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. The data files generated are analyzed using software designated for in vitro diagnostic use. Analysis of the dot plots allows for the classification of abnormal B cells as CLL or another B-cell chronic lymphoproliferative disease.

4. 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	MEM-78[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 panels[1] for a full description of the utility of the antibodies chosen for BD OneFlow B-CLPD T1.

The reagent contains 0.7073% of 2-methyl-4-isothiazolin-3-one (CAS number 2682-20-4) and 0.25% of sodium azide (CAS number 26628-22-8).

	Warning
	H317 May cause an allergic skin reaction. H412 Harmful to aquatic life with long lasting effects. P261 Avoid breathing dust/fume/gas/mist/vapors/spray. P280 Wear protective gloves/protective clothing/eye protection/face protection. P273 Avoid release to the environment. P302+P352 IF ON SKIN: Wash with plenty of water/... P333+P313 If skin irritation or rash occurs: Get medical advice/attention. P362+P364 Take off contaminated clothing and wash it before reuse. 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. EUH210 Safety Data Sheet available on request. Contains 2-methyl-4-isothiazolin-3-one: May produce an allergic reaction.

5. 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.

6. REAGENTS OR MATERIALS REQUIRED BUT NOT PROVIDED

- Templates installer CD for BD OneFlow™ Assays (Catalog No. 659305)

  The OneFlow B-CLPD T1 template is provided on an installer CD. 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 OneFlow B-CLPD T1 template, you will have to order the installer CD the first time you order BD OneFlow B-CLPD T1. The installer CD 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™ Application Guide for B-cell Chronic Lymphoproliferative Diseases* are provided on separate CDs along with the installer CD. The *Application Guides for BD OneFlow™ Assays* CD also contains application guides for other BD OneFlow reagents.

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

– Centrifuge
– 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 (BD FC beads) (Catalog No. 658621)
– BD OneFlow™ LST (Catalog No. 658619)

7. INSTRUMENTS

BD OneFlow B-CLPD T1 is for use on a BD FACSCanto™ II flow cytometer with a 3-laser, 8-color, 4-2H-2V BD default (4-2H-2V) optical configuration, running BD FACSDiva™ software v8.0.1 or later.

8. 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 (for example, BD Vacutainer® tubes). 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. If a longer period of time is desired, each laboratory should validate that specimens processed and stored according to their procedures produce equivalent results to specimens processed immediately after collection. PB[15,16,21] or BM[20,21] specimens collected in anticoagulants may be stored at room temperature for up to 24 hours before testing.

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]

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

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.

9. PROCEDURE

Installing the OneFlow B-CLPD T1 Template

The OneFlow B-CLPD T1 template has to be installed before you run the assay for the first time. Additional 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 templates on both workstations.

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 CD and click the installer icon.

   NOTE If the installer does not start automatically, access it through the CD drive and open it.

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. Eject the installer CD.

Setting up the Cytometer

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. See the *BD FACSDiva™ CS&T IVD Beads* IFU and the *Instrument Setup Guide for BD OneFlow™ Assays* for more information.
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. See the *BD OneFlow™ Setup Beads* IFU and the *Instrument Setup Guide for BD OneFlow™ Assays* for more information.
3. Use BD FC beads and BD FACSDiva software v8.0.1 or later to set fluorescence compensation monthly. See the *BD™ FC Beads 8-color kit for BD OneFlow™ Assays* IFU and the *Instrument Setup Guide for BD OneFlow™ Assays* for more information.

Washing the Specimen

NOTE Before washing the specimen, confirm that the cytometer has been properly set up. We recommend that you confirm that the PMT voltages (PMTVs) are still within their daily target ranges. See the chapter for daily setup in the *Instrument Setup Guide for BD OneFlow™ Assays* for more information.

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.

   NOTE Staining from 3×10^4 to 2.5×10^6 white blood cells gives equivalent results.
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 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 *BD OneFlow™ Application Guide for B-cell Chronic Lymphoproliferative Diseases* 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.

Staining the Specimen

1. 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.

2. For each patient specimen, remove a BD OneFlow B-CLPD T1 tube from the pouch.
3. Place the tubes in a rack, protected from light.
4. 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.

5. 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.

6. Vortex the washed specimen 3–5 seconds to mix well.

7. 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.

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

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

acquired immediately after staining. Protect stained specimens from light until they are acquired.

Setting up the Experiment

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**.

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 BD OneFlow B-CLPD T1 tube you wish to re-run. Click the **Next Tube** button in the **Acquisition Dashboard** to create another tube for that patient. Do not use the new tube icon to create the additional tube to be acquired because the labels and barcode fields will not be populated.

    NOTE 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 B-CLPD T1 acquisition worksheet or another 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.

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

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 B-CLPD T1 acquisition worksheet to exclude debris, if needed.

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

    NOTE The template automatically collects 100,000 total events. Use the menu in the **Acquisition Dashboard** to select a different number of events to acquire, if needed. Collecting total events from 3×10^4 to 2.5×10^6 stained cells gives equivalent results.

7. Inspect the plots on the B-CLPD T1 acquisition worksheet, and adjust the gates as needed.

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

    The CD45 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 B-CLPD T1 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 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 B-CLPD T1 analysis worksheet, and adjust the gates as needed.

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

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

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

NOTE See the *BD OneFlow™ Application Guide for B-cell Chronic Lymphoproliferative Diseases* for examples of dot plots showing populations of normal cells in the B-CLPD T1 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 B-CLPD T1 analysis worksheet and adjust the gate as needed.

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

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

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

7. Perform further analyses as needed.

   NOTE The gates in the dot plots of the B-CLPD T1 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 LST analysis worksheet.

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

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

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

10. Analyze the next sample.

10. PERFORMANCE CHARACTERISTICS

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

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 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 2. 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.

† CD-Chex Plus™ is a trademark of Streck, Inc.

Table 2 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	0.000	0.004	0.000	0.004
Lymphocytes (%Leukocytes)	41.4	0.76	2.23	1.8	5.4
B cells (%Lymphocytes)	13.1	0.14	0.22	1.1	1.7

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

Repeatability

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 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 3. The within-run precision (tube-to-tube repeatability) of the subset percentages was calculated for each cell population.

Table 3 Repeatability of subset percentages

Population	Mean	SD	Upper 95% CL of SD	%CV	Upper 95% CL of %CV
Leukocytes (%SSC Singlets)	100.0	0.007	0.008	0.007	0.008
Lymphocytes (%Leukocytes)	41.4	0.56	0.62	1.4	1.5
B cells (%Lymphocytes)	13.1	0.19	0.20	1.4	1.6

Method Comparison

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:

Overall % agreement = ((a+d)/(a+b+c+d))×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 4.

‡ Sphero is a trademark of Spherotech, Inc.

Table 4 Agreement for B cells needing follow-up as CLL or other B-cell disorder

		Comparator method (EuroFlow system)		
		CLL	Other B-cell disorder	Total
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

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 5.

Table 5 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%

Table 5 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
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. 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 B-CLPD T1 requires experience with leukemia and lymphoma immunophenotyping and classification.

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.

THE PRODUCTS SOLD HEREUNDER ARE WARRANTED ONLY TO CONFORM TO THE QUANTITY AND CONTENTS STATED ON THE LABEL OR IN THE PRODUCT LABELING AT THE TIME OF DELIVERY TO THE CUSTOMER. BD DISCLAIMS HEREBY ALL OTHER WARRANTIES, EXPRESSED OR IMPLIED, INCLUDING WARRANTIES OF MERCHANTABILITY AND FITNESS FOR ANY PARTICULAR PURPOSE AND NONINFRINGEMENT. BD'S SOLE LIABILITY IS LIMITED TO EITHER REPLACEMENT OF THE PRODUCTS OR REFUND OF THE PURCHASE PRICE. BD IS NOT LIABLE FOR PROPERTY DAMAGE OR ANY INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING PERSONAL INJURY, OR ECONOMIC LOSS, CAUSED BY THE PRODUCT.

TROUBLESHOOTING

Problem	Possible Cause	Solution
The resolution between debris and lymphocytes is poor.	Specimen was poorly lysed.	Prepare and stain another specimen.
	Specimen is of poor quality.	Check cell viability.
	Specimen is too old.	Obtain a new specimen and stain it immediately.
	Instrument settings are inappropriate.	Follow proper instrument setup procedures. See the *Instrument Setup Guide for BD OneFlow™ Assays*.
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.

Problem	Possible Cause	Solution
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.
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 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.
Some of the keywords are missing from the statistics box in the analysis worksheet.	BD FACSDiva software did not import all of the keywords into the panel template.	1. Navigate to the analysis worksheet. 2. Right-click the statistics box and select **Edit Stats View**. 3. In the **Header** tab, select the **All** checkbox. 4. Click **OK**.
The statement, **For in vitro diagnostic use**, does not appear in the footer of the analysis worksheet when it is printed.	The paper margins in the printer settings were changed.	1. From the BD FACSDiva software menu bar, select **File > Page Setup**. 2. Ensure that all of the margins are set to 2.54 cm or 1 inch, depending on your default standards. 3. Click **OK**.

REFERENCES

1 van Dongen JJ, Lhermitte L, Böttcher S, et al. EuroFlow antibody panels for standardized *n*-dimensional flow cytometric immunophenotyping of normal, reactive and malignant leukocytes. *Leukemia.* 2012;26:1908-1975.

2 Kalina T, Flores-Montero J, van der Velden VH, et al. EuroFlow standardization of flow cytometer instrument settings and immunophenotyping protocols. *Leukemia.* 2012;26:1986-2010.

3 Nadler LM. B Cell/Leukemia Panel Workshop: Summary and Comments. In: Reinherz EL, Haynes BF, Nadler LM, Bernstein ID, eds. *Leukocyte Typing II: Human B Lymphocytes.* Vol 2. New York, NY: Springer-Verlag; 1986:3-43.

4 Sarfati M, Ishihara H, Delespesse G. CD23 Workshop Panel report. In: Schlossman SF, Boumsell L, Gilks W, et al, eds. *Leucocyte Typing V: White Cell Differentiation Antigens.* New York, NY: Oxford University Press; 1995:530-533.

5 Dörken B, Möller P, Pezzutto A, Schwartz-Albiez R, Moldenhauer G. B-cell antigens: CD10. In: Knapp W, Dörken B, Gilks WR, et al, eds. *Leucocyte Typing IV: White Cell Differentiation Antigens.* New York, NY: Oxford University Press; 1989:33-34.

6 Engel P, Wagner N, Tedder TF. CD79 Workshop report. In: Schlossman SF, Boumsell L, Gilks W, et al, eds. *Leucocyte Typing V: White Cell Differentiation Antigens.* New York, NY: Oxford University Press; 1995:667-670.

7 Ling NR, Maclennan ICM, Mason DY. B-cell and plasma cell antigens: new and previously defined clusters. In: McMichael AJ, Beverley PC, Cobbold S, et al, eds. *Leucocyte Typing III: White Cell Differentiation Antigens.* New York, NY: Oxford University Press; 1987:302-335.

8 Cerný J, Angelisová P, Horejší V. Non-lineage Panel—Analysis by Western blotting. In: Mason D, André P, Bensussan A, et al, eds. *Leucocyte Typing VII: White Cell Differentiation Antigens.* New York, NY: Oxford University Press; 2002:458.

9 Hilgert I, Drbal K. Non-lineage Panel—Analysis by cytofluorometry. In: Mason D, André P, Bensussan A, et al, eds. *Leucocyte Typing VII: White Cell Differentiation Antigens.* New York, NY: Oxford University Press; 2002:459-461.

10 Horejší V, Stockinger H. CD43 Workshop Panel report. In: Kishimoto H, Kikutani H, von dem Borne AEGK, et al, eds. *Leucocyte Typing VI: White Cell Differentiation Antigens.* New York: Garland Publishing, Inc.; 1998:494-497.

11 Cobbold SP, Hale G, Waldmann H. Non-lineage, LFA-1 family, and leucocyte common antigens: new and previously defined clusters. In: McMichael AJ, Beverley PC, Cobbold S, et al, eds. *Leucocyte Typing III: White Cell Differentiation Antigens.* New York, NY: Oxford University Press; 1987:788-803.

12 Appendix C. Summary of antibody names, code numbers, and donor laboratories. In: McMichael AJ, Beverley PC, Cobbold S, et al, eds. *Leucocyte Typing III: White Cell Differentiation Antigens.* New York, NY: Oxford University Press; 1987:988-993.

13 Rothe G, Schmitz G. Consensus protocol for the flow cytometric immunophenotyping of hematopoietic malignancies. *Leukemia*. 1996;10:877-895.

14 Stelzer GT, Marti G, Hurley A, McCoy PJ, Lovett EJ, Schwartz A. US-Canadian consensus recommendations on the immunophenotypic analysis of hematologic neoplasia by flow cytometry: standardization and validation of laboratory procedures. *Cytometry*. 1997;30:214-230.

15 Nicholson JKA, Green TA. Selection of anticoagulants for lymphocyte immunophenotyping: effect of specimen age on results. *J Immunol Methods*. 1993;165:31-35.

16 Paxton H, Bendele T. Effect of time, temperature, and anticoagulant on flow cytometry and hematological values. *Ann NY Acad Sci*. 1993;677:440-443.

17 *Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline—Fourth Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2014. CLSI document M29-A4.

18 Centers for Disease Control. Perspectives in disease prevention and health promotion update: universal precautions for prevention of transmission of human immunodeficiency virus, hepatitis B virus, and other bloodborne pathogens in health-care settings. *MMWR*. 1988;37:377-388.

19 Davis BH, Dasgupta A, Kussick S, Han JY, Estrellado A; on behalf of ICSH/ICCS working group. Validation of cell-based fluorescence assays: practice guidelines from the ICSH and ICCS - part II - preanalytical issues. *Cytometry* Part B 2013;84B:286-290.

20 Stetler-Stevenson M, Greig B, Yuan C. Flow cytometric specimen collection, processing, and reporting. In: Kottke-Marchant K, Davis BH, eds. *Laboratory Hematology Practice*. First Edition. Hoboken, NJ; Wiley-Blackwell Inc.; 2012:105-114.

21 Stetler-Stevenson M, Ahmad E, Barnett D, et al. *Clinical Flow Cytometric Analysis of Neoplastic Hematolymphoid Cells; Approved Guideline—Second Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2007. CLSI document H43-A2.