Immunophenotyping of normal whole blood using a 12-color BD Horizon™ Chroma Research Panel on the BD FACSDuet™ Premium Sample Preparation System integrated with the BD FACSLyric™ Flow Cytometer

Farzad Oreizy, Angela Chen, Bao Nguyen, Chih-Hung Lai, Punam Roka, Beverly Lu, Yang Zeng
BD Biosciences, San Jose, CA

Abstract

Traditional workflows in flow cytometry laboratories involve multiple manual processing steps including pipetting multicolor reagent cocktails, washing, staining and lysing. This involves significant hands-on time and is increasingly challenging as the number of parameters rises. The BD FACSDuet™ Premium Sample Preparation System integrated with the BD FACSLyric™ Flow Cytometer has shown the capacity to reduce the number of error-prone steps while also reducing hands-on time spent acquiring datasets. BD Horizon™ Chroma Reagents are pre-aliquoted, multicolor cocktails in a dried-down, ready-to-use format with increased shelf life that can be made to order based on user design.

In this study, we evaluated a fully automated workflow using the BD FACSDuet™ Premium Sample Preparation System integrated with the BD FACSLyric™ Flow Cytometer to prepare and acquire 20 normal whole blood samples stained with a 12-color BD Horizon™ Chroma Research Dried Panel (CD7 FITC/Anti-Lambda PE/CD34 PerCP-Cy5.5/CD19 PE-Cy7/Anti-Kappa APC/CD5 APC-R700/CD20 APC-H7/CD3 V450/CD45 V500-C/CD8 BV605/CD10 BV711/CD4 BV786) and compared it with manual processing. BD FACSLyric™ Flow Cytometer setup and compensation were completed using BD® CS&T Beads, BD® FC Beads and batch-matched BD dried single-color reagents. Sample preparation was fully automated onboard the BD FACSDuet™ Premium Sample Preparation System by rehydrating the dried cocktail, prewashing fresh whole blood, and transferring to the rehydrated reagent tube, followed by a stain/lyse/wash and automatic transfer to the BD FACSLyric™ Flow Cytometer. Immunophenotyping and characterization was performed for T-cell and B-cell subsets. Overall comparison between automatic and manual sample processing was statistically analyzed and shown in Figure 2 and Table 2.

Methods

Study design:

- Specimen and reagents:
  - 20 normal donor peripheral blood samples (EDTA as anticoagulant) processed and stained with a 12-color BD Horizon™ Chroma Dried Panel in dried-down format
- Method comparison study:
  - Automated method on the BD FACSDuet™ Premium System integrated with the BD FACSLyric™ Flow Cytometer for sample preparation and data acquisition
  - Manual method for sample preparation and data acquisition on the same BD FACSLyric™ Flow Cytometer

Table 1. 12-color BD Horizon™ Chroma Dried Panel

Format	Biomarker	Clone	Cell Population
FITC	CD7	M-T701	T-cell lymphocytes
PE	Lambda	1-155-2	B-cell lymphocytes
PerCP-Cy5.5	CD34	8G12	Early hematopoietic progenitors
PE-Cy7	CD19	SJ25C1	B-cell lymphocytes
APC	Kappa	TB28-2	B-cell lymphocytes
APC-R700	CD5	L17F12	T-cell lymphocytes
APC-H7	CD20	L27	B-cell lymphocytes
BV450	CD3	SK7	T-cell lymphocytes
V500-C	CD45	2D1	Leukocytes
BV605	CD8	SK1	T-cell lymphocytes
BV711	CD10	HI10a	Subset of B-cell lymphocytes and granulocytes
BV786	CD4	SK3	T-cell lymphocytes

Instrument setup:

- The BD FACSLyric™ Flow Cytometer was set up using BD® CS&T Beads. Reference settings were created using BD FC Beads and batch-matched BD dried single-color reagents (CD19 PE-Cy7, CD5 APC-R700, CD20 APC-H7, CD8 BV605, CD10 BV711, CD4 BV786).
- PQC and Assay/tube settings setup were run and passed acceptance criteria before samples were processed and acquired

Assay Setup:

- A user-defined assay was created in BD FACSuite™ Software RUO v1.5 for the comparison study and published to the BD FACSDuet™ Premium System.
- A 2-tube assay was created on the BD FACSDuet™ Premium System for sample preparation
  - The 1st tube is for the pre-wash preparation steps
  - The 2nd tube is for the Stain/Lyse/Wash preparation steps

Results

Figure 1. Workflow description

Automated process

Manual method

Sample Prewash

- Prewash donor whole blood sample (300 µL) using 3 mL of wash buffer on BD FACSDuet™ Premium System with 4x automated wash steps
- Prewash whole blood sample from the same donor (1000 µL) using 10 mL of wash buffer with 3x wash steps

Sample staining and processing

Note: all steps are automated onboard the BD FACSDuet™ Premium System

- Re-hydrate the 12-color BD Horizon ™ Chroma Dried Panel tubes with 50 µL of BD Horizon™ Brilliant Stain Buffer (BSB).
- Transfer 100 µL of prewashed samples to the rehydrated reagent tube to stain cells for 30 min.
- Lyse for 10 min with 1X BD FACSLyse™ Solution followed by centrifugation
- Wash cells with 2 mL of wash buffer 2 times.
- Resuspend cells by adding 450 µL of wash buffer
- Re-hydrate the 12-color BD Horizon™ Chroma Dried Panel tubes with 50 µL of BSB
- Transfer 100 µL of pre-washed samples to the rehydrated reagent tube to stain cells for 30 min.
- Lyse for 10 min with 1X BD FACSLyse™ Solution followed by centrifugation
- Wash cells with 2 mL of wash buffer 2 times
- Resuspend cells by adding 300 µL of wash buffer

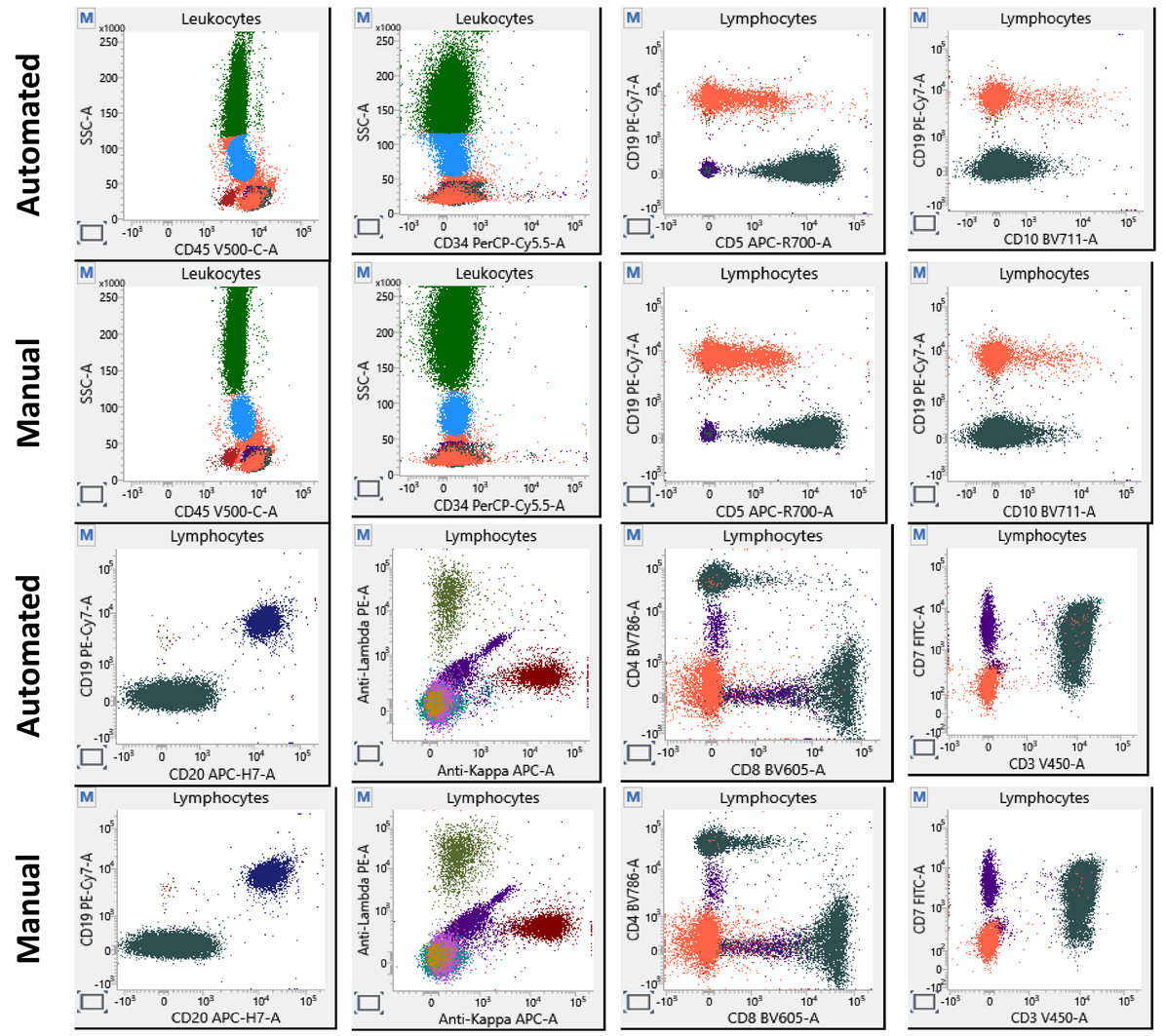
Flow cytometry acquisition

- Worklist was automatically published to the BD FACSLyric™ Flow Cytometer
- Prepared samples were automatically delivered to the integrated BD FACSLyric™ Flow Cytometer for acquisition.
- Manually create worklist on the BD FACSLyric™ Flow Cytometer
- Prepared samples were manually acquired on the same BD FACSLyric™ Flow Cytometer

Data Analysis

- Both automatically and manually collected data were analyzed using BD FACSuite™ Software RUO V1.5.
- Doublets and debris were excluded during gating
- Lymphocyte subsets were further gated and analyzed based on T-cell and B- cell biomarkers.

Figure 2. Dot plot comparison between automated and manual methods using 12-color BD Horizon™ Chroma Dried Panel



Results

Figure 3. Gating Hierarchy

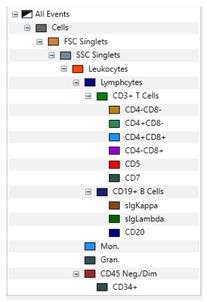


Figure 4. Box plot of cell percentages

Compare automated vs manual processing using normal donor blood (N=20)

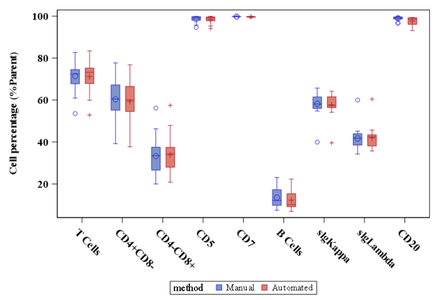


Table 2. Comparison of automated vs manual method on %Cell %Parent of normal peripheral blood (N = 20)

Parameter	Parent	Mean Automated	Mean Manual	Mean %Diff with 95% CI
CD19+ B cells	Lymphocytes	12.2	13.5	-9.5 (-11.8, -7.26)
CD20+ B cells	CD19+ B cells	97.6	99.0	-1.4 (-2.3, -0.5)
Anti-Lambda+ B cells	CD19+ B cells	41.9	41.7	0.5 (-0.5, 1.5)
Anti-Kappa+ B cells	CD19+ B cells	57.8	58.2	-0.7 (-1.5, 0)
CD3+ T cells	Lymphocytes	71.1	71.4	-0.4 (-1.4, 0.6)
CD8+ T cells	CD3+ T cells	33.9	33.3	1.9 (0.6, 3.2)
CD4+ T cells	CD3+ T cells	59.3	60.4	-1.8 (-2.5, -1.1)
CD7+ T cells	CD3+ T cells	99.7	99.8	-0.1 (-0.2, -0.1)
CD5+ T cells	CD3+ T cells	98.4	98.6	-0.2 (-0.2, -0.1)

Conclusions

- We assessed performance of a 12-color BD Horizon™ Chroma Research Dried Panel using 20 normal donor whole blood samples to compare two methods: manual sample processing/acquisition and automated method onboard of the BD FACSDuet™ Premium System integrated with BD FACSLyric™ Flow Cytometer.
- We demonstrated a sample-to-results automated workflow for the dried reagents using a two-tube preparation method on the BD FACSDuet™ Premium System integrated with the BD FACSLyric™ Flow Cytometer.
- The fully automated workflow resulted in comparable dot plot distribution and cell percentages relative to conventional manual method.