

Identification of Human Peripheral Blood Cell Populations with the BD Accuri™ C6 Flow Cytometer

Technical Bulletin

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Introduction

One common task in human blood analysis is to identify populations of **platelets, lymphocytes, monocytes, granulocytes, and eosinophils**. Flow cytometry is a quick and reliable methodology for identifying these populations based on their immunophenotypes (Table 1).

Several features of the BD Accuri™ C6 flow cytometer system make it a good fit for no-wash, differential analysis of human peripheral blood samples (Figure 1). First, the fixed voltage detectors of the BD Accuri C6 simplify data collection and reduce the potential for data loss due to signal over- or under-amplification. Second, the instrument's broad dynamic range makes it easy to analyze populations as varied in size as platelets and eosinophils in the same data file. Finally, BD Accuri™ C6 software's Zoom Tool allows precise control when setting gates.

This technical bulletin explains one method of identifying platelets, T lymphocytes, monocytes, granulocytes, and eosinophils on the BD Accuri C6 using BD Accuri C6 software.



Table 1. Defining immunophenotypes for five blood cell populations.

Population	Immunophenotype
Platelets	CD41 ⁺ CD45 ⁻
T lymphocytes	CD3 ⁺ CD45 ⁺
Monocytes	CD11b ⁺ CD14 ⁺
Granulocytes	CD11b ⁺ CD14 ⁻
Eosinophils	FL1 ⁺⁺ SSC ^{high}

Prerequisites

Software

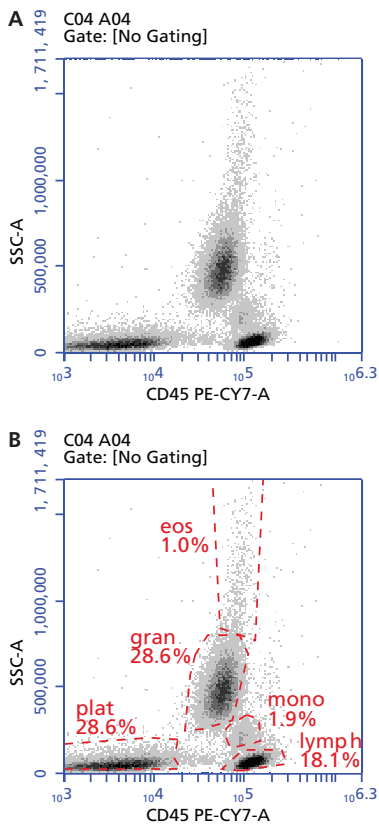
BD Accuri C6 software was used to collect and analyze the data in this example.

Reagents

This method uses directly conjugated antibodies for CD3, CD4, CD8, CD11b, CD14, CD41, and CD45. The recommended BD Biosciences reagents are shown in Table 2.

Table 2. Recommended BD Biosciences reagents for four-part differential analysis.

Marker	Fluorochrome	Clone	Cat. No.
CD3	FITC	UCHT1	555332
CD4	PE	RPA-T4	561843
CD8	APC	RPA-T8	561952
CD11b	PE	ICRF44	561001
CD14	FITC	M5E2	555397
CD41	FITC	HIP8	555466
CD45	PE-Cy™7	HI30	557748

**Figure 1.** Identification of human peripheral blood populations on the BD Accuri C6.

Whole human peripheral blood was stained, red cells were lysed in a no-wash protocol, and events were collected on the BD Accuri C6 flow cytometer. A. BD Accuri C6 software was used to draw an ungated plot of CD45 PE-Cy7 vs SSC-A. B. Gates were drawn and adjusted using the Zoom tool for five populations: eosinophils (eos); platelets (plat); T lymphocytes (lymph); monocytes (mono); and granulocytes (gran).

Sample preparation

BD recommends the following no-wash staining procedure. Alternative protocols should minimize sample handling to reduce cell loss. The final assay can be performed in BD Trucount™ tubes to obtain absolute counts. Perform all pipetting steps as accurately as possible, changing tips between each sample.

1. Collect blood in any standard anticoagulant tube (heparin, sodium citrate, or EDTA). Keep blood at room temperature (RT), rocking gently, until ready to stain.
2. See Table 2 for recommended BD antibodies and Table 3 for a suggested staining tube layout. Select antibodies that are directly conjugated to fluorochromes and titrate prior to use to determine the optimal staining concentration.¹
3. Aliquot 50–100 µL of whole peripheral blood into standard 12 x 75-mm or 1.5-mL microcentrifuge tubes.
4. Add appropriately diluted, directly conjugated antibodies following an experimental plan such as the one in Table 3.

Table 3. Suggested staining tube layout for four-part differential analysis.

Tube	FITC	PE	PE-Cy7	APC
1	–	–	–	–
2	CD41	–	CD45	–
3	–	CD11b	CD45	–
4	–	–	CD45	–
5	–	–	CD45	CD8
6	CD3	–	CD45	–
7	CD3	CD4	CD45	CD8
8	CD14	CD11b	CD45	–

Dashes indicate that no fluorochrome-conjugated antibody was added to a particular tube.

5. Stain cells for 20 minutes at room temperature in the dark.
6. Add red cell lysis buffer of your choice at the manufacturer's recommended dilution. BD recommends BD FACST™ lysing solution (Cat. No. 349202).
7. After adding lysis buffer, vortex each tube *gently*. Place tubes in the dark and allow lysis to proceed for 10 to 20 minutes.
8. If the red cell lysis buffer does not contain a fixative, analyze samples as soon as possible after lysis. Fixed samples can be stored in the dark at 4°C for up to 3 days.

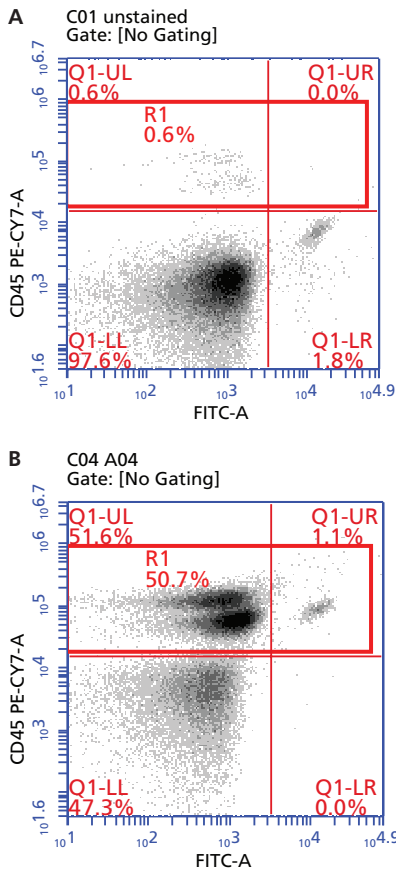


Figure 2. Example of R1 gate placement.

The R1 gate (red box) includes the UL and UR quadrants of the FITC vs PE-Cy7 plot. **A.** In Step 5, use Tube 1 to adjust R1 for PE-Cy7 background. **B.** In Step 7, use Tube 4 to adjust R1 to encompass the CD45 PE-Cy7⁺ population. See Table 3 for tube descriptions.

Data Collection on the BD Accuri C6

Note: Gently resuspend the cells in each sample immediately before placing the tube on the BD Accuri C6 flow cytometer.

1. Power on the BD Accuri C6.
2. Open the BD Accuri C6 software template called **HPB White Cell Population Analysis**. You will find templates for your version of BD Accuri C6 software in the Support > Resources > BD Accuri™ C6 System section of bdbiosciences.com.
3. Verify the following settings in the Collect Tab Control Panel:
 - Threshold: FSC-H = 300,000
 - Run Limit: 100,000 events in R1
 - Fluidics: Fast
4. Using Tube 1, collect at least 200,000 total events and click Pause to stop data collection manually.
5. Adjust R1 on the FITC vs PE-Cy7 plot to contain between 0% and 5% background events (Figure 2A).
6. Using Tube 4 (CD45 PE-Cy7⁺), collect data into a new well, allowing the BD Accuri C6 to stop automatically when it reaches the run limit of 100,000 events in R1.
7. Re-adjust R1, if needed, to encompass the CD45 PE-Cy7⁺ population (Figure 2B).
8. Collect data for all remaining tubes, allowing the BD Accuri C6 to stop automatically at the run limit of 100,000 events in R1.

Data Analysis

1. Verify fluorescence compensation values.
 - a. Open the compensation matrix in BD Accuri C6 software by clicking the Set Color Compensation button.
 - b. Open the C Comp spreadsheet file available in the Support > Resources > BD Accuri™ C6 System section of bdbiosciences.com.
 - c. Copy and paste the Average Compensation Values from the appropriate tab in the C Comp Calculator spreadsheet into the Compensation dialog. (The values are based on your BD Accuri instrument serial number.) For this experiment, use the values in the FITC-PE-PE-Cy7-APC table.
 - d. In the lower left section of the Compensation dialog, under Apply To, select All Samples.
 - e. From the BD Accuri C6 software main menu, select Display > Show Median Statistics.
 - f. Verify the applied compensation values by comparing the median fluorescence channel values of the appropriate negative and positive populations for the data in wells A1–A5. See Section 3.13.2, “Correcting Fluorescence Spillover,” of the *BD Accuri C6 Software User Guide* for a detailed description of this process.
 - g. Adjust compensation values in the BD Accuri C6 software dialog if required.

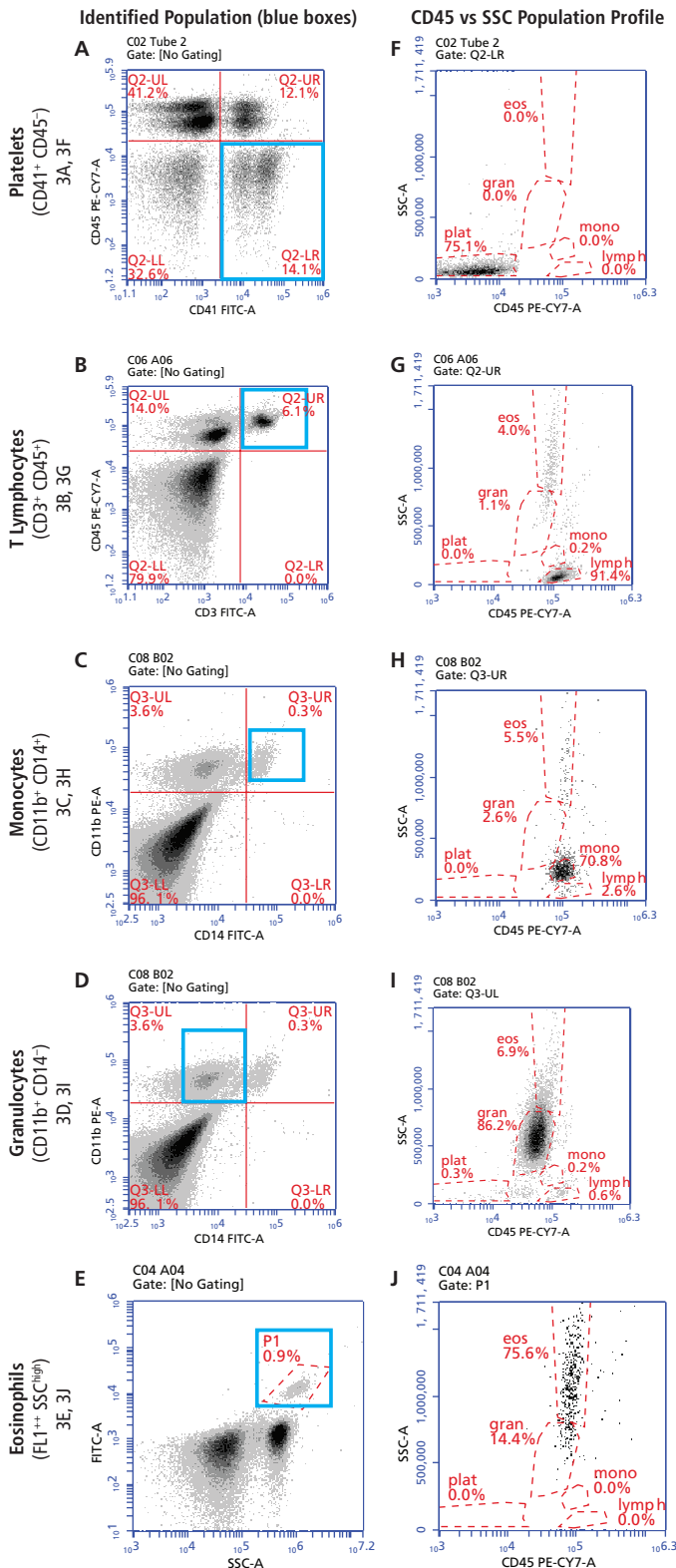


Figure 3. Backgating.

Human peripheral blood populations are first identified by surface markers to aid in adjusting polygons for CD45 vs SSC population identification. A–E. “Backgating” on surface marker or autofluorescence properties to identify specific populations. F–J. The CD45 vs SSC profile of each identified population.

2. Verify the location of the five populations on the CD45 PE-Cy7 vs SSC plot (Plot 3) by backgating on each population’s markers using the following gating procedure (Figure 3). Use the Zoom Tool if necessary to precisely adjust the population gates.

- Platelets: Use Tube 2 data to gate the CD41⁺CD45⁻ cells (platelets, Figure 3A) to Plot 3 (CD45 vs SSC), and adjust the **plat** gate (Figure 3F).
- T lymphocytes: Use Tube 6 data to gate the CD3⁺CD45⁺ cells (T lymphocytes, Figure 3B) to Plot 3, and adjust the **lymph** gate (Figure 3G).
- Monocytes: Use Tube 8 data to gate the CD11b⁺CD14⁻ cells (monocytes, Figure 3C) to Plot 3, and adjust the **mono** gate (Figure 3H).
- Granulocytes: Use Tube 8 data to gate the CD11b⁺CD14⁺ cells (granulocytes, Figure 3D) to Plot 3, and adjust the **gran** gate (Figure 3I).
- Eosinophils: Use Tube 4 data to gate the FL1⁺SSC^{high} cells in gate P1 (eosinophils, Figure 3E) to Plot 3, and adjust the **eos** gate (Figure 3J).

Tip: Use BD Accuri C6 software's Zoom Tool to aid in drawing precise non-overlapping gates on the CD45 vs SSC plot (Figures 4B and 4C).

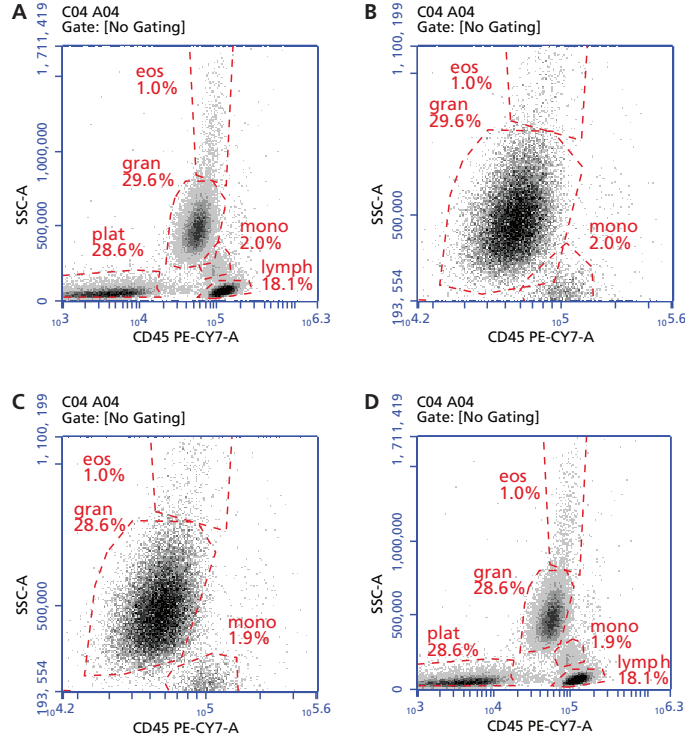


Figure 4. Using the Zoom Tool to adjust gates on five populations of human peripheral blood.

A. Original polygon placement. B. Zoomed plot shows overlap of polygons. C. Adjusted polygons. D. Plot zoomed back to original scale.

¹For a technical reference on proper titration techniques, see: Diamond RA, DeMaggio S (eds). *In Living Color: Protocols in Flow Cytometry and Cell Sorting*. Heidelberg: Springer Verlag; 2000:156-159 (ISBN 3-540-65149-7).

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