

 **BD OneFlow™ LST**
Application Guide

**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
23-21491-00	5/2020	Initial release
23-21491-01	12/2020	Updated troubleshooting to add new QC messages and assay description for the stopping criteria. Updated workflow for contacting technical service.

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Introduction

This chapter covers the following topics:

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

About this guide

This guide covers the acquisition and analysis workflows for the BD OneFlow™ LST assay using BD FACSuite™ Clinical application, and describes the OneFlow™ LST 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 acquiring samples.

Additional information

See the *BD OneFlow™ LST 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 **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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BD OneFlow™ LST Assay

This chapter covers the following topics:

- [About the BD OneFlow™ LST assay \(page 10\)](#)
- [BD OneFlow™ LST workflow \(page 12\)](#)
- [Changing the assay stopping criteria \(page 12\)](#)
- [Reviewing the laboratory report \(page 15\)](#)
- [Adjusting gates \(page 23\)](#)
- [Adding items to the supplemental report \(page 25\)](#)

About the BD OneFlow™ LST assay

BD OneFlow™ LST is a qualitative 8-color (12-antibody) direct immunofluorescence assay for the identification of normal and aberrant mature lymphocyte populations of B-, T-, and NK-cell lineages in peripheral blood, bone marrow, and lymph nodes. It is a screening tube, meant to direct further analysis of the patient specimen.

About the assay

The BD OneFlow™ LST 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™ LST reagent consists of single-use tubes containing a panel of fluorochrome-conjugated antibodies in an optimized dried formulation. The panel comprises the following antibodies:

Antibody	Fluorochrome
CD8	FITC
Anti-Lambda	FITC
CD56	PE
Anti-Kappa	PE
CD5	PerCP-Cy™5.5
CD19	PE-Cy™7
Anti-TCRγ/δ-1	PE-Cy7
CD3	APC
CD38	APC-H7

Antibody	Fluorochrome
CD4	BD Horizon™ V450
CD20	V450
CD45	BD Horizon™ V500-C

The BD OneFlow™ LST reagent is used to stain patient specimens. The stained samples are acquired on the cytometer and then analyzed to identify normal and aberrant mature lymphocyte populations of B, T, and NK lineages.

BD OneFlow™ LST workflow

Workflow steps The following table lists the steps in a typical BD OneFlow™ LST 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™ LST IFU</i> for information.
3	Enter reagent lot and expiration date in the Library. See the <i>BD OneFlow™ LST 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 acquire, 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 15) .
8	Adjust gates, if necessary. See Adjusting gates (page 23) .
9	Add dot plots and gates, if necessary. See Adding items to the supplemental report (page 25) .

Changing the assay stopping criteria

The OneFlow™ LST 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.

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 - LST** 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.

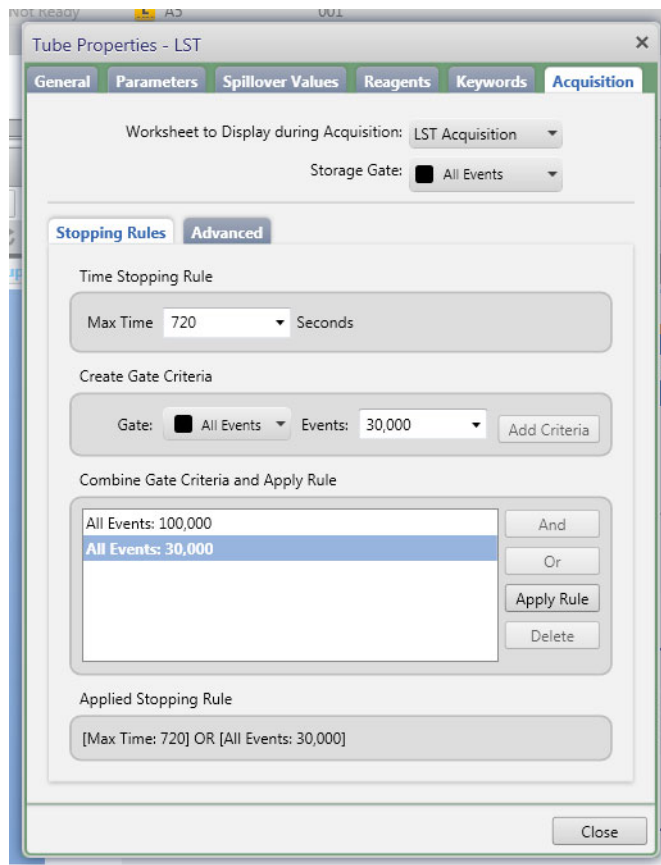
Note: We recommend that you do not increase the acquisition time and risk loss of the sample due to insufficient volume.

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 number of events 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™ LST 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 [Troubleshooting \(page 31\)](#) for information.



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

Acquired Using: Worklist_013	Approved: 5/25/2020 9:30:45	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: LST

Events Acquired	100,000	Sample Type	Blood
Performance QC Date	5/21/2020 16:54:22	Acquisition Date	5/21/2020
Performance QC Status	Pass	Acquisition Start Time	17:06:59
		Acquisition End Time	17:07:10

Results Summary

Population	Parent	# Events	% Parent	% Grandparent	Ratio
All Events		100,000			
Cells	All Events	84,217	84.2		
FSC Singlets	Cells	82,122	97.5	82.1	
SSC Singlets	FSC Singlets	82,100	100.0	97.5	
Leukocytes	SSC Singlets	81,651	99.5	99.4	
Lymphocytes	Leukocytes	24,793	30.4	30.2	
B cells	Lymphocytes	2,417	9.7	3.0	
IgK	B cells	1,317	54.5	5.3	
IgL	B cells	1,091	45.1	4.4	
B cell Ratio - IgK : IgL					1.2
T cells	Lymphocytes	19,753	79.7	24.2	
TCRgd+	T cells	571	2.9	2.3	
TCRgd-	T cells	19,149	96.9	77.2	
CD4+ CD8-	TCRgd-	11,782	61.5	59.6	
CD8+ CD4-	TCRgd-	6,896	36.0	34.9	
CD4+ CD8+	TCRgd-	87	0.5	0.4	
CD4- CD8-	TCRgd-	348	1.8	1.8	
T cell Ratio - CD4 : CD8					1.7
NK cells	NOT B cells OR T cells	2,541	96.9	10.2	
LymphoSUM			99.7		

QC Messages

Showing 0 of 0 QC Messages

- 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™ LST Laboratory Report are provided for analyzing normal and aberrant cell populations in the specimen.

The dot plots on page 2 of the report provide a high level cell analysis.

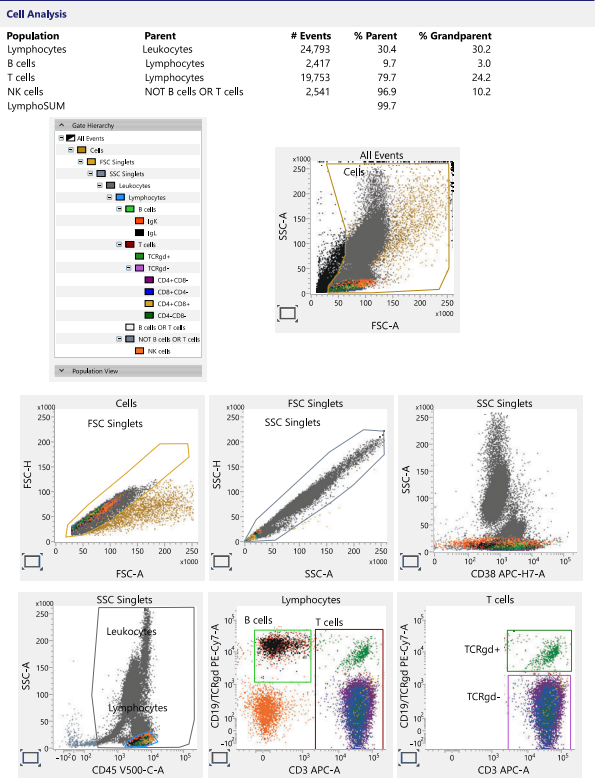
The first three dot plots on the report identify cells, FSC singlets, and SSC singlets. Debris and doublets are excluded by adjusting the gates.

Leukocytes and lymphocytes are identified in the CD45 V500-A vs SSC-A dot plot from the SSC Singlets population.

B cells (CD3⁻CD19⁺) and T cells (CD3⁺) are identified in the CD3 APC-A vs CD19/TCRgd PE-Cy7-A dot plot from the Lymphocytes population.

TCR $\gamma\delta^+$ T cells and TCR $\gamma\delta^-$ T cells are identified in the CD3 APC-A vs CD19/TCRgd PE-Cy7-A dot plot from the T cells population.

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



- 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 are used to analyze B cells. Examine the level of CD20 expression in the CD19/TCRgd PE-Cy7-A vs CD20/CD4 V450-A dot plot.

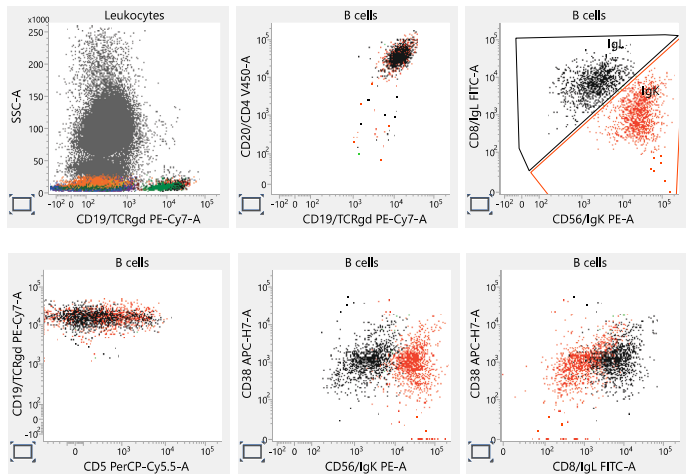
Examine the ratio of Igk- to Igλ-expressing B cells in the CD56/IgK PE-A vs CD8/IgL FITC-A dot plot.

The remaining dot plots further characterize B cells using various markers.

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

B cell Analysis

Population	Parent	# Events	% Parent	% Grandparent	Ratio
B cells	Lymphocytes	2,417	9.7	3.0	
IgK	B cells	1,317	54.5	5.3	
IgL	B cells	1,091	45.1	4.4	
B cell Ratio - IgK : IgL					1.2



- 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 various populations of T cells. TCR γ/δ ⁻ T cells are divided into CD4⁺CD8⁻, CD8⁺CD4⁻, CD4⁺CD8⁺, and CD4⁻CD8⁻ populations in the CD20/CD4 V450-A vs CD8/IgL FITC-A dot plot.

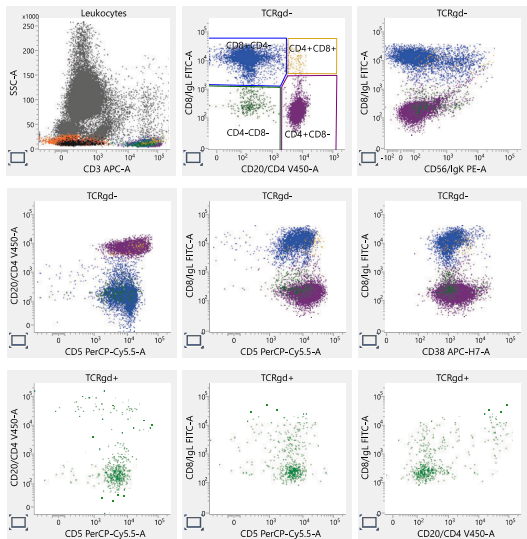
Note: The CD4⁺ and CD8⁺ populations of T cells might trail into the double positive quadrant instead of being discrete

populations. This is a consequence of the panel of antibodies present in BD OneFlow™ LST.

The remaining dot plots further characterize TCR γ/δ^- and TCR γ/δ^+ cells using various markers.

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

T cell Analysis					
Population	Parent	# Events	% Parent	% Grandparent	Ratio
T cells	Lymphocytes	19,753	79.7	24.2	
TCRgd+	T cells	571	2.9	2.3	
TCRgd-	T cells	19,149	96.9	77.2	
CD4+ CD8-	TCRgd-	11,782	61.5	59.6	
CD8+ CD4-	TCRgd-	6,896	36.0	34.9	
CD4+ CD8+	TCRgd-	87	0.5	0.4	
CD4- CD8-	TCRgd-	348	1.8	1.8	
T cell Ratio - CD4 - CD8					1.7



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Assay: OneFlow LST
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- Inspect the dot plots on page 5 of the laboratory report and adjust the gates as needed.

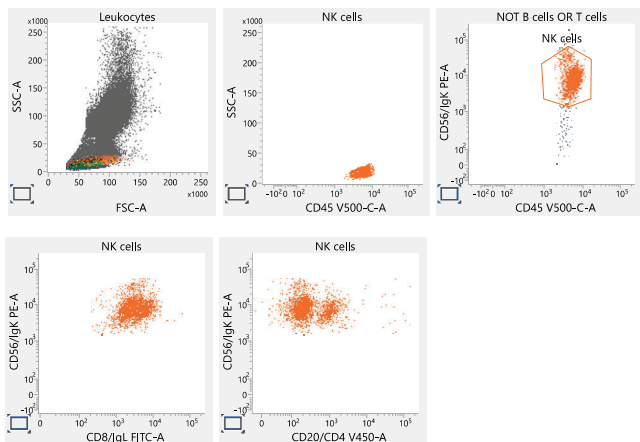
The dot plots on page 5 of the report identify NK cells. NK cells are identified from the NOT T cells OR B cells population in the CD45 V500-C-A vs CD56/IgK PE-A dot plot.

The remaining dot plots further characterize NK cells using various markers.

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

NK cell Analysis

Population	Parent	# Events	% Parent	% Grandparent
NOT B cells OR T cells	Lymphocytes	2,623	10.6	3.2
NK cells	NOT B cells OR T cells	2,541	96.9	10.2



- 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 LST	
CS&T Bead Lot	9420251
CS&T Bead Lot Expiry	12/31/2021 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	123459
Reagent Lot Expiry	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	5/25/2020 9:30:34
Comments:	

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.

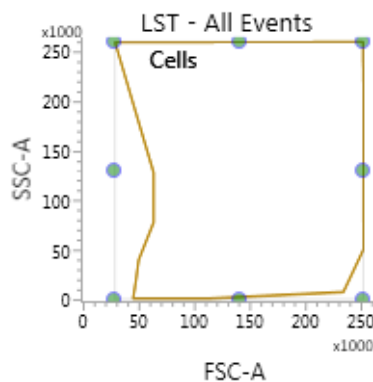
See the *BD FACSLyric™ Clinical System Instructions For Use* for more information and export options.

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.

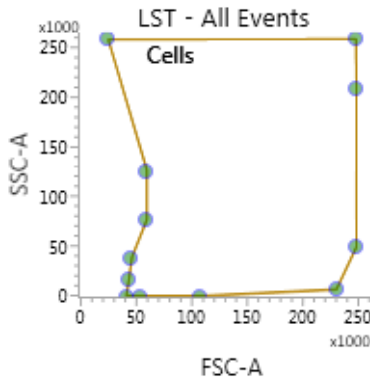


2. Click on one of the circles and drag it to resize the gate.

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.

Adding items to the supplemental report

The OneFlow™ LST 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™ LST 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:

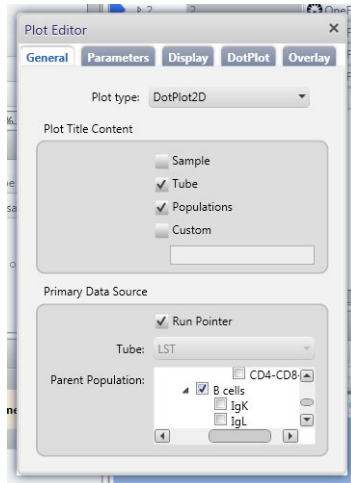
1. Click the **Supplemental Report** tab.
2. In the report menu bar, click the create dot plot icon.
3. Click on the report to add the dot plot.

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

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

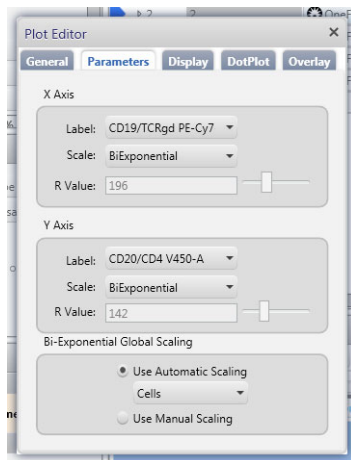
The **Plot Editor** dialog opens.

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

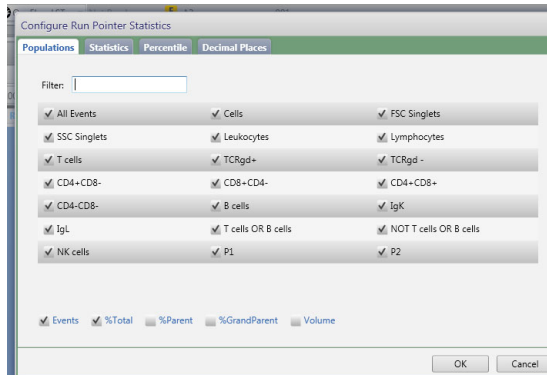


The title of the dot plot will change to show the selected parent population.

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



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



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

Name	P2
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

This chapter covers the following topics:

- [Troubleshooting overview \(page 32\)](#)
- [Problems with cell preparation or staining \(page 32\)](#)
- [Problems using BD OneFlow™ LST \(page 33\)](#)
- [QC messages \(page 35\)](#)

Troubleshooting overview

This chapter lists problems you might encounter when using BD OneFlow™ LST, 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™ LST 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 poorly lysed.	Prepare and stain another specimen.
	Specimen is of poor quality.	Check cell viability.
	Specimen is too old.	Obtain a new specimen and stain it immediately.

Problem	Possible cause	Solution
Staining is dim or fading.	Cell concentration was too high at the staining step.	Check the cell concentration and adjust as needed.
	Washed specimen was not stained within 30 minutes of the last wash.	Repeat staining with a freshly prepared specimen.
	The BD OneFlow™ reagent was exposed to light for too long.	Repeat staining with a new 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™ LST

Problem	Possible cause	Recommended 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) .

Problem	Possible cause	Recommended solution
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">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: LST	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™ LST 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.

Contact information



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