

 **BD OneFlow™ B-CLPD T1**

20 tests per kit—Catalog No. 659293

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1. INTENDED USE

The BD OneFlow™ B-CLPD T1 (B-cell Chronic Lymphoproliferative Diseases Tube 1) shall be used for specimens with B-lineage populations needing further investigation in combination with the BD OneFlow™ LST (Lymphoid Screening Tube). The BD OneFlow™ B-CLPD T1 is intended for flow-cytometric immunophenotyping of B cells in peripheral blood and bone marrow as an aid in the diagnosis of chronic lymphocytic leukemia (CLL) and other B-cell chronic lymphoproliferative diseases. The BD OneFlow™ B-CLPD T1 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 diseases (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

* The EuroFlow trademark and logo and the EuroFlow™ antibody panels are property of the EuroFlow Consortium and cannot be reproduced or published without prior written permission from the EuroFlow coordinator (www.euroflow.org).

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 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™ B-CLPD T1 contains a panel of fluorochrome-conjugated antibodies that, together with antibodies found in BD OneFlow™ LST, can distinguish between specimens of CLL versus other B-cell chronic lymphoproliferative diseases. Analysis of the dot plots allows for the classification of abnormal B cells as CLL or another B-cell chronic lymphoproliferative disease.

4. REAGENT

Reagent Composition

BD OneFlow™ B-CLPD T1 consists of single-use tubes containing the following fluorochrome-conjugated antibodies in an optimized dried formulation. See Table 1.

Table 1 BD OneFlow™ B-CLPD T1 antibody panel

Antibody	Fluorochrome	Clone	Isotype
CD23	FITC	EBVCS-5 (Leu20) ^{3,4}	IgG ₁ , κ
CD10	PE	HI10a ⁵	IgG ₁ , κ
CD79b	PerCP-Cy™5.5 ^a	SN8 ⁶	IgG ₁ , κ
CD19	PE-Cy™7	SJ25-C1 ^{3,7}	IgG ₁ , κ
CD200	APC	MRC OX-104 ^{8,9}	IgG ₁ , κ
CD43	APC-H7	1G10 ¹⁰	IgG ₁ , κ
CD20	V450 ^b	L27 ⁷	IgG ₁ , κ
CD45	V500-C ^b	2D1 (anti-HLe-1) ^{11,12}	IgG ₁ , κ

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

The antibodies in BD OneFlow™ B-CLPD T1 were chosen to work in conjunction with the antibodies in BD OneFlow™ LST to distinguish CLL from other B-cell chronic lymphoproliferative diseases in patient specimens.


CD45, CD19, and CD20 are present in both BD OneFlow™ LST and BD OneFlow™ B-CLPD T1 and serve as backbone markers, allowing for the direct comparison of specimens stained using the two tubes.

CD23, CD200, CD79b, CD43, and CD10 are classification markers and, together with CD5 and CD38 from BD OneFlow™ LST, allow for specimens to be classified as CLL or as other B-cell chronic lymphoproliferative diseases. Anti-Kappa and Anti-Lambda, present in BD OneFlow™ LST, assess the clonality of the B-cell population.

Refer to the article describing the EuroFlow antibody panels¹ for a full description of the utility of the antibodies chosen for BD OneFlow™ B-CLPD T1.

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). Go to 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.
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. To avoid condensation, open the pouch only after it has reached room temperature. Do not remove the desiccant from the reagent pouch.

5. INSTRUMENTS

BD OneFlow™ B-CLPD T1 is for use on the following BD instruments.

Table 2 Recommended BD instruments

Flow cytometer	Setup beads	Setup software	Analysis software
BD FACSLytic™ ^a	BD® CS&T Beads BD® FC Beads 7-Color Kit BD® FC Beads 5-Color Kit	BD FACSuite™ Clinical application v1.4 or later	BD FACSuite™ Clinical application v1.4 or later
BD FACSCanto™ II ^b	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

The BD FACSTM Universal Loader can be used with this product. See the *BD FACSLyric™ Clinical System Instructions For Use* for more information. The Loader can be used with BD FACSuite™ Clinical application v1.5 or later.

6. SPECIMENS

BD OneFlow™ B-CLPD T1 can be used for immunophenotyping by flow cytometry of peripheral blood (PB) or bone marrow (BM) aspirates collected in EDTA or heparin^{13-16,19-21} (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.^{13,14,19}

Specimens should be processed immediately after collection, or up to 24 hours after collection if stored at room temperature (20°C–25°C).^{15,16,20,21} 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.¹³

Samples should be acquired within 60 minutes if kept at room temperature, 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^{17,18} 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™ B-CLPD T1 is provided as single-use tubes in foil pouches. Each kit contains four pouches, each containing five tubes of BD OneFlow™ B-CLPD T1.

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 BD OneFlow™ B-CLPD T1 assay. If you are using the Loader, use BD OneFlow™ Assays Installer I v1.1 to install the assay in BD FACSuite™ Clinical application v1.5 or later. 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 BD OneFlow™ LST assay, you will have to order the installer the first time you order BD OneFlow™ B-CLPD T1. The installer also contains assays for other *BD OneFlow™* reagents.

The *BD OneFlow™ Application Guide for B-cell Chronic Lymphoproliferative Diseases 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 BD OneFlow™ B-CLPD T1 template. The template contains two global worksheets: the BD OneFlow™ B-CLPD T1 Acquisition worksheet and the BD OneFlow™ B-CLPD T1 Analysis worksheet. Unless you already have the current BD OneFlow™ B-CLPD T1 template, you will have to order the installer the first time you order BD OneFlow™ B-CLPD T1. The installer also contains the BD OneFlow™ Setup template and templates for other BD OneFlow reagents.

The *Instrument Setup Guide for BD OneFlow™ Assays* and the *BD OneFlow™ Application Guide for B-cell Chronic Lymphoproliferative Diseases* 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)

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- 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 BD OneFlow™ B-CLPD T1 Assay or Template

The BD OneFlow™ B-CLPD T1 assay, used with BD FACSuite™ Clinical application, or the BD OneFlow™ B-CLPD T1 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 application:

NOTE When you select an assay to install, it will overwrite the BD OneFlow™ B-CLPD T1 assay that was previously installed on the system. If you do not want an existing assay on your computer to be overwritten, do not select that assay from the installer during the installation process.

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**.

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

- Click **Install**.

The assays will be installed in the Library.

- Click **Finish**.

The InstallShield Wizard closes.

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

The ReadMe file opens.

- Click the close box when finished reading it.

- 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.

- 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:\BDEExport\Templates\Panel\BD Panels.

NOTE If your system has only one drive, the templates will be installed in C:\BDEExport\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 application 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. For assay and tube settings setup, select the **Run Setup** and **Generate Reports** checkboxes.
2. Use the BD® FC Beads 7-Color Kit, BD® FC Beads 5-Color Kit, and BD FACSuite™ Clinical application 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 FACSTM 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 540g for 5 minutes at 20°C–25°C.

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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 BD OneFlow™ B-CLPD T1 within 30 minutes of the last wash. Store the washed specimen at 20°C–25°C until you stain it. Make sure that you stain the patient's specimen using both BD OneFlow™ LST and BD OneFlow™ B-CLPD T1. See the *BD OneFlow™ LST IFU* or the appropriate *BD OneFlow™ Application Guide for B-cell Chronic Lymphoproliferative Diseases* for more information.

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™ B-CLPD T1 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.

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15. Write the patient ID on the BD OneFlow™ B-CLPD T1 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 the washed specimen 3–5 seconds to mix well.
17. Add 100 μ L of washed specimen to the tube. Vortex vigorously 3–5 seconds to mix well.

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

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 540g for 5 minutes at 20°C–25°C.
22. Remove the supernatant without disturbing the cell pellet, leaving approximately 50 μ L 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 540g for 5 minutes at 20°C–25°C.
26. Remove the supernatant without disturbing the cell pellet, leaving approximately 50 μ L of residual liquid in the tube.
27. Vortex 3–5 seconds to resuspend the cell pellet.

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

NOTE Samples should be acquired within 60 minutes if kept at room temperature, 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 application navigation bar, click the Library icon.

The Library workspace opens.

2. Expand the **Beads and Reagents** menu and select **Reagents**.
3. Select OneFlow B-CLPD T1 from the **Product Name** list.

The **OneFlow B-CLPD T1** pane opens at the bottom of the page.

4. Click **Add Lot**.

The **Add New Lot** dialog opens.

5. In BD FACSuite™ Clinical application v1.5, click **Scan Barcode** and then scan the barcode on the pouch or tube label.

The Lot ID and expiration date are entered in the appropriate fields.

NOTE In BD FACSuite™ Clinical application v1.4, add the Lot ID and expiration date manually.

6. Select the **Current Lot** checkbox.

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7. 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 application 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. Enter the **Sample ID** for BD 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** or **Universal Loader** from the **Loading Option** menu.

6. If using the Loader, select **30 Tube Rack** or **40 Tube Rack** from the **Carrier Type** 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** dialog, 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** dialog, 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**.

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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 B-CLPD T1** 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™ B-CLPD T1 in the experiment, repeat steps 12–15 to add new specimens. Two **Confirm** dialogs will open, asking if you want to create another BD OneFlow™ B-CLPD T1 acquisition worksheet or another BD OneFlow™ B-CLPD T1 analysis worksheet. Click **Cancel** in each dialog.

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

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17. Highlight the **Product ID** keyword for the appropriate tube, and scan the barcode on the BD OneFlow™ B-CLPD T1 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:

Two versions of the assay are available:

Assay version	Software	Stopping time	Acquisition mode
v1.0	BD FACSuite™ Clinical application v1.4 or later	5 minutes	Manual
v1.1	BD FACSuite™ Clinical application v1.5 or later	3 minutes	Manual, Loader

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™ Application Guide for B-cell Lymphoproliferative Diseases 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 using BD OneFlow™ B-CLPD T1 assay v1.1:

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.

If using the BD FACSTM Universal Loader, vortex tubes immediately before placing them in the Loader racks.

NOTE Make sure that all of the BD OneFlow™ tubes in the rack are acquired within 1 hour. If not, you must validate tubes acquired outside the 1 hour time.

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

The BD OneFlow™ B-CLPD T1 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.

NOTE The assay will automatically collect 100,000 total events. If the assay cannot collect 100,000 total events, acquisition will stop after 3 minutes. A QC message, “All Events gate does not contain the requested 100,000 events” is generated in the Lab Report, and can be ignored if the sample can be analyzed using the events acquired.

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

To acquire the sample using BD OneFlow™ B-CLPD T1 assay v1.0:

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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 BD OneFlow™ B-CLPD T1 Acquisition sheet opens. The acquisition sheet contains dot plots and gates to identify Leukocytes, Lymphocytes, and B cells.

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™ Application Guide for B-cell Chronic Lymphoproliferative Diseases 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™ B-CLPD T1 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.² 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 the appropriate healthcare professional.

7. Inspect the plots on the 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: one to identify leukocytes and the other to identify 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.

NOTE See the *BD OneFlow™ Application Guide for B-cell Chronic Lymphoproliferative Diseases* for examples of dot plots showing populations of normal cells in the 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 Application

NOTE FCS files acquired using BD OneFlow™ B-CLPD T1 assay v1.0 can be opened in BD OneFlow™ B-CLPD T1 assay v1.1.

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

The BD OneFlow™ B-CLPD T1 Laboratory Report opens in the **Laboratory Report** tab.

2. Review the BD OneFlow™ B-CLPD T1 Laboratory Report.

The first page of the laboratory 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 application.

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

The dot plots on page 2 of the laboratory report provide a high level cell analysis, identifying Leukocytes and Lymphocytes.

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.

See the *BD OneFlow™ Application Guide for B-cell Chronic Lymphoproliferative Diseases 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 identify and characterize the B cells in the sample.

5. Inspect page 4 of the laboratory report.

Page 4 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.

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

The BD OneFlow™ B-CLPD T1 Physician Report contains a high level summary of the assay results.

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

See the *BD OneFlow™ Application Guide for B-cell Chronic Lymphoproliferative Diseases 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.

8. Select the **Laboratory Report** tab.

9. Click **E-sign**.

The **E-Signature** dialog opens.

10. Select a user ID.

11. Type your password.

12. (Optional) Enter any comments.

13. Click **Sign**.

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

14. Click **Approve**.

The Laboratory and Physicians Reports are automatically exported to C:\BD Export Clinical. If needed, manually export the Supplemental Report.

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

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™ B-CLPD T1 Analysis** worksheet tab.
 4. Inspect the dot plots on page 1 of the 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 analysis worksheet identify cells, FSC singlets, and SSC singlets. Debris and doublets are excluded by adjusting the gates.

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

NOTE See the *BD OneFlow™ Application Guide for B-cell Chronic Lymphoproliferative Diseases* for examples of dot plots showing populations of normal cells in the analysis worksheet.

5. Examine the B-cell population in the CD19 PE-Cy7-A vs CD20 V450-A dot plot on page 2 of the 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 analysis worksheet.

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

-
7. Perform further analyses as needed.

NOTE The gates in the dot plots of the analysis worksheet are provided for analyzing normal and aberrant cell populations in the specimen.

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 present 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. See the *BD OneFlow™ Application Guide for B-cell Chronic Lymphoproliferative Diseases* for examples of dot plots showing populations of normal cells in the analysis worksheet.

8. Save the BD OneFlow™ B-CLPD T1 analysis worksheet as a PDF.

NOTE The BD OneFlow™ 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 analysis worksheet.
10. 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.

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- 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™ B-CLPD T1 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™ B-CLPD T1 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.

10. PERFORMANCE CHARACTERISTICS

BD FACSLyric™ Flow Cytometer

Precision studies for the reproducibility and repeatability of BD OneFlow™ B-CLPD T1 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™ B-CLPD T1 using control material. Estimates of precision were determined across three BD FACSLyric™ flow cytometers and three operators by acquiring CD-Chex Plus® BC, stained in duplicate by each operator using three lots of BD OneFlow™ B-CLPD T1. Two separate runs were performed by each operator on each of the 5 tested days.

Three 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)	98.63	0.25	0.28	0.25	0.28
Lymphocytes (%Leukocytes)	42.47	0.75	0.86	1.78	2.00
B cells (%Lymphocytes)	12.33	0.15	0.17	1.24	1.39

Table 4 Repeatability of subset percentages

Subset	Mean	SD	Upper 95% CL of SD	%CV	Upper 95% CL of %CV
Leukocytes (%SSC Singlets)	98.63	0.44	0.50	0.45	0.50
Lymphocytes (%Leukocytes)	42.47	1.25	1.43	2.94	3.31
B cells (%Lymphocytes)	12.33	0.57	0.65	4.59	5.15

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 external 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, BD OneFlow™ LST, and BD OneFlow™ B-CLPD T1 acquired on a 10-color BD FACSLyric™ flow cytometer (4-Blue 3-Red 3-Violet) using BD FACSuite™ Clinical application v1.3† and the BD OneFlow™ B-CLPD T1 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™ B-CLPD T1 acquired on a BD FACSCanto™ II flow cytometer (4-2H-2V) using BD FACSDiva™ software v8.0.2 and the BD OneFlow™ B-CLPD T1 template. A total of 93 evaluable PB specimens, 26 evaluable BM specimens and 16 evaluable 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	68	25
BM	23	3

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 58, 49, or 34 minutes of final resuspension, respectively. Samples with aberrant B cell populations were identified as “CLL” or “Other B-CLPD” (including presumptive cases of atypical CLL) using the two systems. Samples were analyzed as “CLL” or “Non-CLL” using the two systems, and compared. “Non-CLL” includes “Other B-CLPD”, abnormal T, abnormal NK, and

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

“normal” (normal for T cells, B cells, and NK cells). Furthermore, samples were analyzed as “Other B-CLPD” or Non-Other B-CLPD” using the two systems, and compared. “Non-Other B-CLPD” includes CLL, abnormal T, abnormal NK, and “normal” (normal for T cells, B cells, and NK cells).

Agreement was calculated as follows:

$$\text{Overall \% agreement} = ((a+d)/(a+b+c+d)) \times 100$$

$$\text{Positive \% agreement} = (a/(a+c)) \times 100$$

$$\text{Negative \% agreement} = (d/(d+b)) \times 100$$

wherein,

a = number of samples identified as “CLL” for both systems,

b = number of samples identified as “CLL” on the BD FACSLyric™ flow cytometer but identified as “Non-CLL” on the BD FACSCanto™ II flow cytometer,

c = number of samples identified as “Non-CLL” on the BD FACSLyric™ flow cytometer but identified as “CLL” on the BD FACSCanto™ II flow cytometer,

d = number of samples identified as “Non-CLL” for both systems.

The results for specimens identified as “CLL” or “Non-CLL” were tabulated. See Table 6.

Table 6 Agreement for specimens identified as “CLL” or “Non-CLL”

		Comparator method (BD FACSCanto™ II flow cytometer)		Total
		CLL	Non-CLL	
Investigational method (BD FACSLyric™ flow cytometer)	CLL	25	0	25
	Non-CLL	0	110	110
Total		25	110	135

Overall % agreement is 100%. The lower 95% confidence limit is 97.81%.

The positive agreement for “CLL” is 100%. The negative agreement for “Non-CLL” is 100%.

Agreement was also calculated using the formulas described previously wherein,

a = number of samples identified as “Other B-CLPD” for both systems,

b = number of samples identified as “Other B-CLPD” on the BD FACSLyric™ flow cytometer but identified as “Non-Other B-CLPD” on the BD FACSCanto™ II flow cytometer,

c = number of samples identified as “Non-Other B-CLPD” on the BD FACSLyric™ flow cytometer but identified as “Other B-CLPD” on the BD FACSCanto™ II flow cytometer,

d = number of samples identified as “Non-Other B-CLPD” for both systems.

The results for specimens identified as “Other B-CLPD” or “Non-Other B-CLPD” were tabulated. See Table 7.

Table 7 Agreement for specimens identified as “Other B-CLPD” or “Non-Other B-CLPD”

		Comparator method (BD FACSCanto™ II flow cytometer)		Total
		Other B-CLPD	Non-Other B-CLPD	
Investigational method (BD FACSLyric™ flow cytometer)	Other B-CLPD	27	0	27
	Non-Other B-CLPD	0	108	108
Total		27	108	135

Overall % agreement is 100%. The lower 95% confidence limit is 97.81%.

The positive agreement for “Other B-CLPD” is 100%. The negative agreement for “Non-Other B-CLPD” is 100%.

Equivalency (BD FACSLyric™ flow cytometer)

A qualitative assessment of the relative fluorescence intensity, either positive or negative, of the indicated markers in aberrant B cells 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. Overall agreement of the two systems in assessing the expression, either positive or negative, of the indicated markers in the specimens was calculated. See Table 8.

Table 8 Agreement for qualitative marker expression on BD FACSLyric™ and BD FACSCanto™ II flow cytometers

Marker	BD OneFlow reagent tube	% Agreement for the expression of marker	Lower 95% CL of % agreement
CD5	LST	98.08	91.20
CD20	LST	100.0	94.4
CD20	B-CLPD T1	100.0	94.4
CD200	B-CLPD T1	100.0	94.4
CD23	B-CLPD T1	100.0	94.4
CD79b	B-CLPD T1	100.0	94.4
CD10	B-CLPD T1	100.0	94.4
CD43	B-CLPD T1	96.15	88.38

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 BD OneFlow™ B-CLPD T1 were performed at BD Biosciences laboratories in San Jose, CA, USA.

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 CD-Chex Plus® BC were stained using three lots of BD OneFlow™ B-CLPD T1 by each operator, and then acquired and analyzed using the BD OneFlow™ B-CLPD T1 template in BD FACSDiva™ software. Three cell populations were identified as being a percentage of the cell populations indicated in Table 9. 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 9 Reproducibility of subset percentages

Population	Mean	SD^a	Upper 95% CL^b of SD	%CV^c	Upper 95% CL of %CV
Leukocytes (%SSC Singlets)	100.00	0.00	0.00	0.00	0.00
Lymphocytes (%Leukocytes)	36.94	1.23	1.40	3.34	3.74
B cells (%Lymphocytes)	12.45	0.19	0.22	1.53	1.71

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 CD-Chex Plus BC were stained using three lots of BD OneFlow™ B-CLPD T1 by each operator, and then acquired and analyzed using the BD OneFlow™ B-CLPD T1 template in BD FACSDiva™ software. Three cell populations were identified as being a percentage of the cell populations indicated in Table 10. The within-run precision (tube-to-tube repeatability) of the subset percentages was calculated for each cell population.

Table 10 Repeatability of subset percentages

Population	Mean	SD	Upper 95% CL of SD	%CV	Upper 95% CL of %CV
Leukocytes (%SSC Singlets)	100.00	0.00	0.00	0.00	0.00
Lymphocytes (%Leukocytes)	36.94	0.40	0.45	1.08	1.21
B cells (%Lymphocytes)	12.45	0.19	0.22	1.52	1.70

Method comparison (BD FACSCanto™ II flow cytometer)

A side-by-side comparison study between the BD OneFlow™ system on the BD FACSCanto™ II flow cytometer and the EuroFlow system on the BD FACSCanto™ II flow cytometer was performed at 4 external clinical sites. The BD OneFlow™ system comprises BD OneFlow™ Setup Beads, BD® FC Beads for compensation, the BD OneFlow™ LST reagent, and the BD OneFlow™ B-CLPD T1 reagent. The EuroFlow reference system comprises Sphero™ Rainbow calibration particles (8 peaks), single color stained cells plus BD® CompBead particles for compensation, and the corresponding EuroFlow reagent cocktails. Both methods used BD FACSDiva™ CS&T IVD beads to perform instrument quality control. Abnormal

mature B-cell populations from 54 patients with CLL and 47 patients with other B-cell disorders, including presumptive cases of atypical CLL, were identified using the two systems, and compared. A total of 70 PB specimens and 31 BM specimens were enrolled in the study. PB and BM specimens were stained within 26 hours of collection. All stained samples were acquired within 66 minutes of staining. Samples with B cells needing follow-up were classified as CLL or another B-cell disorder.

Agreement was calculated as follows:

$$\text{Overall \% agreement} = ((a+d)/(a+b+c+d)) \times 100$$

wherein,

a = number of samples classified as CLL for both systems,

b = number of samples classified as CLL for the BD OneFlow™ system but classified as other B-cell disorder for the EuroFlow system,

c = number of samples classified as other B-cell disorder for the BD OneFlow™ system but classified as CLL for the EuroFlow system, and

d = number of samples classified as other B-cell disorder for both systems.

The results for samples classified as CLL or another B-cell disorder were tabulated. See Table 11.

Table 11 Agreement for B cells needing follow-up as CLL or other B-cell disorder

		Comparator method (EuroFlow system)		Total
		CLL	Other B-cell disorder	
Investigational method (BD OneFlow™ system)	CLL	54	0	54
	Other B-cell disorder	0	47	47
	Total	54	47	101

Overall % agreement is 100%. The lower 95% confidence limit is 97.1%.

Equivalency (BD FACSCanto™ II flow cytometer)

Specimens with CD45⁺CD19⁺ B cells identified as needing follow-up using the BD OneFlow™ LST tube were further characterized according to the expression of specific markers. Specimens were analyzed using the BD OneFlow™ system and the corresponding EuroFlow system described previously. Agreement of the two systems in assessing the expression, either positive or negative, of the indicated markers in aberrant B cells was calculated. See Table 12.

Table 12 Equivalency of the BD OneFlow™ system to the EuroFlow system

Marker	BD OneFlow reagent tube	% Agreement for the expression of marker	Lower 95% CL of % agreement
CD5	LST	98.0%	93.9%
CD20	LST	100.0%	97.1%
CD20	B-CLPD T1	100.0%	97.1%
CD200	B-CLPD T1	100.0%	97.1%
CD23	B-CLPD T1	100.0%	97.1%
CD79b	B-CLPD T1	99.0%	95.4%

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

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.
	The BD OneFlow™ B-CLPD T1 tube was exposed to light for too long.	Repeat staining with a new tube of BD OneFlow™ B-CLPD T1.
	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.	Check the cell concentration of the specimen. If it is too low, concentrate the specimen and rewash it. Repeat staining and acquisition.
	Cytometer is malfunctioning.	Troubleshoot the instrument. See the cytometer IFU for more information.

Problems using BD OneFlow™ B-CLPD T1 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 <i>BD OneFlow™ Application Guide for B-cell Chronic Lymphoproliferative Diseases for BD FACSLyric™ Flow Cytometers</i> .
The FSC-A vs SSC-A dot plot is abnormal.	Cytometer needs adjusting.	Contact BD Biosciences.
The csv file and report are not exported automatically.	The reagent lot number and expiration date were not added to the Library.	<ol style="list-style-type: none"> 1. Add the reagent lot number and expiration date to the Library. 2. Export the csv file and the report PDF manually. See the <i>BD FACSLyric™ Clinical System Instructions For Use</i>.

Problems using BD OneFlow™ B-CLPD T1 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 <i>Instrument Setup Guide for BD OneFlow™ Assays</i> .
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 BD OneFlow™ B-CLPD T1 tube label cannot be scanned.	The barcode on the tube label has been compromised.	Scan the barcode on the BD OneFlow™ B-CLPD T1 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.
The dot plots on the worksheets are missing or the dot plots do not have gates.	The template did not import correctly.	<ol style="list-style-type: none"> 1. Close the current experiment. 2. Create a new experiment. 3. Re-import the BD OneFlow™ B-CLPD T1 template.
Some of the keywords are missing from the statistics box in the analysis worksheet.	BD FACSDiva™ software did not import all of the keywords into the panel template.	<ol style="list-style-type: none"> 1. 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.	<ol style="list-style-type: none"> 1. From the BD FACSDiva™ software menu bar, select File > Page Setup. 2. Ensure that all of the margins are set to 2.54 cm or 1 inch, depending on your default standards. 3. Click OK.

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
23-17184-00	12/2016	Initial release
23-17184-01	7/2018	Revised to use CD10, clone HI10a (updated reagent composition table, precision studies, and reference #5). Updated New Zealand address.
23-17184-02	5/2020	Revised to support using the product on BD FACSLytic™ flow cytometers.
23-17184-03	1/2021	Revised to support using the product with the BD FACSTM Universal Loader.

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