

BD OneFlow™ Application Guide for Acute Leukemias

For BD FACSLyric™ Flow Cytometers



23-21494-00
5/2020



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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

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Introduction

This chapter covers the following topics:

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

About this guide

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

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.

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

Technical support

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*.
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Contacting technical support

To contact technical support:

1. Go to bdbiosciences.com.
2. Select your region.
3. Click the **Support** link 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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BD OneFlow ALOT Assay

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

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.

BD OneFlow ALOT workflow

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 Changing the assay stopping criteria (page 12) .
6	Acquire samples. See the <i>BD FACSLyric™ Clinical System Instructions For Use</i> .
7	Review the laboratory report. See Reviewing the laboratory report (page 14) .
8	Adjust gates, if necessary.
9	Add dot plots and gates, if necessary. See Adding items to the supplemental report (page 24) .

Changing the assay stopping criteria

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

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.

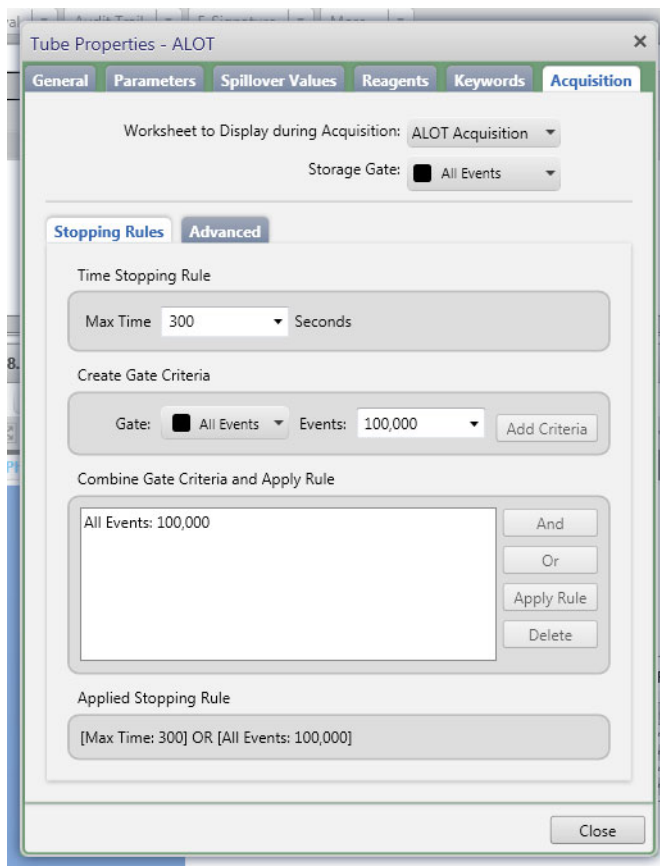
Note: We recommend that you do not increase the acquisition time and risk loss of the sample due to insufficient volume.

6. To change the number of events acquired, in the **Create Gate Criteria** section, click the triangle in the **Events** field.
7. From the menu, select the number of events you want to acquire. 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

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.

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 [QC messages \(page 33\)](#) for information.

 **BD 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 FACSLyric	Cytometer SN: 1234567890	Software: BD FACSuite Clinical v1.4
Operator: Admin User	Director: Mr. J. Smith	Institution: BD
	Department: Reagents & Assays	Address: Limerick Ireland

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

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.

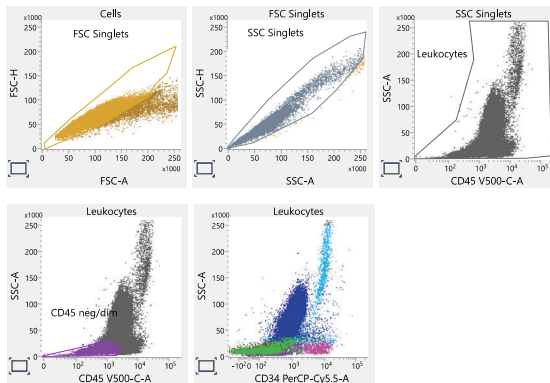
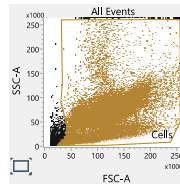
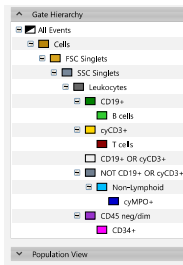
Note: The gates in the dot plots of the OneFlow ALOT Laboratory Report are provided for analyzing normal and aberrant cell populations in the specimen.

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+	53,859	78.3	64.2
CD45 neg/dim	Leukocytes	18,918	21.8	21.8



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- 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⁺ cells are identified from the

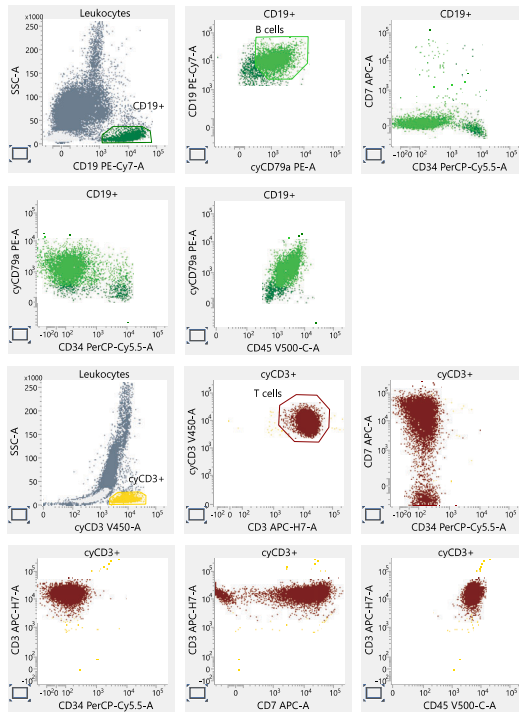
Leukocytes population and then T cells are identified from the cyCD3⁺ population. The cyCD3⁺ cells are further analyzed using various combinations of CD3, CD7, CD34, and CD45.

CD19⁺ cells are identified from the Leukocytes population and then B cells are identified from the CD19⁺ population. The CD19⁺ 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



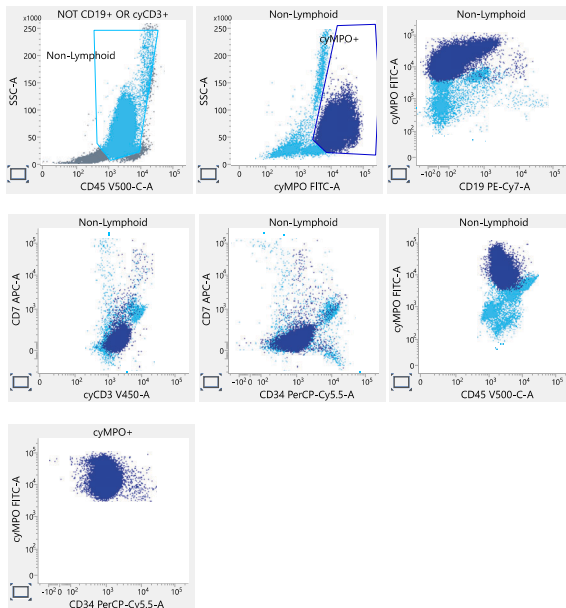
- Inspect the dot plots on page 4 of the laboratory report and adjust the gates as needed.

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.

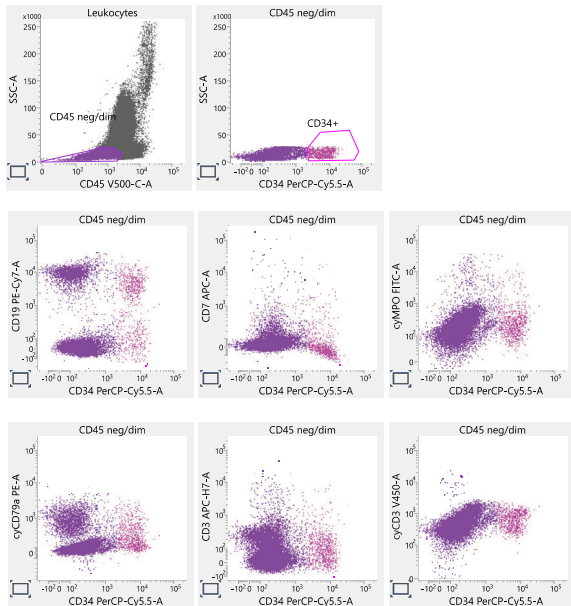
The dot plots on page 5 of the report are used to identify and analyze the CD45 neg/dim cell population. CD45 neg/dim cells are identified from the Leukocytes population and then CD34+ cells are identified from the CD45 neg/dim population.

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	5/21/2020 17:18:47
Comments:	

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Assay: OneFlow ALOT
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8. Select the **Laboratory Report** tab.
9. Click **Approved**.
 The **ESignature** 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.

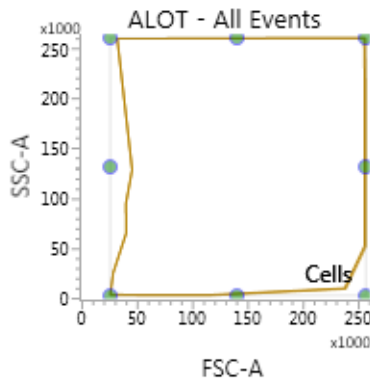
See the *BD FACSLyric™ Clinical System Instructions For Use* for more information and export options.

Adjusting gates

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

To resize or move a gate:

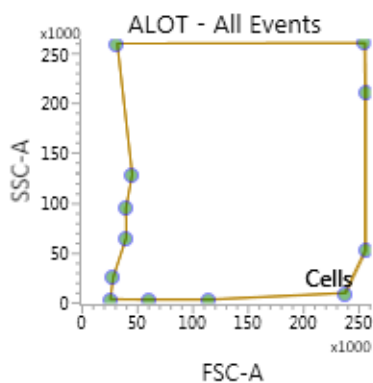
1. Click on a gate in the dot plot so that the gate is in bound mode.



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.

Adding items to the supplemental report

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:

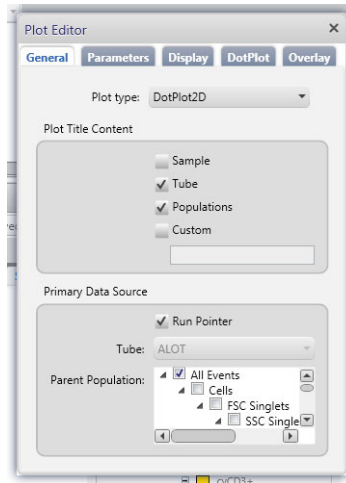
1. Click the **Supplemental Report** tab.
2. In the report menu bar, click the create dot plot icon.
3. Click on the report to add the dot plot.

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.

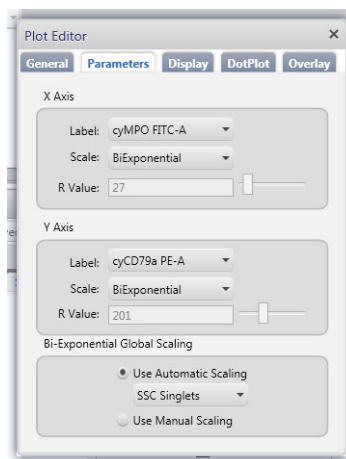
The **Plot Editor** dialog opens.

5. 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.

6. 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	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
CD19 PerCF-Cy5.5-A Mean	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
CD34 PE-Cy5-A Mean	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
CD7 APC-A Mean	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
CD34 APC-PE-A Mean	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
cyCD3 V450-A Mean	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
cyMPO SSC-A Mean	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
Time Mean	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***

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

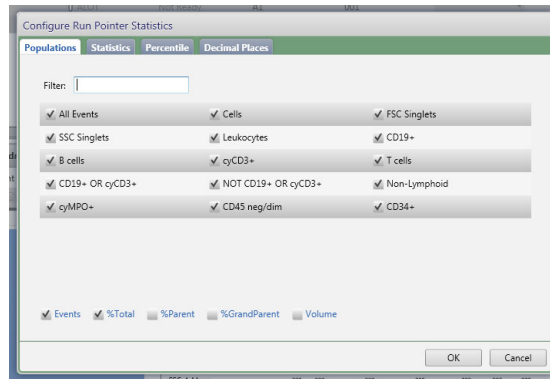
The **Configure Run Pointer Statistics** dialog opens.

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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Troubleshooting

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

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.

Problems with cell preparation or staining

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"> 1. Fix the cells using FIX & PERM Reagent A. 2. Add wash buffer and incubate the tubes for 2 minutes, protected from light. 3. Centrifuge the tubes and proceed to the permeabilization step.
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

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 Changing the assay stopping criteria (page 12) .
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 manually.	<ol style="list-style-type: none"> 1. Add the reagent lot number and expiration date to the Library. 2. Export the csv file and the report PDF manually. See the <i>BD FACSLyric™ Clinical System Instructions For Use</i>.
The Run Pointer Statistics dialog is cropped.	Edit Populations was selected when the statistics box was edited in the Supplemental Report.	Select Edit Statistics when editing the statistics box.

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 Changing the assay stopping criteria (page 12) .
Acquired with expired Performance QC	Daily PQC was not performed during cytometer setup.	Use BD CS&T Beads to perform daily PQC. See the <i>BD FACSLyric™ Instructions for Use</i> .
Acquired without completed Assay Setup	Assay and tube settings setup was not performed during cytometer setup.	Use BD CS&T Beads to perform assay and tube settings setup. See the <i>BD FACSLyric™ 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.
