BD OneFlow™ LST

20 tests per kit—Catalog No. 658619

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1. INTENDED USE

The BD OneFlow™ LST (Lymphoid Screening Tube) is intended for flow-cytometric immunophenotyping of normal and aberrant mature lymphocyte populations of B, T, and NK lineages in peripheral blood, bone marrow, and lymph nodes, as an aid in the diagnosis of hematological disorders. The BD OneFlow LST is designed for use with a suitably equipped BD flow cytometer and software designated for in vitro diagnostic use.

2. SUMMARY AND EXPLANATION

In chronic lymphoproliferative disorders (CLPD), clonogenic events lead to the expansion and accumulation of mature-appearing lymphocytes, which carry a proliferative and/or survival advantage over their normal counterparts.[1] Thus, the detection of phenotypically aberrant and clonal mature lymphocytes is critical to the diagnosis of CLPD.

The EuroFlow™* Consortium designed multicolor antibody panels to fully characterize the cell populations in a patient specimen using immunophenotypic markers that are indicative of normal and abnormal cells.[1] In addition to the optimized multicolor antibody panels, the EuroFlow protocol comprises standardized procedures for cytometer setup, determination of assay settings, sample preparation and staining, sample acquisition, and data analysis.[2]

The single-tube screening panels and multi-tube classification panels fit into the EuroFlow diagnostic algorithm for the identification and classification of hematological disorders. Each tube contains a set of backbone markers and a set of classification markers.[1] Backbone markers are shared across a particular set of panels and are used to normalize the samples so that data files can be combined and analyzed

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as a single large data file. They are markers that identify distinct cell populations in a particular cell lineage. Classification markers have been selected for their diagnostic utility in discriminating between cell types within a given lineage and in classifying the abnormal cell type in the sample.

3. PRINCIPLES OF THE PROCEDURE

Multiparameter flow cytometry is a sensitive and rapid tool for the qualitative and quantitative characterization of cell populations in a specimen. Cells are incubated with fluorochrome-conjugated antibodies which bind to their target molecules. The stained cells can then be analyzed on a single-cell basis. Multiparameter analysis of the data is used to identify the cell populations in the patient specimen and can lead to the identification of an aberrant clonal cell population.

The number of parameters used in flow cytometric immunophenotyping of hematological disorders has increased in recent years. BD OneFlow LST contains a panel of fluorochrome-conjugated antibodies that identify normal and aberrant populations of B, T, and NK lymphocytes. Analysis of the dot plots allows for the identification of normal and abnormal cell populations.

4. REAGENT

Reagent Composition

BD OneFlow LST consists of single-use tubes containing the following fluorochrome-conjugated antibodies in an optimized dried formulation. See Table 1.

Table 1 BD OneFlow LST antibody panel

Antibody	Fluorochrome	Clone	Isotype
CD8	FITC	SK1 (Leu2a)[3]	IgG$_1$, κ

Table 1 BD OneFlow LST antibody panel

Antibody	Fluorochrome	Clone	Isotype
Anti–Lambda	FITC	1-155-2[4]	IgG$_1$, κ
CD56	PE	MY31 (Leu-19)[5,6]	IgG$_1$, κ
Anti-Kappa	PE	TB28-2[4]	IgG$_1$, κ
CD5	PerCP-Cy™5.5[a]	L17F12[7,8]	IgG$_{2a}$, κ
CD19	PE-Cy™7	SJ25-C1[9,10]	IgG$_1$, κ
Anti–TCRγ/δ-1	PE-Cy7	11F2[11,12]	IgG$_1$, κ
CD3	APC	SK7[8]	IgG$_1$, κ
CD38	APC-H7	HB7[9]	IgG$_1$, κ
CD4	V450[b]	SK3 (Leu3a)[3]	IgG$_1$, κ
CD20	V450	L27[9]	IgG$_1$, κ
CD45	V500-C[b]	2D1[13,14]	IgG$_1$, κ

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b. BD Horizon™ V450, BD Horizon™ V500-C

The antibodies in BD OneFlow LST were chosen for their ability to separate normal lymphocytes into their major subpopulations.

CD45 identifies mature lymphocytes and B-cell precursors.

CD3 identifies T cells. CD3 can also be used to identify B cells and NK cells by exclusion.

Anti-TCRγ/δ-1, CD5, CD4, and CD8 can separate T cells into a number of subpopulations.

CD19 and CD20 identify B cells, and together with CD45 can separate B cells into mature B lymphocytes (CD19+, CD20hi, CD45hi) and B-cell precursors (CD19+, CD20−/lo, CD45lo). CD19 and CD20 are also used to identify NK cells by exclusion.

Anti-Kappa and Anti-Lambda can identify normal and clonally expanded populations of B cells expressing Igκ or Igλ on the surface membrane, respectively.

CD38 identifies plasma cells and B-cell precursors. In addition, it is informative in the evaluation of a wide variety of lymphoid malignancies. CD38 can also aid in the identification of NK cells.

CD56 identifies NK cells.

Refer to the article describing the EuroFlow antibody panels[1] for a full description of the utility of the antibodies chosen for BD OneFlow LST.

Precautions

- The reagent contains 0.25 – <1% of 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one (3:1) (CAS number 2682-20-4) and 0.1 – <0.25% of sodium azide (CAS number 26628-22-8). The reagent is classified as hazardous according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS). Visit regdocs.bd.com to download the Safety Data Sheet.

	Warning
⚠	H317: May cause an allergic skin reaction. H412: Harmful to aquatic life with long lasting effects.

	Warning
Prevention	P261: Avoid breathing dust/fume/gas/mist/vapors/spray.
	P272: Contaminated work clothing should not be allowed out of the workplace.
	P280: Wear protective gloves/protective clothing/eye protection/face protection.
	P273: Avoid release to the environment.
Response	P302+P352: IF ON SKIN: Wash with plenty of water/...
	P333+P313: If skin irritation or rash occurs: Get medical advice/attention.
	P321: Specific treatment (see Safety Data Sheet).
	P363: Wash contaminated clothing before reuse.
Disposal	P501: Dispose of contents/container to an appropriate treatment and disposal facility in accordance with applicable laws and regulations, and product characteristics at time of disposal.

Storage and Handling

Store tubes at 2°C–27°C in the foil pouch. Do not freeze the reagent or expose it to direct light at any time during storage or incubation with cells. The dried fluorochrome-conjugated antibodies are stable until the expiration date shown on the pouch and tube labels when stored as directed. Do not use after the expiration date. Once the pouch is opened, the dried fluorochrome-conjugated antibodies are stable for one month when stored as directed.

CAUTION Ensure the pouch is completely resealed after removing a tube. The reagent is very sensitive to moisture. Do not remove the desiccant from the reagent pouch.

5. INSTRUMENTS

BD OneFlow LST is for use on the following BD instruments.

Table 2 Recommended BD instruments

Flow cytometer	Setup beads	Setup software	Analysis software
BD FACSLyric™a	BD® CS&T Beads BD® FC Beads 7-Color Kit BD® FC Beads 5-Color Kit	BD FACSuite™ Clinical software v1.4 or later	BD FACSuite Clinical software v1.4 or later
BD FACSCanto™ IIb	BD FACSDiva™ CS&T IVD Beads BD OneFlow™ Setup Beads BD® FC Beads 8-Color Kit for BD OneFlow™ Assays	BD FACSDiva™ software v8.0.1 or later	BD FACSDiva software v8.0.1 or later

a. 8-color (4-Blue 2-Red 2-Violet), 10-color (4-Blue 3-Red 3-Violet), or 12-color (4-Blue 3-Red 5-Violet)
b. 3-laser, 8-color, 4-2H-2V BD default (4-2H-2V) optical configuration

6. SPECIMENS

BD OneFlow LST can be used for immunophenotyping by flow cytometry of peripheral blood (PB) or bone marrow (BM) aspirates collected in EDTA or heparin[15-18,21-23] (for example, BD Vacutainer® blood collection tubes), and fresh lymph nodes (LN) collected in PBS or cell culture media, such as RPMI. Each type of specimen can have different storage conditions and limitations that should be considered prior to collection and analysis.[15,16,21]

Specimens should be processed immediately after collection, or up to 24 hours after collection if stored at room temperature (20°C–25°C).[17,18,22,23] If a longer period of time is needed, each laboratory should validate that specimens processed and stored according to their procedures produce equivalent results to specimens processed immediately after collection.

Specimens with large numbers of nonviable cells can give erroneous results due to selective loss of populations and to increased nonspecific binding of antibodies to nonviable cells. Viability of specimens should be assessed and a cutoff value established. A cutoff value of at least 80% viable cells has been suggested.[15]

Samples should be acquired immediately after staining or within 1 hour if kept at 2°C–8°C, protected from light. If a longer period of time is needed, 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.

WARNING All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection[19,20] and dispose of with proper precautions in accordance with federal, state, and local regulations. Never pipette by mouth. Wear suitable protective clothing, eyewear, and gloves.

7. REAGENTS AND MATERIALS

Reagents Provided

BD OneFlow LST is provided as single-use tubes in foil pouches. Each kit contains four pouches, each containing five tubes of BD OneFlow LST.

Reagents and Materials Required but Not Provided

For BD FACSLyric flow cytometers:

- BD OneFlow™ Assays Installer I (Catalog No. 664225)

  An installer is required for the OneFlow LST assay. The assay comprises an acquisition sheet, a laboratory report, a physician report, and a supplemental report used for further investigation. Unless you already have the current OneFlow LST assay, you will have to order the installer the first time you order BD OneFlow LST. The installer also contains assays for other BD OneFlow™ reagents.

  The *BD OneFlow™ LST Application Guide for BD FACSLyric™ Flow Cytometers* is provided with the installer. Application guides for other BD OneFlow reagents are also included.

For BD FACSCanto II flow cytometers:

- BD OneFlow™ Assay Templates Installer (Catalog No. 659305)

  An installer is required for the OneFlow LST template. The template contains two global worksheets: the OneFlow LST Acquisition worksheet and the OneFlow LST Analysis worksheet. Unless you already have the current OneFlow LST template, you will have to order the installer the first time you order BD OneFlow LST. The installer also contains the OneFlow Setup template and templates for other BD OneFlow reagents.

  The *Instrument Setup Guide for BD OneFlow™ Assays* and the *BD OneFlow™ LST Application Guide* are provided with the installer. Application guides for other BD OneFlow reagents are also included.

- 15-mL conical polypropylene tubes
- Pasteur pipet
- Serological pipet
- Micropipettor with tips

- Vortex mixer
- Centrifuge
- Wash buffer (filtered PBS + 0.5% BSA + 0.09% or 0.1% sodium azide)
- BD FACS™ Lysing Solution (10X) (Catalog No. 349202)

  See the *BD FACS™ Lysing Solution* instructions for use (IFU) for precautions and warnings.

For BD FACSLyric flow cytometers:

- BD CS&T Beads (Catalog No. 656504 or 656505)
- BD FC Beads 7-Color Kit (Catalog No. 656867)
- BD FC Beads 5-Color Kit (Catalog No. 661564)

For BD FACSCanto II flow cytometers:

- BD FACSDiva 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 (Catalog No. 658621)

8. PROCEDURE

Installing the Assay or Template

The OneFlow LST assay, used with BD FACSuite Clinical software, or the OneFlow LST template, used with BD FACSDiva software, has to be installed before you run the assay for the first time. Additional assays or templates can be installed at the same time, as needed. If you will analyze the FCS files on a different workstation from the one used to acquire the samples, ensure that you install the assays or templates on both workstations.

To install the OneFlow assay in BD FACSuite Clinical software:

1. Insert the installer and click the installer icon.

   The InstallShield Wizard for BD OneFlow Assays opens.

2. Click **Next**.

   The license agreement opens.

3. Select the **I accept the terms in the license agreement** option and click **Next**.

4. To install all of the assays included on the installer, select the **Complete** option and click **Next**.

5. Optional: To install a subset of the assays included on the installer, select the **Custom** option and click **Next**.

   The **Custom Setup** dialog opens.

   - Click the menu to the left of the appropriate assay.
   - From the menu, select **This assay will be installed on your local hard drive**.

6. Click **Install**.

   The assays will be installed in the Library.

7. Click **Finish**.

   The InstallShield Wizard closes.

8. Optional: Double-click the ReadMe file found on the installer.

   The ReadMe file opens.

9. Click the close box when finished reading it.

10. Remove the installer.

To install the OneFlow template in BD FACSDiva software:

NOTE When you select a template to install, it will always overwrite any template with the same name that was previously installed on the system. If you do not want an existing template on your computer to be overwritten, do not select that template from the installer during the installation process.

1. Insert the installer and click the installer icon.

2. Follow the instructions in the dialog.

   The installer will copy and paste the templates in the folder D:\BDExport\Templates\Panel\BD Panels.

   NOTE If your system has only one drive, the templates will be installed in C:\BDExport\Templates\Panel\BD Panels.

   After installation is complete, a dialog opens, summarizing which templates have been successfully copied into the folder.

3. Click **OK** to close the dialog.

4. The installer ReadMe file opens. Click the close box when you have finished reading it.

5. Remove the installer.

Setting up the Cytometer

For BD FACSLyric flow cytometers:

1. Use BD CS&T Beads and BD FACSuite Clinical software v1.4 or later, to perform Characterization QC (CQC) every 6 months or as needed, perform daily Performance QC (PQC), and perform daily assay and tube settings setup.

2. Use the BD FC Beads 7-Color Kit, BD FC Beads 5-Color Kit, and BD FACSuite Clinical software v1.4 or later, to update reference settings every 60 days.

   See the *BD FACSLyric™ Clinical System Instructions For Use*, the *BD FACSLyric™ Clinical Reference System*, and the appropriate reagent IFU for more information.

For BD FACSCanto II flow cytometers:

1. Use BD FACSDiva CS&T IVD Beads (CS&T IVD beads) and BD FACSDiva software v8.0.1 or later, to define the baseline of the cytometer and to run a daily performance check of the cytometer.

2. Use BD OneFlow Setup Beads, lysed washed blood, and BD FACSDiva software v8.0.1 or later, to set photomultiplier tube (PMT) and scatter voltages monthly.

3. Use BD FC Beads and BD FACSDiva software v8.0.1 or later, to set fluorescence compensation monthly.

4. We recommend that you confirm that the PMT voltages (PMTVs) are still within their daily target ranges.

   See the *Instrument Setup Guide for BD OneFlow™ Assays* and the appropriate reagent IFU for more information.

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.

Processing the Specimen

Washing the specimen

NOTE Before washing the specimen, confirm that the cytometer has been properly set up.

1. 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 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 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 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

11. If the pouch is stored refrigerated, allow it to reach room temperature before opening it.

   NOTE The reagent is very sensitive to moisture. To avoid condensation, open the pouch only if it is at room temperature.

12. For each patient specimen, remove a BD OneFlow LST tube from the pouch.

13. Place the tubes in a rack, protected from light.

   Start staining the specimen within one hour of removing a tube from the pouch.

14. Immediately reseal the pouch with any unused tubes.

   NOTE Ensure the pouch is completely resealed after removing a tube. The reagent is very sensitive to moisture. Do not remove the desiccant from the reagent pouch.

15. Write the patient ID on the BD OneFlow LST tube label within the area provided.

   NOTE 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.

16. Vortex washed specimen 3–5 seconds to mix well.

17. Add 100 µL of washed 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.

18. Incubate for 30 minutes at 20°C–25°C, protected from light.

19. Add 2 mL of 1X BD FACS Lysing Solution. Vortex 3–5 seconds to mix well.

20. Incubate for 10 minutes at 20°C–25°C, protected from light.

21. Centrifuge at 540*g* for 5 minutes at 20°C–25°C.

22. Remove the supernatant without disturbing the cell pellet, leaving approximately 50 µL of residual liquid in the tube.

23. Vortex vigorously until the cell pellet is completely resuspended.

24. Add 2 mL of wash buffer to the tube. Vortex 3–5 seconds to mix well.

25. Centrifuge at 540*g* for 5 minutes at 20°C–25°C.

26. Remove the supernatant without disturbing the cell pellet, leaving approximately 50 µL of residual liquid in the tube.

27. Vortex 3–5 seconds to resuspend the cell pellet.

28. Add 200 μL of wash buffer to the tube. Vortex 3–5 seconds to mix well.

   NOTE Samples should be acquired immediately after staining or within 1 hour if kept at 2°C–8°C, protected from light. If a longer period of time is needed, 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.

Setting up the Assay (BD FACSLyric Flow Cytometer)

To add a reagent lot ID and expiration date to the library:

1. From the BD FACSuite Clinical software navigation bar, click the Library icon.

   The Library workspace opens.

2. Expand the **Beads and Reagents** menu and select **Reagents**.

3. Select OneFlow LST from the **Product Name** list.

   The **OneFlow LST** pane opens at the bottom of the page.

4. Click **Add Lot**.

   The **Add New Lot** dialog opens.

5. Manually enter the **Lot ID**, found on the tube label.

6. Click the calendar icon and navigate through the calendar to select the expiration date, found on the tube label.

7. Select the **Current Lot** checkbox.

8. Click **OK.**

The lot ID and expiration date are added to the appropriate columns for the reagent.

NOTE Make sure to add the reagent lot and expiration date prior to acquisition. This has to be done only once for a particular reagent lot.

To create a worklist:

1. From the BD FACSuite Clinical software navigation bar, click the Worklists icon.

The Worklists workspace opens.

2. In the **Manage Worklists** tab, click **New.**

A blank worklist opens in a new tab.

3. In the **Worklist Entries** section, select the appropriate task from the **Task** menu.

4. Manually enter the **Sample ID** for OneFlow reagent tasks.

Do not scan the barcode, found on the tube label, into the software.

NOTE Multiple lots of the same reagent cannot be run on the same worklist.

5. In the **Loading Options** section, select **Manual** from the **Loading Option** menu.

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

Setting up the Experiment (BD FACSCanto II Flow Cytometer)

1. From the menu bar, select **Edit > User Preferences,** then navigate to the **FCS** tab, and select **Export FCS after recording,** to automatically export the FCS files after acquisition. Click **OK.**

2. Confirm that the cytometer is in the default 4-2H-2V configuration.

3. 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.

4. If prompted by the **CST Mismatch** window, select **Use CST Settings**.

5. Rename the experiment according to your laboratory practice.

6. In the **Browser**, right-click **Cytometer Settings > Link Setup** and select the appropriate compensation matrix calculated using BD FC Beads within the past 31 days. Click **Link**.

    See the *BD® FC Beads 8-Color Kit for BD OneFlow™ Assays* IFU or the *Instrument Setup Guide for BD OneFlow™ Assays*.

7. If prompted by the **Cytometer Settings Mismatch** window, select **Overwrite**.

8. Right-click **Cytometer Settings > Unlink From** the previously linked compensation setup. Click **OK**.

    NOTE Unlinking the compensation setup allows updated application settings to be applied while retaining compensation values.

9. In the **Browser**, right-click **Cytometer Settings > Application Settings > Apply** and select the most recent application settings determined within the last 31 days using the BD OneFlow Setup beads. Click **Apply**.

10. A **Confirm** dialog opens. Select **Keep the compensation value**.

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

12. From the menu bar, select **Experiment > New Specimen**.

    The **Panel Templates** dialog opens.

13. Navigate to the **BD Panels** tab and select the OneFlow LST template.

14. Indicate the number of patient specimens you want to acquire using the **Copies** field near the bottom of the **BD Panels** tab. Click **OK**.

15. 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 sample, 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 select **Experiment > New Tube** from the menu bar or use the **New Tube** icon from the **Browser** menu bar to create the additional tube to be acquired because the labels and barcode fields will not be populated.

    NOTE If you want to acquire additional patient samples stained with BD OneFlow LST in the experiment, repeat steps 12–15 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.

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

17. 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.

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

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

Acquiring the Stained Sample

For BD FACSLyric flow cytometers:

The assay will automatically collect 100,000 total events. You cannot append the number of events to collect after acquisition has started. Therefore, if needed, change the number of events to collect before you start acquisition. To change the number of events to collect, see the *BD OneFlow™ LST Application Guide for BD FACSLyric™ Flow Cytometers*. A clinically relevant number of cells can be determined at the discretion of an appropriate healthcare professional.

To acquire the sample:

1. In the **Worklist Controls** bar, select **Run All** from the **Run** menu to run the entire worklist from the beginning.

   Alternatively, to acquire a specific tube, set the run pointer to the sample you want to run and select **Run from Pointer** from the **Run** menu.

2. Vortex each stained tube 3–5 seconds at low speed immediately prior to acquisition.

3. Follow the prompts in the software to load or unload tubes.

   The OneFlow LST Acquisition sheet opens. The acquisition sheet contains dot plots and gates to identify Leukocytes, Lymphocytes, B cells, T cells, NK cells, and their relevant subpopulations.

4. Examine each dot plot on the acquisition sheet.

   NOTE The preview time is 10 seconds and then data is automatically recorded. Do not increase the preview time and risk loss of the sample due to insufficient volume.

5. If it appears that fewer than 100,000 events will be collected, monitor the sample volume and click **Stop Tube** in the **Worklist Controls** bar to stop acquisition before the tube runs dry.

   NOTE The assay will automatically collect 100,000 total events. If the assay cannot collect 100,000 total events, acquisition will stop after 5 minutes. However, make sure you monitor the sample volume and click **Stop Tube** in the **Worklist Controls** bar to stop acquisition before the tube runs dry. To change the stopping criteria, see the *BD OneFlow™ LST Application Guide for BD FACSLyric™ Flow Cytometers*.

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

For BD FACSCanto II flow cytometers:

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.

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

   NOTE The template will automatically collect 100,000 total events. Use the menu in the **Acquisition Dashboard** to select a different number of events to acquire, if needed. A clinically relevant number of cells can be determined at the discretion of an appropriate healthcare professional.

7. Inspect the dot plots on the LST acquisition worksheet and adjust the gates as needed.

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 a consequence of the target values used to create the application settings. The values are specified by the EuroFlow Consortium.

NOTE Enlarge the dot plots while adjusting the gates so you can more readily see the populations of interest. After adjusting the gates, collapse the dot plot to its original size.

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 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.

NOTE See the *BD OneFlow™ LST Application Guide* for examples of the dot plots showing populations of normal cells in the LST acquisition worksheet.

8. Acquire the next sample.

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

Analyzing the Data Using BD FACSuite Clinical Software

1. Set the run pointer to the appropriate sample in the **Worklist Entries** panel.

   The OneFlow LST Laboratory Report opens in the **Laboratory Report** tab.

2. Review the OneFlow LST Laboratory Report.

   The first page of the report shows sample and tube information, population statistics, and QC messages, if generated.

   NOTE Populations with a low number of events might report %Parent or %Grandparent as 0.0%. This is due to rounding the result to a single decimal place in BD FACSuite Clinical software.

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

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

   NOTE Enlarge the dot plots while adjusting the gates so you can more readily see the populations of interest. After adjusting the gates, collapse the dot plot to its original size.

   The dot plots on page 2 of the report provide a high level cell analysis.

   The first three dot plots on the report identify cells, FSC singlets, and SSC singlets. Debris and doublets are excluded by adjusting the gates.

Leukocytes and lymphocytes are identified in the CD45 V500-A vs SSC-A dot plot from the SSC Singlets population.

B cells (CD3−CD19+) and T cells (CD3+) are identified in the CD3 APC-A vs CD19/TCRgd PE-Cy7-A dot plot from the Lymphocytes population.

TCRγδ+ T cells and TCRγδ− T cells are identified in the CD3 APC-A vs CD19/TCRgd PE-Cy7-A dot plot from the T cells population.

See the *BD OneFlow™ LST Application Guide for BD FACSLyric™ Flow Cytometers* for examples of dot plots showing populations of normal cells.

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

   The dot plots on page 3 of the laboratory report are used to analyze B cells. 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.

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

   The dot plots on page 4 of the laboratory report identify various populations of T cells. TCRγ/δ− T cells are divided into CD4+CD8−, CD8+CD4−, CD4+CD8+, and CD4−CD8− populations in the CD20/CD4 V450-A vs CD8/IgL FITC-A dot plot.

   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 TCRγ/δ⁻ and TCRγ/δ⁺ cells using various markers.

6. Inspect the dot plots on page 5 of the laboratory report and adjust the gates as needed.

The dot plots on page 5 of the laboratory report identify NK cells. NK cells are identified from the NOT T cells OR B cells population in the CD45 V500-C-A vs CD56/IgK PE-A dot plot.

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

7. Inspect page 6 of the laboratory report.

Page 6 of the laboratory report includes lot and expiration dates for BD CS&T Beads and the BD OneFlow reagent, reference settings, tube settings, and cytometer configuration.

8. (Optional) Select the **Physician Report** tab to view the report.

The OneFlow LST Physician Report contains a high level summary of the assay results.

9. (Optional) Select the **Supplemental Report** tab to add additional dot plots to further analyze the sample.

See the *BD OneFlow™ LST Application Guide for BD FACSLyric™ Flow Cytometers* for more information.

WARNING Any gated regions deleted in this Supplemental Report are reflected in the Laboratory and Physician Reports. Any gated regions created in this Supplemental Report might be reflected in the Laboratory Report.

WARNING Do not add dot plots or gates to the Laboratory Report or Physician Report. They cannot be deleted and will invalidate the report.

10. Select the **Laboratory Report** tab.

11. Click **Approved**.

    The **ESignature** dialog opens.

12. Select a user ID.

13. Type your password.

14. (Optional) Enter any comments.

15. Click **Sign**.

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

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

Analyzing the Data Using BD FACSDiva Software

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 dot plots on page 1 of the LST analysis worksheet and adjust the gates as needed.

    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 a

consequence of the target values used to create the application settings. The values are specified by the EuroFlow Consortium.

NOTE Enlarge the dot plots while adjusting the gates so you can more readily see the populations of interest. After adjusting the gates, collapse the dot plot to its original size.

The first three dot plots on the LST 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 APC-H7-A vs SSC-A dot plot is included for informational purposes to allow for the visualization of CD38+ cells.

NOTE See the *BD OneFlow™ LST Application Guide* for examples of dot plots showing populations of normal cells.

5. 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. TCRγ/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.

The remaining dot plots further characterize TCRγ/δ− and TCRγ/δ+ cells using various markers.

6. 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⁺CD45⁺. 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.

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.

8. 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.

9. 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 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.

9. LIMITATIONS

- Use of therapeutic monoclonal antibodies in patient treatment can interfere with recognition of target antigens by this reagent. This should be considered when analyzing samples from patients treated in this fashion. BD Biosciences has not characterized the effect of the presence of therapeutic antibodies on the performance of this reagent.
- Use of this reagent for diagnostic evaluation of hematologic disorders should be performed in the context of a thorough immunophenotypic analysis including other relevant markers.
- Use of BD OneFlow LST requires experience with leukemia and lymphoma immunophenotyping and classification. The results should be interpreted by a pathologist, or equivalent professional, in conjunction with other clinical or laboratory findings.
- BD OneFlow LST has not been tested on specimens from patients with minimal residual disease (MRD).
- Avoid using potentially compromised specimens, including clotted, hemolyzed, frozen, or refrigerated specimens.
- BD OneFlow LST has not been tested with the BD FACS™ Universal Loader.

10. PERFORMANCE CHARACTERISTICS

BD FACSLyric Flow Cytometer

Precision studies for the reproducibility and repeatability of
BD OneFlow LST were performed at BD Research Centre Ireland.

Reproducibility and repeatability (BD FACSLyric flow cytometer)

A 5-day study was performed at one site to assess the reproducibility
and repeatability of BD OneFlow LST using control material.
Estimates of precision were determined across three BD FACSLyric
flow cytometers and three operators by acquiring BD® Multi-Check
Control, stained in duplicate by each operator using three lots of
BD OneFlow LST. Two separate runs were performed by each operator
on each of the 5 tested days.

Nine cell populations were identified as being a percentage of the cell
populations indicated in the following tables. The tables present the
mean, standard deviation (SD), coefficient of variation (%CV), and the
upper 95% confidence limit (CL) for reproducibility (operator/
instrument-to-operator/instrument, lot-to-lot, run-to-run, and
day-to-day reproducibility) and repeatability (within-run precision) for
each subset percentage.

Table 3 Reproducibility of subset percentages

Subset	Mean	SD	Upper 95% CL of SD	%CV	Upper 95% CL of %CV
Leukocytes (%SSC Singlets)	99.78	0.13	0.15	0.14	0.15
Lymphocytes (%Leukocytes)	36.56	0.94	1.08	2.58	2.90
T cells (%Lymphocytes)	68.48	1.11	1.26	1.61	1.81
CD4+CD8− cells (%T cells)	66.05	0.30	0.34	0.45	0.50

Table 3 Reproducibility of subset percentages

Subset	Mean	SD	Upper 95% CL of SD	%CV	Upper 95% CL of %CV
CD8+CD4− cells (%T cells)	29.66	0.39	0.44	1.31	1.47
B cells (%Lymphocytes)	12.99	0.34	0.39	2.62	2.95
NK cells (%Lymphocytes)	17.48	1.01	1.16	5.80	6.53
IgK cells (%B cells)	62.57	1.11	1.27	1.78	2.00
IgL cells (%B cells)	33.57	1.53	1.75	4.56	5.12

Table 4 Repeatability of subset percentages

Subset	Mean	SD	Upper 95% CL of SD	%CV	Upper 95% CL of %CV
Leukocytes (%SSC Singlets)	99.78	0.04	0.05	0.04	0.05
Lymphocytes (%Leukocytes)	36.56	0.55	0.63	1.50	1.69
T cells (%Lymphocytes)	68.48	0.47	0.54	0.69	0.78
CD4+CD8− cells (%T cells)	66.05	0.35	0.40	0.53	0.59
CD8+CD4− cells (%T cells)	29.66	0.35	0.40	1.18	1.32
B cells (%Lymphocytes)	12.99	0.24	0.28	1.88	2.11
NK cells (%Lymphocytes)	17.48	0.34	0.38	1.93	2.16
IgK cells (%B cells)	62.57	1.09	1.25	1.75	1.96
IgL cells (%B cells)	33.57	1.16	1.33	3.47	3.89

Method comparison (BD FACSLyric flow cytometer)

A method comparison study between the BD OneFlow system on the BD FACSLyric flow cytometer (Investigational Method) and the BD OneFlow system on the BD FACSCanto II flow cytometer (Comparator Method) was performed at 4 clinical sites. The BD OneFlow system on BD FACSLyric comprises BD CS&T Beads, BD FC Beads 7-Color Kit, BD FC Beads 5-Color Kit, and BD OneFlow LST acquired on a 10-color BD FACSLyric flow cytometer (4-Blue 3-Red 3-Violet) using BD FACSuite Clinical software v1.3[†] and the OneFlow LST assay. The BD OneFlow reference system on BD FACSCanto II comprises BD FACSDiva CS&T IVD Beads, BD OneFlow Setup Beads, BD FC Beads 8-Color Kit for BD OneFlow Assays, and BD OneFlow LST acquired on a BD FACSCanto II flow cytometer (4-2H-2V) using BD FACSDiva software v8.0.2 and the OneFlow LST template. A total of 98 PB specimens, 26 BM specimens and 16 LN specimens were enrolled in the study. Specimens were collected in the anticoagulants shown. See Table 5.

Table 5 Anticoagulants used to collect specimens

Specimen type	Anticoagulant	
	EDTA	Heparin
PB	72	26
BM	23	3

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† A regression study was performed, demonstrating equivalence between BD FACSuite Clinical software v1.3 and v1.4.

For PB, BM, and LN specimens, the first wash step was started within 23, 24, or 23 hours of collection, respectively. Stained PB, BM, and LN samples were acquired within 50, 48, or 31 minutes of final resuspension, respectively. Samples were identified as being "Abnormal" (abnormal for B cells, T cells, or NK cells) or "Normal" (normal for T cells, B cells, and NK cells) using the two systems, and compared.

Agreement was calculated as follows:

Overall % agreement = ((a+d)/(a+b+c+d))×100
Positive % agreement = (a/(a+c))×100
Negative % agreement = (d/(d+b))×100

wherein,

a = number of samples "Abnormal" for both systems,
b = number of samples "Abnormal" on the BD FACSLyric flow cytometer but "Normal" on the BD FACSCanto II flow cytometer,
c = number of samples "Normal" on the BD FACSLyric flow cytometer but "Abnormal" on the BD FACSCanto II flow cytometer,
d = number of samples "Normal" for both systems.

The results for all cell types are shown in Table 6.

Table 6 Agreement for all cells being "Abnormal" or "Normal"

		Comparator method (BD FACSCanto II flow cytometer		
		Abnormal	Normal	Total
Investigational method (BD FACSLyric flow cytometer)	Abnormal	63	0	63
	Normal	0	77	77
	Total	63	77	140

Overall % agreement is 100%. The lower 95% confidence limit is 97.88%.

The positive agreement for "Abnormal" (abnormal for B cells, T cells, and NK cells) is 100%. The negative agreement for "Normal" (normal for T cells, B cells, and NK cells) is 100%.

The results for B cells are shown in Table 7.

Table 7 Agreement for B cells being "Abnormal" or "Normal"

		Comparator method (BD FACSCanto II flow cytometer)		
		Abnormal	Normal	Total
Investigational method (BD FACSLyric flow cytometer)	Abnormal	52	0	52
	Normal	0	88	88
	Total	52	88	140

Overall % agreement is 100%. The lower 95% confidence limit is 97.88%.

The positive agreement for "Abnormal" (abnormal for B cells) is 100%. The negative agreement for "Normal" (normal for B cells) is 100%.

The results for T cells are shown in Table 8.

Table 8 Agreement for T cells being "Abnormal" or "Normal"

		Comparator method (BD FACSCanto II flow cytometer		
		Abnormal	Normal	Total
Investigational method (BD FACSLyric flow cytometer)	Abnormal	9	0	9
	Normal	0	131	131

Table 8 Agreement for T cells being "Abnormal" or "Normal"

		Comparator method (BD FACSCanto II flow cytometer		
		Abnormal	Normal	Total
	Total	9	131	140

Overall % agreement is 100%. The lower 95% confidence limit is 97.88%.

The positive agreement for "Abnormal" (abnormal for T cells) is 100%. The negative agreement for "Normal" (normal for T cells) is 100%.

The results for NK cells are shown in Table 9.

Table 9 Agreement for NK cells being "Abnormal" or "Normal"[a]

		Comparator method (BD FACSCanto II flow cytometer		
		Abnormal	Normal	Total
Investigational method (BD FACSLyric flow cytometer)	Abnormal	2	0	2
	Normal	0	138	138
	Total	2	138	140

a. NK cell malignancies are rare, therefore the expected number of samples is very low.

Overall % agreement is 100%. The lower 95% confidence limit is 97.88%.

The positive agreement for "Abnormal" (abnormal for NK cells) is 100%. The negative agreement for "Normal" (normal for NK cells) is 100%.

Equivalency (BD FACSLyric flow cytometer)

A quantitative assessment of the indicated cell populations, expressed as a percentage of another cell population, was performed for each evaluable specimen enrolled in the method comparison study. Specimens were analyzed using the BD OneFlow system on the BD FACSLyric flow cytometer and the BD FACSCanto II flow cytometer as described previously.

The mean bias for the percentages of the indicated cell populations determined on the BD FACSLyric flow cytometer versus the BD FACSCanto II flow cytometer was calculated. See Table 10.

Table 10 Summary of mean bias for subset percentages

Population	No. of samples	Mean bias	Lower 95% CL of mean bias	Upper 95% CL of mean bias
Leukocytes (%SSC singlets)	134	–0.27	–0.73	0.19
Lymphocytes (%Leukocytes)	134	–0.63	–1.2	–0.06
T cells (%Lymphocytes)	134	–0.17	–0.58	0.24
CD4$^+$CD8$^-$ cells (%T cells)	134	–0.53	–1.03	–0.04
CD8$^+$CD4$^-$ cells (%T cells)	134	0.2	–0.41	0.81
B cells (%Lymphocytes)	134	–0.26	–0.72	0.19
sIgκ$^+$ cells (%B cells)	134	–1.19	–2.1	–0.29
sIgλ$^+$ cells (%B cells)	134	0.7	0.02	1.38
NK cells (%Lymphocytes)	134	0.45	–0.07	0.98

The results of the method comparison and equivalency studies indicate that the two systems are substantially equivalent.

BD FACSCanto II Flow Cytometer

Precision studies for the reproducibility and repeatability of the BD OneFlow LST were performed at BD Biosciences laboratories in San Jose, CA.

Reproducibility (BD FACSCanto II flow cytometer)

Two operators performed two separate runs per day over a period of eight days, alternating the runs on two BD FACSCanto II flow cytometers. For each run, duplicate samples of BD Multi-Check Control were stained using three lots of BD OneFlow LST by each operator, and then acquired and analyzed using the OneFlow LST template in BD FACSDiva software. Nine cell populations were identified as being a percentage of the cell populations indicated in Table 11. The reproducibility of the subset percentages was calculated for each cell population. Reproducibility comprises four components: operator/instrument-to-operator/instrument, lot-to-lot, run-to-run, and day-to-day reproducibility.

Table 11 Reproducibility of subset percentages

Population	Mean	SD[a]	Upper 95% CL[b] of SD	%CV[c]	Upper 95% CL of %CV
Leukocytes (%SSC Singlets)	100.0	0.01	0.03	0.01	0.03
Lymphocytes (%Leukocytes)	37.9	0.5	1.1	1.2	2.9
T cells (%Lymphocytes)	70.3	0.2	0.3	0.3	0.5
CD4+CD8− cells (%T cells)	63.4	0.5	1.5	0.8	2.4
CD8+CD4− cells (%T cells)	25.1	1.1	3.2	4.4	12.8
B cells (%Lymphocytes)	14.8	0.2	1.0	1.5	6.5
NK cells (%Lymphocytes)	14.8	0.3	0.8	1.9	5.6
smIgκ+ cells (%B cells)	59.4	0.2	3.1	0.3	5.2
smIgλ+ cells (%B cells)	40.6	0.2	2.8	0.4	6.8

40

a. SD = Standard deviation
b. CL = Confidence limit
c. %CV = Coefficient of variation

Repeatability (BD FACSCanto II flow cytometer)

Two operators performed two separate runs per day over a period of eight days, alternating the runs on two BD FACSCanto II flow cytometers. For each run, duplicate samples of BD Multi-Check control were stained using three lots of BD OneFlow LST by each operator, and then acquired and analyzed using the OneFlow LST template in BD FACSDiva software. Nine cell populations were identified as being a percentage of the cell populations indicated in Table 12. The within-run precision (tube-to-tube repeatability) of the subset percentages was calculated for each of the cell populations.

Table 12 Repeatability of subset percentages

Population	Mean	SD	Upper 95% CL of SD	%CV	Upper 95% CL of %CV
Leukocytes (%SSC Singlets)	100.0	0.03	0.03	0.03	0.03
Lymphocytes (%Leukocytes)	37.9	0.5	0.5	1.3	1.4
T cells (%Lymphocytes)	70.3	0.4	0.4	0.5	0.6
CD4$^+$CD8$^-$ cells (%T cells)	63.4	0.5	0.6	0.8	0.9
CD8$^+$CD4$^-$ cells (%T cells)	25.1	0.7	0.8	3.0	3.2
B cells (%Lymphocytes)	14.8	0.3	0.3	1.8	2.0
NK cells (%Lymphocytes)	14.8	0.3	0.3	2.1	2.3
smIgκ$^+$ cells (%B cells)	59.4	0.8	0.8	1.3	1.4
smIgλ$^+$ cells (%B cells)	40.6	0.8	0.8	1.9	2.1

Method comparison (BD FACSCanto II flow cytometer)

A side-by-side comparison study between the BD OneFlow LST system on the BD FACSCanto II flow cytometer and the EuroFlow LST system on the BD FACSCanto II flow cytometer was performed at 3 external clinical sites. The BD OneFlow LST system comprises BD OneFlow Setup Beads, BD FC Beads for compensation, and the BD OneFlow LST reagent. The EuroFlow LST reference system comprises Sphero™ Rainbow calibration particles (8 peaks), single color stained cells plus BD® Multicolor CompBeads for compensation, and the EuroFlow LST reagent cocktail. Both methods used BD FACSDiva CS&T IVD beads to perform instrument quality control. Abnormal mature lymphocyte populations from 81 patients with B-cell malignancies, 35 patients with T-cell malignancies, and 6 patients with NK-cell malignancies were identified using the two systems, and compared. In addition, 9 samples were identified as being from other lineages, for example, plasma cell disorders or bi-phenotypic samples. Cell populations from 76 negative samples, including 19 healthy donors, were identified using the two systems. A total of 123 PB specimens, 53 BM specimens, and 31 fresh LN specimens were enrolled in the study. PB and BM specimens were stained within 24 hours of collection. LN specimens were stained within 6 hours of collection. All stained samples were acquired within 1 hour of staining. Samples were identified as being "Follow-up needed" or "No follow-up needed" using the two systems, and compared.

Agreement was calculated as follows:

Overall % agreement = ((a+d)/(a+b+c+d))×100

wherein,

a = number of samples "Follow-up needed" for both systems,
b = number of samples "Follow-up needed" for the BD OneFlow system but "No follow-up needed" for the EuroFlow system,
c = number of samples "No follow-up needed" for the BD OneFlow system but "Follow-up needed" for the Euroflow system, and
d = number of samples "No follow-up needed" for both systems.

The results for all cell types are shown in Table 13.

Table 13 Agreement for all cells being "Follow-up needed" or "No follow-up needed"

| | | Comparator method (Euroflow LST cocktail) | | |
		Follow-up needed	No follow-up needed	Total
Investigational method (BD OneFlow LST)	Follow-up needed	131	0	131
	No follow-up needed	0	76	76
	Total	131	76	207

Overall % agreement is 100%. The lower 95% confidence limit is 98.6%.

The results for T cells are shown in Table 14.

Table 14 Agreement for T cells being "Follow-up needed" or "No follow-up needed"

| | | Comparator method (Euroflow LST cocktail) | | |
		Follow-up needed	No follow-up needed	Total
Investigational method (BD OneFlow LST)	Follow-up needed	35	0	35
	No follow-up needed	0	172	172
	Total	35	172	207

Overall % agreement is 100%. The lower 95% confidence limit is 98.6%.

The results for B cells are shown in Table 15.

Table 15 Agreement for B cells being "Follow-up needed" or "No follow-up needed"

		Comparator method (Euroflow LST cocktail)		
		Follow-up needed	No follow-up needed	Total
Investigational method (BD OneFlow LST)	Follow-up needed	81	0	81
	No follow-up needed	0	126	126
	Total	81	126	207

Overall % agreement is 100%. The lower 95% confidence limit is 98.6%.

The results for NK cells are shown in Table 16.

Table 16 Agreement for NK cells being "Follow-up needed" or "No follow-up needed"[a]

		Comparator method (Euroflow LST cocktail)		
		Follow-up needed	No follow-up needed	Total
Investigational method (BD OneFlow LST)	Follow-up needed	6	0	6
	No follow-up needed	0	201	201
	Total	6	201	207

a. NK cell malignancies are rare, therefore the expected number of samples is very low.

Overall % agreement is 100%. The lower 95% confidence limit is 98.6%.

Equivalency (BD FACSCanto II flow cytometer)

Peripheral blood, bone marrow, and lymph node specimens collected at 3 external clinical laboratories were obtained from patients with T-cell, B-cell, or NK-cell abnormalities, or with no hematological abnormalities. Specimens were analyzed using the BD OneFlow LST system and the EuroFlow LST system described previously.

The bias for leukocytes identified as being a percentage of SSC singlets is summarized in Table 17.

Table 17 Summary of bias for Leukocytes (%SSC singlets)

Population	No. of samples	Average bias	Lower 95% CL of average bias	Upper 95% CL of average bias
Leukocytes (%SSC singlets)	207	−1.1%	−1.7%	−0.6%

The remaining eight cell populations were identified as being a percentage of the cell populations indicated in Table 18. Deming regression statistics indicate that the results obtained using the two systems are substantially equivalent.

Table 18 Equivalency of the BD OneFlow system to the EuroFlow system

Population	No. of samples	Intercept	Slope	Lower 95% CL of slope	Upper 95% CL of slope
Lymphocytes (%Leukocytes)	207	–0.68	1.01	1.00	1.02
T cells (%Lymphocytes)	207	1.07	0.99	0.98	1.00
CD4+CD8− cells (%T cells)	207	–0.64	1.01	1.00	1.02
CD8+CD4− cells (%T cells)	207	–0.45	1.00	0.99	1.01
B cells (%Lymphocytes)	207	–0.01	1.00	0.99	1.00
NK cells (%Lymphocytes)	207	0.81	0.92	0.84	1.00
smIgκ+ cells (%B cells)	206[a]	2.24	1.00	0.97	1.02
smIgλ+ cells (%B cells)	206[a]	–2.04	1.00	0.98	1.03

a. One patient specimen had 0 and 2 B-cell events for the two systems, respectively. Since smIgκ and smIgλ are defined as a percentage of B-cell events in this study, they could not be defined in one system, and therefore could not be included in the quantitative analysis for smIgκ and smIgλ for that specimen.

11. TROUBLESHOOTING

Problems with cell preparation or staining

Problem	Possible Cause	Solution
The resolution between debris and lymphocytes is poor.	Specimen was poorly lysed.	Prepare and stain another specimen.
	Specimen is of poor quality.	Check cell viability.
	Specimen is too old.	Obtain a new specimen and stain it immediately.
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 tube of 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.

Problems using BD OneFlow LST on BD FACSLyric flow cytometers:

Problem	Possible Cause	Solution
Not enough cells of interest are acquired.	Cell concentration was too low.	Resuspend fresh specimen at a higher concentration. Repeat staining and acquisition.
	The default setting of 100,000 events acquired is too low.	Change the number of events acquired. Repeat staining and acquisition. See the *BD OneFlow™ LST Application Guide For the BD FACSLyric™ Flow Cytometer*.

Problem	Possible Cause	Solution
The FSC-A vs SSC-A dot plot is abnormal.	Cytometer needs adjusting.	Contact BD Biosciences.
The csv file and report are not exported automatically.	The reagent lot number and expiration date were not added to the Library manually.	1. Add the reagent lot number and expiration date to the Library. 2. Export the csv file and the report PDF manually. See the *BD FACSLyric™ Clinical System Instructions For Use.*

Problems using BD OneFlow LST on BD FACSCanto II flow cytometers:

Problem	Possible Cause	Solution
The resolution between debris and lymphocytes is poor.	Instrument settings are inappropriate.	Follow proper instrument setup procedures. See the *Instrument Setup Guide for BD OneFlow™ Assays.*
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**.
The barcode on the BD OneFlow LST tube label cannot be scanned.	The barcode on the tube label has been compromised.	Scan the barcode on the BD OneFlow LST pouch label into the **Product ID** keyword field in the **Experiment Layout**. Next, manually enter a semicolon (;) followed by the six-digit tube-specific ID, found adjacent to the barcode on the tube label, after the last digit of the barcode.
The dot plots on the worksheets are missing or the dot plots do not have gates.	The template did not import correctly.	1. Close the current experiment. 2. Create a new experiment. 3. Re-import the OneFlow LST template.

Problem	Possible Cause	Solution
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. In the **Browser**, set the current tube pointer to the tube that you are analyzing. 2. Navigate to the analysis worksheet. 3. Right-click the statistics box and select **Edit Stats View**. 4. In the **Header** tab, select the **All** checkbox. 5. 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**.

WARRANTY

Unless otherwise indicated in any applicable BD general conditions of sale for non-US customers, the following warranty applies to the purchase of these products.

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HISTORY

Revision	Date	Change made
23-15586-00	7/2014	Initial release
23-15586-01	12/2016	Revised for new OneFlow LST template.
23-15586-02	5/2020	Revised to support using the product on BD FACSLyric flow cytometers.