor. The %diff of MFI between lots were generated and 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.

To evaluate lot-to-lot performance of the 12-color panel, two cocktails of BD GMP antibodies were prepared using different lots of each antibody-conjugate and tested on the BD FACSDuet™ Premium Sample Preparation System integrated with the BD FACSLyric™ Flow Cytometer using whole blood from two healthy donors. Based on dot plot distribution, both cocktails showed comparable ability to identify and differ the targeted cell populations. %Parent results of various cell populations were generated for each lot and compared. The percent difference of %Parent between 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.

Method

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 specimens 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.
- Reagent: 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

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	SJ25C1	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	HI10a	BV711	2.5
CD4	SK3	BV786	5

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

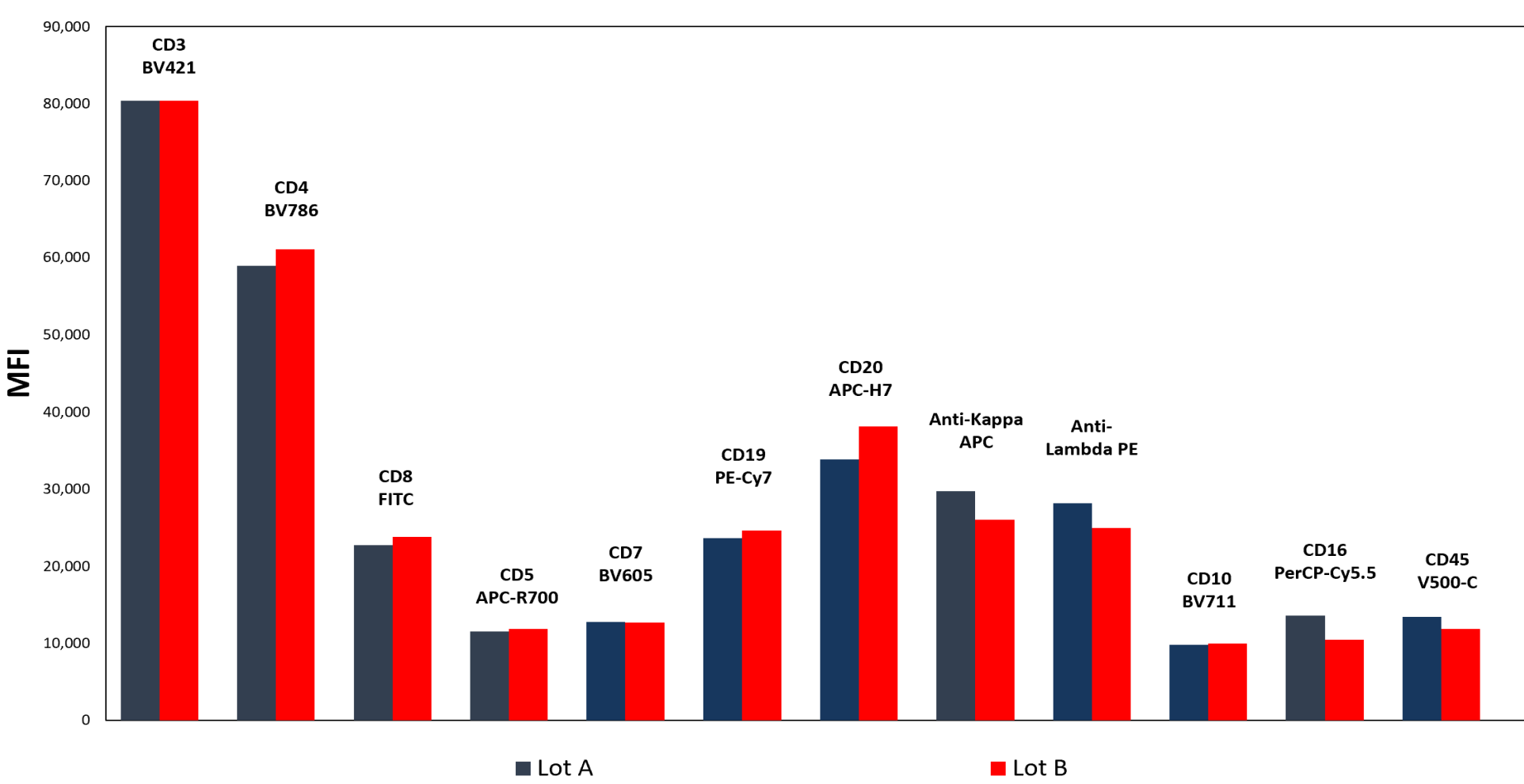
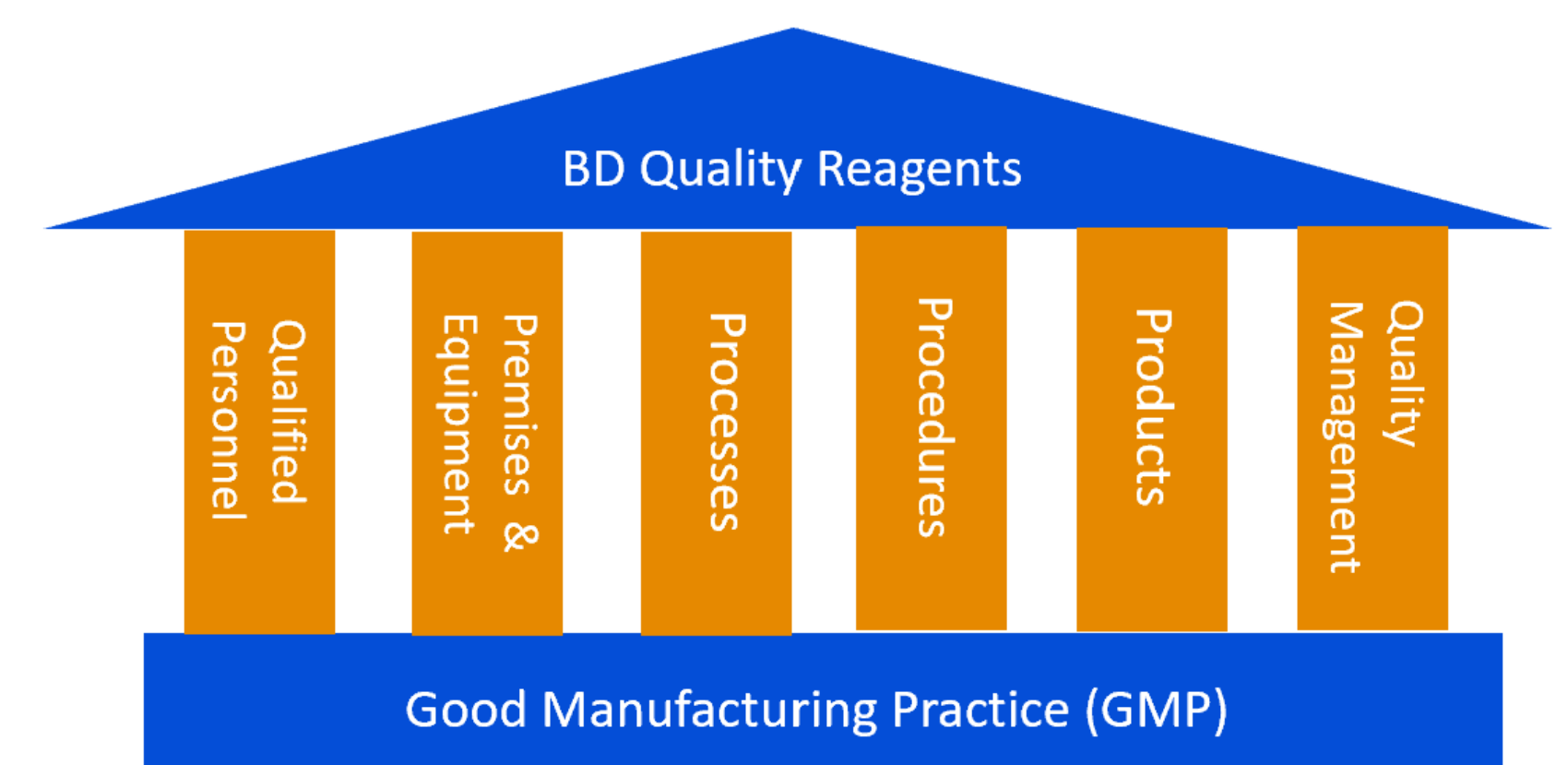


Figure 1: MFI comparison for the two reagent lots using antibody specific cell populations

- 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 1 2D plot of SSC vs CD3 BV421.
- For the CD10 BV711 stained cells, granulocytes were used to show Median Fluorescence Intensity (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

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

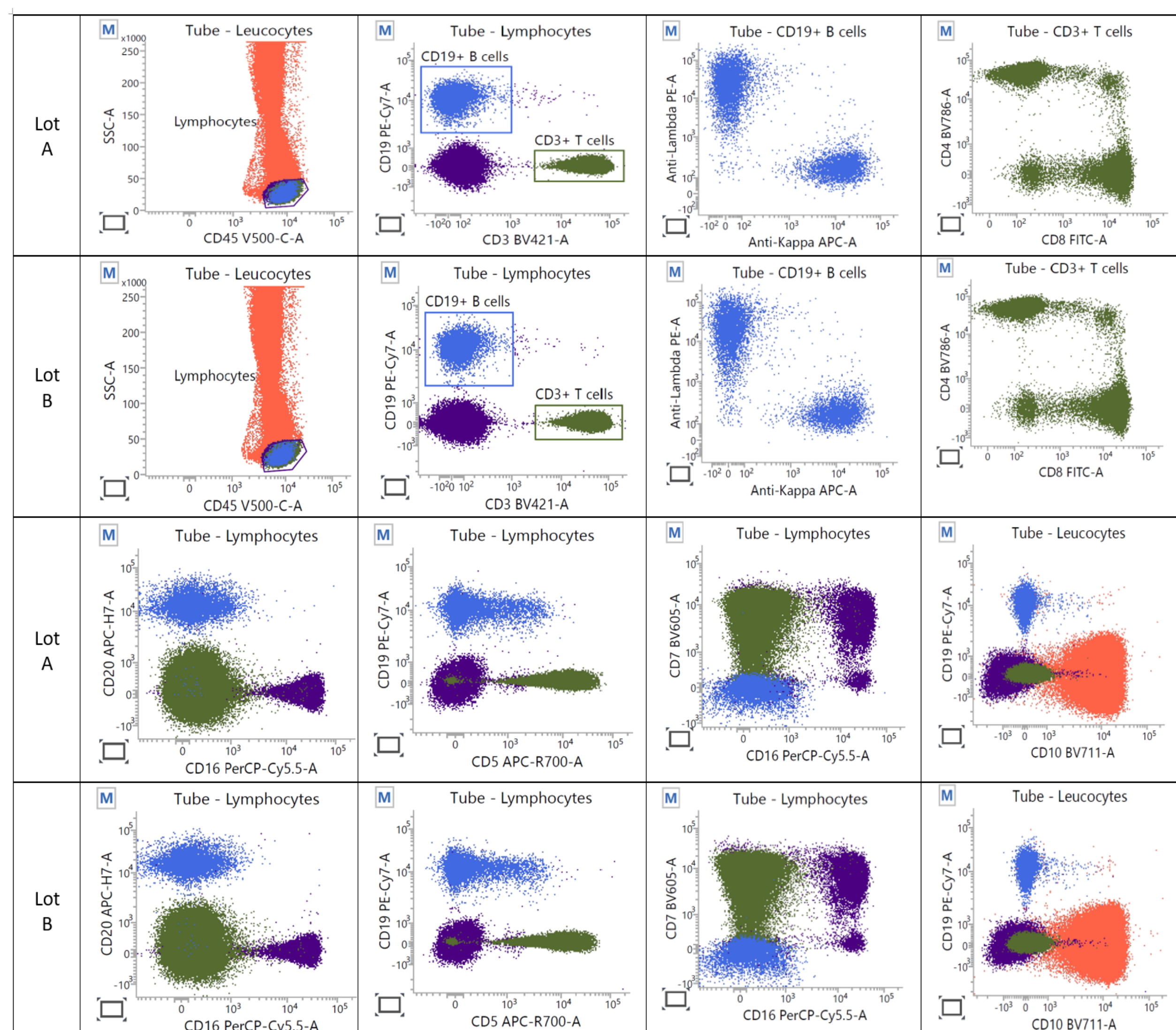


Figure 2: Dot plot comparison between the two lots of the 12-color antibody cocktail

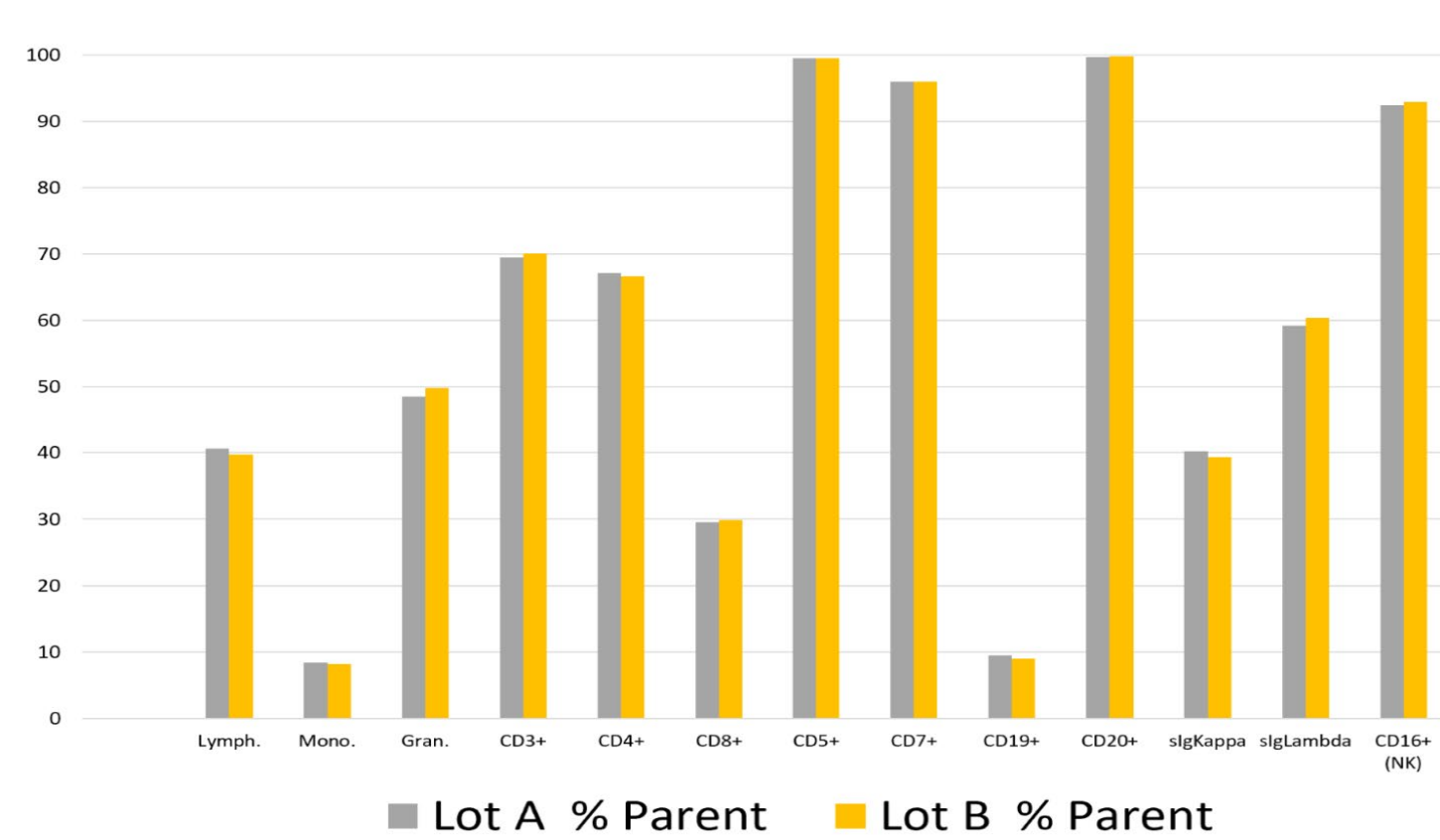


Figure 3: Percent Parent of each cell population for the two reagent lots.

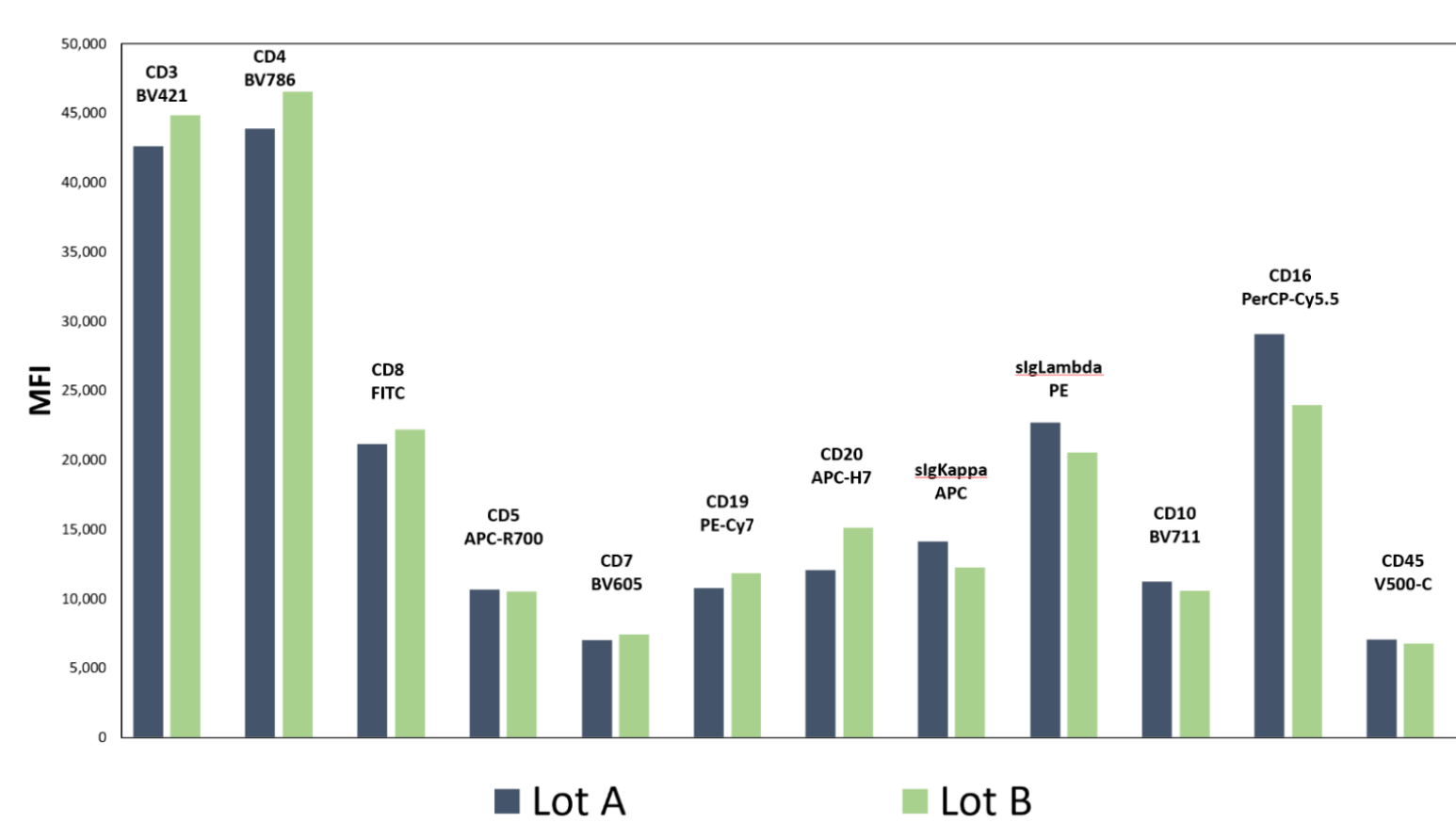


Figure 4: MFI comparison for the two reagent lots using antibody specific cell populations

Cell population	Donor 1	Donor 2	Average %diff
Lymph.	2.01	0.06	0.98
Mon.	2.44	6.57	2.06
Gran.	2.39	0.17	1.11
CD3+	0.83	0.60	0.11
CD4+	0.70	0.67	0.69
CD8+	1.07	3.71	2.39
CD5+	0.07	0.03	0.02
CD7+	0.02	0.59	0.28
CD19+	6.38	4.47	0.96
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	0.10

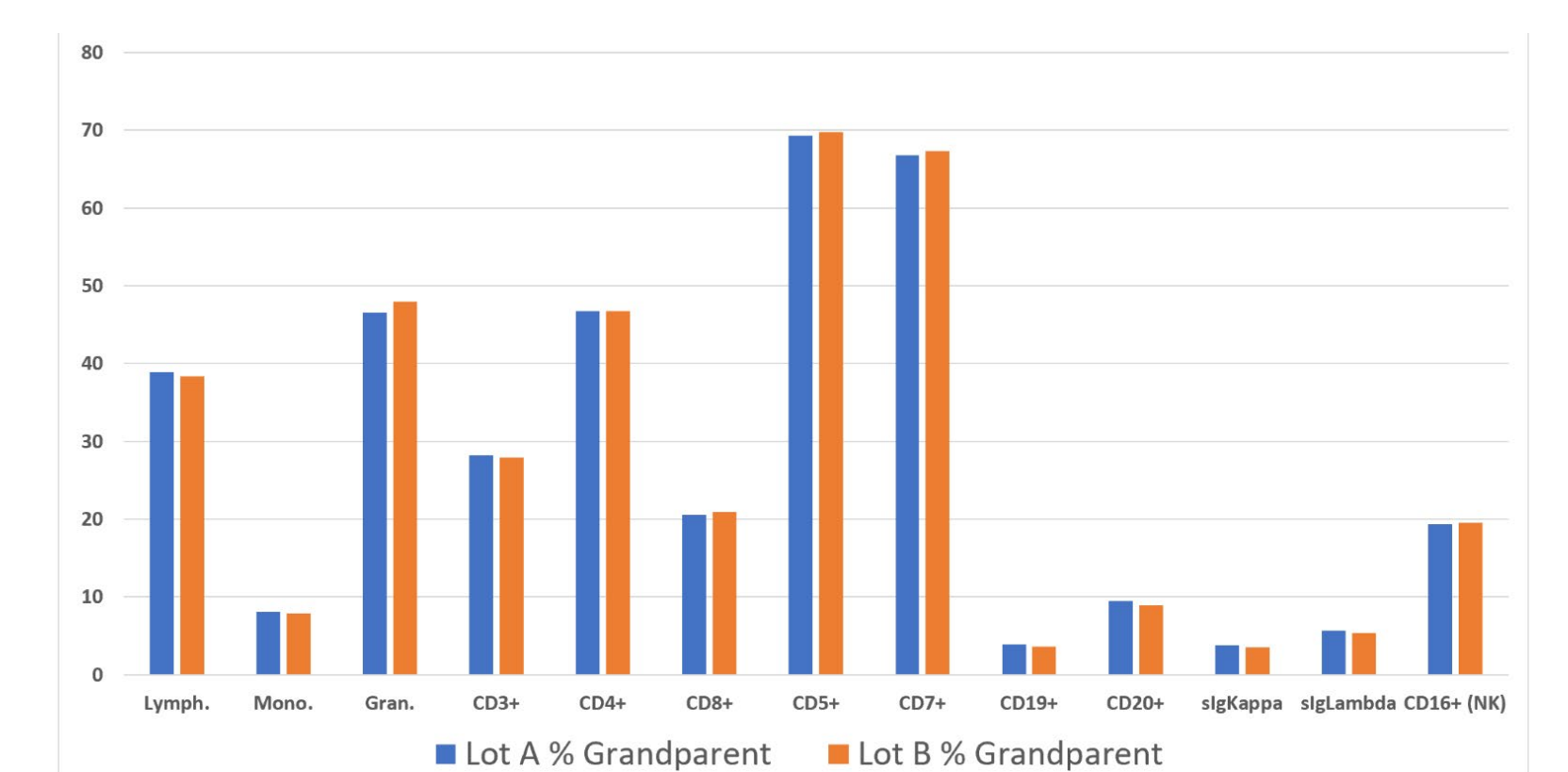


Figure 5: Percent Grandparent of each cell population for the two reagent lots.

Conclusions

- 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.

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