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

For BD OneFlow™ LST and BD OneFlow™ B-CLPD T1

IVD CE

23-17211-01
11/2019

Becton, Dickinson and Company
BD Biosciences
2350 Qume Drive
San Jose, CA 95131 USA

EC REP **Benex Limited**
Pottery Road, Dun Laoghaire
Co. Dublin, Ireland
Tel +353.1.202.5222
Fax +353.1.202.5388

BD Biosciences
European Customer Support
Tel +32.2.400.98.95
Fax +32.2.401.70.94
help.biosciences@europe.bd.com

Australian and New Zealand Distributors:

Becton Dickinson Pty Ltd.
66 Waterloo Rd
Macquarie Park NSW 2113
Australia

Becton Dickinson Ltd.
14b George Bourke Drive
Mt Wellington, Auckland, 1060
New Zealand

bdbiosciences.com
ClinicalApplications@bd.com

Copyrights

Trademarks

Regulatory information

The BD FACSCanto II flow cytometer is a Class 1 Laser Product.

For In Vitro Diagnostic Use.

History

Revision	Date	Change made
23-17211-00	9/2016	Initial release
23-17211-01	11/2019	Removed CD from the installer description. Removed the cell range. Updated Australian and New Zealand addresses.

Contents

1

Overview

This chapter covers the following topics:

Overview of the BD OneFlow system

About the system

The BD OneFlow™ system provides a comprehensive set of reagents and protocols to reproducibly set up the flow cytometer and stain patient specimens. The consistent instrument setup and sample staining enable you to acquire and analyze patient specimens for immunophenotyping of normal and aberrant cell populations in a manner compatible with that prescribed by the EuroFlow™ Consortium.

The BD OneFlow™ reagents are used to stain patient specimens. The stained samples are acquired on the cytometer and then analyzed. BD OneFlow™ B-CLPD T1 is used in combination with BD OneFlow™ LST as an aid in the diagnosis of chronic lymphocytic leukemia (CLL) and other B-cell chronic lymphoproliferative diseases.

Materials needed

- BD OneFlow LST
  - Catalog No. 658619
- BD OneFlow B-CLPD T1
  - Catalog No. 659293
- BD FACSDiva™ CS&T IVD beads (CS&T IVD beads)
  - Catalog No. 656046 or 656047
- BD OneFlow™ Setup Beads
  - Catalog No. 658620
- BD® FC Beads 8-color kit for BD OneFlow™ Assays (BD FC beads)
  - Catalog No. 658621
- Templates installer for BD OneFlow Assays
  - Catalog No. 659305
- BD FACS™ lysing solution
  - Catalog No. 349202

- BD FACSCanto™ II flow cytometer with a 3-laser, 8-color, 4-2H-2V BD default optical configuration, running BD FACSDiva™ software v8.0.1 or later

Workflows for the BD OneFlow reagents

Before you begin

We recommend that you set up the cytometer before you start staining the specimen. Perform the daily performance check and ensure that the cytometer has passed, confirm that the photomultiplier tube voltages (PMTVs) are within their daily target ranges, set up the experiment, and import the appropriate OneFlow template(s). See the *Instrument Setup Guide for BD OneFlow™ Assays* and Setting up the experiment (page 16).

Specimen preparation

Task	Reagents or materials	Template needed	Outcome
Washing the specimen	Patient specimen Wash buffer	None	Washed patient specimen is ready for staining.
Staining the specimen	The appropriate BD OneFlow reagent tube(s) BD FACS lysing solution Wash buffer	None	Stained patient specimen is ready for acquisition.

Sample acquisition

Task	Reagents or materials	Template needed	Outcome
Setting up the experiment	None	The appropriate tube-specific OneFlow template	The tube-specific OneFlow template is imported into an experiment, and application settings are applied.
Acquiring the stained sample	Stained patient sample	The appropriate BD OneFlow Acquisition worksheet	The FCS file is generated.

Data analysis

Task	Reagents or materials	Template needed	Outcome
Analyzing the data using BD FACSDiva software	FCS file for patient sample	The appropriate BD OneFlow Analysis worksheet	CLL is distinguished from other types of B-CLPD.

2

Specimen preparation

This chapter covers the following topics:

Washing the specimen

About the specimens

This procedure works for peripheral blood and bone marrow specimens collected in EDTA- or heparin-containing blood collection tubes (for example, BD Vacutainer® tubes).

BD OneFlow LST contains antibodies which recognize Igκ and Igλ found on the surface of B cells. Therefore, to avoid interference from serum antibodies found in the specimen, you must prewash the specimen three times before you stain it using BD OneFlow LST. We recommend that you wash the samples in the same manner before staining them with BD OneFlow B-CLPD T1.

Washing the specimen

1. For each specimen, label a 15-mL conical tube with the specimen ID.
2. Invert the specimen in the collection tube 10 times to mix well.
3. Add 300 µL of the patient specimen to the labeled conical tube.

   Note: The washing procedure should provide sufficient washed patient specimen to stain using both BD OneFlow LST and BD OneFlow B-CLPD T1.
4. Add 10 mL of wash buffer (filtered PBS + 0.5% BSA + 0.09% or 0.1% sodium azide).
5. Invert the tube 3–5 times to mix well.
6. Centrifuge at 540*g* for 5 minutes at 20°C–25°C.
7. Remove the supernatant without disturbing the cell pellet.
8. Vortex the tube until no cell aggregates remain before adding wash buffer.
9. Repeat steps 4–8 twice for a total of three washes.

10. Resuspend the cell pellet in 200 µL of wash buffer to give a final volume of approximately 300 µL.

    Note: Start staining the specimen using the appropriate BD OneFlow reagent tube within 30 minutes of the last wash. Store the washed specimen at 20°C–25°C until you stain it.

Staining the specimen

About the dried reagent

The BD OneFlow reagent tubes are very sensitive to moisture. To avoid condensation, open the pouches only if they are at room temperature. Ensure the pouch is completely resealed after removing a tube. Do not remove the desiccant from the reagent pouch.

Write the current date on the pouch label when it is first opened. Use the tubes from that pouch within one month before opening the next pouch.

Diluting BD FACS lysing solution

Dilute the 10X concentrate 1:10 with room temperature (20°C–25°C) deionized water. The prepared solution is stable for 1 month when stored in a glass or high density polyethylene (HDPE) container at room temperature.

Staining the specimen

1. If the pouches are stored refrigerated, allow them to reach room temperature before opening them.
2. For each patient specimen, remove the appropriate BD OneFlow reagent tube from its pouch.
3. Place the tubes in a rack, protected from light.
4. Immediately reseal the pouch with any unused tubes.
5. Write the patient ID on the appropriate tube label within the area provided.
6. Vortex the washed specimen 3–5 seconds to mix well.

7. Add 100 µL of washed patient specimen to the tube. Vortex vigorously 3–5 seconds to mix well.

   If less than 100 µL of specimen is used, add wash buffer to a final volume of 100 µL.

   Note: Do not wipe the outside of the tube with ethanol or isopropanol because the ink on the printed label can run.

8. Incubate for 30 minutes at 20°C–25°C, protected from light.
9. Add 2 mL of 1X BD FACS lysing solution to each tube. Vortex 3–5 seconds to mix well.
10. Incubate for 10 minutes at 20°C–25°C, protected from light.
11. Centrifuge at 540*g* for 5 minutes at 20°C–25°C.
12. Remove the supernatant without disturbing the cell pellet, leaving approximately 50 µL of residual liquid in the tube.
13. Vortex vigorously until the cell pellet is completely resuspended.
14. Add 2 mL of wash buffer (filtered PBS + 0.5% BSA + 0.09% or 0.1% sodium azide) to each tube. Vortex 3–5 seconds to mix well.
15. Centrifuge at 540*g* for 5 minutes at 20°C–25°C.
16. Remove the supernatant without disturbing the cell pellet, leaving approximately 50 µL of residual liquid in the tube.
17. Vortex 3–5 seconds to resuspend the cell pellet.
18. Add 200 µL of wash buffer to each tube. Vortex 3–5 seconds to mix well.

Next step

Specimens should be acquired immediately after staining. If a longer period of time is desired, each laboratory should validate that stained specimens acquired after being held under their storage conditions produce equivalent results to specimens acquired immediately after staining. Protect stained specimens from light until they are acquired.

This page intentionally left blank

3

Sample acquisition

This chapter covers the following topics:

Setting up the experiment

About linking and unlinking compensation

When you create a new experiment you must apply the correct application settings. Before applying the correct application settings, you first link the appropriate compensation matrix to the experiment and then unlink the compensation matrix. Unlinking the compensation matrix allows updated application settings to be applied, thus giving PMTVs that will result in correct target median fluorescence intensity (MFI) values, while retaining compensation values. When you apply the application settings, you keep the compensation values.

Before you begin

1. In BD FACSDiva software v.8.0.1 or later, ensure that cytometer warmup is complete, fluidics startup has been performed, and that the cytometer is in the default 4-2H-2V configuration.
2. Verify that the daily performance check was completed and passed for the default 4-2H-2V configuration using CS&T IVD beads within the past 24 hours. See the *Instrument Setup Guide for BD OneFlow™ Assays*.
3. Recommended: confirm that the PMTVs are still within their daily target ranges. See the chapter for daily setup in the *Instrument Setup Guide for BD OneFlow™ Assays*.
4. Make sure that you have installed the appropriate templates. See the *Instrument Setup Guide for BD OneFlow™ Assays* or the Instructions for Use for the appropriate BD OneFlow reagent.

Setting up the experiment

1. Create a new experiment.
   a. From the menu bar, select **Experiment > New Experiment > Blank Experiment**. Click **OK**.

      Note: You can also create an experiment directly from the **Browser** using the **Experiment** icon.

b. If prompted by the **CST Mismatch** dialog, select **Use CST Settings**.

c. Rename the experiment according to your laboratory practice.

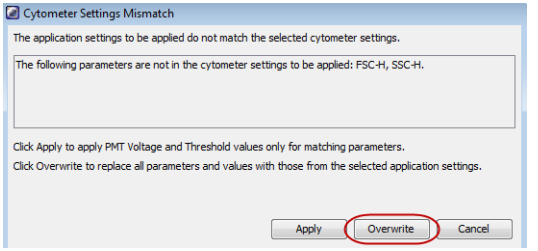
2. Link compensation.

a. In the **Browser**, right-click **Cytometer Settings.**

b. From the menu, select **Link Setup**.

c. Select the appropriate compensation matrix calculated using BD FC beads within the past 31 days. Click **Link**.

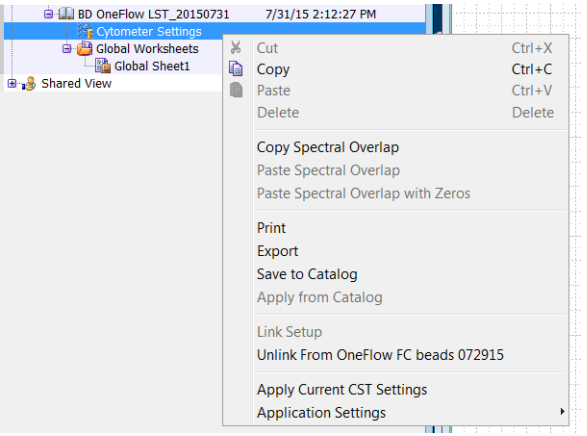
d. If prompted by the **Cytometer Settings Mismatch** dialog, select **Overwrite**.



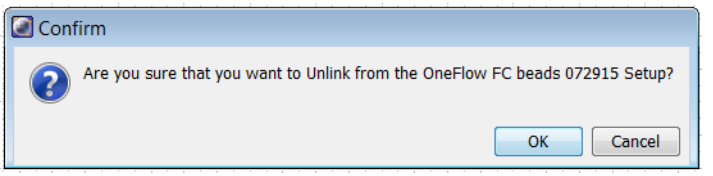
3. Unlink compensation.

a. In the **Browser**, right-click **Cytometer Settings.**

b. From the menu, select **Unlink From** and select the previously linked compensation setup.



c. From the **Confirm** dialog that opens, click **OK** to unlink from the previously linked compensation setup.



4. Apply application settings.

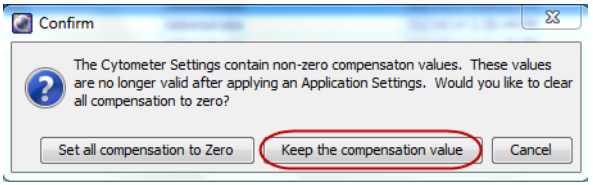
   a. In the **Browser**, right-click **Cytometer Settings**.

   b. From the menu, select **Application Settings** > **Apply**.

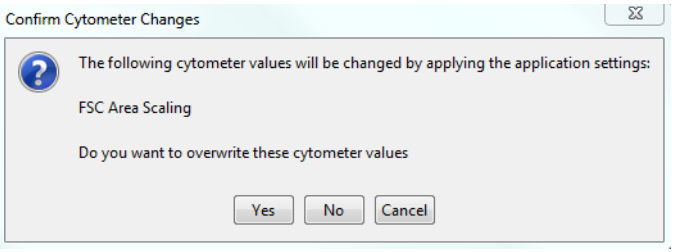
   c. Select the most recent application settings. Click **Apply**.

   Note: Confirm that the most recent application settings were created within the past 31 days using BD OneFlow Setup Beads. The application settings are created in the monthly setup as described in the *Instrument Setup Guide for BD OneFlow™ Assays*.

d. When prompted by the **Confirm** dialog, select **Keep the compensation value**.



e. If prompted by the **Confirm Cytometer Changes** dialog, click **Yes** to overwrite the cytometer values for **FSC Area Scaling**.



5. Import the appropriate tube-specific OneFlow template.

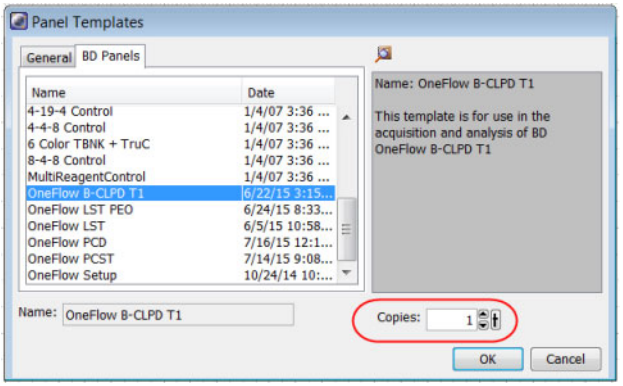
   a. Select the experiment in the **Browser** and then select **Experiment > New Specimen** from the menu bar**.**

      The **Panel Templates** dialog opens.

   b. Navigate to the **BD Panels** tab and select the appropriate OneFlow template.

      Note: Make sure that you select the template for the BD OneFlow tube that you are acquiring.

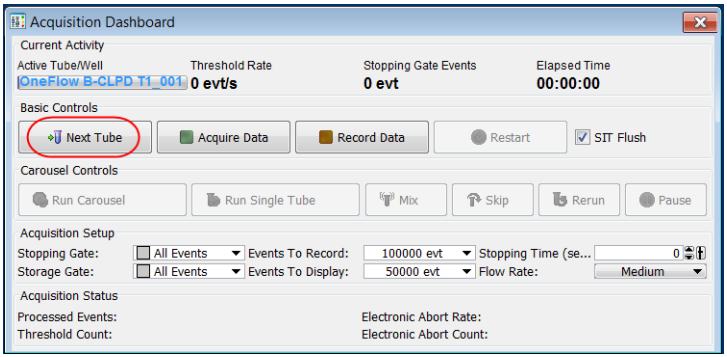
c. Indicate the number of patient specimens you want to acquire using the **Copies** field.



d. Click **OK**.

e. Rename each specimen, for example, with the appropriate patient ID in front of the specimen name.

Note: If you have to re-run a particular patient specimen, set the current tube pointer to the tube you wish to re-run. Click **Next Tube** in the **Acquisition Dashboard** to create another tube for that patient. Do not use the new tube icon to create the additional tube to be acquired because the labels and barcode fields will not be populated.



Note: You can import more than one template into an experiment, however, you can only import one template at a time. Repeat step 5 for each tube-specific OneFlow template that you want to import into the experiment.

6. Confirm that all of the voltages are the same as those set as application settings.

   a. In the **Browser,** select the application settings that you want to confirm.

   b. In the **Inspector,** navigate to the **Parameters** tab to view the voltages in the application settings.

   c. From the menu bar, select **Cytometer** > **Catalogs**.

      The **Catalogs** dialog opens.

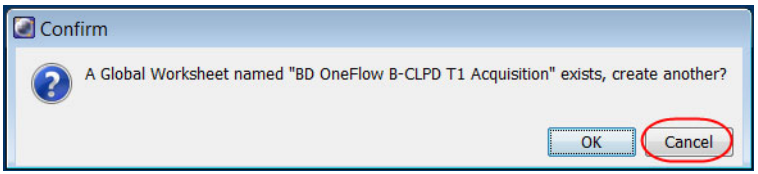
   d. Navigate to the **Application Settings** tab.

   e. Select the application settings used in the current experiment. Click **View**.

   f. Confirm that the voltages in the catalog are the same as those in the application settings.

   g. Click **Close** in the **Catalogs** dialog.

Note: If you want to acquire additional patient samples in the experiment, repeat step 5 to add new specimens. Two **Confirm** dialogs will open asking if you want to create another acquisition worksheet or another analysis worksheet. Click **Cancel** in each dialog.

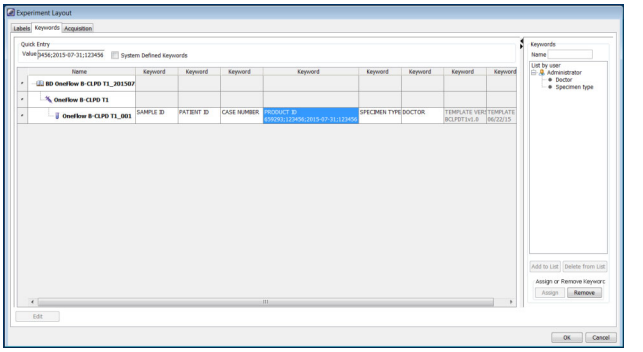


7. Scan the barcode on the tube label into the **Product ID** keyword field.

   a. From the menu bar, select **Experiment** > **Experiment Layout** and navigate to the **Keywords** tab.

b. Highlight the **Product ID** keyword for the appropriate tube, and scan the barcode on the BD OneFlow tube label.

Note: If you cannot scan the barcode on the tube label, see Troubleshooting.



c. Manually add the appropriate information to the remaining keywords, as needed.

d. Click **OK** to close the **Experiment Layout**.

Acquiring the stained sample

Acquiring the tube

1. In the **Browser**, expand the appropriate specimen and set the current tube pointer to that tube.
2. Select the appropriate BD OneFlow acquisition worksheet tab.
3. Vortex the stained tube 3–5 seconds at low speed.
4. Install the tube on the cytometer. Adjust the flow rate to **Medium** in the **Acquisition Dashboard**. Click **Acquire Data**.
5. Verify that the population is on scale and adjust the gate in the first plot of the acquisition worksheet to exclude debris, if needed.

6. Click **Record Data** in the **Acquisition Dashboard** to collect total events.

   Note: The template automatically collects 100,000 total events. Use the menu in the **Acquisition Dashboard** to select a different number of events to acquire, if needed.

Inspecting the LST acquisition worksheet

1. Select the **BD OneFlow LST Acquisition** worksheet tab.
2. Inspect the dot plots on the LST acquisition worksheet and adjust the gates as needed.

   The FSC-A vs SSC-A dot plot is used to identify cells.

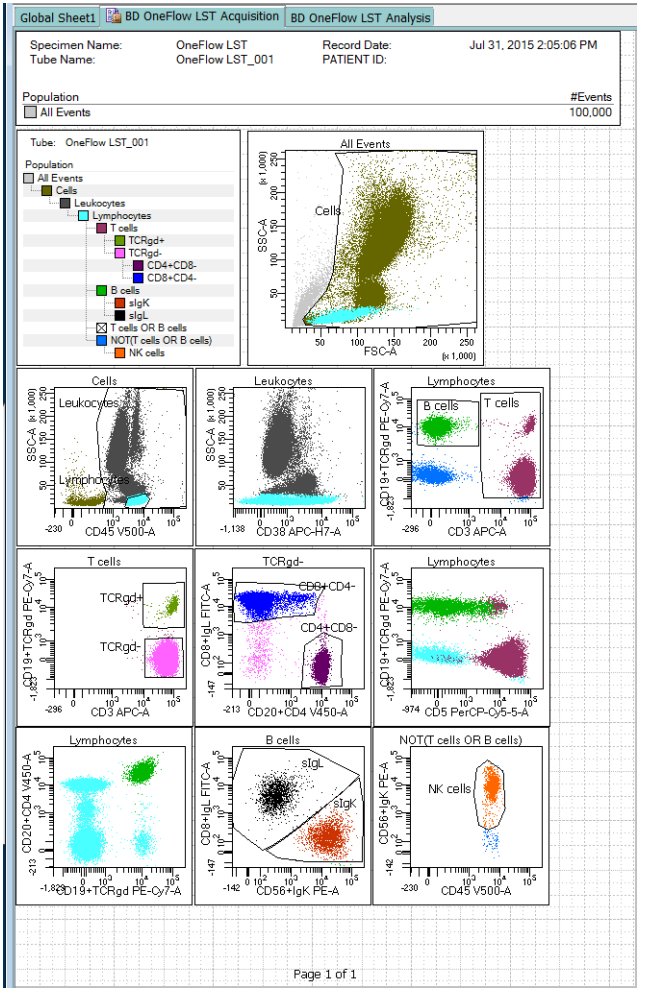
   The CD45 V500-A vs SSC-A dot plot contains two gates: one to identify leukocytes and the other to identify lymphocytes. T cells and B cells are identified in the CD3 APC-A vs CD19+TCRgd PE-Cy7-A dot plot from the lymphocyte population.

   T cells are divided into TCRγ/δ$^+$ and TCRγ/δ$^-$ populations in the CD3 APC-A vs CD19+TCRgd PE-Cy7-A dot plot from the T-cell population. TCRγ/δ$^-$ cells are divided into CD8$^+$CD4$^-$ and CD4$^+$CD8$^-$ populations in the CD20+CD4 V450-A vs CD8+IgL FITC-A dot plot.

   Igκ- and Igλ-expressing B cells are identified in the CD56+IgK PE-A vs CD8+IgL FITC-A dot plot from the B-cell population.

   NK cells are identified from the NOT(T cells OR B cells) population in the CD45 V500-A vs CD56+IgK PE-A dot plot.

The remaining dot plots do not contain gates and are included to ensure that the antibodies can stain cells in the specimen, therefore serving as an internal quality control for the tube.



3. Continue until all of the tubes have been acquired.

4. From the menu bar, select **File > Export > Experiments**, and select the **Directory Export** option. Click **OK**.

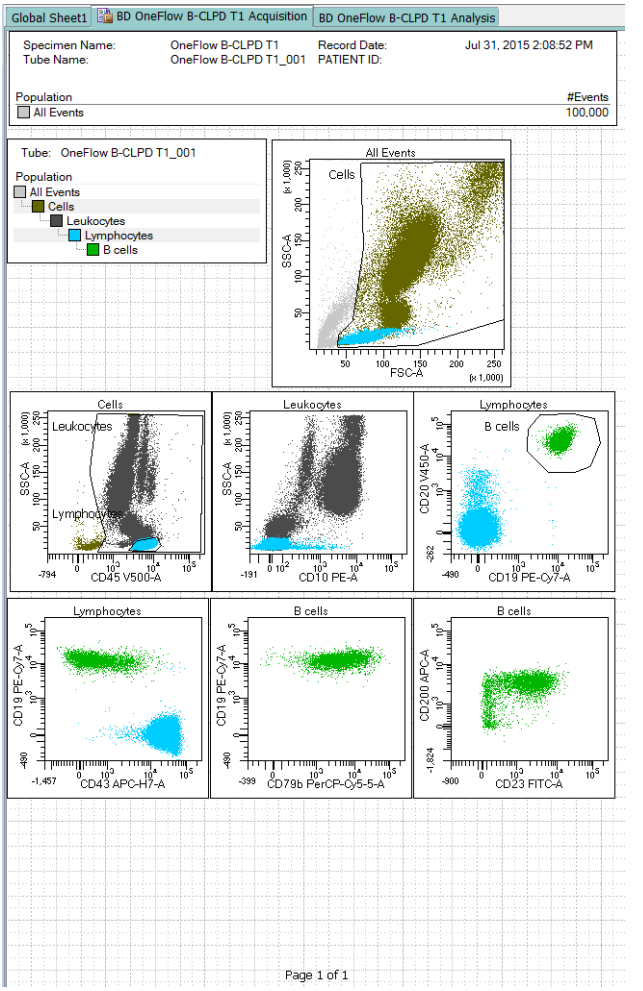
Inspecting the B-CLPD T1 acquisition worksheet

1. Select the **BD OneFlow B-CLPD T1 Acquisition** worksheet tab.
2. Inspect the plots on the B-CLPD T1 acquisition worksheet and adjust the gates as needed.

   The FSC-A vs SSC-A dot plot is used to identify cells.

   The CD45-A vs SSC-A dot plot contains two gates to identify leukocytes and lymphocytes. B cells are identified in the CD19 PE-Cy7-A vs CD20 V450-A dot plot from the lymphocyte population.

   The remaining dot plots do not contain gates and are included to ensure that the antibodies can stain cells in the specimen, therefore serving as an internal quality control for the tube.



3. Continue until all of the tubes have been acquired.
4. From the menu bar, select **File > Export > Experiments**, and select the **Directory Export** option. Click **OK**.

4

Data analysis

This chapter covers the following topic:

Analyzing the data using BD FACSDiva software

About the dot plots

Some of the dot plots might look different from those in other experiments. The initial FSC-A vs SSC-A dot plot to identify cells and eliminate debris may appear compressed. This is due to the FSC and SSC target values used to create the application settings. The values are specified by the EuroFlow Consortium.

Analyzing BD OneFlow LST

1. From the menu bar, select **File > Import > Experiments**.
2. Select the experiment that you want to analyze. Click **Import**.

   The experiment with the associated acquisition and analysis worksheets opens.
3. Select the **BD OneFlow LST Analysis** worksheet tab.
4. Inspect the plots on page 1 of the LST analysis worksheet and adjust the gates as needed.

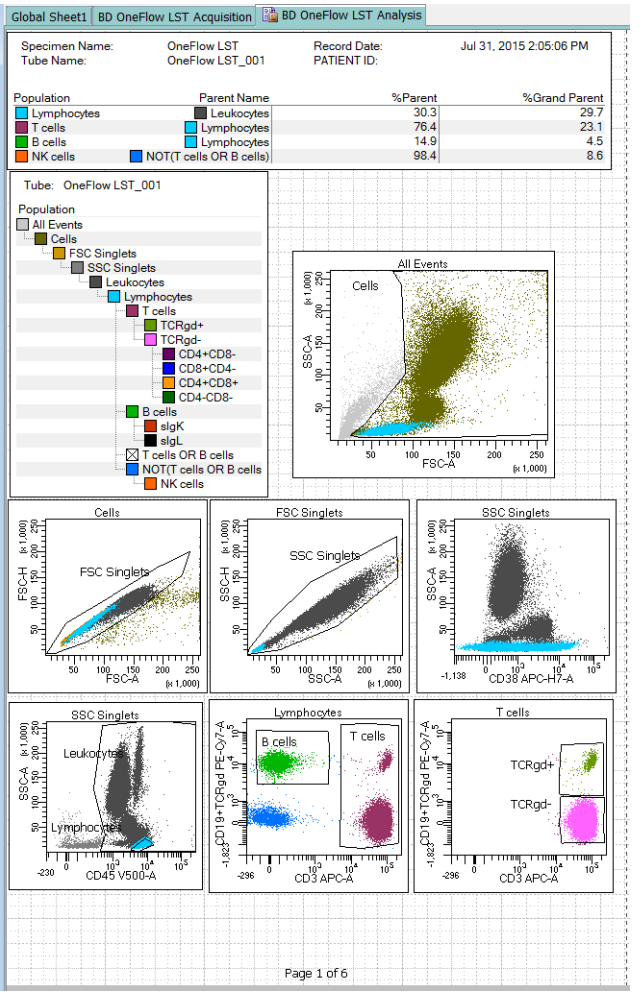
   Note: Enlarge the plot while adjusting the gates so you can more readily see the populations of interest.

   The first three dot plots on page 1 of the analysis worksheet identify cells, FSC singlets, and SSC singlets. Debris and doublets are excluded by adjusting the gates.

   Examine the leukocyte and lymphocyte populations in the CD45 V500-A vs SSC-A dot plot.

   Examine the B-cell and T-cell populations in the CD3 APC-A vs CD19+TCRgd PE-Cy7-A dot plot from the lymphocyte population. Examine the TCRγ/δ^+ and TCRγ/δ^- populations in the CD3 APC-A vs CD19+TCRgd PE-Cy7-A dot plot from the T-cell population. The CD38 FITC-A vs SSC-A dot plot is included for informational purposes to allow for the visualization of $CD38^+$ cells.

Note: These are examples of normal peripheral blood. Patient samples and bone marrow may look different.



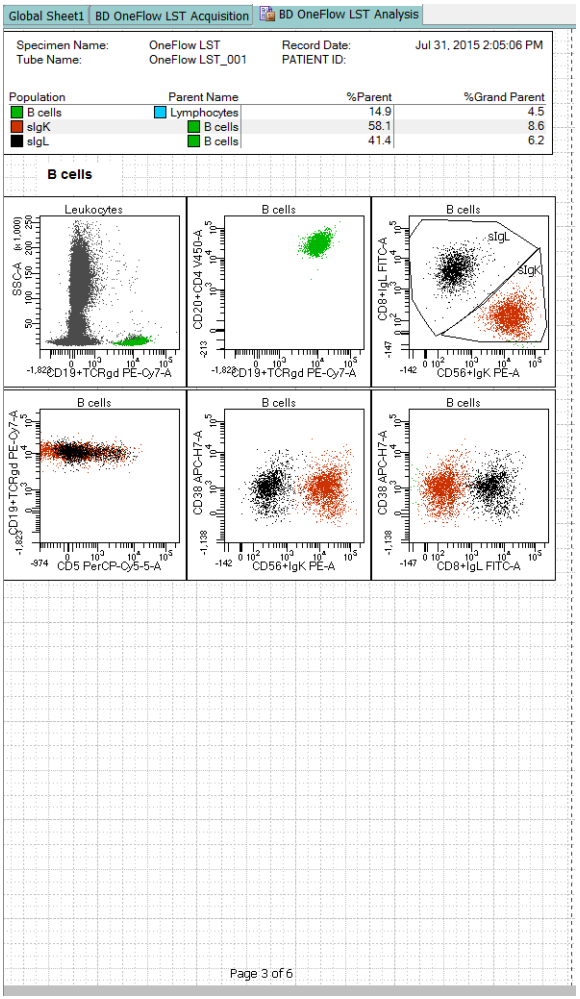
5. Inspect the dot plots on page 3 of the LST analysis worksheet and adjust the gates as needed.

   The dot plots on page 3 of the analysis worksheet identify B cells. B cells are initially identified as being $CD3^{-}CD19^{+}$.

Examine the level of CD20 expression in the CD19+TCRgd PE-Cy7-A vs CD20+CD4 V450-A dot plot.

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

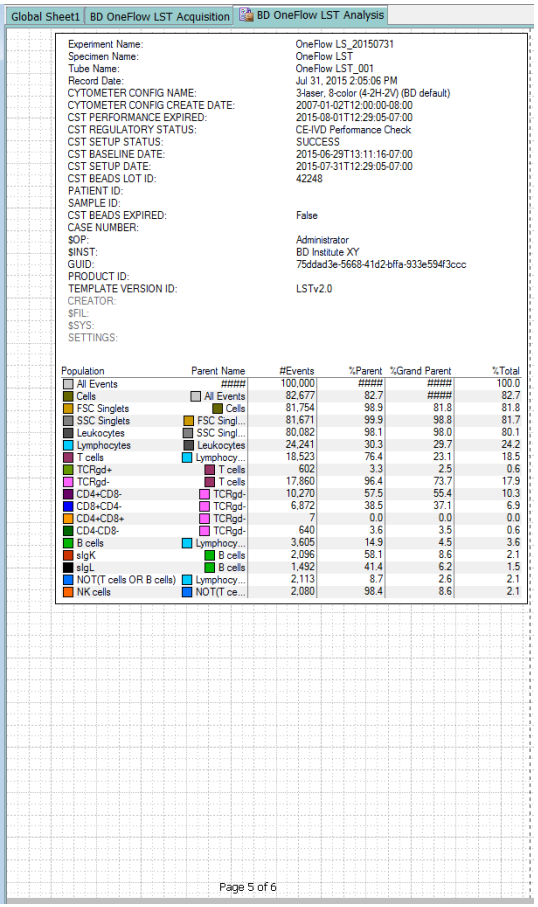
The remaining dot plots further characterize B cells using various markers.



Note: The dot plots on page 2 of the LST analysis worksheet identify T cells. The dot plots on page 4 of the LST analysis worksheet identify NK cells. See the *BD OneFlow™ LST Application Guide* for dot plots showing normal populations of T cells and NK cells.

6. Examine the results in the statistics box on page 5 of the LST analysis worksheet.

   Confirm that all of the keywords are present in the statistics box. If any of the keywords are missing, see Troubleshooting.


Global Sheet1 | BD OneFlow LST Acquisition | BD OneFlow LST Analysis

Experiment Name:	OneFlow LS_20150731
Specimen Name:	OneFlow LST
Tube Name:	OneFlow LST_001
Record Date:	Jul 31, 2015 2:05:06 PM
CYTOMETER CONFIG NAME:	3-laser, 8-color (4-2H-2V) (BD default)
CYTOMETER CONFIG CREATE DATE:	2007-01-02T12:00:00-08:00
CST PERFORMANCE EXPIRED:	2015-08-01T12:29:05-07:00
CST REGULATORY STATUS:	CE-IVD Performance Check
CST SETUP STATUS:	SUCCESS
CST BASELINE DATE:	2015-06-29T13:11:16-07:00
CST SETUP DATE:	2015-07-31T12:29:05-07:00
CST BEADS LOT ID:	42248
PATIENT ID:	
SAMPLE ID:	
CST BEADS EXPIRED:	False
CASE NUMBER:	
$OP:	Administrator
$INST:	BD Institute XY
GUID:	75ddad3e-5668-41d2-bffa-933e594f3ccc
PRODUCT ID:	
TEMPLATE VERSION ID:	LSTv2.0
CREATOR:	
$FIL:	
$SYS:	
SETTINGS:	

Population	Parent Name	#Events	%Parent	%Grand Parent	%Total
All Events	#####	100,000	#####	#####	100.0
Cells	All Events	82,677	82.7	#####	82.7
FSC Singlets	Cells	81,754	98.9	81.8	81.8
SSC Singlets	FSC Singl...	81,671	99.9	98.8	81.7
Leukocytes	SSC Singl...	80,082	98.1	98.0	80.1
Lymphocytes	Leukocytes	24,241	30.3	29.7	24.2
T cells	Lymphocy...	18,523	76.4	23.1	18.5
TCRgd+	T cells	602	3.3	2.5	0.6
TCRgd-	T cells	17,860	96.4	73.7	17.9
CD4+CD8-	TCRgd-	10,270	57.5	55.4	10.3
CD8+CD4-	TCRgd-	6,872	38.5	37.1	6.9
CD4+CD8+	TCRgd-	7	0.0	0.0	0.0
CD4-CD8-	TCRgd-	640	3.6	3.5	0.6
B cells	Lymphocy...	3,605	14.9	4.5	3.6
slgK	B cells	2,096	58.1	8.6	2.1
slgL	B cells	1,492	41.4	6.2	1.5
NOT(T cells OR B cells)	Lymphocy...	2,113	8.7	2.6	2.1
NK cells	NOT(T ce...	2,080	98.4	8.6	2.1

Page 5 of 6

7. Perform further analyses as needed.

   Note: The gates in the dot plots of the LST analysis worksheet are provided for analyzing normal and aberrant cell populations in the specimen. See the *BD OneFlow™ LST Application Guide* for dot plots showing normal populations of T cells and NK cells.

8. Save the LST analysis worksheet as a PDF.

   Note: The LST analysis worksheet is a global worksheet. Any gates that are adjusted when analyzing a sample on a global worksheet will be changed in previously analyzed files. Previously saved PDFs won't change, but if you go back to a previously analyzed global worksheet, you will have to readjust the gates so they match what they were before.

9. Optional: click **Print** to print the LST analysis worksheet.

10. Analyze the next sample.

Analyzing BD OneFlow B-CLPD T1

1. From the menu bar, select **File > Import > Experiments**.

2. Select the experiment that you want to analyze. Click **Import**.

   The experiment with the associated acquisition and analysis worksheets opens.

3. Select the **BD OneFlow B-CLPD T1 Analysis** worksheet tab.

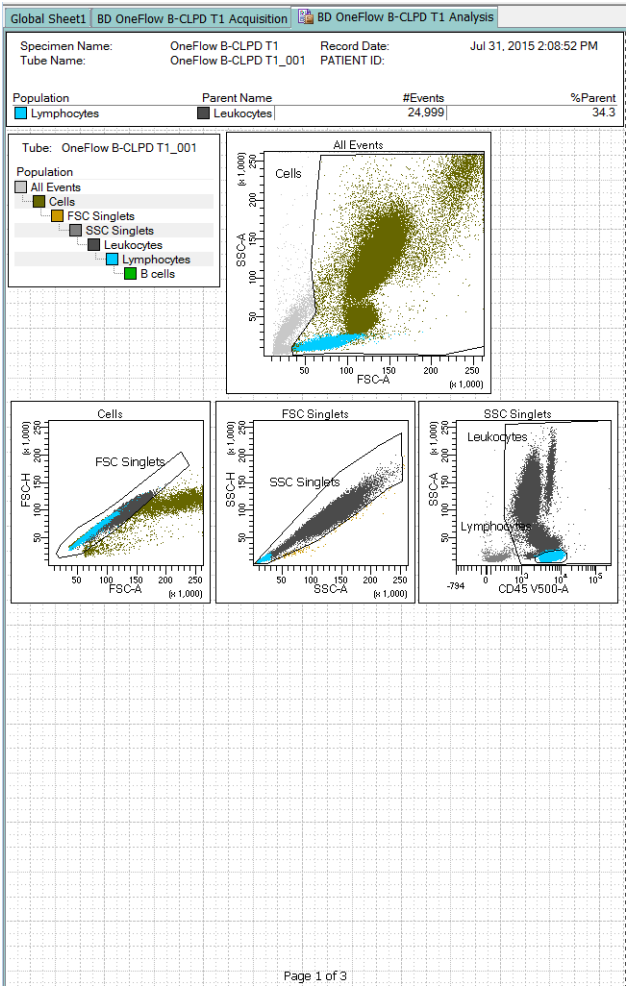
4. Inspect the plots on page 1 of the B-CLPD T1 analysis worksheet and adjust the gates as needed.

   Note: Enlarge the dot plot while adjusting the gates so you can more readily see the populations of interest.

   The first three dot plots on page 1 of the B-CLPD T1 analysis worksheet identify cells, FSC singlets, and SSC singlets. Debris and doublets are excluded by adjusting the gates.

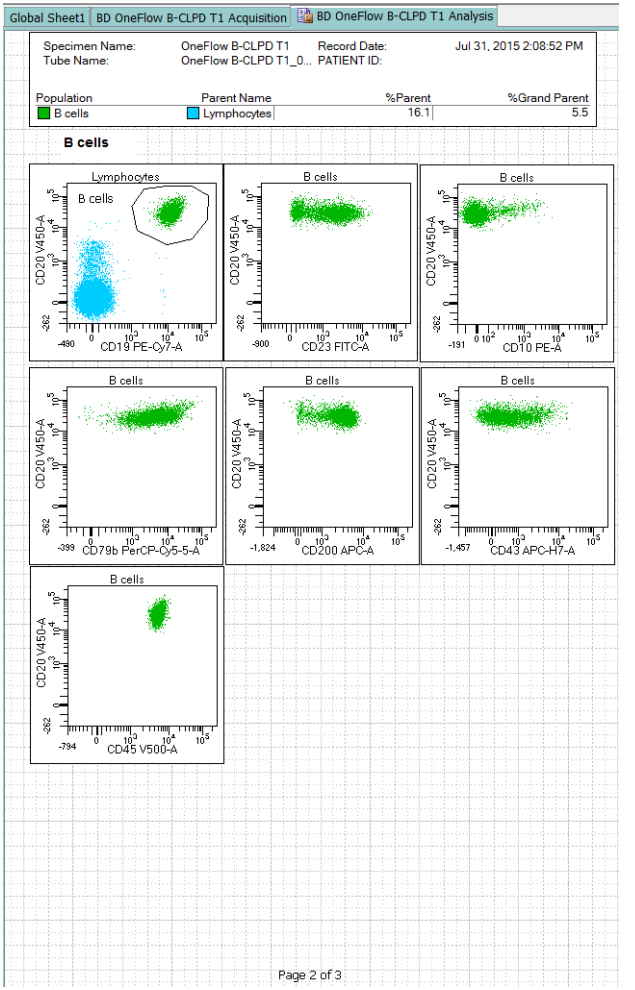
   Examine the leukocyte and lymphocyte populations in the CD45 V500-A vs SSC-A dot plot.

Note: These are examples of normal peripheral blood. Patient samples and bone marrow may look different.



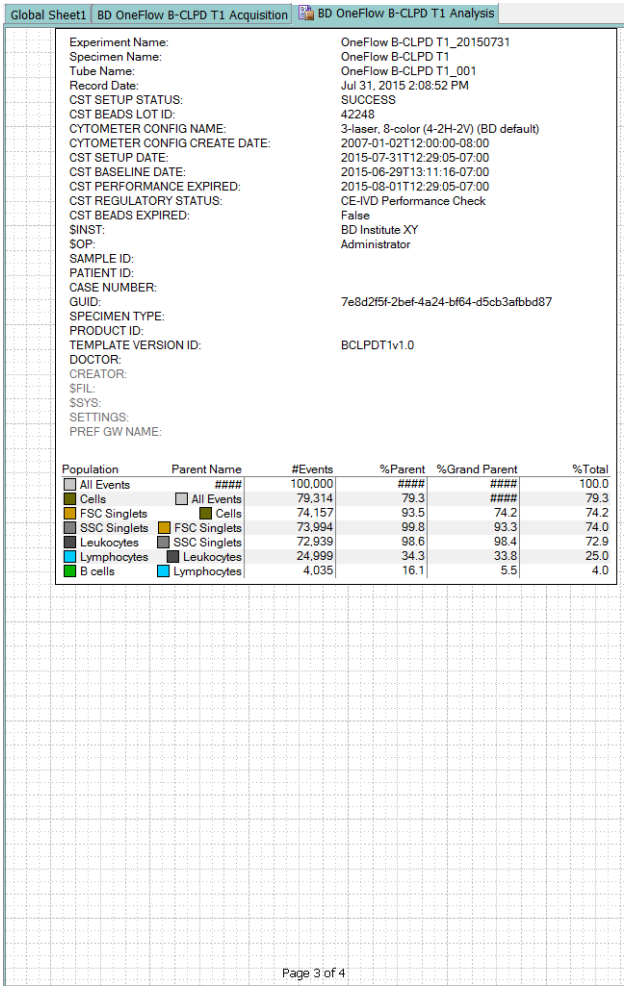
5. Examine the B-cell population in the CD19 PE-Cy7-A vs CD20 V450-A dot plot on page 2 of the B-CLPD T1 analysis worksheet and adjust the gate as needed.

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



6. Examine the results in the statistics box on page 3 of the B-CLPD T1 analysis worksheet.

Confirm that all of the keywords are present in the statistics box. If any of the keywords are missing, see Troubleshooting.


Global Sheet1 | BD OneFlow B-CLPD T1 Acquisition | BD OneFlow B-CLPD T1 Analysis

Experiment Name:	OneFlow B-CLPD T1_20150731
Specimen Name:	OneFlow B-CLPD T1
Tube Name:	OneFlow B-CLPD T1_001
Record Date:	Jul 31, 2015 2:08:52 PM
CST SETUP STATUS:	SUCCESS
CST BEADS LOT ID:	42248
CYTOMETER CONFIG NAME:	3-laser, 8-color (4-2H-2V) (BD default)
CYTOMETER CONFIG CREATE DATE:	2007-01-02T12:00:00-08:00
CST SETUP DATE:	2015-07-31T12:29:05-07:00
CST BASELINE DATE:	2015-06-29T13:11:16-07:00
CST PERFORMANCE EXPIRED:	2015-08-01T12:29:05-07:00
CST REGULATORY STATUS:	CE-IVD Performance Check
CST BEADS EXPIRED:	False
$INST:	BD Institute XY
$OP:	Administrator
SAMPLE ID:	
PATIENT ID:	
CASE NUMBER:	
GUID:	7e8d2f5f-2bef-4a24-bf64-d5cb3afbbd87
SPECIMEN TYPE:	
PRODUCT ID:	
TEMPLATE VERSION ID:	BCLPDT1v1.0
DOCTOR:	
CREATOR:	
$FIL:	
$SYS:	
SETTINGS:	
PREF GW NAME:	

Population	Parent Name	#Events	%Parent	%Grand Parent	%Total
All Events	####	100,000	####	####	100.0
Cells	All Events	79,314	79.3	####	79.3
FSC Singlets	Cells	74,157	93.5	74.2	74.2
SSC Singlets	FSC Singlets	73,994	99.8	93.3	74.0
Leukocytes	SSC Singlets	72,939	98.6	98.4	72.9
Lymphocytes	Leukocytes	24,999	34.3	33.8	25.0
B cells	Lymphocytes	4,035	16.1	5.5	4.0

Page 3 of 4

Note: When evaluating a patient sample you must analyze FCS files acquired from the patient sample stained with BD OneFlow LST as well as with BD OneFlow B-CLPD T1. The markers found in BD OneFlow B-CLPD T1 are used in conjunction with

CD5, CD38, Anti-Kappa, and Anti-Lambda, present in BD OneFlow LST, to differentially diagnose CLL versus other B-cell chronic lymphoproliferative diseases.

7. Perform further analyses as needed.
8. Save the B-CLPD T1 analysis worksheet as a PDF.

   Note: The B-CLPD T1 analysis worksheet is a global worksheet. Any gates that are adjusted when analyzing a sample on a global worksheet will be changed in previously analyzed files. Previously saved PDFs won't change, but if you go back to a previously analyzed global worksheet, you will have to readjust the gates so they match what they were before.

9. (Optional) Click **Print** to print the B-CLPD T1 analysis worksheet.
10. Analyze the next sample.

5

Troubleshooting

This chapter covers the following topics:

Templates do not import correctly

You may observe that templates do not import correctly. For example, there might not be dot plots in the global worksheet, the plots from the wrong worksheet appear when you import a panel template, or the imported panel template does not include tubes.

If you suspect that the templates did not import correctly:

1. Close the current experiment.
2. Create a new experiment.
3. Re-import the panel template.

Problems using a BD OneFlow reagent

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.
	Instrument settings are inappropriate.	Follow proper instrument setup procedures. See the *Instrument Setup Guide for BD OneFlow™ Assays*.

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.
Some of the dot plots are dimmed.	FSC-H and SSC-H were not selected when the application settings were created.	Check that FSC-H and SSC-H are selected on the **Parameters** tab of the **Inspector**.

Problem	Possible cause	Solution
The barcode on the tube label cannot be scanned.	The barcode on the tube label has been compromised.	Scan the barcode on the BD OneFlow pouch label into the **Product ID** keyword field in the **Experiment Layout**. Next, after the last digit of the barcode, manually enter a semicolon (;) followed by the six-digit tube-specific ID, found adjacent to the barcode on the tube label.
Some of the keywords are missing from the statistics box in the analysis worksheet.	BD FACSDiva software did not import all of the keywords into the panel template.	1. Navigate to the analysis worksheet. 2. Right-click the statistics box and select **Edit Stats View**. 3. In the **Header** tab, select the **All** checkbox. 4. Click **OK**.
The statement, **For in vitro diagnostic use**, does not appear in the footer of the analysis worksheet when it is printed.	The paper margins in the printer settings were changed.	1. From the BD FACSDiva software menu bar, select **File > Page Setup**. 2. Ensure that all of the margins are set to 2.54 cm or 1 inch, depending on your default standards. 3. Click **OK**.