Stem Cell Enumeration SOP 4:
Analysis of Data Acquired on the BD FACSCanto™ II Cytometer

Purpose

To analyze and review the data acquired on the BD FACSCanto™ II flow cytometer.

Scope

This procedure applies to the clinical laboratory environment with the BD FACSCanto II flow cytometer for the purpose of CD34 enumeration using whole blood specimens, bone marrow (fresh or thawed), cord blood (fresh or thawed), and leucopheresis (fresh or thawed) specimens. We recommend that all personnel who operate the instrument be sufficiently trained to fully perform and implement this guideline.

Equipment Required

BD FACSCanto II workstation

Materials Required

Biohazard safety manual
Biohazard sharps waste container
Personal protective equipment (PPE)
- Protective gloves
- Protective eyewear
- Closed-toe shoes
- Lab coat

Completed worklist or data files

Procedure

1. Open BD FACSCanto™ clinical software.
2. Log in with the appropriate user name and password. Wait for the cytometer to connect to the software.
3. To analyze data within a worklist:
   a. Select **File > Open Worklist**.
   b. Navigate to the worklist containing data to be analyzed and click **Open**.
4. To analyze data from multiple days:
   a. Select **File > New Analysis Worklist**.

   b. Click **Add Data Files from Directory**.
   c. Navigate to the folder containing the data files.
   d. Click **Open**.
5. To analyze individual data files:

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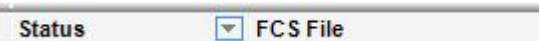   a) Select **File > New Analysis Worklist**.
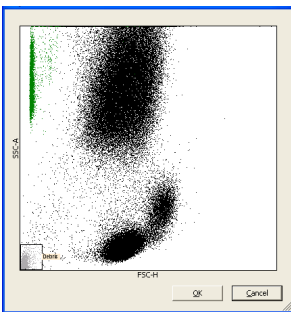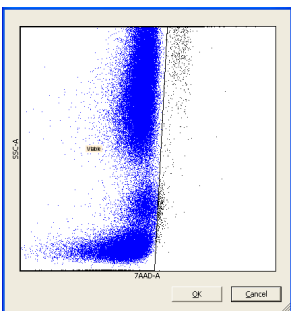
    b) Click **Add Data File**.

    c) Navigate to the folder containing the data files and select the files to be analyzed.

    d) Click **Open**.

6. Click in the **Status** column for the first sample to open the laboratory report.
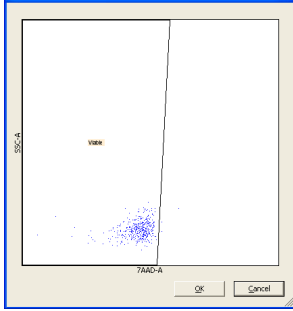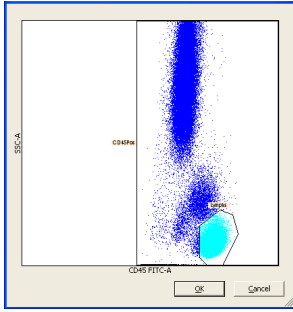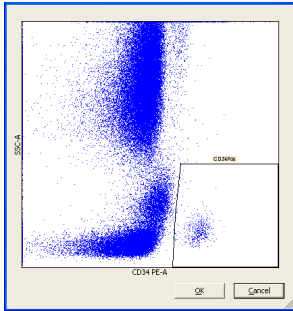
    Status   FCS File

7. The following table explains the template gating strategy. For details about specific gating examples, see the *BD Stem Cell Enumeration Application Guide for BD FACSCanto II Flow Cytometers*, document 23-11196-01.

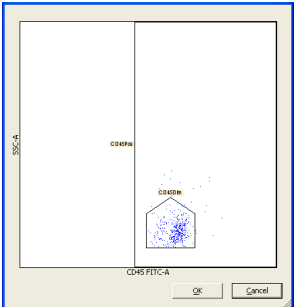| Step	Description	Dot Plot	Plot Information	Action
1.	Exclude debris from the plots displaying the cells.	**Plot 6 (FSC vs SSC)** **Gate name:** Debris	• All ungated events are displayed. • Events in the Debris gate appear gray. • Beads appear green in the upper-left corner.	The Debris gate encompasses events at the lower-left corner of the plot. Adjust if necessary.
2.	Identify the viable cells in plot 8.	**Plot 8 (7-AAD vs SSC)** **Gate name:** Viable	• All ungated events (excluding debris) are displayed. • Events within the Viable gate appear blue. **Note:** This viable gate is the same gate as shown in plot 7.	Confirm that the Viable gate encompasses only 7-AAD⁻ events.

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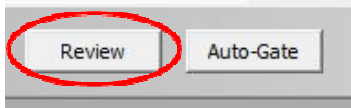| Step	Description	Dot Plot	Plot Information	Action
3.	Identify the viable cells in plot 7.	**Plot 7 (7-AAD vs SSC)** **Gate name:** Viable	• All CD34+ events are shown, viable and non-viable. • All events are blue. **Note:** This viable gate is the same gate as shown in plot 8.	Confirm that the Viable gate encompasses only 7-AAD− events. This is the only place that non-viable CD34 events will be displayed (7-AAD+).
4.	Identify the lymphocytes.	**Plot 1 (CD45 vs SSC)** **Gate names:** CD45Pos and Lymphocytes	• All events excluding beads and debris are displayed. • CD45+ events appear blue. • Lymphocytes appear light blue.	Adjust the CD45Pos gate to include all events. The left edge of the gate should include all dim CD45 cells. Avoid platelet streaks if present.
5.	Identify CD34+ events among the viable CD45+ events in plot 1.	**Plot 2 (CD34 vs SSC)** **Gate name:** CD34Pos	• Viable CD45+ events from plot 1 are displayed. • CD34+ events are enclosed by the gate in the lower right of the plot.	Adjust the CD34Pos gate to include the cluster of events at the lower right of the plot. Do not reduce the height of the gate. **Note:** Non-viable CD34 events are not displayed.

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Step	Description	Dot Plot	Plot Information	Action
6.	Identify the CD45+ dim events among the viable CD45+ events in plot 1.	**Plot 3 (CD45 vs SSC)** SSC-A / CD45Pos / CD45Dim / CD45 FITC-A / OK Cancel **Gate name:** CD45Dim	• All CD45+ from the CD34 gated cells in plot 2 are displayed. • The rectangle gate is for CD45+ events. • The polygon gate is for CD45dim events.	Confirm that the CD45Dim gate encompasses the cluster of cells in the CD45Pos gate. **Note:** The height of the gate is the same as plot 1. The gate is set by the algorithm on total CD34 cells, which is necessary for CD34 viability estimation.

8. Look at the QC messages at the end of the report to ensure validity.
9. Click the **Review** button, select **Current User** or **Other User,** and enter your password.

10. Click the right arrow to continue to the next sample.

11. Repeat steps 7 through 10 for each additional sample.
12. Once all samples have been analyzed, select **File > Save** to save changes to the worklist.
13. If using an analysis worklist, select **File > Save As** and enter a name for the analysis worklist.
14. Click **Save**.
15. Select **File > Print all Lab Reports**, if needed.

References

BD FACSCanto™ II Instructions for Use, document 23-12882-01.

BD FACSCanto™ Clinical Software Reference Manual, document 23-14529-00.

BD Stem Cell Enumeration Application Guide for BD FACSCanto II Flow Cytometers, document 23-11196-01.

BD™ Stem Cell Enumeration Kit technical data sheet, document 23-7867-04, available at www.bdbiosciences.com.

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