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

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



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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
23-17211-00	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.

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Overview

This chapter covers the following topics:

- Overview of the BD OneFlow system (page 6)
- Workflows for the BD OneFlow reagents (page 7)

Overview of the BD OneFlow system

About the system	The BD OneFlow TM 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 TM Consortium.
	The BD OneFlow TM reagents are used to stain patient specimens. The stained samples are acquired on the cytometer and then analyzed. BD OneFlow TM B-CLPD T1 is used in combination with BD OneFlow TM 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*TM *Assays* and Setting up the experiment (page 16).

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.

Specimen preparation

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.

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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	Thi spec coll	s procedure works for peripheral blood and bone marrow cimens collected in EDTA- or heparin-containing blood ection tubes (for example, BD Vacutainer® tubes).
	BD four from the LST man	OneFlow LST contains antibodies which recognize Ig κ and Ig λ nd on the surface of B cells. Therefore, to avoid interference n serum antibodies found in the specimen, you must prewash specimen three times before you stain it using BD OneFlow T. We recommend that you wash the samples in the same nner 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 μ L 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 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 μ L of wash buffer to give a final volume of approximately 300 μ L.

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	he BD OneFlow reagent tubes are very sensitive to moisture. To roid condensation, open the pouches only if they are at room mperature. Ensure the pouch is completely resealed after moving a tube. Do not remove the desiccant from the reagent puch.
	Trite the current date on the pouch label when it is first opened. se the tubes from that pouch within one month before opening he next pouch.
Diluting BD FACS lysing solution	ilute the 10X concentrate 1:10 with room temperature (20°C– 5°C) deionized water. The prepared solution is stable for 1 month hen stored in a glass or high density polyethylene (HDPE) ontainer at room temperature.
Staining the specimen	If the pouches are stored refrigerated, allow them to reach room temperature before opening them.
	For each patient specimen, remove the appropriate BD OneFlow reagent tube from its pouch.
	Place the tubes in a rack, protected from light.
	Immediately reseal the pouch with any unused tubes.
	Write the patient ID on the appropriate tube label within the area provided.
	Vortex the washed specimen 3-5 seconds to mix well.

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 $\mu L.$

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.
- 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.
- 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 μ L 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

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Sample acquisition

This chapter covers the following topics:

- Setting up the experiment (page 16)
- Acquiring the stained sample (page 22)

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 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.

- 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 1 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.

Cytometer Settings Mismatch	
The application settings to be applied do not match	a the selected cytometer settings.
The following parameters are not in the cytometer	r settings to be applied: FSC-H, SSC-H.
Click Apply to apply PMT Voltage and Threshold va Click Overwrite to replace all parameters and value	lues only for matching parameters. es with those from the selected application settings.
	Apply Overwrite Cancel

- 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 OneFlowTM 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.

General BD Panels			
Name	Date		Name: OneFlow B-CLPD T1
4-19-4 Control 4-4-8 Control 5 Color TBNK + TruC 3-4-8 Control 4-48 Control	1/4/07 3:36 1/4/07 3:36 1/4/07 3:36 1/4/07 3:36 1/4/07 3:36	*	This template is for use in the acquisition and analysis of BD OneFlow B-CLPD T1
DneFlow B-CLPD T1	6/22/15 3:15		
DneFlow LST PEO DneFlow LST DneFlow PCD DneFlow PCST DneFlow Setup	6/24/15 8:33 6/5/15 10:58 7/16/15 12:1 7/14/15 9:08 10/24/14 10:	4 III	
me: OneFlow B-CLPD T1		1	Copies: 1

- 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.

Acquisition Day	bboard		
Current Activity Active Tube/Well	Threshold Rate	Stopping Gate Events 0 evt	Elapsed Time 00:00:00
Basic Controls	Acquire Data	Record Data	Restart II SIT Flush
Carousel Controls	Run Single Tube	Mix P S	kip 🖪 Rerun 🕒 Pause
Acquisition Setup Stopping Gate: Storage Gate:	All Events V Events To Rec	cord: 100000 evt • Sto play: 50000 evt • Flo	opping Time (se 0 \$) w Rate: Medium V
Acquisition Status Processed Events: Threshold Count:		Electronic Abort Rate: Electronic Abort Count:	

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.

uid	k Entry									\$ Keywords
	Name Name	Keyword	Keyword	Keyword	Keyword	Keyword	Keyword	Keyword	Keyword	Ust by user B . Administrator
	BD OneFlow B-CLPD T1_20150							_		Doctor Specimen type
L	Coneflow B-CLPD T1									
	0neflow 8-CLPD T1_001	SAMPLE ID	PATIENT ID	CASE NUMBER	PRODUCT ID 659293:123456:2015-07-31:12345	SPECIMEN TYP	EDOCTOR	DCLPDT1v1.0	06/22/15	
										Add to List Delete from Assign or Remove Keye

- 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	1.	Select the BD OneFlow LST Acquisition worksheet tab.	
acquisition worksheet	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 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.	
		Igk- and Ig λ -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

D T1 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. 26 BD OneFlow Application Guide for B-cell Chronic Lymphoproliferative Diseases



- 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 28)

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 ce and eliminate debris may appear compressed. This is due to the FSC and SSC target values used to create the application setting The values are specified by the EuroFlow Consortium.		
Analyzing	1.	From the menu bar, select File > Import > Experiments.	
BD OneFlow LSI	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 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 Ig κ - to Ig λ -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*TM *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.

	eert BD Offerlow LST	riequisition ca	-			
	Experiment Name:		Uner	10W L5_201507.	31	
	Specimen Name:		Uner	low LS I		
	Tube Name:		OneF	low LST_001		
	Record Date:		Jul 31	1, 2015 2:05:06	PM	
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	CYTOMETER CONFIG C	REATE DATE:	2007	-01-02T12:00:00	-08:00	
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	COT RECOLATOR TOTAL	105.	CLIN		CHECK	
	CST SETUP STATUS:		5000	LE55		
	CST BASELINE DATE:		2015	-06-29113:11:16	-07:00	
	CST SETUP DATE:		2015	-07-31T12:29:05	-07:00	
	CST BEADS LOT ID:		4224	8		
	PATIENT ID:					
	SAMPLE ID:					
	COT DEADO EVDIDED.		Entra			
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	SINST:		BD In	stitute XY		
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	TEMPLATE VERSION IL	<i>.</i>	LOIV	2.0		
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	CD4+CD8+ CD4+CD8+ CD4+CD8+ CD4+CD8+ CD4+CD8+ CD4+CD8+ CD4+CD8+ CD4+CD8+ CD4+CD8+ CD4+CD8+ CD4+CD8- D4+CD8- D4+CD8- D4+CD8- D4+CD8- D4+CD8+ D4+C08+ D4+C08+ D4+C08+C08+ D4+C08+C08+C08+C08+C08+C08+C08+C08+C08+C08	Leukocytes Lymphocy T cells T cells T CRgd- TCRgd- TCRgd- TCRgd-	24,241 18,523 602 17,860 10,270 6,872 7 640 2,605	30.3 76.4 3.3 96.4 57.5 38.5 0.0 3.6 14 9	29.7 23.1 2.5 73.7 55.4 37.1 0.0 3.5	24. 18. 0. 17. 10. 6. 0. 0.
	T cells T CRgd+ TCRgd- CD4+CD8- CD4+CD8- CD4+CD8+ CD4+CD8- CD4-CD8- CD4-CD8- CD4-CD8-	Leukocytes Lymphocy T cells T CRgd- TCRgd- TCRgd- TCRgd- TCRgd- TCRgd-	24,241 18,523 602 17,860 10,270 6,872 7 640 3,605	30.3 76.4 3.3 96.4 57.5 38.5 0.0 3.6 14.9	29.7 23.1 2.5 73.7 55.4 37.1 0.0 3.5 4.5	24. 18. 0. 17. 10. 6. 0. 0. 3.
	T cells T cells CD4+CD8- CD4+CD8- CD4+CD8- CD4+CD8- CD4-CD8- B cells B cells	Leukocytes Lymphacy T cels T cels T CRgd- T CRgd- T CRgd- T CRgd- T CRgd- CRgd- Lymphacy B cels	24,241 18,523 602 17,860 10,270 6,872 7 640 3,605 2,096	30.3 76.4 3.3 96.4 57.5 38.5 0.0 3.6 14.9 58.1	29.7 23.1 2.5 73.7 55.4 37.1 0.0 3.5 4.5 8.6	24. 18. 0. 17. 10. 6. 0. 0. 0. 3. 2.
	T cells T cells TCRgd+ CD4+CD8- CD4+CD8- CD4+CD8- CD4+CD8- CD4+CD8- SlgK slgL	Leukocytes Lymphocy T cels T CRgd- TCRgd- TCRgd- TCRgd- TCRgd- TCRgd- B cels B cels	24,241 18,523 602 17,860 10,270 6,872 7 640 3,605 2,096 1,492	30.3 76.4 3.3 96.4 57.5 38.5 0.0 3.6 14.9 58.1 41.4	29.7 23.1 2.5 73.7 55.4 37.1 0.0 3.5 4.5 8.6 8.6 6.2	24. 18. 0, 17. 10. 6. 0, 0. 3. 3. 2. 1.
	T cells T CRgd+ CD4+CD8- CD4+CD8- CD4+CD8- CD4+CD8- B cells 8 lgL NOT(T cells OR B cells	Leukocytes Lymphacy T cels T cels T CRgd- TCRgd- TCRgd- TCRgd- TCRgd- TCRgd- DCRG- DCRG-	24,241 18,523 602 17,860 10,270 6,872 7 640 3,605 2,096 1,492 2,113	30.3 764 3.3 964 57.5 38.5 0.0 3.6 14.9 58.1 41.4 8.7	29.7 23.1 2.5 73.7 55.4 37.1 0.0 3.5 4.5 8.6 62 2.6	24. 18. 0. 17. 10. 6. 0. 0. 3. 2. 1. 2.
	T cells T CRgd+ CD4+CD8- CD4+CD8- CD4+CD8- CD4+CD8- B cells sigK sigK sigK sigK sigK sigK sigK sigK sigK sigK sigK NK cells	Leukocytes Lymphocy T cels T CRgd- T CRgd- T CRgd- T CRgd- T CRgd- Lymphocy B cels Upmphocy NOT (T ce	24,241 18,523 602 17,860 10,270 6,872 7 640 3,605 2,096 1,492 2,113 2,080	30.3 76.4 3.3 96.4 57.5 38.5 0.0 3.6 14.9 58.1 41.4 41.4 8.7 98.4	29.7 23.1 2.5 5.4 37.1 0.0 3.5 4.5 8.6 6.2 2.6 6 8.6	24. 18 0. 17 10. 6. 0. 0. 3. 2. 1. 2. 2. 2.
	T cells T Cells T CRgd+ CD4+CD8+ CD4+CD8+ CD4+CD8+ CD4+CD8+ D CD4+CD8+ B cells sigL NOT(T cells OR B cells NK cells	Leukocytes Lymphocy T cels T cels T CRgd- TCRgd- TCRgd- TCRgd- TCRgd- Lymphocy B cels) Lymphocy NOT(T ce	24,241 18,523 602 17,860 10,270 6,872 7 640 3,605 2,096 1,492 2,113 2,080	30.3 76.4 3.3 96.4 57.5 38.5 0.0 3.6 14.9 58.1 41.4 8.7 98.4	29.7 23.1 2.5 73.7 55.4 37.1 0.0 3.5 4.5 6 6.2 2.6 8.6 8.6	24. 18. 0. 17. 10. 6. 0. 0. 3. 2. 1. 2. 2.
	T cells T CRgd+ TCRgd+ CD4+CD8- CD4+CD8- CD4+CD8- B cells sigk sigk NOT(T cells OR B cells	Leukocytes Lymphocy T cels T cels T CRgd- T CRgd- T CRgd- T CRgd- T CRgd- B cels B cels B cels D Lymphocy NOT(T ce	24,241 18,523 602 17,860 10,270 6,872 7 640 3,605 2,096 1,492 2,113 2,080	30.3 76.4 3.3 96.4 57.5 38.5 0.0 3.6 14.9 58.1 41.4 8.1 41.4 8.7 98.4	29.7 23.1 2.5 73.7 55.4 37.1 0.0 3.5 4.5 4.5 8.6 6.2 2.6 6 8.6	24. 18. 0, 17. 10. 6. 0, 0, 3. 2. 1. 2. 2.
	T cells T Chg4 T Chg4 CD4+CD8 CD4+CD8- CD4+CD8- CD4+CD8- B cells sigK sigL NOTT cells OR B cells NK cells	Leukoytes Lymphoz T cels T cels T CRgd- T CRgd- T CRgd- T CRgd- T CRgd- T CRgd- B cels) Lymphozy NOT(T ce	24,241 18,523 602 17,860 10,270 6,872 7 640 3,605 2,096 1,492 2,113 2,080	30.3 76.4 3.3 96.4 57.5 38.5 0.0 3.6 14.9 58.1 41.4 8.7 98.4	29.7 23.1 2.5 73.7 55.4 37.1 0.0 3.5 4.5 8.6 6.2 2.6 8.6	24. 18. 0. 17. 10. 6. 0. 0. 0. 3. 2. 1. 2. 2.
	T cello TCRgd- TCRgd- CD4-CD8- CD4-CD8- CD4-CD8- CD4-CD8- CD4-CD8- CD4-CD8- CD4-CD8- SigK sigK sigL NK cells	Leukoytes Lymphozy T cels T cels T CRgd- T CRgd- T CRgd- T CRgd- T CRgd- T CRgd- S cels S cels Lymphozy NOT(T ce	24,241 18,523 602 17,860 10,270 6,872 7 640 3,605 2,096 1,492 2,113 2,080	30.3 76.4 3.3 96.4 57.5 38.5 0.0 3.6 14.9 58.1 41.4 8.7 98.4	29.7 23.1 2.5 73.7 55.4 37.1 0.0 3.5 4.5 8.6 6.2 2.6 8.6	24. 18. 0, 17. 10. 6. 0. 0. 3. 2. 1. 2. 2.
	T cells T CRg4 T CRg4 CD4+CD8- CD4+CD8- CD4+CD8- CD4+CD8- B cells slgK slgL NCT cells OR B cells	Leukoytes Lymphacy T cels T Cels T CRgd- TCRgd- TCRgd- TCRgd- TCRgd- Lymphocy B cels B cels B cels	24,241 18,523 602 17,860 10,270 6,872 7 7 640 3,605 2,096 1,492 2,113 2,080	30.3 76.4 3.3 96.4 57.5 38.5 0.0 3.6 14.9 58.1 41.4 8.7 98.4	29.7 23.1 2.5 73.7 55.4 37.1 0.0 3.5 4.5 8.6 6.2 2.6 8.6	24. 18. 0. 17. 10. 6. 0. 0. 0. 0. 0. 0. 0. 0. 0. 0. 0. 0. 0.
	Totlogia TCRgd- TCRgd- CD4+CD8- CD4+CD8- CD4+CD8- CD4+CD8- CD4-CD8- B cells sigk sigL NOT(T cels OR B cells NK cells	Leukoytes Lymphoz T cels T cels T CRgd- T CRgd- T CRgd- T CRgd- T CRgd- T CRgd- S cels S cels D Lymphoz NOT(T ce	24,241 18,523 602 17,860 10,270 6,872 7 640 3,605 2,096 1,492 2,113 2,080	00.3 76.4 3.3 96.4 57.5 38.5 0.0 3.6 14.9 58.1 41.4 8.7 98.4	297 231 25 737 554 371 00 35 455 86 62 266 86	24. 18. 0. 17. 10. 6. 0. 0. 3. 2. 1. 2. 2.
	T cells TCRgd- TCRgd- CD4-CD8- CD4-CD8- CD4-CD8- CD4-CD8- CD4-CD8- CD4-CD8- CD4-CD8- CD4-CD8- CD4-CD8- B cells algK algK NOTT cells OR B cells	Leukoytes Lymphacy T cels T Cels T CRgd- TCRgd- TCRgd- TCRgd- TCRgd- Lymphocy B cels B cels B cels	24,241 18,523 602 17,860 10,270 6,872 7 7 640 3,805 2,096 1,492 2,113 2,080	00.3 76.4 3.3 96.4 57.5 38.5 0.0 3.6 14.9 58.1 41.4 8.7 98.4	297 231 25 737 554 371 00 35 45 8.6 62 2.6 8.6 8.6	24. 18. 0. 17. 10. 6. 0. 0. 3. 2. 2. 2.
	T CrBgd+ TCRgd+ TCRgd+ CD4+CD8- CD4+CD8- CD4+CD8- CD4+CD8- CD4-CD8- B cells algK algK algK NOT(T cells OR B cells	Leukoptes Lymohacy T cels T CRgd- T CRgd- T CRgd- T CRgd- T CRgd- T CRgd- T CRgd- T CRgd- T CRgd- B cels B cels D Lymphacy NOT(T ce	24,241 18,523 602 17,860 10,270 6,872 7 640 3,605 2,096 1,492 2,113 2,080	00,3 76,4 3,3 96,4 57,5 38,5 0,0 3,6 14,9 58,1 41,4 8,7 98,4	297 231 25 737 554 371 00 35 455 86 62 266 86	24, 18, 0, 17, 10, 6, 0, 0, 0, 3, 2, 1, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2,
	T cclad T Cftad+ T Cftad+ T Cftad- CD4+CD8- CD4+CD8- CD4+CD8- CD4-CD8- B cells SigN NK cells	Leukortes Lymphocy T cels T CRgd- TCRgd- TCRgd- TCRgd- TCRgd- Lymphocy B cels Lymphocy	24,241 18,523 602 17,860 10,270 6,872 7 640 3,605 2,096 1,492 2,113 2,080	00,3 76,4 3,3 96,4 57,5 38,5 0,0 0,3 6,0 14,9 58,1 41,4 8,7 98,4	297 231 2.5 73.7 55.4 37.1 0.0 3.5 4.5 8.6 62 2.2.6 8.6	24. 18. 0. 17. 10. 6. 0. 0. 0. 0. 0. 0. 0. 0. 0. 0. 0. 0. 0.
	T Crads TCRgds TCRgds CD4+CD8 CD4+CD8 CD4+CD8 CD4+CD8 CD4+CD8 B cells sigk sigk NOT(T cells OR B cells NK cells	Leukocytes Lymphocy T cells T CRgd- TCRgd- TCRgd- TCRgd- TCRgd- B cells B cells B cells NOT(T ce	24.241 18.523 602 17.860 10.270 6.872 7 640 3.605 2.996 1.492 2.113 2.080	30,3 76,4 3,3 96,4 57,5 38,5 0,0 3,6 14,9 58,1 41,4 8,7 98,4	297 231 257 737 554 371 0.0 355 45 86 62 26 886	24 18 0 17 10 6 0 0 0 3 2 1 1 2 2 1 2
	T cclag - TCRgd+ TCRgd+ CD4+CD8- CD4+CD8- CD4+CD8- CD4+CD8- CD4+CD8- B cells algK algK algK NOT(T cells OR B cells	Leukoptes Lymphocy T cels T CRgd- TCRgd- TCRgd- TCRgd- TCRgd- Lymphocy B cels Lymphocy	24,241 18,523 602 17,860 10,270 6,872 7 640 3,605 2,096 1,492 2,113 2,080	30.3 76.4 3.3 96.4 57.5 0.0 3.6 14.9 58.1 41.4 8.7 98.4	297 231 255 737 554 371 0.0 355 4.5 8.6 6.2 2.6 8.6	24 18 0 17 10 0 0 0 3 3 2 2 1 2 2 2
	T ccells TCRg4+ TCRg4+ CD4+CD8- CD4+CD8- CD4+CD8- CD4+CD8- CD4+CD8- CD4+CD8- B cells sigl. NOTT cells OR B cells	Leukoptes Lymphoz T cels T CRg4- TCRg4 TCRg4 TCRg4 CCRg4 Lymphoz NOT(T ce	24,241 18,523 602 017,260 10,270 6,872 7 7 640 3,605 2,096 1,492 2,113 2,080	00.3 76.4 76.4 77.5 80.0 3.6 14.9 58.1 41.4 87.7 98.4	237 231 255 737 554 371 00 3.5 455 6.6 6.2 2.2 6 8.6	24 18 0 17 10 6 0 0 0 3 2 1 1 2 2 1 1 2 2
	T Cr6gd+ TCRgd+ TCRgd+ CD4+CD8- CD4+CD8- CD4+CD8- CD4-CD8- B cells algK algK algK NOT(T cells OR B cells	Leukocytes Lymphocy T cells T Cells T CRgd- T CRgd- T CRgd- T CRgd- T CRgd- T CRgd- B cells B cells B cells B cells	24,241 18,523 602 17,8800 10,270 6,872 77 640 3,605 2,096 1,492 2,113 2,080	00.3 76.4 96.4 97.5 00.0 3.6 14.9 9.5 8.1 41.4 8.7 98.4	23.7 23.1 2.5 73.7 55.4 37.1 00 3.5 8.6 6.2 2.6 8.6	24 18 0 17 10 6 0 0 0 3 3 2 2 1 2 2
	T cclad TCRad+ TCRad+ CD4+CD8- CD4+CD8- CD4+CD8- CD4-CD8- B cells sigK sigI VIT cells NK cells	Leukoytes Lymphocy T cells T CRgd- T CRgd- T CRgd- T CRgd- T CRgd- T CRgd- T CRgd- T CRgd- Notropy CRgd- B cells B cells B cells	24,241 18,523 602 17,8600 10,270 6,877 6,00 3,605 2,096 1,492 2,113 2,080	0.3 76.4 3.3 86.4 57.5 88.5 88.5 14.9 58.1 41.4 8.7 98.4	29.7 23.1 7,5 7,5 55.4 37.1 0.0 3.5 8.6 6.2 2.6 8.6 8.6	24 18 0, 17, 10, 0, 0, 0, 3, 2, 1, 2, 2, 1, 2, 2, 1, 2, 2, 1, 2, 2, 1, 2, 2, 1, 2, 2, 1, 2, 2, 1, 2, 2, 1, 2, 2, 2, 2, 2, 1, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2,
	T CR84+ TCR84+ TCR84+ CD4+CD8- CD4+CD8- CD4+CD8- CD4+CD8+ CD4+CD8+ CP4+CD8+ GP4+CD8+ GP4+CD8+ GP4+CD8+ Style style NOTT cells OR B cells	Leukoytes Lymphocy T Cells T Cell	24,241 18,523 602 17,860 10,270 6,872 7 6,40 3,605 2,066 1,492 2,113 2,080	30.3 76.4 3.3 96.4 57.5 38.5 5.8 5.5 0.0 0.0 6.0 14.9 55.1 41.4 8.7 98.4	297 2311 25 7377 554 3711 00 35 45 86 62 266 86 86	24 24 18 0 17 10 0 0 0 0 3 2 1 1 2 2 2
	T collo Jus T CRgd+ T CRgd+ CD4+CD8- CD4+CD8- CD4+CD8- CD4-CD8- B cells a JgK a JgK a JgK NK cells	Leukoptes Umphocy T cells T cells	24,241 18,523 602 17,860 10,270 6,877 6,07 6,07 6,07 3,605 2,096 1,452 2,113 2,080	00.3 76.4 96.4 57.5 00 3.6 14.9 95.8 14.9 95.8 14.9 98.4	237 2311 25 7377 554 3711 00 35 45 86 62 26 6 86	24 18 0, 17, 10, 0, 0, 0, 3, 3, 2, 1, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2,
	T ccells (J-20 TCRad+ TCRad+ CD4+CD8- CD4+CD8- CD4+CD8- CD4+CD8- CD4+CD8- B cells S cells S cells NK cells	Leukoytes Umphocy T Cells T Cells	24,241 18,523 602 17,860 10,270 6,872 7 6,40 3,605 2,066 2,066 2,060	30.3 76.4 3.3 96.4 57.5 38.5 7.5 0.0 0.0 0.6 14.9 58.1 41.4 41.4 41.4 98.4	297 2311 25 7377 554 554 554 554 554 554 652 266 86 86 86 86	24 24 8 0 17 10 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
	T Crayd- TCRgd- TCRgd- CD4-CD8- CD4-CD8- CD4-CD8- CD4-CD8- B cells sigK sigK sigL NOT(T cells OR B cells	Leukostes Lymohocy Toels	24,241 18,523 602 17,860 10,270 6,872 7 640 3,605 2,096 1,492 2,113 2,080	30.3 764 3.3 964 575 88.5 7.5 88.5 0.0 3.6 14.9 58.1 41.4 8.7 98.4	297 2311 25 7377 554 3711 00 35 45 86 62 266 86	24 24 0 0 17 10 6 0 0 0 3 2 1 2 2 2 2 2 2 2 2 2 2 2 2 2
	T Crad+ TCRad+ TCRad+ CD4+CD8- CD4+CD8- CD4+CD8- CD4+CD8- CD4+CD8- B cells sigL B cells sigL NOT(T cells OR B cells	Leukostes Lymohocy T cels T cels	24,241 18,523 602 17,860 10,270 6,872 7 640 3,605 2,066 1,452 2,113 2,080	30.3 764 33 964.4 57.5 38.5 7.5 0.0 3.6 14.9 58.1 41.4 8.7 98.4	237 2311 25 7377 554 3711 00 35 45 86 62 26 8 86	24 24 18 0 7 10 6 0 0 0 0 0 0 0 0 0 0 0 0 0
	T ccele T CRad+ T CRad+ T CRad+ CD4+CD8- CD4+CD8- CD4+CD8- CD4+CD8- CD4+CD8- B cells ■ sigl. NK cells	Leukostes Lymohocy. T cols T CRd- T C	24,241 18,523 602 17,860 10,270 6,872 7 3,666 2,266 2,262 1,422 1,422 1,422 1,422 1,422 1,422 1,422 1,422 1,421 2,080	30.3 764 3.3 964 57.5 38.5 38.5 0.0 3.6 14.9 5.6 1 4.9 7 8.4 9 8.4	23.7 23.1 2.5 73.7 55.4 37.1 0.0 3.5 4.5 8.6 6.6 8.6 8.6 8.6	24 18 18 0 7 10 6 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
	T Crad- TCRgd+ TCRgd+ CD4+CD8- CD4+CD8- CD4+CD8- CD4+CD8- D4+CD8- B cells algL NOT(T cells OR B cells NK cells	Leukoytes Lymohovy- T Cells T Cells T Cells T Charles T Charles T Charles T	24,241 18,523 602 17,860 10,270 6,872 7 640 3,605 2,096 1,492 2,113 2,080	30.3 764 33 964.4 57.5 38.5 5.8 57.5 0.0 0.3 6.0 14.9 581.1 41.4 87.7 98.4	237 231 25 737 554 371 00 35 45 62 26 86 86 86 86	24 18 10 10 10 10 10 10 10 10 10 10
	T Crain TCRad+ TCRad+ CD4+CD8- CD4+CD8- CD4+CD8- CD4-CD8- B cells sigK sigI HCT cells HK cells	Leukostes Lymohocy T. cels T. cel	24,241 18,523 602 17,860 10,270 6,872 7 640 3,665 2,086 2,086 2,086 2,080	30.3 764 3.3 964.4 957.5 3855 0.0 3.6 14.9 9.5 81.1 41.4 8.7 9.84	237 231 25 73.7 55.4 37.1 00 35 45 86 62 26 6 86	
	T CrBd+ TCRd+ TCRd+ TCRd- CD4+CD8- CD4+CD8- CD4+CD8- CD4+CD8- CD4+CD8- CP4+CD8- GP4+CD8- GP4+CD8- GP4+CD8- HOTT Cells OR B cells	Leukoytes Lymohocy Toels T	24,241 18,523 602 17,860 10,270 6,877 870 3,605 3,605 2,096 1,452 2,080	00.3 764.3 3.3 964.4 77.5 38.5 0.0 0.3 6 14.9 264.4 8.7 98.4	23.7 23.1 2.5 73.7 55.4 37.1 0.0 3.5 4.5 8.6 8.6 8.6 8.6	24 18 0 0 17 7 10 6 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
	T Crad+ TCRad+ TCRad+ CD4+CD8- CD4+CD8- CD4+CD8- CD4+CD8- CD4+CD8- B cells algL NC(TCcals OR B cells algL NC(TCcals OR B cells	Leukostes Lymohocy T Celis T Celis T Celis T Chigd- T Chi	24,241 18,523 602 17,860 6,877 6,40 3,605 2,096 1,492 2,113 2,080	30.3 76.4 3.3 96.4 7.5 8.5 8.5 9.0 9.6 9.6 9.5 9.5 9.5 9.5 9.5 9.5 9.5 9.5 9.5 9.5	237 231 25 737 554 371 005 45 45 45 45 45 45 45 45 45 45 45 45 45	24, 18, 0, 17, 10, 6, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,
	T colls (Job TCRad+ TCRad+ TCRad+ CD4+CD8- CD4+CD8- CD4+CD8- CD4+CD8- CD4+CD8- CD4+CD8- CD4+CD8- B cells S cells S cells NK cells	Leukostes Lymphory TroBis TroBis TrCBid T	24,241 18,523 602 17,860 6,872 6,872 6,872 6,872 2,086 1,482 2,113 2,080	30.3 764 3.3 964 57.5 38.5 0.0 3.6 14.9 561 4.4 7 88.4 98.4	23.7 23.1 2.5 73.7 55.4 37.1 0.0 3.5 4.5 8.6 2.6 8.6 8.6 8.6 8.6	24 18:4 00 177: 10 6:0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
	T CRad+ TCRad+ TCRad+ CD4+CD8- CD4+CD8- CD4+CD8- CD4+CD8- CD4+CD8- CD4+CD8- CD4+CD8- CD4+CD8- CD4+CD8- CD4+CD8- CD4+CD8- CD4- CD4-CD8- CD4-CD8- CD4-CD8- CD4-CD8- CD4-CD8- CD4-CD8- CD4-CD8- CD4-CD8- CD4-CD8- CD4-CD8- CD4-CD8- CD4-CD8- CD4-CD8- CD4-CD8- CD4- CD4-CD8- CD4- CD4- CD4-CD8- CD4- CD4- CD4- CD4- CD4- CD4- CD4- CD4	Leukoytes Lymohovy- Toelis Toelis Toelis Toelis Toelis Toelis Toelis Toelis Toelis Toelis Beelis Beelis Beelis Umphovy- NoT(foe)	24,241 18,523 602 17,860 602 7,767 6,877 6,7 6,87 2,096 1,492 2,113 2,080	30.3 76.4 3.3 96.4 77.5 88.5 0.0 0.4 9.9 1.5 9.1 9.1 9.1 9.1 9.1 9.1 9.1 9.1 9.1 9.1	237 231 25 737 554 371 00 355 45 62 26 86 86 86	
	T CrBq+ TCRq+ TCRq+ CD4+CD8- CD4+CD8- CD4+CD8- CD4+CD8- B cells sigt HCCD8- HCCD8- CD4+CD8- B cells sigt HCCD8- HCCD8- CD4- CD4-CD8- CD4-CD8- CD4-CD8- CD4- CD4- CD4- CD4- CD4- CD4- CD4- CD4	Leukostes Lymohocy T cels T cels	24,241 18,523 602 17,860 602 7,760 6,877 6,40 3,605 2,096 1,492 2,113 2,080	30.3 76.4 3.3 96.4 67.5 0.6 1.6 1.6 1.6 1.6 1.6 1.6 1.6 1.6 1.6 1	237 231 25 737 554 371 05 45 86 62 26 86 86	24, 18, 0, 17, 10, 6, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,
	T ccele (Jos TCRad+ TCRad+ CD4+CD8- CD4+CD8- CD4+CD8- CD4+CD8- CD4+CD8- CD4+CD8- CD4+CD8- B cels B cels HOTT cels OR B cels NK cels	Leukostes Lymphory, Tradis Tra	24,241 18,523 602 17,860 10,270 6.877 670 3.665 2,096 1.4522 2,133 2,080	30.3 76.4 3.3 96.4 57.5 38.5 0.0 3.6 14.9 56.4 8.7 98.4	23.1 23.1 2.5 73.7 55.4 37.1 0.0 3.5 4.5 8.2 2.2 2.2 2.2 8.6 8.6	24, 18, 0, 17,7, 10, 6, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,

	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 <i>BD OneFlow</i> TM <i>LST</i> <i>Application Guide</i> 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	1.	From the menu bar, select File > Import > Experiments.
Analyzing BD OneFlow B-CLPD T1	1. 2.	From the menu bar, select File > Import > Experiments. Select the experiment that you want to analyze. Click Import.
Analyzing BD OneFlow B-CLPD T1	1. 2.	From the menu bar, select File > Import > Experiments. Select the experiment that you want to analyze. Click Import. The experiment with the associated acquisition and analysis worksheets opens.
Analyzing BD OneFlow B-CLPD T1	1. 2. 3.	From the menu bar, select File > Import > Experiments. Select the experiment that you want to analyze. Click Import. The experiment with the associated acquisition and analysis worksheets opens. Select the BD OneFlow B-CLPD T1 Analysis worksheet tab.
Analyzing BD OneFlow B-CLPD T1	1. 2. 3. 4.	From the menu bar, select File > Import > Experiments. Select the experiment that you want to analyze. Click Import. The experiment with the associated acquisition and analysis worksheets opens. Select the BD OneFlow B-CLPD T1 Analysis worksheet tab. Inspect the plots on page 1 of the B-CLPD T1 analysis worksheet and adjust the gates as needed.
Analyzing BD OneFlow B-CLPD T1	1. 2. 3. 4.	From the menu bar, select File > Import > Experiments. Select the experiment that you want to analyze. Click Import. The experiment with the associated acquisition and analysis worksheets opens. Select the BD OneFlow B-CLPD T1 Analysis worksheet tab. 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.
Analyzing BD OneFlow B-CLPD T1	1. 2. 3. 4.	From the menu bar, select File > Import > Experiments. Select the experiment that you want to analyze. Click Import. The experiment with the associated acquisition and analysis worksheets opens. Select the BD OneFlow B-CLPD T1 Analysis worksheet tab. 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.



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 On	eFlow B-CLPD T1 Acq	uisition 🏙 BD (DneFlow B-CLPD	T1 Analysis	
Experiment Speciment Tube Name Record Dat CST BEADC CYTOMETE CYTOMETE CST SETUF CST SETUF CST SETUF CST PERFC CST PERFC CST PERFC CST REGU CST BASEL CST PERFC SINST SAMPLE ID PATLENT ID CASE NUM GUID:	Name: Jame: STATUS: LOT ID: R CONFIG CREATE D/ DATE: INDE DATE: INTRANCE EXPIRED: JATORY STATUS: EXPIRED:	NTE:	OneFlow B-CLPI OneFlow B-CLPI OneFlow B-CLPI Jul 31, 2015 2:00 SUCCESS 8UCCESS 8UCCESS 82248 3-laser.8-color (2007-01-02T12: 2015-06-29T13: 2015-06-29T14: 2015-06-29T13: 2015-06-29T14:	D T1_20150731 D T1_001 3:52 PM 4-2H-2V) (BD defi 00:00-08:00 29:05-07:00 11:16-07:00 29:05-07:00 ance Check	ault) bbd87
SPECIMEN PRODUCT TEMPLATE DOCTOR: CREATOR: SFIL: SSYS: SETTINGS: PREF GWN	TYPE: D: VERSION ID: IAME:		BCLPDT1v1.0		
Population	Parent Name	#Events	%Parent	%Grand Parent	%Total
All Events Cells FSC Sing SSC Sing Leukocyte Lymphocy B cells	#### All Events ets Cells lets FSC Singlets res SSC Singlets tes Leukocytes Lymphocytes	100.000 79.314 74.157 73.994 72.939 24.999 4.035	#### 79.3 93.5 99.8 98.6 34.3 16.1	#### #### 74.2 93.3 98.4 33.8 5.5	100.0 79.3 74.2 74.0 72.9 25.0 4.0

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 (page 38)
- Problems using a BD OneFlow reagent (page 38)

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 TM 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.	 Navigate to the analysis worksheet. Right-click the statistics box and select Edit Stats View. In the Header tab, select the All checkbox. 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.	 From the BD FACSDiva software menu bar, select File > Page Setup. Ensure that all of the margins are set to 2.54 cm or 1 inch, depending on your default standards. Click OK.