



**BD OneFlow™**

# **Application Guide for Acute Leukemias**

**For BD FACSLyric™ Flow  
Cytometers**

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## Laser safety information

The BD FACSLyric flow cytometer is a Class 1 Laser Product.

## Regulatory information

For In Vitro Diagnostic Use.

## History

| Revision    | Date    | Change made                                                                                                                                           |
|-------------|---------|-------------------------------------------------------------------------------------------------------------------------------------------------------|
| 23-21494-00 | 5/2020  | Initial release                                                                                                                                       |
| 23-21494-01 | 12/2020 | Updated troubleshooting to add new QC messages and assay description for the stopping criteria.<br>Updated workflow for contacting technical service. |

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# 1

## Introduction

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This chapter covers the following topics:

- [About this guide \(page 6\)](#)
- [Technical support \(page 7\)](#)

## About this guide

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**What's in this guide** This guide covers the acquisition and analysis workflows for the BD OneFlow™ ALOT assay using BD FACSuite™ Clinical application and describes the OneFlow™ ALOT Laboratory Report. It also includes assay-specific troubleshooting information.

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**Assumptions** This guide assumes that you have read the *BD FACSLyric™ Clinical System Instructions For Use (IFU)* and the *BD FACSLyric™ Clinical Reference System* and that you are familiar with running the software and cytometer. The documents provide details on performing quality control (QC), filling out the worklist, and running samples.

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**Additional information** See the *BD OneFlow™ ALOT IFU* for information on preparing samples.

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## Technical support

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### Before contacting technical support

Try the following options for answering technical questions and solving problems:

- Read the section of this guide specific to the operation you are performing.
  - See the assay-specific troubleshooting section of this guide for specific problems.
  - See the troubleshooting section of the *BD FACSLyric™ Clinical System Instructions For Use* and the *BD FACSLyric™ Clinical Reference System*.
- 

### Contacting technical support

**To contact technical support:**

1. Go to [bdbiosciences.com](http://bdbiosciences.com).
2. Select your region.
3. Click **Support**.
4. Expand your country for details for your local region.

When contacting BD Biosciences, have the following information available:

- The product name, part number, serial number, and details of recent system performance
  - The test you are performing
  - Any error messages
-

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# 2

## BD OneFlow™ ALOT Assay

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This chapter covers the following topics:

- [About the BD OneFlow™ ALOT Assay \(page 10\)](#)
- [BD OneFlow™ ALOT workflow \(page 11\)](#)
- [Changing the assay stopping criteria \(page 12\)](#)
- [Reviewing the laboratory report \(page 14\)](#)
- [Adjusting gates \(page 22\)](#)
- [Adding items to the supplemental report \(page 24\)](#)

## About the BD OneFlow™ ALOT Assay

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BD OneFlow™ ALOT is a qualitative 8-color direct immunofluorescence assay for immunophenotyping of aberrant immature populations of hematopoietic cells (lymphoid and non-lymphoid lineage) in bone marrow and peripheral blood. It is a screening tube, meant to direct further analysis of the patient specimen.

### About the assay

The BD OneFlow™ ALOT assay consists of a Laboratory Report, which contains dot plots and gates to identify the cell populations of interest, a Physician Report which summarizes the results, a Supplemental Report which can be used as a workspace to add dot plots and gates to the analysis, and tube settings that are used to reach target median fluorescence intensity (MFI) for cell populations, ensuring compatibility with the EuroFlow design.

The BD OneFlow™ ALOT reagent consists of single-use tubes containing a panel of fluorochrome-conjugated antibodies in an optimized dried formulation. The antibodies are divided between two tubes: BD OneFlow™ ALOT (S) contains antibodies that recognize surface markers and BD OneFlow™ ALOT (C) contains antibodies that recognize cytoplasmic markers. The panel comprises the following antibodies:

| Antibody | Tube | Fluorochrome       |
|----------|------|--------------------|
| CD34     | S    | PerCP-Cy™5.5       |
| CD19     | S    | PE-Cy™7            |
| CD7      | S    | APC                |
| CD3      | S    | APC-H7             |
| CD45     | S    | BD Horizon™ V500-C |
| MPO      | C    | FITC               |
| CD79a    | C    | PE                 |
| CD3      | C    | BD Horizon™ V450   |

The BD OneFlow™ ALOT reagent is used to stain patient specimens. The stained samples are acquired on the cytometer and then analyzed to identify aberrant immature hematopoietic populations of lymphoid and non-lymphoid lineages.

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## BD OneFlow™ ALOT workflow

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**Workflow steps**      The following table lists the steps in a typical assay workflow.

| Step | Description                                                                                                                                                               |
|------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1    | Perform daily instrument Performance QC (PQC) and assay and tube settings setup using BD® CS&T Beads. See the <i>BD FACSLyric™ Clinical System Instructions For Use</i> . |
| 2    | Prepare the patient specimens. See the <i>BD OneFlow™ ALOT IFU</i> for information.                                                                                       |
| 3    | Enter reagent lot and expiration date in the Library. See the <i>BD OneFlow™ ALOT IFU</i> for information.                                                                |
| 4    | Create worklist. See the <i>BD FACSLyric™ Clinical System Instructions For Use</i> .                                                                                      |
| 5    | Optional: change the number of events to collect, if needed. See <a href="#">Changing the assay stopping criteria (page 12)</a> .                                         |
| 6    | Acquire samples. See the <i>BD FACSLyric™ Clinical System Instructions For Use</i> .                                                                                      |
| 7    | Review the laboratory report. See <a href="#">Reviewing the laboratory report (page 14)</a> .                                                                             |
| 8    | Adjust gates, if necessary.                                                                                                                                               |
| 9    | Add dot plots and gates, if necessary. See <a href="#">Adding items to the supplemental report (page 24)</a> .                                                            |

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## Changing the assay stopping criteria

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The OneFlow™ ALOT assay acquires 100,000 total events by default. If the OneFlow™ assay v1.1 is unable to acquire 100,000 total events, acquisition will stop after 3 minutes. OneFlow™ assay v1.0 will stop acquisition after 5 minutes. You can change the number of events acquired or the acquisition time, as needed.

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### Procedure

To change the stopping criteria:

1. In the worklist, ensure that the run pointer is at the sample for which you want to change the number of events acquired.
2. Click the triangle next to the entry number to expand the sample.

The run pointer will move to the newly expanded tube.

3. Right-click the run pointer and select **Tube Properties** from the menu.

The **Tube Properties - ALOT** dialog opens.

4. Navigate to the **Acquisition** tab.
5. To change the acquisition time, in the **Time Stopping Rule** section, select the desired maximum time using the menu.

**Note:** We recommend that you do not increase the acquisition time and risk loss of the sample due to insufficient volume.

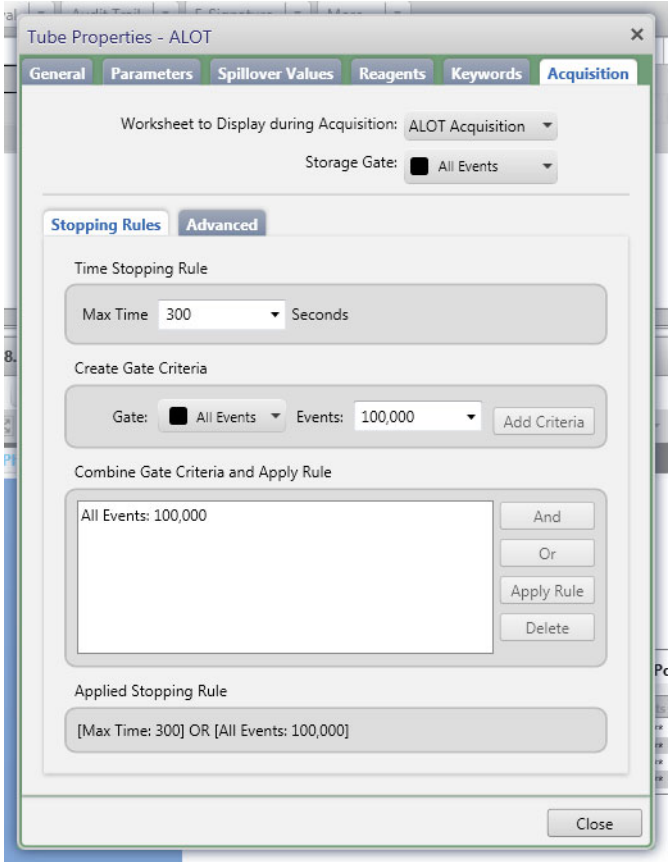
6. To change the number of events acquired, in the **Create Gate Criteria** section, select or enter the number of events you want to acquire in the **Events** field.

7. Click **Add Criteria**.

The selected number of events is added in the **Combine Gate Criteria and Apply Rule** section.

8. In the **Combine Gate Criteria and Apply Rule** section, select the number of events you want to acquire. Click **Apply Rule**.

The selected maximum acquisition time and number of events acquired will show in the **Applied Stopping Rule** section.



- 9. Click Close.

**Note:** The new stopping criteria will apply only to the selected tube, not to other tubes in the worklist.



## Reviewing the laboratory report

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The OneFlow™ ALOT Laboratory Report contains assay and patient-specific information, cell population statistics, QC messages, dot plots with gates to guide the analysis of the sample, and instrument QC information.

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### Viewing the laboratory report

1. Click the **Laboratory Report** tab to open the report.
2. Review page 1 of the laboratory report.
  - a. Review the information about the sample, cytometer, and tube for accuracy.
  - b. Review the assay results showing the cell population statistics.

- c. Review any QC messages to address potential issues and determine whether they affect the results. See [Troubleshooting \(page 29\)](#) for information.

**OneFlow ALOT: Laboratory Report**

**Sample ID:** 1234  
**Sample Name:** abcd  
**Case Number:** 12345678

|                              |                               |                                     |
|------------------------------|-------------------------------|-------------------------------------|
| Acquired Using: Worklist_013 | Approved: 5/21/2020 17:18:49  | Entry Status: Approved              |
| Cytometer: BD FACSLytic      | Cytometer SN: 1234567890      | Software: BD FACSuite Clinical v1.4 |
| Operator: Admin User         | Director: Mr. J. Smith        | Institution: BD                     |
|                              | Department: Reagents & Assays | Address: Limerick<br>Ireland        |

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**Tube Name:** ALOT

|                       |                    |                        |             |
|-----------------------|--------------------|------------------------|-------------|
| Events Acquired       | 100,000            | Sample Type            | Bone Marrow |
| Performance QC Date   | 5/21/2020 16:54:22 | Acquisition Date       | 5/21/2020   |
| Performance QC Status | Pass               | Acquisition Start Time | 17:06:14    |
|                       |                    | Acquisition End Time   | 17:06:25    |

---

**Results Summary**

| Population               | Parent                         | # Events | % Parent | % Grandparent |
|--------------------------|--------------------------------|----------|----------|---------------|
| All Events               |                                | 100,000  |          |               |
| Cells                    | All Events                     | 90,004   | 90.0     |               |
| FSC Singlets             | Cells                          | 87,049   | 96.7     | 87.0          |
| SSC Singlets             | FSC Singlets                   | 86,968   | 99.9     | 96.6          |
| Leukocytes               | SSC Singlets                   | 86,964   | 100.0    | 99.9          |
| CD19+                    | Leukocytes                     | 6,029    | 6.9      | 6.9           |
| B cells (CD19+ cyCD79a+) | CD19+                          | 5,382    | 89.3     | 6.2           |
| cyCD3+                   | Leukocytes                     | 9,583    | 11.0     | 11.0          |
| T cells (cyCD3+CD3+)     | cyCD3+                         | 9,549    | 99.6     | 11.0          |
| Non-Lymphoid             | Leukocytes NOT CD19+ OR cyCD3+ | 55,859   | 78.3     | 64.2          |
| cyMPO+                   | Non-Lymphoid                   | 49,185   | 88.1     | 68.9          |
| CD45 neg/dim             | Leukocytes                     | 18,918   | 21.8     | 21.8          |
| CD34+                    | CD45 neg/dim                   | 826      | 4.4      | 0.9           |

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**QC Messages**

Showing 0 of 0 QC Messages

For In Vitro Diagnostic Use.      Assay: OneFlow ALOT      Page: 1 of 6      Printed: 5/21/2020 17:19:07

- 3. Inspect the dot plots on page 2 of the laboratory report, and adjust the gates as needed.

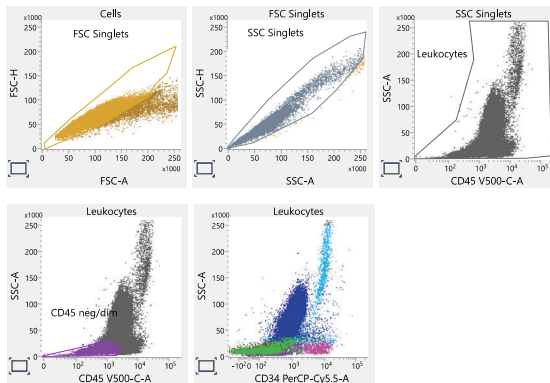
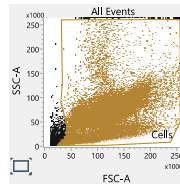
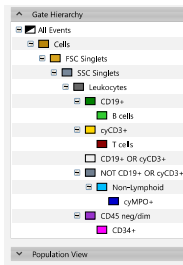
**Note:** The gates in the dot plots of the OneFlow™ ALOT Laboratory Report are provided for analyzing normal and aberrant cell populations in the specimen.

The dot plots on page 2 of the report identify the major cell populations. First, debris is removed and the singlet populations are identified, followed by the Leukocytes and CD45 neg/dim cells.

Sample ID: 1234  
 Sample Name: abcd  
 Case Number: 12345678

**Cell Analysis**

| Population   | Parent                          | # Events | % Parent | % Grandparent |
|--------------|---------------------------------|----------|----------|---------------|
| Leukocytes   | SSC Singlets                    | 86,964   | 100.0    | 99.9          |
| CD19+        | Leukocytes                      | 6,029    | 6.9      | 6.9           |
| cyCD3+       | Leukocytes                      | 9,583    | 11.0     | 11.0          |
| Non-Lymphoid | Leukocytes, NOT CD19+ OR cyCD3+ | 55,859   | 78.3     | 64.2          |
| CD45 neg/dim | Leukocytes                      | 18,918   | 21.8     | 21.8          |



For In Vitro Diagnostic Use.

Assay: OneFlow ALOT  
 Page 2 of 6

Printed: 5/21/2020 17:19:07

- Inspect the dot plots on page 3 of the laboratory report, and adjust the gates as needed.

The dot plots on page 3 of the report identify and analyze T cells and B cells. cyCD3<sup>+</sup> cells are identified from the



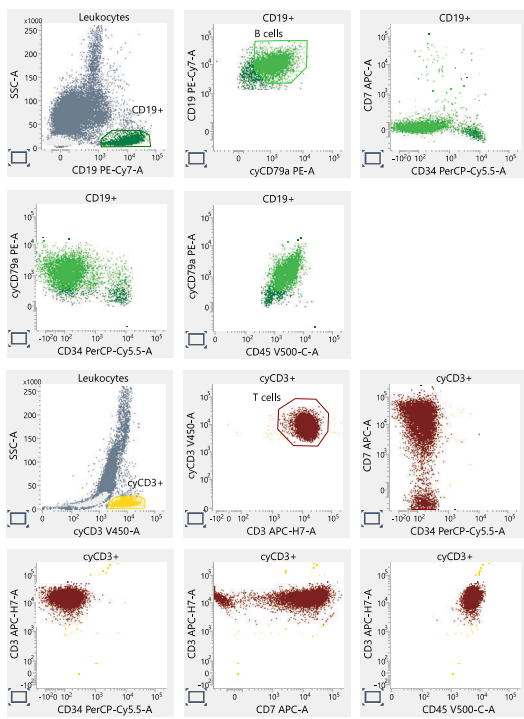
Leukocytes population and then T cells are identified from the cyCD3<sup>+</sup> population. The cyCD3<sup>+</sup> cells are further analyzed using various combinations of CD3, CD7, CD34, and CD45.

CD19<sup>+</sup> cells are identified from the Leukocytes population and then B cells are identified from the CD19<sup>+</sup> population. The CD19<sup>+</sup> cells are further analyzed using various combinations of CD7, CD34, CD79a, and CD45.

Sample ID: 1234  
 Sample Name: abcd  
 Case Number: 12345678

**B cell & T cell Analysis**

| Population | Parent | # Events | % Parent | % Grandparent |
|------------|--------|----------|----------|---------------|
| B cells    | CD19+  | 5,382    | 89.3     | 6.2           |
| T cells    | cyCD3+ | 9,549    | 99.6     | 11.0          |



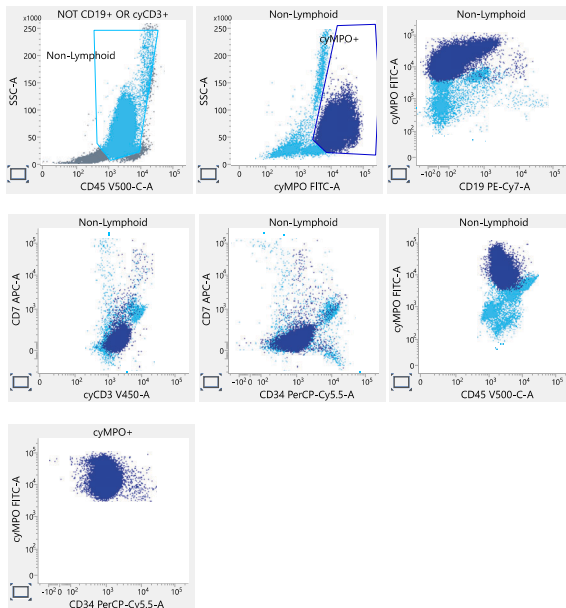
- Inspect the dot plots on page 4 of the laboratory report and adjust the gates as needed.

The dot plots on page 4 of the report identify and analyze non-lymphoid cells. Non-lymphoid cells are identified from the NOT cyCD3+ OR CD19+ population and then the cyMPO+ cells are identified from the Non-Lymphoid population. The non-lymphoid cells are further characterized using MPO, CD19, CD7, cyCD3, CD34, and CD45.

Sample ID: 1234  
 Sample Name: abcd  
 Case Number: 12345678

**Non-Lymphoid cell Analysis**

| Population   | Parent                         | # Events | % Parent | % Grandparent |
|--------------|--------------------------------|----------|----------|---------------|
| Non-Lymphoid | Leukocytes NOT CD19+ OR cyCD3+ | 55,859   | 78.3     | 64.2          |
| cyMPO+       | Non-Lymphoid                   | 49,185   | 88.1     | 68.9          |



6. Inspect the dot plots on page 5 of the laboratory report and adjust the gates as needed.

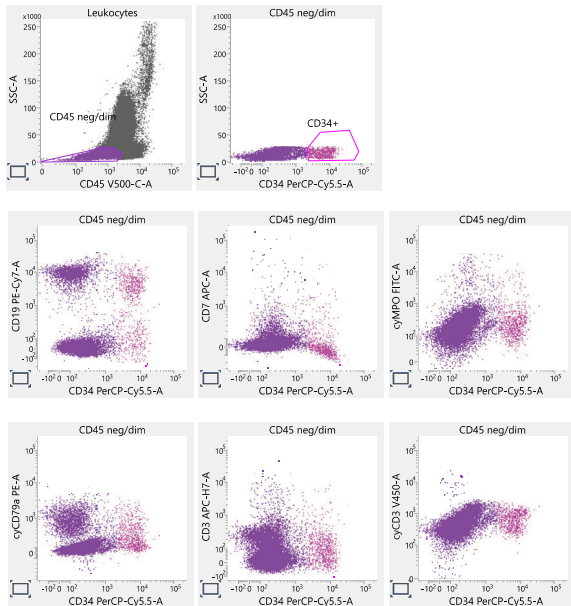
The dot plots on page 5 of the report are used to identify and analyze the CD45 neg/dim cell population. CD45 neg/dim cells are identified from the Leukocytes population and then CD34+ cells are identified from the CD45 neg/dim population.

The remaining dot plots further characterize the CD45 neg/dim cells for CD34 expression in combination with each of the remaining markers.

Sample ID: 1234  
 Sample Name: abcd  
 Case Number: 12345678

**CD45 neg/dim cell Analysis**

| Population   | Parent       | # Events | % Parent | % Grandparent |
|--------------|--------------|----------|----------|---------------|
| CD45 neg/dim | Leukocytes   | 18,918   | 21.8     | 21.8          |
| CD34+        | CD45 neg/dim | 826      | 4.4      | 0.9           |



7. Inspect page 6 of the laboratory report.

Page 6 of the report includes lot and expiration dates for BD® CS&T Beads and the BD OneFlow™ reagent,

reference settings, tube settings, and cytometer configuration.

Sample ID: 1234  
Sample Name: abcd  
Case Number: 12345678

| BD OneFlow ALOT                     |                        |
|-------------------------------------|------------------------|
| CS&T Bead Lot                       | 9420251                |
| CS&T Bead Lot Expiry                | 12/31/2021 0:00:00     |
| Characterization QC Date            | 3/2/2020 16:04:36      |
| LW Reference Settings Creation date | 3/3/2020 10:18:34      |
| LW Reference Settings Modified date | 5/18/2020 16:22:44     |
| Reagent Lot (S)                     | 123457                 |
| Reagent Lot Expiry (S)              | 6/29/2020              |
| Reagent Lot (C)                     | 123456                 |
| Reagent Lot Expiry (C)              | 6/29/2020              |
| Tube Settings                       | BD OneFlow Settings_v1 |
| Cytometer Configuration             | 4-Blue 3-Red 5-Violet  |
| Keyword 1                           | Keyword 1              |
| Keyword 2                           | Keyword 2              |

Signature: Admin User  
Comments: 5/21/2020 17:18:47

8. Select the **Laboratory Report** tab.
9. Click **E-sign**.  
The **E-Signature** dialog opens.
10. Select a user ID.
11. Type your password.
12. (Optional) Enter any comments.

13. Click **Sign**.

The signer's user ID, date and time, and comments are added to the E-signature box in all three reports.

The Laboratory and Physicians Reports are automatically exported to C:\BD Export Clinical. If needed, manually export the Supplemental Report.

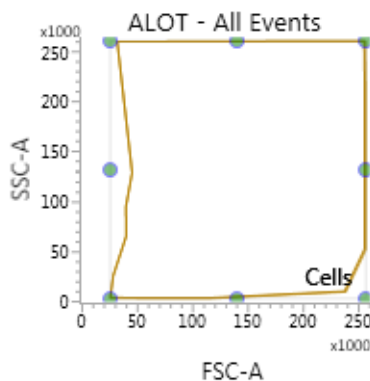
See the *BD FACSLyric™ Clinical System Instructions For Use* for more information and export options.

## Adjusting gates

The provided gates can be adjusted as needed to encompass the population of interest.

To resize or move a gate:

1. Click on a gate in the dot plot so that the gate is in bound mode.

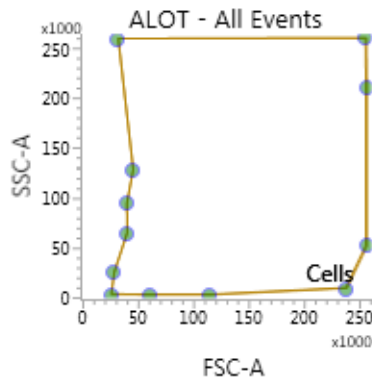


2. Click on one of the circles and drag it to resize the gate.

3. Click on one of the lines between the circles and drag it to move the gate.
4. Click inside any of the circles to rotate the gate.
5. Click the dot plot to exit bound mode.

**To adjust the shape of a gate:**

1. Double-click on a gate in the dot plot so that the gate is in vertex mode.



2. Click on one of the circles and drag it to reshape the gate.
3. Click on one of the lines between the circles and drag it to move the gate.
4. Click inside any of the circles to rotate the gate.
5. Click the dot plot to exit vertex mode.

See the *BD FACSLyric™ Clinical Reference System* for more information.

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## Adding items to the supplemental report

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The OneFlow™ ALOT Laboratory Report includes dot plots and gates to analyze the cell populations in the sample. However, you can add dot plots, gates, and statistics to the OneFlow™ ALOT Supplemental Report if you want to identify additional cell populations.



**Warning** Any gated regions deleted in this Supplemental Report are reflected in the Laboratory and Physician Reports.

Any gated regions created in this Supplemental Report might be reflected in the Laboratory Report.

---

### Procedures

#### To add a dot plot and gate to the report:

1. Click the **Supplemental Report** tab.
2. In the report menu bar, click the create dot plot icon.
3. Click on the report to add the dot plot.

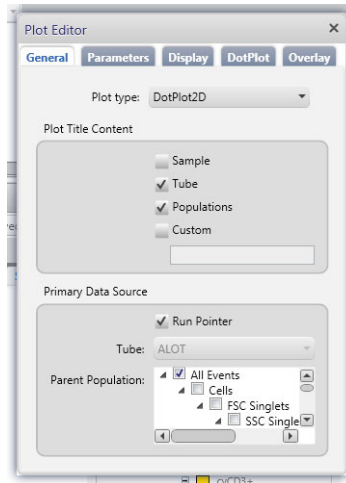
An FSC-A vs SSC-A dot plot named **ALOT - All Events** is added to the report.

4. Right-click on the dot plot and select **Properties** from the menu.

The **Plot Editor** dialog opens.

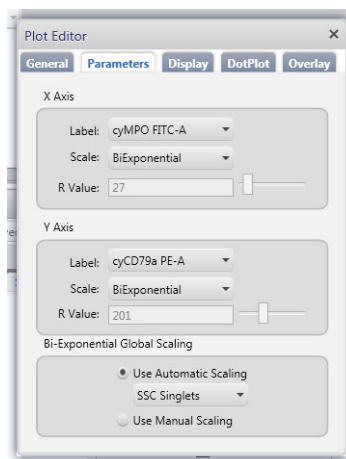


- On the **General** tab in the **Primary Data Source** section, select the **Parent Population** that you want to use.



The title of the dot plot will change to show the selected parent population.

- Click the **Parameters** tab and select the **Label** you want to use for the X Axis and Y Axis from the menu.



The labels of the axes will change to show the selected labels.

7. Click the close box to close the **Plot Editor** dialog.
8. Click the icon for the type of gate you want to add.
9. Draw the gate to encompass the population of interest.
10. Adjust the gate, as needed.

**To add statistics to the report:**

1. In the report menu bar, click to the right of the statistics icon and select **Run Pointer Statistics** to add a statistics box for the selected tube only.
2. Click the statistics icon and then click on the **Supplemental Report**.

A statistics box for the designated tube is added to the report.

| Name                    | All Events | Cells | FSC Singlets | SSC Singlets | Leukocytes | CD19+ | B cells | cyCD3+ | T cells | CD19+ OR cyCD3+ | NOT CD19+ OR cyCD3+ | Non-Lymphoid | cyMPO+ | CD45 neg/dim | CD34+ |
|-------------------------|------------|-------|--------------|--------------|------------|-------|---------|--------|---------|-----------------|---------------------|--------------|--------|--------------|-------|
| Events                  | ***        | ***   | ***          | ***          | ***        | ***   | ***     | ***    | ***     | ***             | ***                 | ***          | ***    | ***          | ***   |
| % Total                 | ***        | ***   | ***          | ***          | ***        | ***   | ***     | ***    | ***     | ***             | ***                 | ***          | ***    | ***          | ***   |
| FSC-A Mean              | ***        | ***   | ***          | ***          | ***        | ***   | ***     | ***    | ***     | ***             | ***                 | ***          | ***    | ***          | ***   |
| SSC-A Mean              | ***        | ***   | ***          | ***          | ***        | ***   | ***     | ***    | ***     | ***             | ***                 | ***          | ***    | ***          | ***   |
| cyMPO FITC-A Mean       | ***        | ***   | ***          | ***          | ***        | ***   | ***     | ***    | ***     | ***             | ***                 | ***          | ***    | ***          | ***   |
| cyCD79a PE-Cy5-A Mean   | ***        | ***   | ***          | ***          | ***        | ***   | ***     | ***    | ***     | ***             | ***                 | ***          | ***    | ***          | ***   |
| CD34 PerCP-Cy5.5-A Mean | ***        | ***   | ***          | ***          | ***        | ***   | ***     | ***    | ***     | ***             | ***                 | ***          | ***    | ***          | ***   |
| CD34 PE-Cy5-A Mean      | ***        | ***   | ***          | ***          | ***        | ***   | ***     | ***    | ***     | ***             | ***                 | ***          | ***    | ***          | ***   |
| CD7 APC-A Mean          | ***        | ***   | ***          | ***          | ***        | ***   | ***     | ***    | ***     | ***             | ***                 | ***          | ***    | ***          | ***   |
| CD34 APC-PE-A Mean      | ***        | ***   | ***          | ***          | ***        | ***   | ***     | ***    | ***     | ***             | ***                 | ***          | ***    | ***          | ***   |
| cyCD3 VAPD-A Mean       | ***        | ***   | ***          | ***          | ***        | ***   | ***     | ***    | ***     | ***             | ***                 | ***          | ***    | ***          | ***   |
| cyMPO SSC-A Mean        | ***        | ***   | ***          | ***          | ***        | ***   | ***     | ***    | ***     | ***             | ***                 | ***          | ***    | ***          | ***   |
| Time Mean               | ***        | ***   | ***          | ***          | ***        | ***   | ***     | ***    | ***     | ***             | ***                 | ***          | ***    | ***          | ***   |

3. Right-click the statistics box and select **Edit Statistics**.

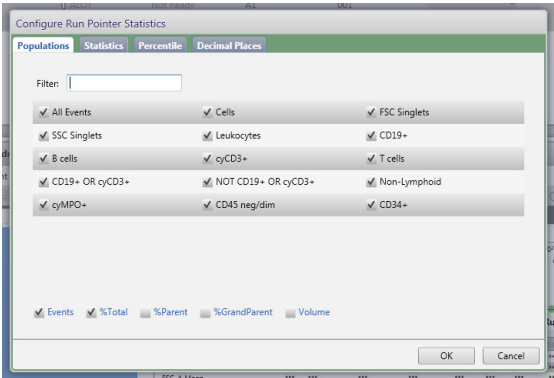
The **Configure Run Pointer Statistics** dialog opens.

4. On the **Statistics** tab, clear the parameters to exclude in the statistics.

Clear the top checkbox to clear all of the parameters.

5. On the **Populations** tab, individually clear all of the unneeded populations.

6. Select the **%Parent** and **%Grandparent** checkboxes, as needed.



7. The statistics box is reconfigured to show only the specified information.

| 1234:ALOT RunPointerStatistics |     |
|--------------------------------|-----|
| Name                           | P1  |
| Events                         | *** |
| % Total                        | *** |
| % Grandparent                  | *** |
| % Parent                       | *** |

**Note:** The statistics for the new gated population will not be shown on the laboratory report.

**Note:** The custom statistics box will not be exported to Laboratory Information Systems (LIS) or exported within the csv file.

**To export the custom statistics box:**

1. Right-click the statistics box.
2. Select **Export Statistics** and the appropriate format.

The **Save As** dialog opens.

3. Click **Save**.

The statistics box is saved as a csv file in C:\BD Export Clinical.

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# 3

## Troubleshooting

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This chapter covers the following topic:

- [Troubleshooting overview \(page 30\)](#)
- [Problems with cell preparation or staining \(page 30\)](#)
- [Problems using BD OneFlow™ ALOT \(page 32\)](#)
- [QC messages \(page 33\)](#)

## Troubleshooting overview

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This chapter lists problems you might encounter when using BD OneFlow™ ALOT, QC messages that might be generated, and provides recommended solutions.

### Additional troubleshooting information

The *BD FACSLyric™ Clinical Reference System* contains additional troubleshooting information covering the cytometer, setup and QC, software QC messages, and general software troubleshooting. The *BD OneFlow™ ALOT IFU* also contains troubleshooting information related to the reagent and sample staining.

If, after reading through the possible problems and solutions and checking the other sources of troubleshooting information, you still have questions, contact BD Biosciences Technical Support. See [Technical support \(page 7\)](#) for information.

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## Problems with cell preparation or staining

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| Problem                                                  | Possible cause                               | Solution                                                                                                                                                                    |
|----------------------------------------------------------|----------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| The resolution between debris and lymphocytes is poor.   | Specimen was over-permeabilized.             | Repeat staining; incubate the tube in FIX & PERM Reagent B for 15 minutes only.                                                                                             |
|                                                          | Specimen is of poor quality.                 | Check cell viability.                                                                                                                                                       |
|                                                          | Specimen is too old.                         | Obtain a new specimen and stain it immediately.                                                                                                                             |
| The cytoplasmic staining (cyMPO, cyCD79a, cyCD3) is dim. | The cells were not completely permeabilized. | Repeat staining; carefully measure the specimen volumes in the cell fixation and permeabilization steps such that the ratio of fixed sample to FIX & PERM Reagent B is 1:1. |

| Problem                        | Possible cause                                            | Solution                                                                                                                                                                                                                                                              |
|--------------------------------|-----------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Cells clump after being fixed. | Cells were not completely resuspended before fixing them. | Vortex tubes until no cell aggregates remain before adding FIX & PERM Reagent A.                                                                                                                                                                                      |
|                                | Cells were not thoroughly washed after fixing them.       | <ol style="list-style-type: none"> <li>1. Fix the cells using FIX &amp; PERM Reagent A.</li> <li>2. Add wash buffer and incubate the tubes for 2 minutes, protected from light.</li> <li>3. Centrifuge the tubes and proceed to the permeabilization step.</li> </ol> |
| Staining is dim or fading.     | Cell concentration was too high at the staining step.     | Check the cell concentration and adjust as needed.                                                                                                                                                                                                                    |
|                                | The BD OneFlow™ tube was exposed to light for too long.   | Repeat staining with a new BD OneFlow™ tube.                                                                                                                                                                                                                          |
|                                | Stained cells were stored too long before acquiring them. | Repeat staining with a fresh specimen and acquire it promptly.                                                                                                                                                                                                        |
| Few or no cells are recorded.  | Cell concentration was too low.                           | Resuspend fresh specimen at a higher concentration. Repeat staining and acquisition.                                                                                                                                                                                  |
|                                | Cytometer is malfunctioning.                              | Troubleshoot the instrument. See the cytometer IFU for more information.                                                                                                                                                                                              |

## Problems using BD OneFlow™ ALOT

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| Problem                                                 | Possible cause                                                                                      | Solution                                                                                                                                                                                                                                            |
|---------------------------------------------------------|-----------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Not enough cells of interest are acquired.              | Cell concentration was too low.                                                                     | Resuspend fresh specimen at a higher concentration. Repeat staining and acquisition.                                                                                                                                                                |
|                                                         | The default setting of 100,000 events acquired is too low.                                          | Change the number of events acquired. Repeat staining and acquisition. See <a href="#">Changing the assay stopping criteria (page 12)</a> .                                                                                                         |
| The FSC-A vs SSC-A dot plot is abnormal.                | Cytometer needs adjusting.                                                                          | Contact BD Biosciences.                                                                                                                                                                                                                             |
| The csv file and report are not exported automatically. | The reagent lot number and expiration date were not added to the Library.                           | <ol style="list-style-type: none"> <li>1. Add the reagent lot number and expiration date to the Library.</li> <li>2. Export the csv file and the report PDF manually. See the <i>BD FACSLyric™ Clinical System Instructions For Use</i>.</li> </ol> |
| The <b>Run Pointer Statistics</b> dialog is cropped.    | <b>Edit Populations</b> was selected when the statistics box was edited in the Supplemental Report. | Select <b>Edit Statistics</b> when editing the statistics box.                                                                                                                                                                                      |

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## QC messages

Review any QC messages to address potential issues and determine whether they affect the results.

| QC message                                                | Possible cause                                                          | Recommended solution                                                                                                                                                                                                 |
|-----------------------------------------------------------|-------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| All Events gate does not contain requested 100,000 events | Cell concentration was too low.                                         | Determine whether there are enough events to make a decision. If needed, resuspend fresh specimen at a higher concentration. Repeat staining and acquisition.                                                        |
|                                                           | The default setting of 100,000 events acquired is too high.             | Determine whether there are enough events to make a decision. If needed, change the number of events acquired. Repeat staining and acquisition. See <a href="#">Changing the assay stopping criteria (page 12)</a> . |
| Acquired with expired Performance QC                      | Daily PQC was not performed during cytometer setup.                     | Use BD <sup>®</sup> CS&T Beads to perform daily PQC. See the <i>BD FACSLyric<sup>™</sup> Instructions for Use</i> .                                                                                                  |
| Acquired without completed Assay Setup                    | Assay and tube settings setup was not performed during cytometer setup. | Use BD <sup>®</sup> CS&T Beads to perform assay and tube settings setup. See the <i>BD FACSLyric<sup>™</sup> Instructions for Use</i> .                                                                              |

| QC message                                               | Possible cause                                                             | Recommended solution                                                                                                                                                       |
|----------------------------------------------------------|----------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Acquired with expired reagent: ALOT                      | The wrong reagent lot and expiration date are in the Library.              | Confirm that the lot and expiration date in the Library match those on the tube label. If necessary, enter the correct information, re-stain the specimen, and acquire it. |
|                                                          | The reagent is past the expiration date.                                   | Repeat staining with a new tube that has not expired. Ensure that the new lot and expiration date are entered in the Library and acquire the sample.                       |
| Acquired with expired Reference Settings                 | The reference settings are expired.                                        | Use BD® FC Beads to update the reference settings. See the <i>BD FACSLyric™ Clinical Reference System</i> .                                                                |
| Acquired with modified tube settings or spillover values | The voltages, compensation, or threshold were adjusted during acquisition. | Repeat staining with a new tube and acquire the sample without making adjustments during acquisition.                                                                      |

If you are running OneFlow™ ALOT assay v1.1 in BD FACSuite™ Clinical application v1.5, these additional QC messages might be shown.

| QC message      | Possible cause                                                  | Recommended solution                                                                                                          |
|-----------------|-----------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------|
| Gate(s) added   | A new gate(s) was created during acquisition or analysis.       | Determine whether the additional gate(s) help make a decision. If needed, remove the added gate(s) before signing the report. |
| Gate(s) deleted | An existing gate(s) was deleted during acquisition or analysis. | Repeat staining with a new tube and acquire the sample with the default gates.                                                |

| QC message                            | Possible cause                                                            | Recommended solution                                                                                                                                                     |
|---------------------------------------|---------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Additional plot(s) created            | A new plot(s) was created during acquisition or analysis.                 | Determine whether the additional plot(s) help make a decision. If needed, remove the added plot(s) before signing the report.                                            |
| Plot(s) removed                       | An existing plot(s) was deleted during acquisition or analysis.           | Repeat staining with a new tube and acquire the sample with the default plots.                                                                                           |
| Acquired with modified stopping rules | The default settings for stopping rules were adjusted during acquisition. | Determine whether there are enough events to make a decision. If needed, repeat staining and acquire the sample without adjusting the stopping rules during acquisition. |

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## Contact information

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