

BD OneFlow™ LST Application Guide



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Regulatory information

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

For In Vitro Diagnostic Use.

History

Revision	Date	Change made
23-16165-00	7/2014	Initial release
23-16165-01	9/2016	Updated with the LST v2.0 acquisition and analysis worksheets

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Overview

This chapter covers the following topics:

- [Overview of the BD OneFlow system \(page 6\)](#)
- [Workflows for the BD OneFlow LST \(page 7\)](#)

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™ LST tube 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.

-
- Materials needed**
- BD OneFlow LST
 - Catalog No. 658619
 - 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 CD for BD OneFlow™ Assays
 - Catalog No. 659305
 - BD FACSTM 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 LST

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	BD OneFlow LST BD FACS lysing solution Wash buffer	None	Stained patient specimen is ready for acquisition.

Sample acquisition

Task	Reagents or materials	Template needed	Outcome
Import the OneFlow LST template.	None	OneFlow LST	The OneFlow LST template is imported into an experiment and application settings are applied.
Acquiring the stained sample	Stained patient sample	BD OneFlow LST 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	BD OneFlow LST Analysis worksheet	Patient lymphocyte populations are identified.

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Specimen preparation

This chapter covers the following topics:

- [Washing the specimen \(page 10\)](#)
- [Staining the specimen \(page 11\)](#)

Washing the specimen

About the specimens

This procedure works for peripheral blood (PB), bone marrow (BM), and lymph node (LN) specimens. Collect PB and BM specimens in EDTA- or heparin-containing blood collection tubes (for example, BD Vacutainer® tubes). For LN tissue, follow your current lab protocol to prepare a single-cell suspension. Staining from 3×10^4 to 4×10^6 white blood cells using BD OneFlow LST gives equivalent results.

BD OneFlow LST contains antibodies which recognize Ig κ and Ig λ 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.

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 μ L of the patient specimen to the labeled conical tube.
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 540g 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 BD OneFlow LST 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

BD OneFlow LST is very sensitive to moisture. To avoid condensation, open the pouch only if it is 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 all of the tubes from that pouch before opening the next one.

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 pouch is stored refrigerated, allow it to reach room temperature before opening it.
2. Open the pouch and remove a tube for each patient specimen.
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 BD OneFlow LST 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 540g 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 540g 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.

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Sample acquisition

This chapter covers the following topics:

- [Setting up the experiment \(page 14\)](#)
- [Acquiring the stained sample \(page 20\)](#)

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 it and then unlink the compensation matrix. Unlinking the compensation matrix allows updated application settings to be applied, thus giving photomultiplier tube voltages (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 value.

Before you begin

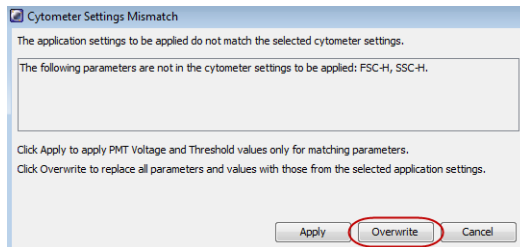
1. In BD FACSDiva software v.8.0.1, or later, ensure that cytometer warmup is complete, fluidics startup has been performed, and that the cytometer is in the default 4-2H-2V configuration.
 2. Verify that the daily performance check was completed and passed for the default 4-2H-2V configuration using 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 OneFlow LST template. See the *Instrument Setup Guide for BD OneFlow™ Assays* or the *BD OneFlow™ LST Instructions for Use*.
-

Setting up the experiment

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

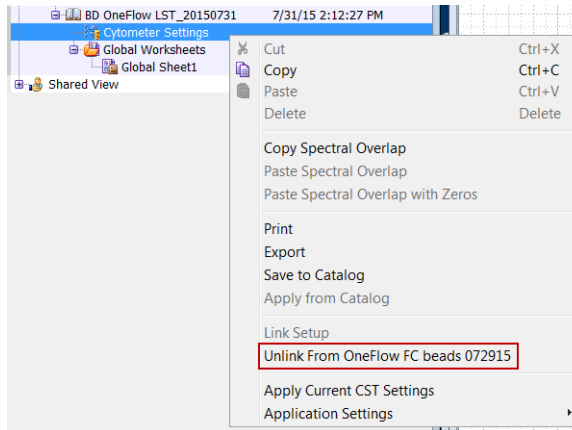
Note: Alternatively, create an experiment using the **Experiment** icon in the **Browser**.
 - b. If prompted by the **CST Mismatch** window, select **Use CST Settings**.

- 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 created using BD FC beads within the past 31 days. Click **Link**.
 - d. If prompted by the **Cytometer Settings Mismatch** window, select **Overwrite**.

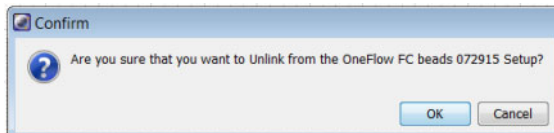


3. Unlink compensation.
 - a. In the **Browser**, right-click **Cytometer Settings**.

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



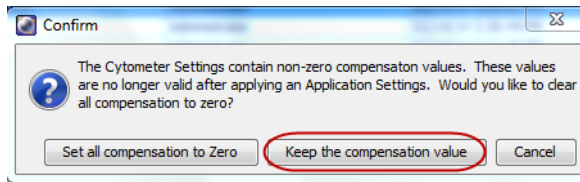
- c. From the **Confirm** dialog that opens, click **OK** to unlink from the previously linked compensation setup.



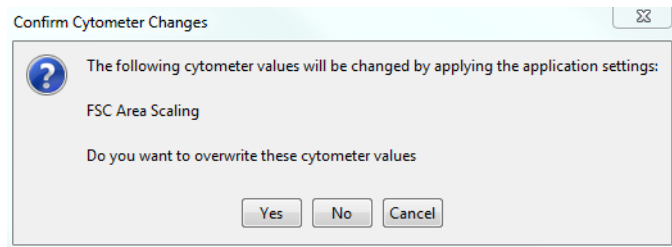
4. Apply application settings.
 - a. In the **Browser**, right-click **Cytometer Settings**.
 - b. From the menu, select **Application Settings > Apply**.
 - c. Select the most recent application settings. Click **Apply**.

Note: Confirm that the most recent application settings were created within the past 31 days using the BD OneFlow Setup beads. They 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**.

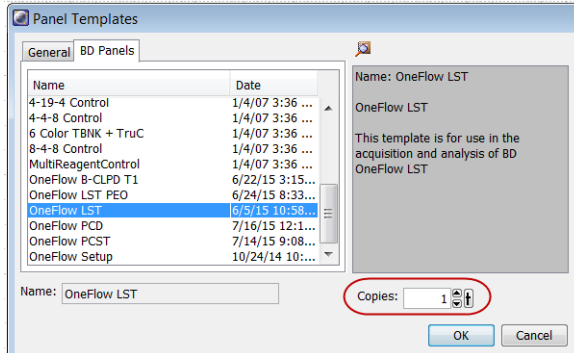


- e. If prompted by the **Confirm Cytometer Changes** dialog, click **Yes** to overwrite the cytometer values for **FSC Area Scaling**.



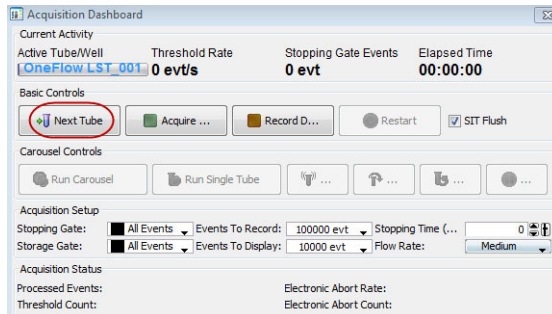
5. Import the OneFlow LST template.
- Select the experiment in the **Browser** and then select **Experiment > New Specimen** from the menu bar. The **Panel Templates** dialog opens.
 - Navigate to the **BD Panels** tab and select the OneFlow LST template.

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



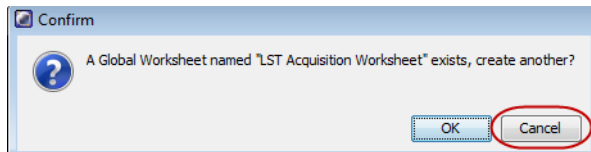
- d. Click **OK**.
- e. Rename each specimen, for example, with the appropriate patient ID in front of the specimen name.

Note: If you have to rerun a particular patient specimen, set the current tube pointer to the OneFlow LST tube you wish to rerun. 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.



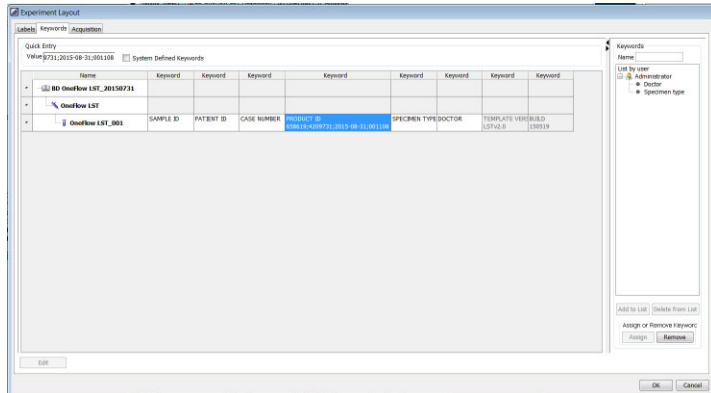
6. Confirm that all of the voltages are the same as those set as application settings.
 - a. In the **Browser**, select the application settings that you want to confirm.
 - b. In the **Inspector**, navigate to the **Parameters** tab to view the voltages in the application settings.
 - c. From the menu 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 LST Acquisition worksheet or another LST 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 LST 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 **BD OneFlow LST 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 LST acquisition worksheet to exclude debris, if needed.

- 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. Collecting total events from 3×10^4 to 4×10^6 stained cells gives equivalent results.

Inspecting the LST acquisition worksheet

- Select the **BD OneFlow LST Acquisition** worksheet tab.
- 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.

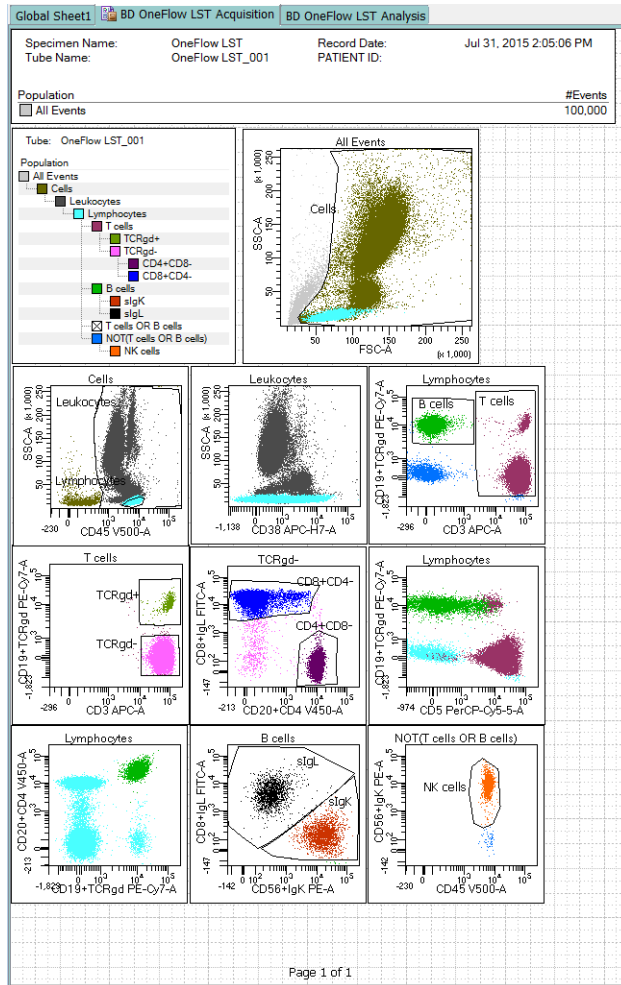
The CD45 V500-A vs SSC-A dot plot contains two gates to identify leukocytes and lymphocytes. T cells and B cells are identified in the CD3 APC-A vs CD19+TCRgd PE-Cy7-A dot plot from the lymphocyte population.

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

Ig λ^- and Ig κ -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**.
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Data analysis

This chapter covers the following topic:

- [Analyzing the data using BD FACSDiva software \(page 26\)](#)

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.

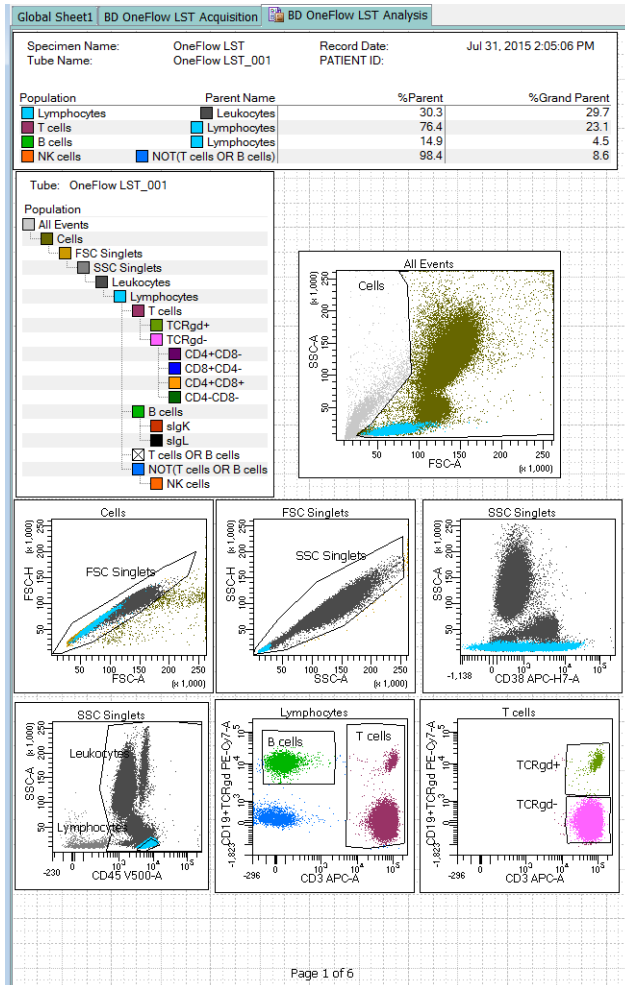
Note: Enlarge the plot while adjusting the gates so you can more readily see the populations of interest.

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 TCR γ/δ^+ and TCR γ/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, bone marrow, and lymph nodes may look different.



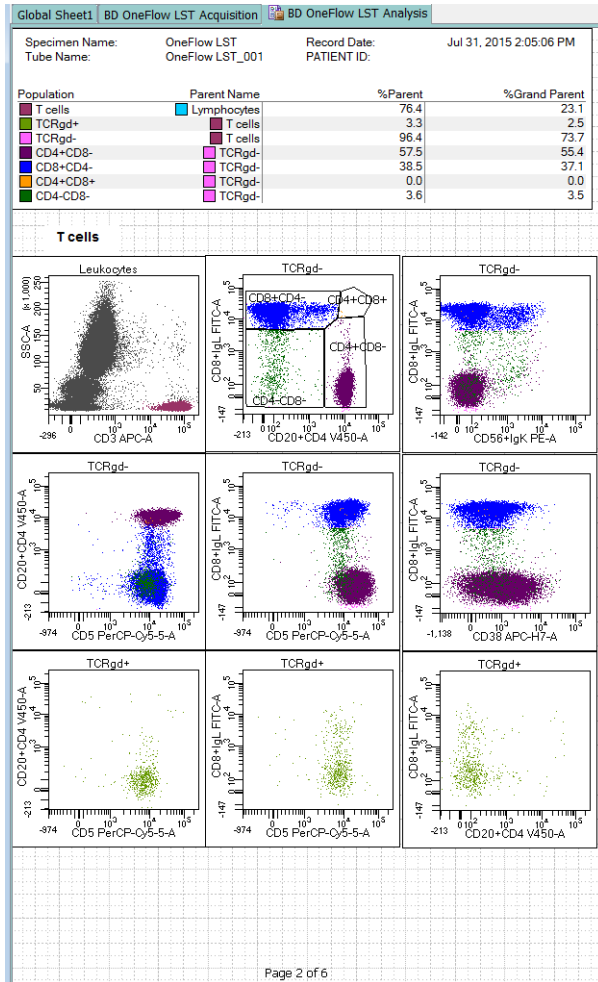
- Inspect the dot plots on page 2 of the LST analysis worksheet, and adjust the gates as needed.

The dot plots on page 2 of the analysis worksheet identify various populations of T cells. TCR γ δ ⁻ T cells are divided into CD8⁺CD4⁻, CD4⁺CD8⁺, 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.



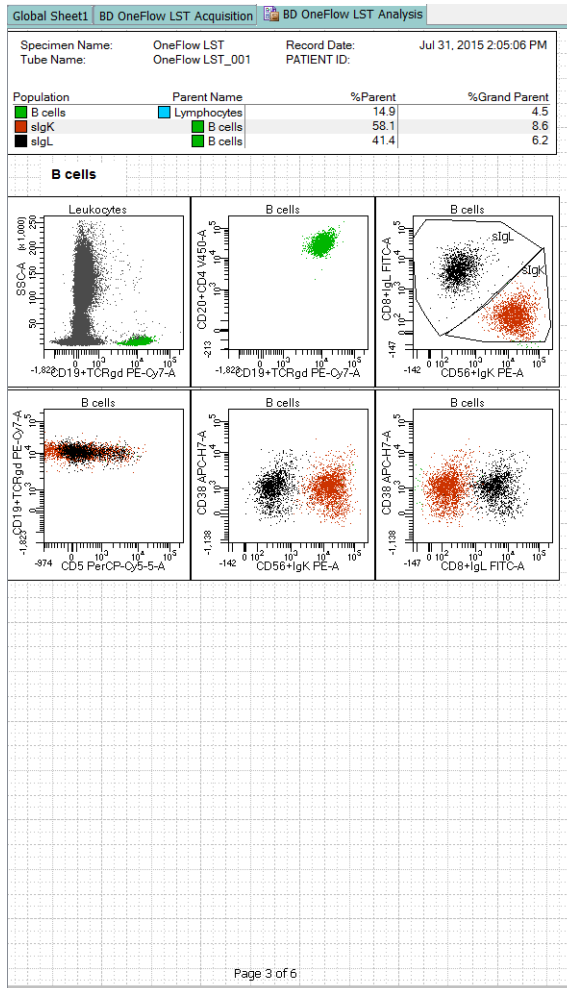
- Inspect the dot plots on page 3 of the LST analysis worksheet and adjust the gates as needed.

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

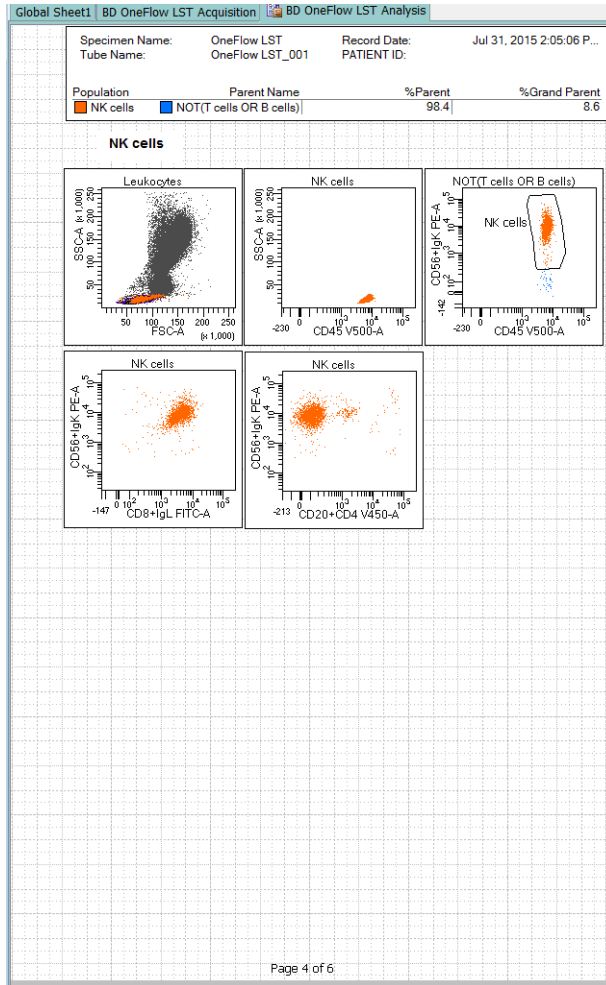
The remaining dot plots further characterize B cells using various markers.



7. Inspect the dot plots on page 4 of the LST analysis worksheet and adjust the gates as needed.

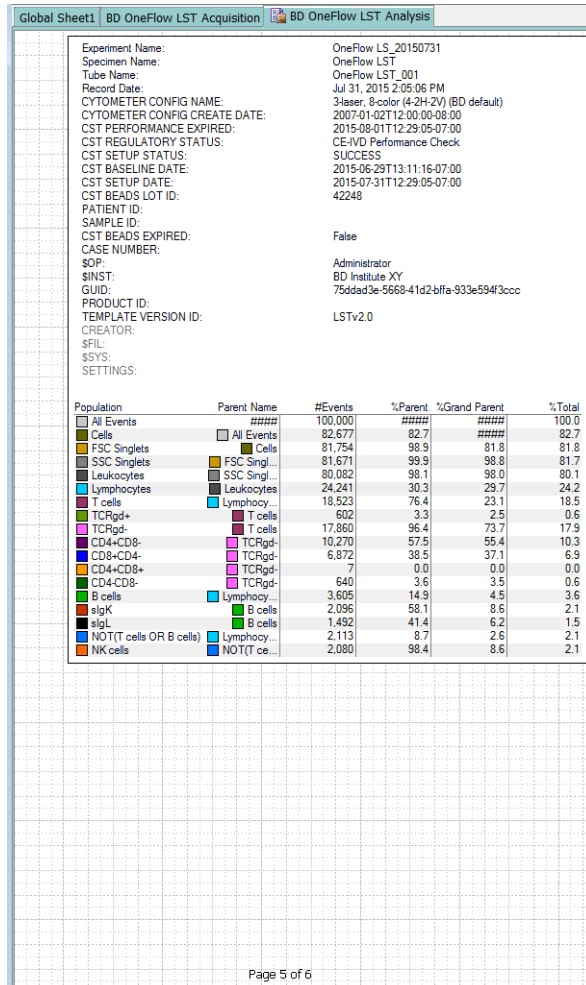
The dot plots on page 4 of the analysis worksheet identify NK cells. NK cells are identified from the NOT(T cells OR B cells) population in the CD45 V500-A vs CD56+IgK PE-A dot plot.

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



- 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.



- 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.

10. 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.

11. (Optional) Click **Print** to print the LST analysis worksheet.
 12. Analyze the next sample.
-

5

Troubleshooting

This chapter covers the following topics:

- [Templates do not import correctly \(page 36\)](#)
- [Problems using BD OneFlow LST \(page 36\)](#)

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 BD OneFlow LST

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 <i>Instrument Setup Guide for BD OneFlow™ Assays</i> .

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.
	BD OneFlow LST was exposed to light for too long.	Repeat staining with a new BD OneFlow LST.
	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.	<ol style="list-style-type: none"> 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.	<ol style="list-style-type: none"> 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.