BD OneFlow™ ALOT

BD OneFlow™ ALOT—Catalog No. 660228—10 Tests

23-18878(04)
2023-10
English

CE 2797 IVD

1. INTENDED USE

The BD OneFlow™ ALOT (Acute Leukemia Orientation Tube) is intended for in vitro diagnostic use for qualitative flow-cytometric immunophenotyping of immature hematopoietic cell populations (lymphoid and myeloid lineages) on a BD flow cytometer equipped with:

- A 488-nm blue laser, a 640-nm red laser, and a 405-nm violet laser
- The ability to detect forward scatter (FSC) and side scatter (SSC)
- At least eight-color fluorescence
- Software to acquire and analyze the data

BD OneFlow™ ALOT is used as an aid in the screening of hematologically abnormal patients having, or suspected of having, acute lymphoblastic leukemia or acute myeloid leukemia. BD OneFlow™ ALOT can be used with peripheral whole blood and bone marrow specimens collected in EDTA or heparin. The results should be interpreted by a pathologist or equivalent professional in conjunction with other clinical or laboratory findings.

Specific antigens contained in BD OneFlow™ ALOT are listed as follows:

Tube	FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	APC-H7	V450	V500-C
ALOT	cyMPO[a]	cyCD79a	CD34	CD19	CD7	CD3	cyCD3	CD45
a. cy refers to cytoplasmic								

2. SUMMARY OF THE TEST

Acute leukemias are a heterogeneous group of diseases characterized as having a clonal (neoplastic) population of immature hematopoietic cells in the peripheral blood (PB) or bone marrow (BM).[1] There are two major classes of acute leukemias: lymphoid precursor leukemias and acute myeloid leukemias (AML). The lymphoid precursor leukemias are divided into B-cell and T-cell precursor lymphoblastic leukemias (BCP-ALL and T-ALL, respectively). In addition, a small number of neoplasms do not fit into any of these categories because they either show no clear expression of markers indicative of a particular lineage, or they express markers specific to more than one lineage. They include acute undifferentiated leukemia (AUL) and mixed phenotype acute leukemia (MPAL).

The sample acquisition can be automated using the optional BD FACS™ Universal Loader when used with the BD FACSLyric™ flow cytometer. This assay is not for automated sample preparation. Data analysis can be performed using a pre-defined template and gating, however, reviewing all plots and adjusting gating manually by the user are recommended.

Principle of Operation

The reagent tubes that comprise BD OneFlow™ ALOT are composed of monoclonal antibodies, each conjugated to a specific fluorochrome. The specimen is added to the reagent tube and incubated, allowing each fluorochrome-conjugated monoclonal antibody in the reagent to bind to a specific antigen. After incubation, the FIX&PERM® Cell Fixation and Permeabilization Kit is used to fix the stained cells in suspension and to permeabilize the cell membranes. Cells are acquired on a BD flow cytometer using the instrument software. During acquisition, the cells travel past the laser beam and scatter the laser light. The stained cells fluoresce. These scatter and fluorescence signals, detected by the instrument, provide information about the cell's size, internal complexity, and relative fluorescence intensity. The instrument software is used to analyze the data and report the result.

3. REAGENT

Reagent Composition

BD OneFlow™ ALOT consists of two single-use tubes containing fluorochrome-conjugated antibodies in an optimized dried formulation. The BD OneFlow™ ALOT (S) tube contains antibodies that recognize cell surface antigens, and the BD OneFlow™ ALOT (C) tube contains antibodies that recognize cytoplasmic antigens. All of the antibodies have IgG1 heavy chains and kappa light chains. The antibody clone names are shown beneath the antibody.

Table 1 Antibody composition of BD OneFlow™ ALOT

Reagent	FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	APC-H7	V450[a]	V500-C[a]
ALOT (S)	–	–	CD34 (8G12)[2]	CD19 (SJ25-C1)[3,4]	CD7 (M-T701)[5]	CD3 (SK7)[6,7]	–	CD45 (2D1)[8,9]
ALOT (C)	cyMPO (MPO-7)[10]	CD79a (HM57)[11]	–	–	–	–	CD3 (UCHT-1)[8,12]	–

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

The antibodies in the BD OneFlow™ ALOT tubes were chosen for their ability to identify and characterize aberrant immature populations of hematopoietic cells.

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

Precautions

- The reagent tubes contain 0.70% 2-methyl-4-isothiazolin-3-one (CAS number 2682-20-4). These reagents are classified as hazardous according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) and Regulation (EC) No 1272/2008.

	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. P273: Avoid release to the environment. P280: Wear protective gloves/protective clothing/eye protection/face protection.
Response	P302+P352: IF ON SKIN: Wash with plenty of soap and water. P333+P313: If skin irritation or rash occurs: Get medical advice/attention. P362+P364: Take off contaminated clothing and wash it before reuse.
Disposal	P501: Dispose of contents/container to an approved facility in accordance with local, regional, national and international regulations.

- Go to regdocs.bd.com/regdocs/sdsSearch to download the Safety Data Sheet.

Storage and Handling

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

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

4. INSTRUMENTS

The recommended BD systems are outlined. See the following table. See the corresponding reagent or instrument user documentation for details.

Table 2 Recommended BD systems

Flow cytometer	Setup beads	Setup software	Analysis software
BD FACSLyric™[a]	BD® CS&T Beads BD® FC Beads 7-Color Kit BD® FC Beads 5-Color Kit BD® FC Beads 2-Color Kit (12-color instrument)	BD FACSuite™ Clinical application v1.4 or later[b]	BD FACSuite™ Clinical application v1.4 or later
BD FACSCanto™ II[c]	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

Flow cytometer	Setup beads	Setup software	Analysis software
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. The Loader can be used with BD FACSuite™ Clinical application v1.5 or later c. 3-laser, 8-color, 4-2H-2V BD default (4-2H-2V) optical configuration			

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

5. SPECIMEN COLLECTION AND PREPARATION

BD OneFlow™ ALOT can be used for immunophenotyping by flow cytometry of peripheral blood (PB) or bone marrow (BM) aspirates.

- Collect PB specimens aseptically by venipuncture in BD Vacutainer® K2 or K3 EDTA blood collection tubes, or equivalent, or in lithium or sodium heparin.[13]
- Collect BM specimens in BD Vacutainer® K2 or K3 EDTA blood collection tubes, or equivalent, or in lithium or sodium heparin.

  We recommend that you follow guidelines described in consensus protocols for flow cytometric immunophenotyping of hematopoietic malignancies.[14,15]
- Specimens should be processed up to 24 hours 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. A minimum viability of 75% is recommended.[16]
- Samples should be acquired within 60 minutes of staining if kept at room temperature, protected from light.

  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.

Interfering Conditions

Substances present in the specimen might interfere with the assay:

- Use of therapeutic monoclonal antibodies in patient treatment, such as Rituximab and Alemtuzumab, can interfere with recognition of target antigens by this reagent or deplete the clinically relevant cell populations. 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.
- Avoid using potentially compromised specimens, including clotted, hemolyzed, frozen, or refrigerated specimens.

The table lists the substances that were tested for interference with BD OneFlow™ ALOT.

Testing for interference was performed in accordance with CLSI guidelines.[19] There was no detectable interference at the following concentrations.

Table 3 Test concentrations for exogenous interferents

Exogenous interferent	Concentration tested
Acetaminophen	0.156 mg/mL
Acetylsalicylic acid (Aspirin)	0.03 mg/mL
Albuterol	0.000045 mg/mL
Guaifenesin	0.0045 mg/mL
Ibuprofen	0.219 mg/mL
Oseltamivir phosphate	0.000399 mg/mL

Table 4 Test concentrations for endogenous interferents

Endogenous interferent	Highest expected concentration
Hemoglobin or hemolysate	10 mg/mL
Albumin or total protein	60 mg/mL
Bilirubin, conjugated	0.4 mg/mL
Bilirubin, unconjugated	0.4 mg/mL
Triglycerides	15 mg/mL
Erythrocytes	6×10^6 cells/µL

6. PROCEDURE

Reagents and Materials

Reagents and materials provided

BD OneFlow™ ALOT is provided as single-use tubes in foil pouches. Each kit contains four pouches:

- two pouches, each containing five tubes of BD OneFlow™ ALOT (S)
- two pouches, each containing five tubes of BD OneFlow™ ALOT (C)

Reagents and materials required but not provided

For BD FACSLyric™ flow cytometers:

- BD OneFlow™ Assays Installer II (Catalog No. 664226)

  An installer is required for the BD OneFlow™ ALOT 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™ ALOT assay, you will have to order the installer for BD OneFlow™ Assays the first time you order BD OneFlow™ ALOT.

  The *BD OneFlow™ Application Guide for Acute Leukemias for BD FACSLyric™ Flow Cytometers* is provided with the installer.

For BD FACSCanto™ II flow cytometers:

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

  An installer is required for the BD OneFlow™ ALOT template. The template contains two global worksheets: the BD OneFlow™ ALOT Acquisition worksheet and the BD OneFlow™ ALOT Analysis worksheet. Unless you already have the current BD OneFlow™ ALOT template, you will have to order the installer the first time you order BD OneFlow™ ALOT. 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 Acute Leukemias* are provided with the installer.

- 15-mL conical polypropylene tubes
- 40-µm cell strainer (if needed for processing BM specimens)
- Pasteur pipet
- Serological pipet
- Micropipettor with tips
- Vortex mixer
- Centrifuge
- Wash buffer (filtered phosphate buffered saline (PBS) + 0.5% bovine serum albumin (BSA) + 0.09% or 0.1% sodium azide)
- FIX&PERM® Cell Fixation and Permeabilization kit
- (Optional) BD FACS™ Universal Loader

NOTE Labs must validate any deviations from the following procedures.

Installing the Assay or Template

The BD OneFlow™ Assay Installer, used with BD FACSuite™ Clinical application, or the BD OneFlow™ Assay Template Installer, 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 BD OneFlow™ assay in BD FACSuite™ Clinical application:

NOTE When you select an assay to install, it will overwrite the BD OneFlow™ 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**.
4. To install all of the assays included on the installer, select the **Complete** option and click **Next**.
5. Optional: To install a subset of the assays included on the installer, select the **Custom** option and click **Next**.

   The **Custom Setup** dialog opens.

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

6. Click **Install**.

   The assays will be installed in the Library.

7. Click **Finish**.

   The InstallShield Wizard closes.

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

   The ReadMe file opens.

9. Click the close box when finished reading it.
10. Remove the installer.

To install the OneFlow template in BD FACSDiva™ software:

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

1. Insert the installer and click the installer icon.
2. Follow the instructions in the dialog.

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

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

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

3. Click **OK** to close the dialog.
4. The installer ReadMe file opens. Click the close box when you have finished reading it.
5. Remove the installer.

Setting up the Cytometer

For BD FACSLyric™ flow cytometers:

1. Use BD® CS&T Beads and BD FACSuite™ Clinical 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. In addition, use the BD® FC Beads 2-Color Kit to set up a 12-color instrument.

   See the *BD FACSLyric™ 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.

Staining the Specimen

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

1. If the pouches are stored refrigerated, allow them to reach room temperature before opening them.

   NOTE The reagent is very sensitive to moisture. To avoid condensation, open the pouches only if they are at room temperature.
2. For each patient specimen, remove a BD OneFlow™ ALOT (S) tube from its pouch. Do not remove the BD OneFlow™ ALOT (C) tube from its pouch at this time.
3. Place the tubes in a rack, protected from light.

   Start staining the specimen within one hour of removing a tube from the pouch.
4. Immediately reseal the pouch with any unused tubes.

   NOTE Ensure that 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.

   The reagent is very sensitive to light. Start staining the specimen immediately.
5. Write the patient ID on the 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. Prepare each specimen as described.
   - For PB specimens, invert the collection tube 10 times to mix well.
   - For BM specimens, invert the collection tube 10 times to mix well. Verify that the specimen is not lipemic and does not have particulate matter in it.
     – If needed, pass sufficient specimen through a 40-µm cell strainer.
7. Add 50 µL of wash buffer and 50 µL of unwashed specimen to the BD OneFlow™ ALOT (S) tube.

   NOTE Staining from 5×10^5 to 4×10^7 white blood cells/mL gives equivalent results.
8. Vortex vigorously 3–5 seconds to mix well.
9. Incubate for 30 minutes at 20–25 °C, protected from light.
10. Add 1.5 mL of wash buffer. Vortex vigorously 3–5 seconds to mix well.
11. Add an additional 1.5 mL of wash buffer. Vortex gently to mix.
12. Centrifuge at 540*g* for 5 minutes at 20–25 °C.
13. Remove the supernatant without disturbing the cell pellet, leaving approximately 50 µL of residual liquid in the tube.
14. Vortex vigorously until the cell pellet is completely resuspended.
15. Add 100 µL of FIX&PERM Reagent A (fixation solution) to the tube. Vortex vigorously 3–5 seconds to mix well.
16. Incubate for 15 minutes at 20–25 °C, protected from light.
17. Add 1.5 mL of wash buffer. Vortex vigorously 3–5 seconds to mix well.
18. Add an additional 1.5 mL of wash buffer. Vortex gently to mix.
19. Centrifuge at 540*g* for 5 minutes at 20–25 °C.
20. Remove the supernatant without disturbing the cell pellet, leaving approximately 50 µL of residual liquid in the tube.
21. Vortex vigorously until the cell pellet is completely resuspended.

    NOTE If you are unable to obtain a single-cell suspension, see Troubleshooting.
22. Measure the volume in each tube using a pipet and add wash buffer to give a final volume of 100 µL in each tube. Vortex 3–5 seconds to mix well.

NOTE It is important to have a final volume of 100 µL in each tube so that all of the cells will be completely permeabilized in steps 25–28.

23. Remove the appropriate number of BD OneFlow™ ALOT (C) tubes from the pouch and reseal the pouch immediately.

    NOTE Ensure that 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.

    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 one.

24. Write the patient ID on the BD OneFlow™ ALOT (C) tube label within the area provided.
25. Add 100 µL of FIX&PERM Reagent B (permeabilization solution) to the BD OneFlow™ ALOT (C) tube.
26. Transfer 100 µL of the sample from the BD OneFlow™ ALOT (S) tube to the corresponding BD OneFlow™ ALOT (C) tube.

    NOTE Make sure that the patient ID numbers on the two tubes are the same.

27. Vortex the BD OneFlow™ ALOT (C) tube vigorously 3–5 seconds to mix well.
28. Incubate for 15 minutes at 20–25 °C, protected from light.
29. Add 1.5 mL of wash buffer. Vortex vigorously 3–5 seconds to mix well.
30. Add an additional 1.5 mL of wash buffer. Vortex gently to mix.
31. Centrifuge at 540*g* for 5 minutes at 20–25 °C.
32. Remove the supernatant without disturbing the cell pellet, leaving approximately 50 µL of residual liquid in the tube.
33. Vortex 3–5 seconds to resuspend the cell pellet.
34. Add 250 µL of wash buffer to the tube to give a final volume of 300 µL. Vortex 3–5 seconds to mix well.

    NOTE Samples should be acquired within 60 minutes of staining if kept at room temperature, protected from light.

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 ALOT from the **Product Name** list.

   The **OneFlow ALOT** 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.

   Make sure to add the Lot ID and expiration date for both tubes, BD OneFlow™ ALOT (S) and BD OneFlow™ ALOT (C).

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

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

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 reagent cannot be run on the same worklist.

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

   See the *BD FACSLyric™ 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 4-2H-2V BD default 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** and select 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 ALOT 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 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™ ALOT acquisition worksheet or another BD OneFlow™ ALOT 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™ ALOT (C) tube label.

    NOTE If you cannot scan the barcode on the tube label, see Troubleshooting on page 24.
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 Acute Leukemias 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™ ALOT 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 FACS™ 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™ ALOT Acquisition sheet opens. The acquisition sheet contains dot plots and gates to identify Cells, Leukocytes, and $CD45^{neg/dim}$.

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™ System Instructions For Use* for more information.

To acquire the sample using BD OneFlow™ ALOT assay v1.0:

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 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™ ALOT Acquisition sheet opens. The acquisition sheet contains dot plots and gates to identify Cells, Leukocytes, and $CD45^{neg/dim}$.

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 Acute Leukemias for BD FACSLyric™ Flow Cytometers*.

   See the *BD FACSLyric™ 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™ ALOT 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 an appropriate healthcare professional.

7. Inspect the dot 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 from the Cells population is used to identify leukocytes.

   The CD45 V500-A vs SSC-A dot plot from the Leukocytes population is used to identify the $CD45^{neg/dim}$ population.

   The remaining dot plots do not contain gates and are included to assess staining of the $CD45^{neg/dim}$ population with all of the antibodies, therefore serving as an internal quality control for the tube.

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

8. Continue until all of the tubes have been acquired.
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™ ALOT assay v1.0 can be opened in BD OneFlow™ ALOT assay v1.1.

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

   The BD OneFlow™ ALOT Laboratory Report opens in the **Laboratory Report** tab.

2. Review the BD OneFlow™ ALOT 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.

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 FSC Singlets, SSC Singlets, Leukocytes, and $CD45^{neg/dim}$ cells.

   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 Acute Leukemias 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 analyze the T cells and B cells in the sample.

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

   The dot plots on page 4 of the laboratory report are used to identify and analyze the Non-Lymphoid cells in the sample.

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

   The dot plots on page 5 of the laboratory report are used to identify and analyze the $CD45^{neg/dim}$ cells in the sample.

7. Inspect page 6 of the laboratory report.

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

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

   The BD OneFlow™ ALOT Physician Report contains a high level summary of the assay results.

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

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

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

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

10. Select the **Laboratory Report** tab.
11. Click **E-Sign**.

    The **E-Signature** dialog opens.

12. Select a user ID.
13. Type your password.
14. (Optional) Enter any comments.
15. 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.

16. 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™ 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™ ALOT 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 page 1 of the analysis worksheet identify cells, FSC singlets, and SSC singlets. Debris and doublets are excluded by adjusting the gates.

   Examine the Leukocytes population in the CD45 V500-A vs SSC-A dot plot from the SSC Singlets gate.

   Examine the $CD45^{neg/dim}$ population in the CD45 V500-A vs SSC-A dot plot from the Leukocytes gate.

   The CD34 PerCP-Cy5.5-A vs SSC-A dot plot does not have a gate and is used for informational purposes.

   NOTE See the *BD OneFlow™ Application Guide for Acute Leukemias* for examples of dot plots showing populations of normal cells in the analysis worksheet.
5. Inspect the dot plots identifying T cells on the top half of page 2 of the analysis worksheet, and adjust the gates as needed.

   Cells expressing cytoplasmic CD3 ($cyCD3^+$) are identified in the cyCD3 V450-A vs SSC-A dot plot from the Leukocytes population.

   T cells are identified in the CD3 APC-H7-A vs cyCD3 V450-A dot plot from the $cyCD3^+$ population. The $cyCD3^+$ cells are characterized in the remaining dot plots.
6. Inspect the dot plots identifying B cells on the bottom half of page 2 of the analysis worksheet, and adjust the gates as needed.

   $CD19^+$ cells are identified in the CD19 PE-Cy7-A vs SSC-A dot plot from the Leukocytes population.

   B cells are identified in the cyCD79a PE-A vs CD19 PE-Cy7-A dot plot from the $CD19^+$ population. The $CD19^+$ cells are characterized in the remaining dot plots.
7. Inspect the dot plots identifying non-lymphoid cells on page 3 of the analysis worksheet, and adjust the gates as needed.

   Non-lymphoid cells are identified in the CD45 V500-A vs SSC-A dot plot from the NOT($cyCD3^+$ OR $CD19^+$) population.

   Cells expressing cytoplasmic myeloperoxidase ($cyMPO^+$) are identified in the cyMPO FITC-A vs SSC-A dot plot from the Non-Lymphoid population. The non-lymphoid cells are characterized in the remaining dot plots.
8. Inspect the dot plots identifying $CD45^{neg/dim}$ cells on page 4 of the analysis worksheet, and adjust the gates as needed.

   $CD45^{neg/dim}$ cells are identified in the CD45 V500-A vs SSC-A dot plot from the Leukocytes population.

$CD45^{neg/dim}CD34^{+}$ cells are identified in the CD34 PerCP-Cy5.5-A vs SSC-A dot plot from the $CD45^{neg/dim}$ population.

The remaining dot plots present the expression of each of the markers in combination with CD34 for the $CD45^{neg/dim}$ population.

9. Examine the results in the statistics box on page 5 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 on page 24.

10. 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. Aberrant cell populations will require further analysis.

11. Save the BD OneFlow™ ALOT analysis worksheet as a PDF.

    NOTE The BD OneFlow™ ALOT 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 will not 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.

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

7. RESULTS

Representative Data

See the appropriate application guide to see laboratory reports showing dot plots from a hematologically normal adult sample stained with each reagent.

For BD FACSLyric™ flow cytometers:

- *BD OneFlow™ Application Guide for Acute Leukemias for BD FACSLyric™ Flow Cytometers*

For BD FACSCanto™ II flow cytometers:

- *BD OneFlow™ Application Guide for Acute Leukemias*

8. LIMITATIONS

- 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™ ALOT 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™ ALOT has not been tested on specimens from patients with minimal residual disease (MRD).

9. PERFORMANCE CHARACTERISTICS

Specimen handling and collection (AOB/AOS)

A study was performed to assess the Age of Blood (AOB) and Age of Stain (AOS) using BD OneFlow™ ALOT. The stability of EDTA- or heparin-anticoagulated peripheral blood or bone marrow specimens was evaluated by assessing the combined effect of:

- AOB: time duration between specimen collection and staining
- AOS: time duration between completion of stained sample processing and acquisition with the flow cytometer.

Specimens were tested up to 24 hours post collection and stained samples were tested up to 3 hours post stain. All samples were maintained at room temperature before staining or acquisition.

Based on the results of this study, we recommend staining samples within 24 hours of collection and acquiring samples within 1 hour of staining if the sample is kept at 20–25 °C.

BD FACSLyric™ Flow Cytometer

Method comparison, BD FACSLyric™ vs BD FACSCanto™ II 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 3 clinical sites. The BD OneFlow™ system on BD FACSLyric™ comprises BD® CS&T Beads, BD® FC Beads 7-Color Kit, BD® FC Beads 5-Color Kit, and BD OneFlow™ ALOT 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™ ALOT assay. (A regression study was performed, demonstrating equivalence between BD FACSuite™ Clinical application v1.3 and v1.4.) 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™ ALOT acquired on a BD FACSCanto™ II flow cytometer (4-2H-2V) using BD FACSDiva™ software v8.0.2 and the BD OneFlow™ ALOT template. A total of 26 evaluable PB specimens and 28 evaluable BM specimens were enrolled in the study. Specimens were collected in the anticoagulants shown. See the following table.

Table 5 Anticoagulants used to collect specimens

Specimen type	Anticoagulant	
	EDTA	Heparin
PB (lymphoid)	12	2
PB (non-lymphoid)	10	1
PB (mixed phenotype acute leukemia)	1	0
PB (total)	23	3
BM (lymphoid)	7	0
BM (non-lymphoid)	21	0
BM (total)	28	0

All enrolled specimens were stained within 23 hours of collection. All stained samples were acquired within 33 minutes of final resuspension. Samples with populations of aberrant immature hematopoietic cells were identified as being non-lymphoid or lymphoid using the two systems, and compared. Samples identified as being non-lymphoid included specimens with AUL. Samples identified as being lymphoid included 19 B-lymphoid lineage and 2 T-lymphoid lineage specimens.

Agreement was calculated as follows:

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

Positive % agreement = (a/(a+c))×100

Negative % agreement = (d/(d+b))×100

wherein,

a = number of samples identified as non-lymphoid for both systems,

b = number of samples identified as non-lymphoid on the BD FACSLyric™ flow cytometer but identified as lymphoid on the BD FACSCanto™ II flow cytometer,

c = number of samples identified as lymphoid on the BD FACSLyric™ flow cytometer but identified as non-lymphoid on the BD FACSCanto™ II flow cytometer,

d = number of samples identified as lymphoid for both systems.

The results for samples identified as non-lymphoid or lymphoid were tabulated. One evaluable specimen was excluded from the table because it was a mixed phenotype acute leukemia. See the following table.

Table 6 Agreement for aberrant immature hematopoietic cells being lymphoid or non-lymphoid

		Comparator method (BD FACSCanto™ II flow cytometer)		
		Non-lymphoid	Lymphoid	Total
Investigational method (BD FACSLyric™ flow cytometer)	Non-lymphoid	32	0	32
	Lymphoid	0	21	21
	Total	32	21	53

Overall % agreement is 100%. The lower 95% confidence limit is 95.75%.

The positive agreement for specimens identified as non-lymphoid is 100%. The negative agreement for specimens identified as lymphoid is 100%.

Equivalency (BD FACSLyric™ flow cytometer)

A qualitative assessment of the cell populations for the presence or absence of critical BD OneFlow™ ALOT markers 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 the following table.

Table 7 Agreement for qualitative marker expression on BD FACSLyric™ and BD FACSCanto™ II flow cytometers

Marker	**No. of specimens**	**% Overall agreement for the expression of marker**	**Lower 95% CL of % overall agreement**
$CD45^{neg/dim}$	54	100%	95.83%
$CD45^{neg/dim}$ and $cyCD3^{+}$	54	100%	95.83%
$CD45^{neg/dim}$ and $CD3^{+}$	54	100%	95.83%
$CD45^{neg/dim}$ and $CD19^{+}$	54	100%	95.83%
$CD45^{neg/dim}$ and $cyCD79a^{+}$	54	100%	95.83%
$CD45^{neg/dim}$ and $cyMPO^{+}$	54	100%	95.83%

Marker	No. of specimens	% Overall agreement for the expression of marker	Lower 95% CL of % overall agreement
$CD45^{neg/dim}$ and $CD7^{+}$	54	100%	95.83%
$CD45^{neg/dim}$ and $CD34^{+}$	54	100%	95.83%

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

Method comparison, BD FACS™ Universal Loader vs manual acquisition

A study was performed at one site to demonstrate equivalency between acquisition using the BD FACS™ Universal Loader and manual acquisition. Bone marrow specimens from normal and abnormal subjects were stained using a minimum of 3 lots of BD OneFlow™ ALOT. For each specimen, 10 stained replicates were acquired either manually or using the BD FACS™ Universal Loader (using the 30-tube and 40-tube racks).

The mean, absolute bias, and 95% confidence interval (CI) for acquisition using the BD FACS™ Universal Loader vs manual acquisition were determined for the indicated populations. See the following table.

Table 8 BD FACS™ Universal Loader vs manual acquisition for BD OneFlow™ ALOT

Cell Population	N	Mean		Absolute % bias (95% CI)
		Loader	Manual	
Leukocytes (%SSC singlets)	24	99.98	99.97	0.01 (–0.01, 0.03)
$CD19^{+}$ cells (%Leukocytes)	24	15.10	15.00	0.11 (–0.02, 0.23)
B cells (%$CD19^{+}$)	24	77.68	77.06	0.63 (–0.24, 1.49)
$cyCD3^{+}$ cells (%Leukocytes)	24	13.91	13.72	0.20 (–0.05, 0.45)
T cells (%$cyCD3^{+}$)	24	93.42	92.59	0.83 (0.19, 1.48)
$cyMPO^{+}$ cells (%Non-lymphoid)	24	78.46	77.72	0.75 (0.12, 1.37)
$CD34^{+}$ cells (%Leukocytes)	24	6.71	6.67	0.04 (–0.06, 0.14)

The results were also assessed qualitatively by a flow cytometry expert for concordance between samples acquired manually and samples acquired using the Loader. All samples stained with BD OneFlow™ ALOT showed 100% agreement.

Precision, within-site, control material (BD FACSLyric™ flow cytometer)

A 21-day single-site precision study was performed to assess the within-site precision (repeatability and reproducibility) of BD OneFlow™ ALOT using control material. Estimates of precision were determined across three BD FACSLyric™ flow cytometers and a minimum of three operators by acquiring CD-Chex CD34®, stained in duplicate by each operator using three lots of BD OneFlow™ ALOT. Two separate runs were performed by each operator on each of the 21 tested days.

Eight cell populations were identified as being a percentage of the parent population. Repeatability (within-run variability) and reproducibility (variability between runs, between days, between operators, between lots, and between instruments) were estimated. A total of 828 samples were analyzed. The mean, standard deviation (SD), and coefficient of variation (%CV) is presented for each population. In addition, the one-sided 95% confidence limit (Upper SD or Upper %CV) for the total system precision was calculated.

Table 9 Summary of within-site precision of subset percentages for BD OneFlow™ ALOT

Population (% of positive)	Mean	Repeatability		Reproducibility		Total precision	
		SD	%CV	SD	%CV	Upper SD	Upper %CV
$CD45^+$	34.73	1.11	3.20	2.12	0.06	2.83	8.16
$CD19^+$	4.55	0.09	2.08	0.33	0.07	0.36	7.90
$cyCD3^+$	23.71	0.66	2.77	0.93	0.04	1.42	5.98
$CD3^+$	96.48	1.60	1.66	6.96	0.07	7.67	7.95
$CD7^+$	89.29	0.75	0.84	6.40	0.07	6.92	7.76
$cyCD79a^+$	95.11	2.15	2.26	1.63	0.02	2.83	2.98
$CD34^+$	1.57	0.05	3.34	0.16	0.10	0.22	13.87
$cyMPO^+$	94.17	3.24	3.44	4.75	0.05	6.07	6.45

Precision, multi-site, control material (BD FACSLyric™ flow cytometer)

Multi-site precision was evaluated across three sites using one lot of BD OneFlow™ ALOT to stain three replicates of CD-Chex CD34®. Two runs per day were acquired on a BD FACSLyric™ flow cytometer by one operator per site over a period of 5 days.

Twelve cell populations were identified as being a percentage of the parent population. Repeatability (within-run variability) and reproducibility (variability between runs, between days, and between sites) were estimated. The mean, standard deviation (SD), and coefficient of variation (%CV) is presented for each population. A total of 90 samples were analyzed. In addition, the one-sided 95% confidence limit (Upper SD or Upper %CV) for the total system precision was calculated.

Table 10 Summary of multi-site precision of subset percentages for BD OneFlow™ ALOT

Population (% of positive)	Mean	Repeatability		Reproducibility		Total precision	
		SD	%CV	SD	%CV	Upper SD	Upper %CV
$CD45^+$	35.97	0.64	1.77	0.49	0.01	0.97	2.70
$CD19^+$	4.00	0.07	1.80	0.04	0.01	0.10	2.43

Population (% of positive)	Mean	Repeatability		Reproducibility		Total precision	
		SD	%CV	SD	%CV	Upper SD	Upper %CV
cyCD3$^+$	27.08	1.29	4.75	0.85	0.03	1.97	7.27
CD3$^+$	93.22	2.03	2.17	2.44	0.03	5.51	5.91
CD7$^+$	86.78	2.23	2.57	1.48	0.02	3.42	3.94
cyCD79a$^+$	97.19	0.64	0.66	0.58	0.01	1.10	1.13
CD34$^+$	1.64	0.04	2.52	0.09	0.05	0.23	13.86
cyMPO$^+$	93.49	0.81	0.87	0.89	0.01	1.63	1.74

Detection capability, limit of detection (BD FACSLyric™ flow cytometer)

The detection capability of BD OneFlow™ ALOT was assessed. An abnormal specimen was added to a hematologically normal bone marrow specimen such that the abnormal population was at 0%, 0.02%, 0.05%, or 0.1% of all events of the normal specimen. Two sets of abnormal and normal specimens and 2 lots of BD OneFlow™ ALOT were evaluated in the study.

Table 11 Detection capability of BD OneFlow™ ALOT

Target population	Expected % abnormal cells	N[a]	Number disease samples	Mean actual % abnormal cells (95% CI[b])	SD of bias (Upper 95% CI)	Trueness (95% lower bound)
CD45$^{neg/dim}$ CD34$^+$ cyMPO$^+$	0.000	20	0	0.000 (0.000, 0.001)	N/A[c]	N/A
	0.020	20	20	0.017 (0.016, 0.018)	0.0023 (0.0043)	100 (88.1)
	0.050	20	20	0.044 (0.043, 0.046)	0.0032 (0.0059)	100 (88.1)
	0.100	20	20	0.092 (0.090, 0.094)	0.0038 (0.0071)	100 (88.1)

a. N = Number of replicates
b. CI = Confidence interval
c. N/A = Not applicable

Based on the results, a detection capability of 0.03% of all events is recommended for BD OneFlow™ ALOT.

BD FACSCanto™ II Flow Cytometer

Method comparison, BD FACSCanto™ II flow cytometer vs EuroFlow system

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 5 external clinical sites. The BD OneFlow™ system comprises BD OneFlow™ Setup Beads, BD® FC Beads for compensation, and the BD OneFlow™ ALOT reagent. The EuroFlow reference system comprises Sphero™ Rainbow calibration particles (8 peaks), single color stained cells for compensation, and the corresponding

EuroFlow reagent cocktails. Both methods used BD FACSDiva™ CS&T IVD beads to perform instrument quality control. Aberrant immature hematopoietic cell populations from 93 patients were identified using the two systems, and compared. A total of 37 PB specimens and 56 BM specimens were enrolled in the study. PB and BM specimens were stained within 24 hours of collection. The stained samples were acquired within 45 minutes of staining. Samples with populations of immature hematopoietic cells were identified as being non-lymphoid or lymphoid. Samples identified as being non-lymphoid included specimens with AUL. Samples identified as being lymphoid included 37 B-lymphoid lineage and 7 T-lymphoid lineage specimens.

Agreement was calculated as follows:

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

wherein,

a = number of samples identified as non-lymphoid for both systems,

b = number of samples identified as non-lymphoid for the BD OneFlow™ system but identified as lymphoid for the EuroFlow system,

c = number of samples identified as lymphoid for the BD OneFlow™ system but identified as non-lymphoid for the Euroflow system, and

d = number of samples identified as lymphoid for both systems.

The results for samples identified as non-lymphoid or lymphoid were tabulated. See the following table.

Table 12 Agreement for aberrant immature hematopoietic cells being lymphoid or non-lymphoid

		Comparator method (EuroFlow system)		
		Non-lymphoid	Lymphoid	Total
Investigational method (BD OneFlow™ system)	Non-lymphoid	49	0	49
	Lymphoid	0	44	44
	Total	49	44	93

Overall % agreement is 100%. The lower 95% confidence limit is 96.8%.

Equivalency (BD FACSCanto™ II flow cytometer)

A qualitative assessment of the cell populations for the presence or absence of critical BD OneFlow™ ALOT markers was performed for each specimen. Specimens were analyzed using the BD OneFlow™ system and the corresponding EuroFlow system 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 the following table.

Table 13 Equivalency of the BD OneFlow™ system to the EuroFlow system

Marker	**% Overall agreement for the expression of marker**	**Lower 95% CL of % overall agreement**
$CD45^{neg/dim}$	100%	96.8%
$CD45^{neg/dim}$ and cyCD3	100%	96.8%
$CD45^{neg/dim}$ and CD3	100%	96.8%
$CD45^{neg/dim}$ and CD19	100%	96.8%

Marker	% Overall agreement for the expression of marker	Lower 95% CL of % overall agreement
$CD45^{neg/dim}$ and cyCD79a	100%	96.8%
$CD45^{neg/dim}$ and cyMPO	100%	96.8%

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

Precision (BD FACSCanto™ II flow cytometer)

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

Two operators each performed two separate runs per day on either of two BD FACSCanto™ II flow cytometers. The operators switched to the other instrument each day over a period of eight days. For each run, duplicate samples of CD-Chex CD34® were stained using three lots of BD OneFlow™ ALOT by each operator, and then acquired and analyzed using the BD OneFlow™ ALOT template in BD FACSDiva™ software.

Reproducibility

Three cell populations were identified as being a percentage of the cell populations indicated in the table. 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 14 Reproducibility of subset percentages

Population	Mean	SD[a]	Upper 95% CL[b] of SD	%CV[c]	Upper 95% CL of %CV
T cells (% leukocytes)	29.0	0.2	0.6	0.7	1.9
B cells (% leukocytes)	5.7	0.1	0.6	2.3	10.0
$cyMPO^+$ cells (% leukocytes)	54.8	1.1	4.8	2.0	8.8

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

Repeatability

Three cell populations were identified as being a percentage of the cell populations indicated in the table. The within-run precision (tube-to-tube repeatability) of the subset percentages was calculated for each cell population.

Table 15 Repeatability of subset percentages

Population	Mean	SD	Upper 95% CL of SD	%CV	Upper 95% CL of %CV
T cells (% leukocytes)	29.0	0.5	0.5	1.7	1.9
B cells (% leukocytes)	5.7	0.1	0.2	2.5	2.8

Population	Mean	SD	Upper 95% CL of SD	%CV	Upper 95% CL of %CV
cyMPO$^+$ cells (% leukocytes)	54.8	0.8	0.9	1.4	1.6

10. TROUBLESHOOTING

Problems with cell preparation or staining

Problem	Possible Cause	Solution
The resolution between debris and cells is poor.	Specimen was over-permeabilized.	Repeat staining. Incubate the tube in FIX&PERM Reagent B for 15 minutes only.
	Specimen is of poor quality.	Check cell viability.
	Specimen is too old.	Obtain a new specimen and stain it immediately.
The cytoplasmic staining (cyMPO, cyCD79a, cyCD3) is dim.	The cells were not completely permeabilized.	Repeat staining. Carefully measure the specimen volumes in the cell fixation and permeabilization steps such that the ratio of fixed sample to FIX&PERM Reagent B is 1:1.
Cells clump after being fixed.	Cells were not completely resuspended before fixing them.	Vortex tubes until no cell aggregates remain before adding FIX&PERM Reagent A. If needed, gently pipette the sample up and down until no cell aggregates remain.
	Cells were not thoroughly washed after fixing them.	Incubate the tubes for 2 minutes in the dark in wash buffer after they have been fixed using FIX&PERM Reagent A.
Staining is dim or fading.	Cell concentration was too high at the staining step.	Check the cell concentration and adjust as needed.
	The BD OneFlow™ tube was exposed to light for too long.	Repeat staining with a new BD OneFlow™ tube.
	Stained cells were stored too long before acquiring them.	Repeat staining with a fresh specimen and acquire it promptly.
Few or no cells are recorded.	Cell concentration was too low.	Resuspend fresh specimen at a higher concentration. Repeat staining and acquisition.
	Cytometer is malfunctioning.	Troubleshoot the instrument. See the cytometer instructions for use for more information.

Problems using the reagent on BD FACSLyric™ flow cytometers:

Problem	Possible Cause	Solution
Not enough cells of interest are acquired.	Cell concentration was too low.	Resuspend fresh specimen at a higher concentration. Repeat staining and acquisition.
	The default setting of 100,000 events acquired is too low.	Change the number of events acquired. Repeat staining and acquisition. See the *BD OneFlow™ Application Guide for Acute Leukemias for BD FACSLyric™ Flow Cytometers.*
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.	1. Add the reagent lot number and expiration date to the Library. 2. Export the csv file and the report PDF manually. See the *BD FACSLyric™ System Instructions For Use*.

Problems using the reagent on BD FACSCanto™ II flow cytometers:

Problem	Possible Cause	Solution
The resolution between debris and lymphocytes is poor.	Instrument settings are inappropriate.	Follow proper instrument setup procedures. See the *Instrument Setup Guide for BD OneFlow™ Assays*.
Some of the dot plots are dimmed.	FSC-H and SSC-H were not selected when the application settings were created.	Check that FSC-H and SSC-H are selected on the **Parameters** tab of the **Inspector**.
The barcode on the BD OneFlow™ ALOT (C) tube label cannot be scanned.	The barcode on the tube label has been compromised.	Scan the barcode on the BD OneFlow™ ALOT (C) 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.	1. Close the current experiment. 2. Create a new experiment. 3. Re-import the BD OneFlow™ ALOT template.

Problem	Possible Cause	Solution
Some of the keywords are missing from the statistics box in the analysis worksheet.	BD FACSDiva™ software did not import all of the keywords into the panel template.	1. In the **Browser**, set the current tube pointer to the tube that you are analyzing. 2. Navigate to the analysis worksheet. 3. Right-click the statistics box and select **Edit Stats View**. 4. In the **Header** tab, select the **All** checkbox. 5. Click **OK**.
The statement, **For in vitro diagnostic use**, does not appear in the footer of the analysis worksheet when it is printed.	The paper margins in the printer settings were changed.	1. From the BD FACSDiva™ software menu bar, select **File** > **Page Setup**. 2. Ensure that all of the margins are set to 2.54 cm or 1 inch, depending on your default standards. 3. Click **OK**.

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. Greaves MF, Titley I, Colman SM, et al. CD34 cluster workshop report. In: Schlossman SF, Boumsell L, Gilks W, et al, eds. *Leucocyte Typing V: White Cell Differentiation Antigens.* Vol 1. New York, NY: Oxford University Press; 1995;840-846.
3. 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.
4. 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.
5. Reiter C. Cluster report: CD7. In: Knapp W, Dörken B, Gilks WR, et al, eds. *Leucocyte Typing IV: White Cell Differentiation Antigens*. New York, NY: Oxford University Press; 1989:341-342.
6. Haynes BF. Summary of T-cell studies performed during the Second International Workshop and Conference on Human Leukocyte Differentiation Antigens. In: Reinherz EL, Haynes BF, Nadler LM, Bernstein ID, eds. *Leukocyte Typing II: Human T Lymphocytes.* Vol 1. New York, NY: Springer-Verlag; 1986:3-30.
7. Knowles RW. Immunochemical analysis of the T-cell-specific antigens. In: Reinherz EL, Haynes BF, Nadler LM, Bernstein ID, eds. *Leukocyte Typing II: Human T Lymphocytes.* Vol 1. New York, NY: Springer-Verlag; 1986:259-288.
8. 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.
9. 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.
10. Pulford KA, Erber WN, Crick JA, et al. Use of monoclonal antibody against human neutrophil elastase in normal and leukaemic myeloid cells. *J Clin Pathol*. 1988;41:853-860.
11. 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; 1995:667-679.

12. McMichael AJ, Gotch FM. T-cell antigens: new and previously defined clusters. In: McMichael AJ, Beverley PCL, Cobbold S, et al, eds. *Leucocyte Typing III: White Cell Differentiation Antigens*. New York, NY: Oxford University Press; 1987:31-62.
13. *Collection of Diagnostic Venous Blood Specimens*, *7th ed.* Wayne, PA: Clinical and Laboratory Standards Institute; 2017. CLSI document GP41.
14. Rothe G, Schmitz G. Consensus protocol for the flow cytometric immunophenotyping of hematopoietic malignancies. *Leukemia*. 1996;10:877-895.
15. 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.
16. *Clinical Flow Cytometric Analysis of Neoplastic Hematolymphoid Cells; Approved Guideline—Second Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2007. CLSI document H43-A2.
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 and Prevention. 2007 Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Healthcare Settings. https://www.cdc.gov/infectioncontrol/guidelines/isolation/index.html. Accessed March 12, 2019.
19. *Interference Testing in Clinical Chemistry; Approved Guideline—Third Edition.* Wayne, PA: Clinical and Laboratory Standards Institute; 2018. CLSI document EP07-A3.

NOTICE

EU Only: Users shall report any serious incident related to the device to the Manufacturer and National Competent Authority.

Outside EU: Contact your local BD representative for any incident or inquiry related to this device.

Refer to the Eudamed website: https://ec.europa.eu/tools/eudamed for Summary of Safety and Performance.

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.

PATENTS AND TRADEMARKS

For US patents that may apply, see bd.com/patents.

McCarthy Boulevard, Milpitas, California 95035, USA, and any money paid for the material will be refunded.

HISTORY

Revision	Date	Changes made
23-18878-01	2020-05	Revised to support using the product on BD FACSLyric™ flow cytometers.
23-18878-02	2021-01	Revised to support using the product with the BD FACS™ Universal Loader.
23-18878(03)	2023-01	Updated to meet requirements for Regulation (EU) 2017/746.
23-18878(04)	2023-10	Updated legal manufacturer address. Added EU and Swiss importer addresses and importer symbol. Updated precautionary statements to align with SDS. Updated symbols glossary.

SYMBOLS GLOSSARY

Please refer to product labeling for applicable symbols.

Symbol	Meaning
	Manufacturer
	Authorized representative in the European Community
	Authorised representative in Switzerland
	Date of manufacture
	Use-by date
	Batch code
	Catalogue number
	Serial number
	Sterile
	Sterilized using aseptic processing techniques
	Sterilized using ethylene oxide
	Sterilized using irradiation
	Sterilized using steam or dry heat
	Do not resterilize
	Non-sterile
	Do not use if package is damaged and consult *instructions for use*
	Sterile fluid path
	Sterile fluid path (ethylene oxide)
	Sterile fluid path (irradiation)
	Fragile, handle with care
	Keep away from sunlight
	Keep dry
	Lower limit of temperature
	Upper limit of temperature
	Temperature limit
	Humidity limitation
	Biological risks
	Do not re-use
	Consult *instructions for use* or consult electronic *instructions for use*
	Caution
	Contains or presence of natural rubber latex
	In vitro diagnostic medical device
	Negative control
	Positive control
	Contains sufficient for <*n*> tests
	For IVD performance evaluation only
	Non-pyrogenic
	Patient number
	This way up
	Do not stack

Symbol	Meaning
	Single sterile barrier system
	Contains or presence of phthalate: combination of bis(2-ethylhexyl) phthalate (DEHP) and benzyl butyl phthalate (BBP)
	Collect separately Indicates separate collection for waste of electrical and electronic equipment required.
	CE marking; Signifies European technical conformity
	Device for near-patient testing
	Device for self-testing
	This only applies to US: "Caution: Federal Law restricts this device to sale by or on the order of a licensed practitioner."
	Country of manufacture "CC" shall be replaced by either the two letter or the three letter country code.
	Collection time
	Cut
	Peel here
	Collection date
	Keep away from light
	Hydrogen gas is generated
	Perforation
	Start panel sequence number
	End panel sequence number
	Internal sequence number
	<Box #> / <Total Boxes>
	Medical device
	Contains hazardous substances
	Ukrainian conformity mark
	Meets FCC requirements per 21 CFR Part 15
	UL product certification for US and Canada
	Unique device identifier
	Importer
	Place patient label in framed area only
	Magnetic resonance (MR) safe
	Magnetic resonance (MR) conditional
	Magnetic resonance (MR) unsafe
This Product Contains Dry Natural Rubber	This Product Contains Dry Natural Rubber
For Export Only	For Export Only

Note: Text layout in symbols is determined by label design.

L006715(09) 2023-08

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