 **BD OneFlow™**
**Application Guide for
Plasma Cell Disorders**

**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
23-21493-00	5/2020	Initial release
23-21493-01	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

What's in this guide This guide covers the acquisition and analysis workflows for the BD OneFlow™ PCST and BD OneFlow™ PCD assays using BD FACSuite™ Clinical software and describes the OneFlow™ PCST Laboratory Report and the OneFlow™ PCD 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™ PCST IFU* and *BD OneFlow™ PCD 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 **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™ PCST and BD OneFlow™ PCD Assays

This chapter covers the following topics:

- [About the Assays \(page 10\)](#)
- [Assay workflow \(page 12\)](#)
- [Changing the assay stopping criteria \(page 13\)](#)
- [Reviewing the laboratory report \(page 15\)](#)
- [Adjusting gates \(page 25\)](#)
- [Adding items to the supplemental report \(page 27\)](#)

About the Assays

Purpose of the assay

BD OneFlow™ PCST and BD OneFlow™ PCD are qualitative 8-color direct immunofluorescence assays for immunophenotyping plasma cells in bone marrow. The tubes are used together to identify and characterize normal and abnormal plasma cells.

About the assay

Each 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™ PCST 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™ PCST (S) contains antibodies that recognize surface markers and BD OneFlow™ PCST (C) contains antibodies that recognize cytoplasmic markers. The panel comprises the following antibodies:

Antibody	Tube	Fluorochrome
CD38	S	FITC
CD56	S	PE
β2-Microglobulin	S	PerCP-Cy™5.5
CD19	S	PE-Cy™7
CD45	S	BD Horizon™ V450
CD138	S	BD Horizon™ V500-C
Anti-Kappa	C	APC
Anti-Lambda	C	APC-H7

The BD OneFlow™ PCD 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
CD38	FITC
CD28	PE
CD27	PerCP-Cy5.5
CD19	PE-Cy7
CD117	APC
CD81	APC-H7
CD45	BD Horizon™ V450
CD138	BD Horizon™ V500-C

The reagents are used to stain patient specimens. The stained samples are acquired on the cytometer and then analyzed to characterize populations of plasma cells.

Assay workflow

Workflow steps

The following table lists the steps in a typical workflow for running the BD OneFlow™ PCST and BD OneFlow™ PCD assays.

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. You can stain a patient's specimen with BD OneFlow™ PCST and BD OneFlow™ PCD concurrently or sequentially. See the <i>BD OneFlow™ PCST IFU</i> and the <i>BD OneFlow™ PCD IFU</i> for information.
3	Enter reagent lot and expiration date in the Library. See the <i>BD OneFlow™ PCST IFU</i> and the <i>BD OneFlow™ PCD 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 13) .
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 25)
9	Add dot plots and gates, if necessary. See Adding items to the supplemental report (page 27) .

Changing the assay stopping criteria

Each 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** dialog for the appropriate tube 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.

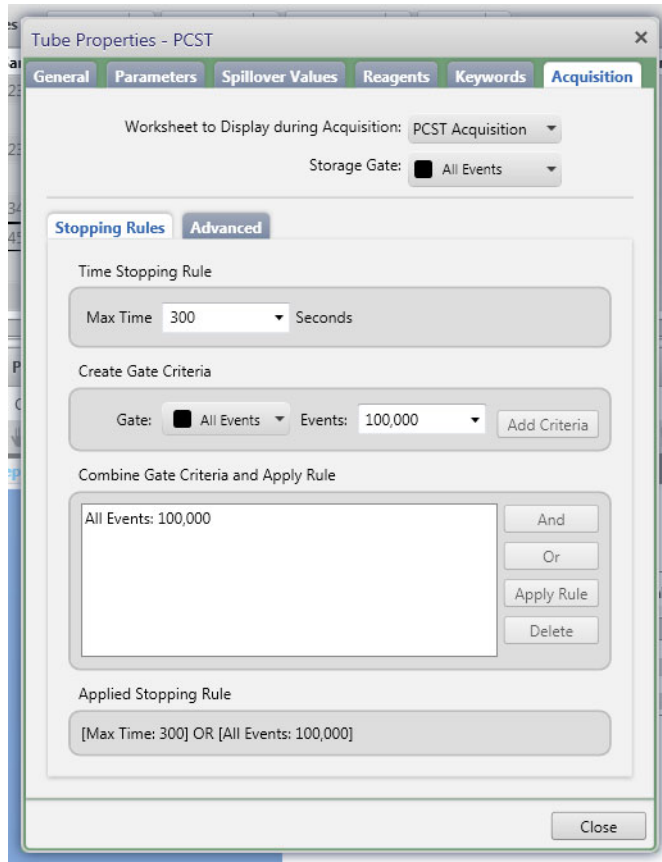
6. To change the number of events acquired, in the **Create Gate Criteria** section, select or enter the number of events you want to acquire in the **Events** field.

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

Viewing the OneFlow™ PCST 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 \(page 33\)](#) for information.

OneFlow PCST: Laboratory Report

Sample ID: 12345
Sample Name: abcd
Case Number: 0123456

Acquired Using: Worklist_012	Approved: 5/18/2020 17:37:48	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: PCST

Events Acquired: 100,000	Sample Type: Bone Marrow
Performance QC Date: 5/18/2020 15:55:59	Acquisition Date: 5/18/2020
Performance QC Status: Pass	Acquisition Start Time: 16:40:16
	Acquisition End Time: 16:40:28

Results Summary

Population	Parent	# Events	% Parent	% Grandparent	Ratio
All Events		100,000			
Cells	All Events	94,417	94.4		
FSC Singlets	Cells	93,453	99.0	93.5	
SSC Singlets	FSC Singlets	93,181	99.7	98.7	
CD38+	SSC Singlets	176	0.2	0.2	
Plasma cells	CD38+	163	92.6	0.2	
cylgK+	Plasma cells	125	76.7	71.0	
cylgL+	Plasma cells	34	20.9	19.3	
Plasma cell Ratio - cylgK : cylgL					3.7
B cells	SSC Singlets	2,756	3.0	2.9	

QC Messages

Showing 0 of 0 QC Messages

For: In Vitro Diagnostic Use. Assay: OneFlow PCST Printed: 5/18/2020 17:38:07
Page 1 of 4

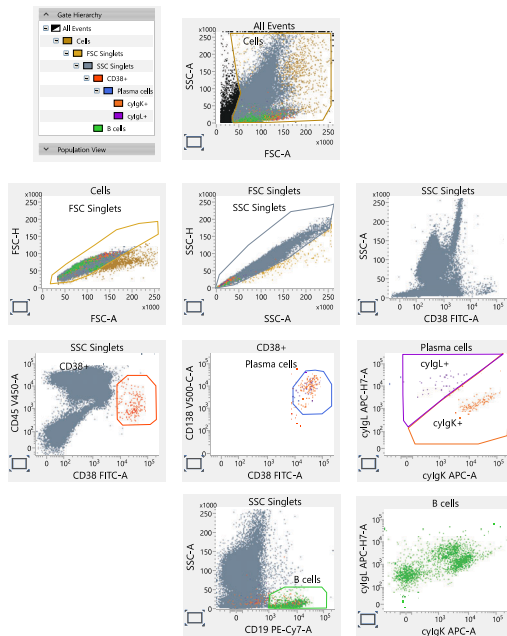
3. Inspect the dot plots on page 2 of the laboratory report, and adjust the gates as needed.

Note: The gates in the dot plots of the OneFlow™ PCST 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 B cells.

Sample ID: 12345
Sample Name: abcd
Case Number: 0123456

Cell Analysis				
Population	Parent	# Events	% Parent	% Grandparent
CD38+	SSC Singlets	176	0.2	0.2
Plasma cells	CD38+	163.0	92.6	0.2
B cells	SSC Singlets	2,756.0	3.0	2.9



For In Vitro Diagnostic Use.

Assay: OneFlow PCST
Page: 2 of 4

Printed: 5/18/2020 17:38:07

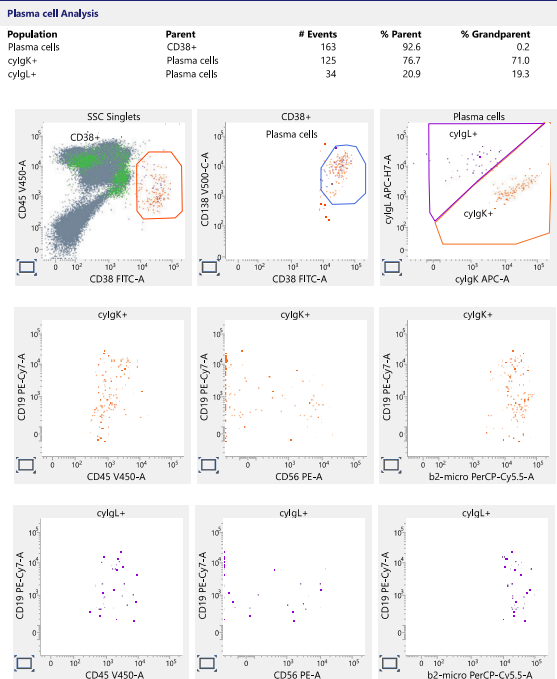
- Inspect the dot plots on page 3 of the laboratory report, and adjust the gates as needed.

The dot plots on page 3 of the report identify and analyze plasma cells. CD38⁺ cells are identified in the CD38 FITC-A vs CD45 V450-A dot plots from the SSC Singlets population. Plasma cells are identified in the CD38 FITC-A vs CD138 V500-C-A dot plot from the CD38⁺ cell population.

Assess the clonality of the plasma cells in the cyIgK APC-A vs cyIgL APC-H7-A dot plot.

The kappa- and lambda-expressing cells are further characterized according to the levels of CD19, CD45, CD56, and β 2-microglobulin expression in the remaining dot plots.

Sample ID: 12345
 Sample Name: abcd
 Case Number: 0123456



For In Vitro Diagnostic Use.

Assay: OneFlow PCST
 Page 3 of 4

Printed: 5/18/2020 17:38:07

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 PCST

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)	123451
Reagent Lot Expiry (S)	6/29/2020
Reagent Lot (C)	123452
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	5/18/2020 17:37:44
Comments:	

For In-Vitro Diagnostic Use.

Assay: OneFlow PCST
 Page 4 of 4

Printed: 5/18/2020 17:38:07

6. Select the **Laboratory Report** tab.
7. Click **E-sign**.
 The **E-Signature** 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.

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.

Viewing the OneFlow™ PCD Laboratory Report

To review the OneFlow™ PCD Laboratory Report:

1. Navigate to the worklist manager and select the task for the same patient specimen stained with BD OneFlow™ PCD.
2. Click the **Laboratory Report** tab to open the report.
3. 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 \(page 33\)](#) for information.

 **OneFlow PCD: Laboratory Report**

Sample ID: 12345
Sample Name: abcd
Case Number: 0123456

Acquired Using: Worklist_012	Approved: 5/18/2020 17:40:16	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: PCD

Events Acquired: 100,000	Sample Type: Bone Marrow
Performance QC Date: 5/18/2020 15:55:59	Acquisition Date: 5/18/2020
Performance QC Status: Pass	Acquisition Start Time: 16:39:21
	Acquisition End Time: 16:39:33

Results Summary

Population	Parent	# Events	% Parent	% Grandparent
All Events		100,000		
Cells	All Events	90,932	90.9	
FSC Singlets	Cells	87,822	96.6	87.8
SSC Singlets	FSC Singlets	87,241	99.3	95.9
CD38+	SSC Singlets	17	0.0	0.0
Plasma cells	CD38+	11	64.7	0.0
B cells	SSC Singlets	916	1.0	1.0

QC Messages

Showing 0 of 0 QC Messages

For: In Vitro Diagnostic Use. Assay: OneFlow PCD Page 1 of 4 Printed: 5/18/2020 17:40:47

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

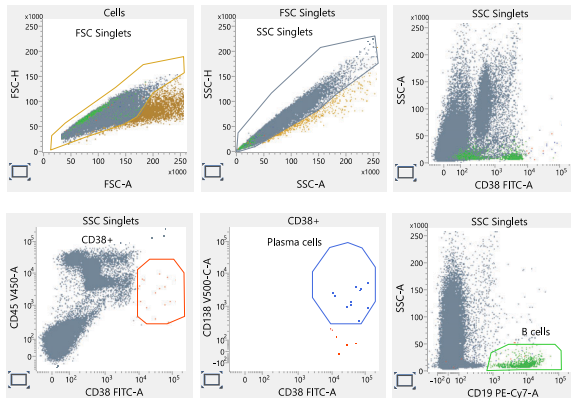
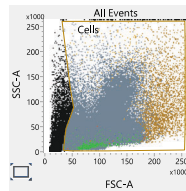
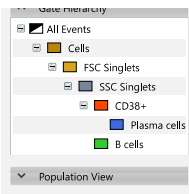
Note: The gates in the dot plots of the OneFlow™ PCD Laboratory Report are provided for analyzing normal and aberrant cell populations in the specimen.

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 CD38⁺ cells, plasma cells, and B cells.

Sample ID: 12345
 Sample Name: abcd
 Case Number: 0123456

Cell analysis

Population	Parent	#Events	% Parent	% Grandparent
CD38+	SSC Singlets	17	0.0	0.0
Plasma cells	CD38+	11	64.7	0.0
B cells	SSC Singlets	916	1.0	1.0



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

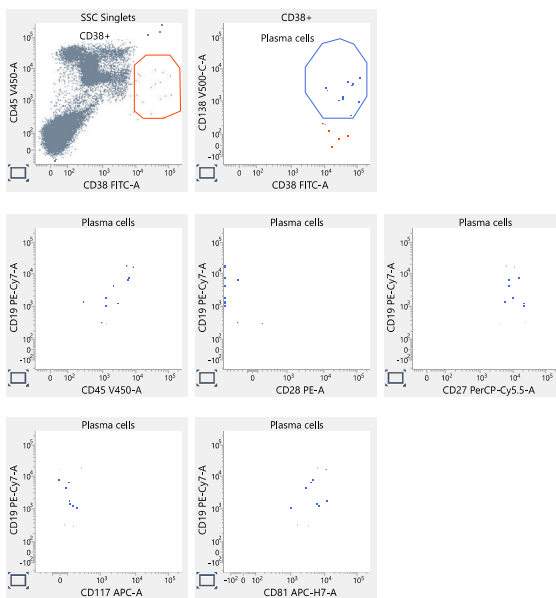
The dot plots on page 3 of the report analyze plasma cells. The first two dot plots are repeated from page 2. Plasma cells are

further characterized according to levels of CD45, CD28, CD27, CD117, and CD81 expression in the remaining dot plots.

Sample ID: 12345
Sample Name: abcd
Case Number: 0123456

Plasma cell analysis

Population	Parent	# Events	% Parent	% Grandparent
Plasma cells	CD38+	11	64.7	0.0



6. 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 PCD	
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	123450
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 17:40:17
Comments	

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

12. 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.

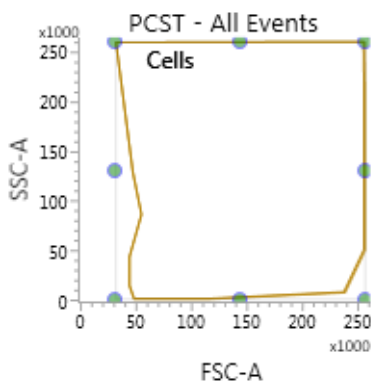
See the *BD FACSLyric™ Clinical System Instructions For Use* for more information and export options.

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.

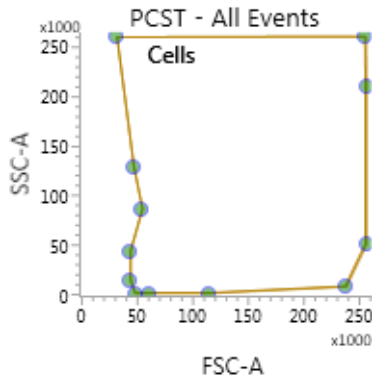


2. Click on one of the circles and drag it to resize the gate.
3. Click on one of the lines between the circles and drag it to move the gate.

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™ 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™ 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:

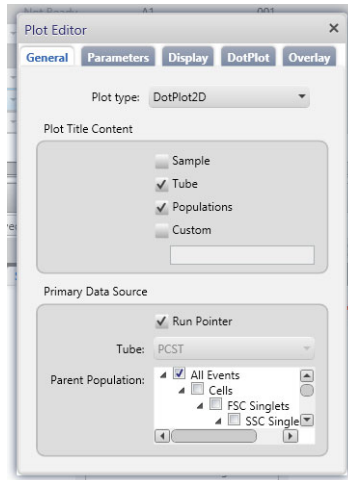
1. Click the **Supplemental Report** tab.
2. In the report menu bar, click the create dot plot icon.
3. Click on the report to add the dot plot.

An FSC-A vs SSC-A dot plot showing All Events for the appropriate tube is added to the report.

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

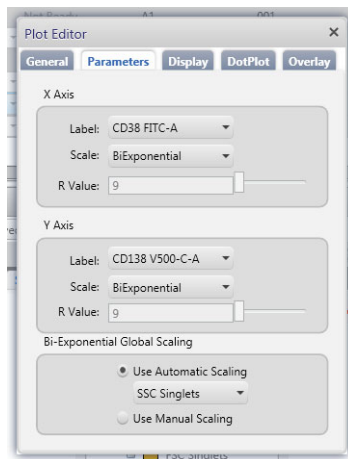
The **Plot Editor** dialog opens.

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.

4456:PCST RunPointerStatistics										
Name	All Events	Cells	FSC Singlets	SSC Singlets	CD38+	Plasma cells	cyIgK+	cyIgL+	B cells	P1
Events	###	###	###	###	###	###	###	###	###	###
% Total	###	###	###	###	###	###	###	###	###	###
FSC-A Mean	###	###	###	###	###	###	###	###	###	###
SSC-A Mean	###	###	###	###	###	###	###	###	###	###
CD38 FITC-A Mean	###	###	###	###	###	###	###	###	###	###
CD56 PE-A Mean	###	###	###	###	###	###	###	###	###	###
b2-micro PerCP-Cy5.5-A Mean	###	###	###	###	###	###	###	###	###	###
CD19 PE-Cy7-A Mean	###	###	###	###	###	###	###	###	###	###
cyIgK APC-A Mean	###	###	###	###	###	###	###	###	###	###
cyIgL APC-H7-A Mean	###	###	###	###	###	###	###	###	###	###
CD45 V450-A Mean	###	###	###	###	###	###	###	###	###	###
CD138 V500-C-A Mean	###	###	###	###	###	###	###	###	###	###
Time Mean	###	###	###	###	###	###	###	###	###	###

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

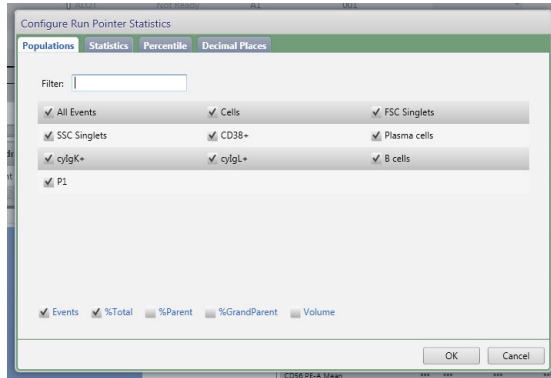
The **Configure Run Pointer Statistics** dialog opens.

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.

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

This chapter covers the following topic:

- [Troubleshooting overview \(page 34\)](#)
- [Problems with cell preparation or staining \(page 34\)](#)
- [Problems using BD OneFlow™ PCST and BD OneFlow™ PCD \(page 36\)](#)
- [QC messages \(page 37\)](#)

Troubleshooting overview

This chapter lists problems you might encounter when using the BD OneFlow™ reagent, 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™ PCST IFU* and the *BD OneFlow™ PCD IFU* also contain 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 cells 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.

Problem	Possible cause	Solution
The cytoplasmic staining (Igκ and Igλ) 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.
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.	Incubate the tubes for 2 minutes in the dark in wash buffer after they have been fixed using FIX & PERM Reagent A.
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 BD OneFlow™ tube.
	Cells were not acquired within 1 hour of staining.	Repeat staining with a fresh specimen and acquire 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™ PCST and BD OneFlow™ PCD

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 13) .
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 13) .
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: PCST (or PCD)	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 a OneFlow™ assay v1.1 in BD FACSuite™ Clinical application v1.5 or later, 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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