BD OneFlow™ Application Guide for B-cell Chronic Lymphoproliferative Diseases

For BD FACSLyric™ Flow Cytometers

IVD CE

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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
23-21492-00	5/2020	Initial release

Contents

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Introduction

This chapter covers the following topics:

About this guide

What's in this guide

This guide covers the acquisition and analysis workflows for the BD OneFlow™ B-CLPD T1 assay using BD FACSuite™ Clinical software and describes the BD OneFlow B-CLPD T1 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™ B-CLPD T1* 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*.

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 B-CLPD T1 Assay

This chapter covers the following topics:

About the BD OneFlow B-CLPD T1 Assay

Purpose of the assay

BD OneFlow B-CLPD T1 is a qualitative 8-color direct immunofluorescence assay for immunophenotyping B cells in peripheral blood and bone marrow. It is used in combination with BD OneFlow™ LST to distinguish between chronic lymphocytic leukemia (CLL) and other B-cell chronic lymphoproliferative diseases.

About the assay

The BD OneFlow B-CLPD T1 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 B-CLPD T1 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
CD23	FITC
CD10	PE
CD79b	PerCP-Cy™5.5
CD19	PE-Cy™7
CD200	APC
CD43	APC-H7
CD20	BD Horizon™ V450
CD45	BD Horizon™ V500-C

The BD OneFlow B-CLPD T1 reagent is used to stain patient specimens. The stained samples are acquired on the cytometer and then analyzed to characterize populations of B cells.

BD OneFlow B-CLPD T1 workflow

Workflow steps

The following table lists the steps in a typical BD OneFlow B-CLPD T1 assay workflow.

Step	Description
1	Perform daily instrument Performance QC (PQC) and assay and tube settings setup using BD® CS&T Beads. See the *BD FACSLyric™ Clinical System Instructions For Use*.
2	Prepare the patient specimens. See the *BD OneFlow™ B-CLPD T1* IFU for information.
3	Enter reagent lot and expiration date in the Library. See the *BD OneFlow™ B-CLPD T1* IFU for information.
4	Create worklist. See the *BD FACSLyric™ Clinical System Instructions For Use*.
5	Optional: change the number of events to collect, if needed. See Changing the assay stopping criteria (page 12).
6	Acquire samples. See the *BD FACSLyric™ Clinical System Instructions For Use*.
7	Review the laboratory report. See Reviewing the laboratory report (page 14).
8	Adjust gates, if necessary. See Adjusting gates (page 19)
9	Add dot plots and gates, if necessary. See Adding items to the supplemental report (page 21).

Changing the assay stopping criteria

The OneFlow B-CLPD T1 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.

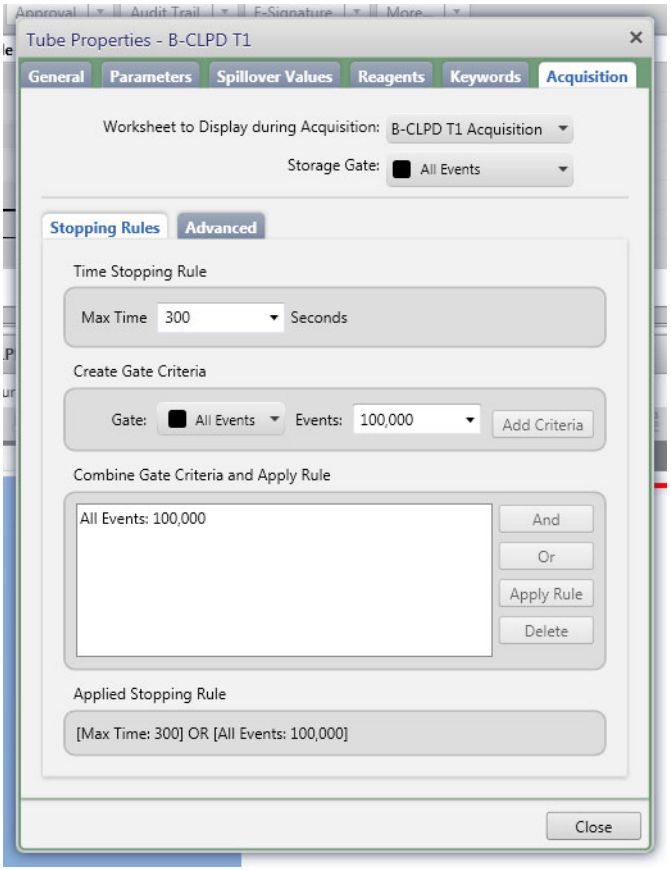
   The run pointer will move to the newly expanded tube.
3. Right-click the run pointer and select **Tube Properties** from the menu.

   The **Tube Properties - B-CLPD T1** 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 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.

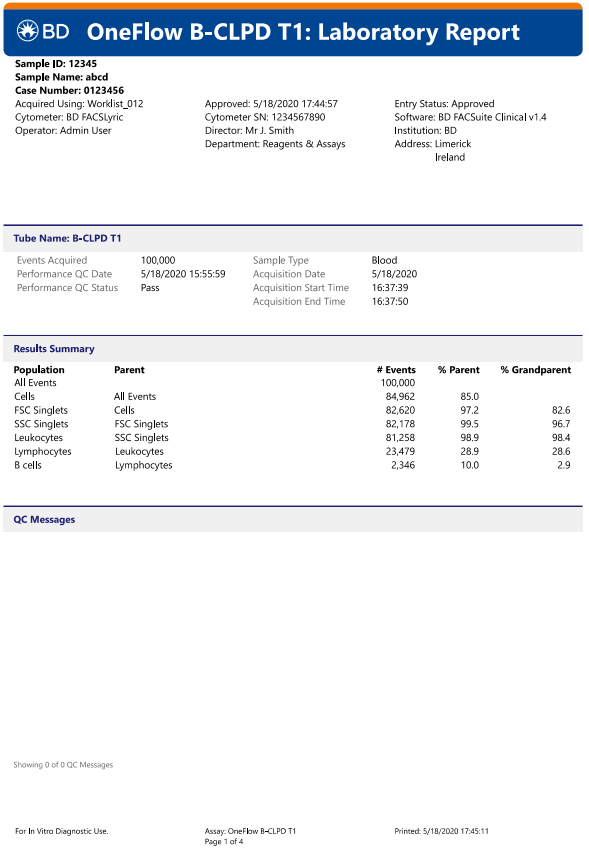
Reviewing the laboratory report

The OneFlow B-CLPD T1 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 lab 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 30) for information.



BD OneFlow B-CLPD T1: Laboratory Report

Sample ID: 12345
Sample Name: abcd
Case Number: 0123456
Acquired Using: Worklist_012
Cytometer: BD FACSLyric
Operator: Admin User

Approved: 5/18/2020 17:44:57
Cytometer SN: 1234567890
Director: Mr J. Smith
Department: Reagents & Assays

Entry Status: Approved
Software: BD FACSuite Clinical v1.4
Institution: BD
Address: Limerick
Ireland

Tube Name: B-CLPD T1

Events Acquired	100,000	Sample Type	Blood
Performance QC Date	5/18/2020 15:55:59	Acquisition Date	5/18/2020
Performance QC Status	Pass	Acquisition Start Time	16:37:39
		Acquisition End Time	16:37:50

Results Summary

Population	Parent	# Events	% Parent	% Grandparent
All Events		100,000		
Cells	All Events	84,962	85.0	
FSC Singlets	Cells	82,620	97.2	82.6
SSC Singlets	FSC Singlets	82,178	99.5	96.7
Leukocytes	SSC Singlets	81,258	98.9	98.4
Lymphocytes	Leukocytes	23,479	28.9	28.6
B cells	Lymphocytes	2,346	10.0	2.9

QC Messages

Showing 0 of 0 QC Messages

For In Vitro Diagnostic Use.

Assay: OneFlow B-CLPD T1
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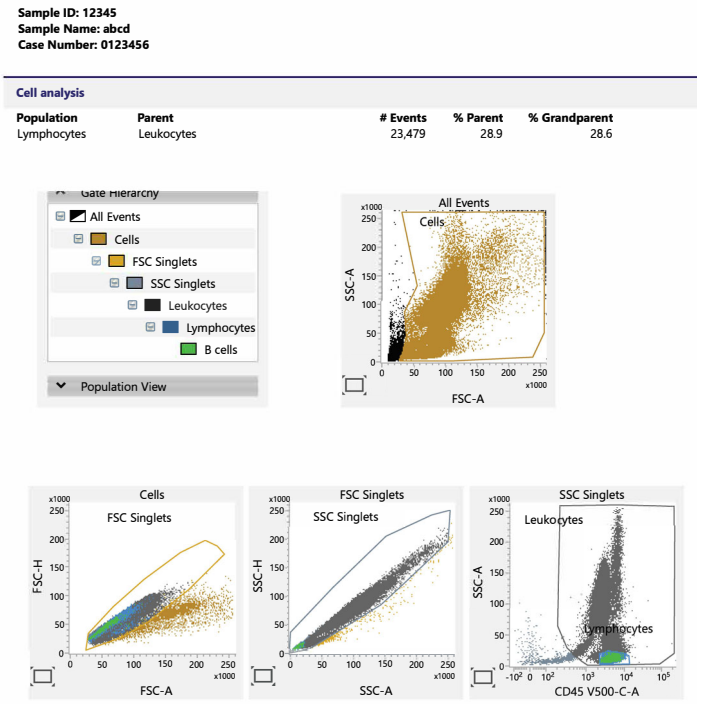
Printed: 5/18/2020 17:45:11

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 B-CLPD T1 Laboratory Report are provided for analyzing normal and aberrant cell populations in the specimen.

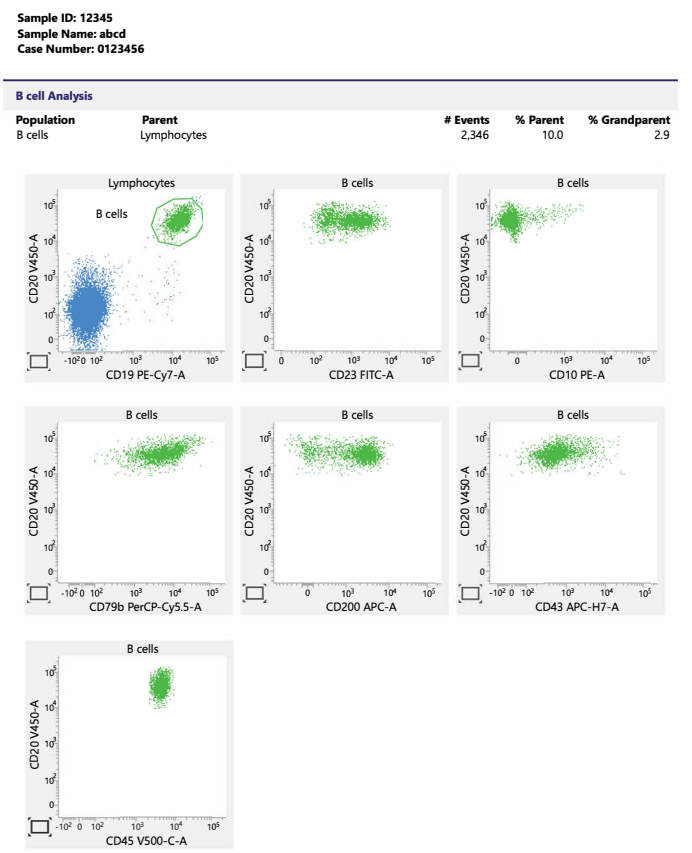
The dot plots on page 2 of the report identify the major cell populations. First, debris is removed and the singlet populations are identified, followed by the Leukocytes and Lymphocytes.



4. 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 B cells. B cell are identified in the CD19 PE-Cy7-A vs CD20 V450-A dot plot from the Lymphocytes population.

The B-cells are further characterized according to the levels of CD23, CD10, CD79b, CD200, CD43, and CD45 expression in the remaining dot plots.



5. Inspect page 4 of the laboratory report.

Page 4 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: 12345
Sample Name: abcd
Case Number: 0123456

BD OneFlow B-CLPD T1

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	123458
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 5/18/2020 17:44:54
Comments:

For In Vitro Diagnostic Use. Assay: OneFlow B-CLPD T1 Printed: 5/18/2020 17:45:11
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6. Select the **Laboratory Report** tab.
7. Click **Approved**.

   The **ESignature** dialog opens.
8. Select a user ID.
9. Type your password.
10. (Optional) Enter any comments.

11. Click **Sign**.

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

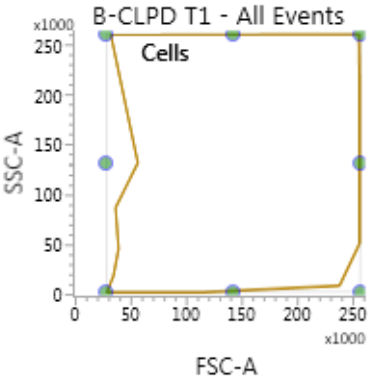
    See the *BD FACSLyric™ Clinical System Instructions For Use* for more information and export options.

Adjusting gates

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

To resize or move a gate:

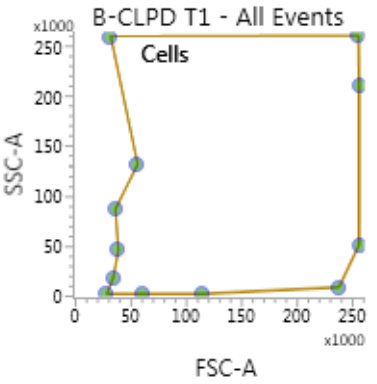
1. Click on a gate in the dot plot so that the gate is in bound mode.



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 B-CLPD T1 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 B-CLPD T1 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

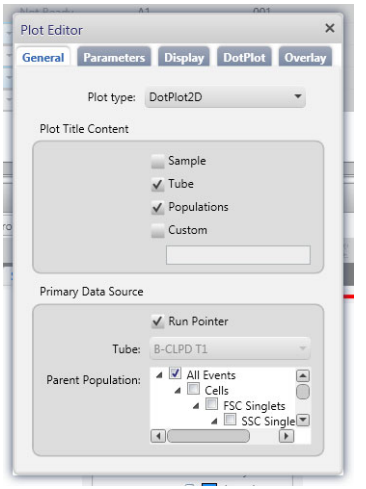
To add a dot plot and gate to the report:

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 **B-CLPD T1 - All Events** is added to the report.
4. Right-click on the dot plot and select **Properties** from the menu.

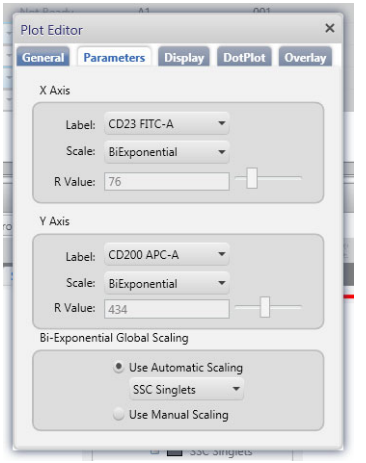
   The **Plot Editor** dialog opens.

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.

2234:B-CLPD T1 RunPointerStatistics

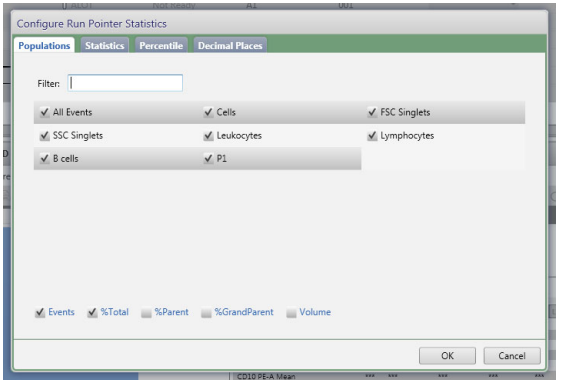
Name	All Events	Cells	FSC Singlets	SSC Singlets	Leukocytes	Lymphocytes	B cells	P1
Events	***	***	***	***	***	***	***	***
% Total	***	***	***	***	***	***	***	***
FSC-A Mean	***	***	***	***	***	***	***	***
SSC-A Mean	***	***	***	***	***	***	***	***
CD23 FITC-A Mean	***	***	***	***	***	***	***	***
CD10 PE-A Mean	***	***	***	***	***	***	***	***
CD79b PerCP-Cy5.5-A Mean	***	***	***	***	***	***	***	***
CD19 PE-Cy7-A Mean	***	***	***	***	***	***	***	***
CD200 APC-A Mean	***	***	***	***	***	***	***	***
CD43 APC-H7-A Mean	***	***	***	***	***	***	***	***
CD20 V450-A Mean	***	***	***	***	***	***	***	***
CD45 V500-C-A Mean	***	***	***	***	***	***	***	***
Time Mean	***	***	***	***	***	***	***	***

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

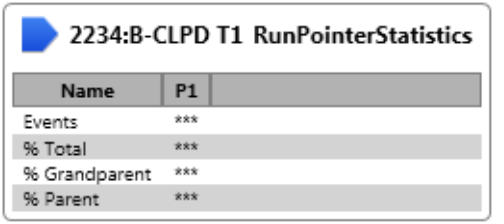
   The **Configure Run Pointer Statistics** dialog opens.
4. On the **Statistics** tab, clear the parameters to exclude in the statistics.

   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.



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

This chapter lists problems you might encounter when using BD OneFlow B-CLPD T1, 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™ B-CLPD T1* 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 B-CLPD T1

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.

Problem	Possible cause	Solution
The csv file and report are not exported automatically.	The reagent lot number and expiration date were not added to the Library manually.	1. Add the reagent lot number and expiration date to the Library. 2. Export the csv file and the report PDF manually. See the *BD FACSLyric™ Clinical System Instructions For Use*.
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 *BD FACSLyric™ Instructions for Use*.

QC message	Possible cause	Recommended solution
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 *BD FACSLyric™ Instructions for Use*.
Acquired with expired reagent: B-CLPD T1	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 *BD FACSLyric™ Clinical Reference System*.
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.