

BD OneFlow™ Plasma Cell Disorders Panel

BD OneFlow™ PCST—Catalog No. 659912—10 Tests

BD OneFlow™ PCD—Catalog No. 659913—10 Tests

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English



1. INTENDED USE

The BD OneFlow™ Plasma Cell Disorders Panel is intended for in vitro diagnostic use for qualitative flow-cytometric immunophenotyping of plasma cell populations 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

The BD OneFlow™ Plasma Cell Disorders Panel is used as an aid in the differential diagnosis of hematologically abnormal patients having, or suspected of having, plasma cell disorders. The BD OneFlow™ Plasma Cell Disorders Panel can be used with bone marrow specimens collected in EDTA or heparin tubes. The results should be interpreted by a pathologist or equivalent professional in conjunction with other clinical or laboratory findings.

The BD OneFlow™ Plasma Cell Disorders Panel comprises the following BD OneFlow™ tubes:

- BD OneFlow™ PCST (Plasma Cell Screening Tube)
- BD OneFlow™ PCD (Plasma Cell Disorders Tube)

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

	FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	APC-H7	V450 ^a	V500-C ^a
PCST	CD38	CD56	β2-microglobulin	CD19	cyIgκ ^b	cyIgλ	CD45	CD138
PCD	CD38	CD28	CD27	CD19	CD117	CD81	CD45	CD138

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

b. cy refers to cytoplasmic

2. SUMMARY OF THE TEST

Plasma cell disorders (PCD) are a group of diseases most often characterized as having a clonal (neoplastic) population of plasma cells in the bone marrow (BM).¹ The cells may secrete a clonal immunoglobulin which can be detected in the circulation. These disorders comprise several distinct diseases, including multiple myeloma and monoclonal gammopathy of undetermined significance. The multicolor antibody panels are used to characterize the cell populations in a patient specimen using immunophenotypic markers that are indicative of normal and abnormal cells.

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 the BD OneFlow™ Plasma Cell Disorders Panel 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

The BD OneFlow™ Plasma Cell Disorders Panel is composed of three single-use tubes containing the following fluorochrome-conjugated antibodies in an optimized dried formulation. BD OneFlow™ PCST is composed of two reagent tubes: BD OneFlow™ PCST (S) contains antibodies that recognize cell surface antigens, and BD OneFlow™ PCST (C) contains antibodies that recognize cytoplasmic antigens. BD OneFlow™ PCD is composed of one reagent tube which contains antibodies that recognize cell surface antigens. All of the antibodies have IgG₁ heavy chains and kappa light chains with the exception of β2-Microglobulin, which has IgM heavy chains. The antibody clone names are shown in the following table in the row beneath the antibody.

Table 1 Antibody composition of BD OneFlow™ PCST and BD OneFlow™ PCD

Reagent	FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	APC-H7	V450	V500-C
PCST (S)	CD38 (HB7) ²	CD56 (MY31) ^{3,4}	β2-Micro- globulin (TÜ99) ⁵	CD19 (SJ25- C1) ^{2,6}	–	–	CD45 (2D1) ^{7,8}	CD138 (MI15) _{9,10}
PCST (C)	–	–	–	–	Anti- Kappa (TB28-2) ¹¹	Anti- Lambda (1-155-2) ¹¹	–	–
PCD	CD38 (HB7)	CD28 (L293) ¹²	CD27 (L128) ¹³	CD19 (SJ25-C1)	CD117 (104D2) ¹⁴	CD81 (JS81) ¹⁵	CD45 (2D1)	CD138 (MI15)


The antibodies in the reagent tubes were chosen for their ability to identify and characterize plasma cells.

Refer to the article describing the EuroFlow antibody panels¹ for a full description of the utility of the antibodies chosen for the reagent tubes.

Precautions

- The reagent tubes contain 0.66% 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.
	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 six months 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 FACSLytic™ ^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

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 the 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

The BD OneFlow™ Plasma Cell Disorders Panel can be used for immunophenotyping by flow cytometry of bone marrow (BM) aspirates.

- 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.^{16,17}
- 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.¹⁸

- 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^{19,20} and dispose of with proper precautions in accordance with federal, state, and local regulations. Never pipette by mouth. Wear suitable protective clothing, eyewear, and gloves.

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.

6. PROCEDURE

Reagents and Materials

Reagents and materials provided

The reagent tubes are provided as single-use tubes in foil pouches.

BD OneFlow™ PCST:

- Two pouches, each containing five tubes of BD OneFlow™ PCST (S)
- Two pouches, each containing five tubes of BD OneFlow™ PCST (C)

BD OneFlow™ PCD:

- Two pouches, each containing five tubes of BD OneFlow™ PCD

Reagents and materials required but not provided

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

An installer is required to use the BD OneFlow™ reagents with BD FACSuite™ Clinical application. The assays installer includes an acquisition sheet, a laboratory report, a physician report, and a supplemental report used for sample analysis and results reporting. Unless you already have the current BD OneFlow™ Assays Installer, you will have to order the installer the first time you order the reagent.

The *BD OneFlow™ Application Guide for Plasma Cell Disorders for BD FACSLyric™ Flow Cytometers* is provided with the installer.

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

An installer is required to use the BD OneFlow™ reagents with BD FACSDiva™ software. The templates installer includes two global worksheets: the BD OneFlow™ Acquisition worksheet and the BD OneFlow™ Analysis worksheet. Unless you already have the current BD OneFlow™ Assay Templates Installer, you will have to order the installer the first time you order the reagent. The installer also contains the OneFlow Setup template.

The *Instrument Setup Guide for BD OneFlow™ Assays* and the *BD OneFlow™ Application Guide for Plasma Cell Disorders* are provided with the installer.

- 15-mL conical polypropylene tubes
- 40-µm cell strainer, if needed
- Pasteur pipet
- Serological pipet
- Micropipettor with tips
- Vortex mixer
- Centrifuge
- Wash buffer (filtered PBS + 0.5% 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 the 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**.

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

The **Custom Setup** dialog opens.

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

The assays will be installed in the Library.

- Click **Finish**.

The InstallShield Wizard closes.

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

The ReadMe file opens.

- Click the close box when finished reading it.

- Remove the installer.

To install the OneFlow template in BD FACSDiva™ software:

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

- Insert the installer and click the installer icon.
- Follow the instructions in the dialog.

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

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

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

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

Setting up the Cytometer

For BD FACSLyric™ flow cytometers:

- 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.
- 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:

- 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.
- 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.
- 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.

Processing the Specimen

Washing the specimen

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

1. For each tube to be stained, label a 15-mL conical tube with the specimen ID.
2. Invert the specimen in the collection tube 10 times to mix well. Verify that the specimen is not lipemic and does not have particulate matter in it.
3. If needed, pass sufficient specimen through a 40- μ m cell strainer.
4. Add 300 μ L of the specimen to the labeled conical tube.
5. Add 10 mL of wash buffer (filtered PBS + 0.5% BSA + 0.09% or 0.1% sodium azide).
6. Invert the tube 3–5 times to mix well.
7. Centrifuge at 540g for 5 minutes at 20–25 °C.
8. Remove the supernatant without disturbing the cell pellet.
NOTE If the cell pellet is disturbed, centrifuge the tube again and then remove the supernatant without disturbing the cell pellet.
9. Vortex the tube until no cell aggregates remain in the residual wash buffer.
10. Repeat steps 5–9 twice for a total of three washes.
11. Resuspend the cell pellet in wash buffer to give a final volume of 300 μ L.
12. Vortex vigorously 3–5 seconds to completely resuspend the cell pellet.

NOTE Start staining the specimen within 30 minutes of the last wash. Store the washed specimen at 20–25 °C until you stain it.

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™ PCST (S) tube and a BD OneFlow™ PCD tube from its pouch and reseal the pouch immediately. Do not remove the BD OneFlow™ PCST (C) tube 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 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. Use the tubes within six months of opening the pouch.
5. Write the patient ID on the tube label within the area provided.
6. Vortex washed specimen 3–5 seconds to mix well.
7. Add 50 μ L of wash buffer and 50 μ L of washed specimen to each tube. Vortex vigorously 3–5 seconds to mix well.
NOTE Staining from 5×10^5 to 8×10^7 white blood cells/mL gives equivalent results.
8. Incubate for 30–35 minutes at 20–25 °C in the dark.
9. Add 1.5 mL of wash buffer. Vortex vigorously 3–5 seconds to mix well.
10. Add an additional 1.5 mL of wash buffer. Vortex gently to mix.
11. Centrifuge at 540g for 5 minutes at 20–25 °C.
12. Remove the supernatant without disturbing the cell pellet, leaving approximately 50 μ L of residual liquid in the tube.
13. Vortex vigorously until the cell pellet is completely resuspended.

14. Add 100 μ L of FIX&PERM[®] Reagent A (fixation solution) to each tube. Vortex vigorously 3–5 seconds to mix well.
NOTE The BD OneFlow[™] PCD tube does not stain intracellular markers. However, it is critical to perform the fixation and permeabilization steps to allow a direct comparison of the staining results with those obtained using the BD OneFlow[™] PCST tube.
15. Incubate for 15 minutes at 20–25 °C in the dark.
16. Add 1.5 mL of wash buffer. Vortex vigorously 3–5 seconds to mix well.
17. Add an additional 1.5 mL of wash buffer. Vortex gently to mix.
18. Centrifuge at 540g for 5 minutes at 20–25 °C.
19. Remove the supernatant without disturbing the cell pellet, leaving approximately 50 μ L of residual liquid in the tube.
20. Vortex vigorously until the cell pellet is completely resuspended.
NOTE If you are unable to obtain a single-cell suspension, see Troubleshooting.
21. Measure the volume in each tube using a pipet and add wash buffer to give a final volume of 100 μ L in each tube. Vortex 3–5 seconds to mix well.
NOTE It is important to have a final volume of 100 μ L in each tube so that all of the cells will be completely permeabilized in steps 24–27.
22. Remove the appropriate number of BD OneFlow[™] PCST (C) tubes from the pouch and reseal the pouch immediately.
CAUTION 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. Use the tubes within six months of opening the pouch.
23. Write the patient ID on the BD OneFlow[™] PCST (C) tube label within the area provided.
24. Add 100 μ L of FIX&PERM[®] Reagent B (permeabilization solution) to the BD OneFlow[™] PCST (C) tube and to the BD OneFlow[™] PCD tube.
25. Transfer 100 μ L of the sample from the BD OneFlow[™] PCST (S) tube to the corresponding BD OneFlow[™] PCST (C) tube.
NOTE Make sure that the patient ID numbers on the two tubes are the same.
26. Vortex the tubes vigorously 3–5 seconds to mix well.
27. Incubate for 15 minutes at 20–25 °C in the dark.
28. Add 1.5 mL of wash buffer. Vortex vigorously 3–5 seconds to mix well.
29. Add an additional 1.5 mL of wash buffer. Vortex gently to mix.
30. Centrifuge at 540g for 5 minutes at 20–25 °C.
31. Remove the supernatant without disturbing the cell pellet, leaving approximately 50 μ L of residual liquid in the tube.
32. Vortex 3–5 seconds to resuspend the cell pellet.
33. Add 250 μ L of wash buffer to the tube to give a final volume of 300 μ L. 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 the appropriate BD OneFlow[™] reagent from the **Product Name** list.
The **OneFlow reagent** pane opens at the bottom of the page.
4. Click **Add Lot**.
The **Add New Lot** dialog opens.

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

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

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

6. Select the **Current Lot** checkbox.
7. Click **OK**.

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

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

To create a worklist:

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

The Worklists workspace opens.

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

A blank worklist opens in a new tab.

3. In the **Worklist Entries** section, select the appropriate task from the **Task** menu.
4. Enter the **Sample ID** for BD OneFlow™ reagent tasks.

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

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

5. In the **Loading Options** section, select **Manual** or **Universal Loader** from the **Loading Option** menu.
6. If using the Loader, select **30 Tube Rack** or **40 Tube Rack** from the **Carrier Type** menu.

NOTE Confirm that the order of samples in the rack matches the order entered in the software when setting up the Loader.

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 default 4-2H-2V configuration.
3. From the menu bar, select **Experiment > New Experiment > Blank Experiment**. Click **OK**.

NOTE You can also create an experiment directly from the **Browser** using the **Experiment** icon.

4. If prompted by the **CST Mismatch** window, select **Use CST Settings**.
5. Rename the experiment according to your laboratory practice.
6. In the **Browser**, right-click **Cytometer Settings > Link Setup** and select the appropriate compensation matrix calculated using BD® FC Beads within the past 31 days. Click **Link**.

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

7. If prompted by the **Cytometer Settings Mismatch** window, select **Overwrite**.
 8. Right-click **Cytometer Settings > Unlink From** 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 appropriate OneFlow template.
14. Indicate the number of patient specimens you want to acquire using the **Copies** field near the bottom of the **BD Panels** tab. Click **OK**.
15. Rename each specimen, for example, with the appropriate patient ID in front of the specimen name.
NOTE If you have to re-run a particular patient sample, set the current tube pointer to the tube you wish to re-run. Click **Next Tube** in the **Acquisition Dashboard** to create another tube for that patient. Do not select **Experiment > New Tube** from the menu bar or use the **New Tube** icon from the **Browser** menu bar to create the additional tube to be acquired because the labels and barcode fields will not be populated.
NOTE If you want to acquire additional patient samples stained with the BD OneFlow™ reagent in the experiment, repeat steps 12–15 to add new specimens. Two **Confirm** dialogs will open asking if you want to create another acquisition worksheet or another analysis worksheet. Click **Cancel** in each dialog.
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™ tube label.
NOTE If you cannot scan the barcode on the tube label, see Troubleshooting.
18. Manually add the appropriate information to the remaining keywords, as needed.
19. Click **OK** to close the **Experiment Layout**.

Acquiring the Stained Sample

For BD FACSLyric™ flow cytometers:

Two versions of the assay are available:

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

The assay will automatically collect 100,000 total events. You cannot append the number of events to collect after acquisition has started. Therefore, if needed, change the number of events to collect before you start acquisition. To change the number of events to collect, see the *BD OneFlow™ Application Guide for Plasma Cell Disorders 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 the BD OneFlow™ 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™ Acquisition sheet opens. The acquisition sheet contains dot plots and gates to identify Leukocytes, Lymphocytes, and the relevant cell populations for the assay.

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 the BD OneFlow™ 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 stained tube 3–5 seconds at low speed immediately prior to acquisition.
3. Follow the prompts in the software to load or unload tubes.

The BD OneFlow™ Acquisition sheet opens. The acquisition sheet contains dot plots and gates to identify Leukocytes, Lymphocytes, and the relevant cell populations for the assay.

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 Plasma Cell Disorders 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 appropriate **BD OneFlow™ Acquisition** worksheet tab.
3. Vortex the stained tube 3–5 seconds at low speed.
4. Install the tube on the cytometer. Adjust the flow rate to **Medium** in the **Acquisition Dashboard**. Click **Acquire Data**.
5. Verify that the population is on scale and adjust the gate in the first plot of the acquisition worksheet to exclude debris, if needed.
6. Click **Record Data** in the **Acquisition Dashboard** to collect total events.

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

7. Inspect the dot plots on the 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 remaining dot plots either contain gates to identify the relevant cell populations for the assay, or 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 appropriate *BD OneFlow™ Application Guide* for examples of the dot plots showing populations of normal cells in the acquisition worksheet.

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

Analyzing the Data Using BD FACSuite™ Clinical Application

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

To analyze a sample stained using BD OneFlow™ PCST:

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

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

2. Review the BD OneFlow™ PCST 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 Cells, FSC Singlets, SSC Singlets, and B 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. Use the CD45 V500-C-A vs SSC-A density plot when adjusting the Leukocytes and Lymphocytes gates.

See the *BD OneFlow™ Application Guide for Plasma Cell Disorders for BD FACSLytic™ Flow Cytometers* for examples of dot plots showing populations of normal cells.

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

The dot plots on page 3 of the laboratory report are used to analyze the plasma cells in the sample. Dot plots and gates are provided to identify CD38⁺ cells, Plasma cells, cyIgL⁺, and cyIgK⁺ cells. The remaining dot plots characterize the cyIgL⁺ and cyIgK⁺ populations.

5. Inspect page 4 of the laboratory report.

Page 4 of the laboratory report includes lot and expiration dates for BD[®] CS&T Beads and the BD OneFlow™ reagent, reference settings, tube settings, and cytometer configuration.

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

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

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- (Optional) Select the **Supplemental Report** tab to add additional dot plots to further analyze the sample. See the *BD OneFlow™ Application Guide for Plasma Cell Disorders 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.

- Select the Laboratory Report tab.
- Click **E-sign**.

The **E-Signature** dialog opens.

- Select a user ID.
- Type your password.
- (Optional) Enter any comments.
- Click **Sign**.

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

- 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.

To analyze a sample stained using BD OneFlow™ PCD:

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

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

- Review the BD OneFlow™ PCD 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.

- 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 Cells, FSC Singlets, SSC Singlets, CD38⁺ cells, Plasma cells, and B 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. Use the CD45 V500-C-A vs SSC-A density plot when adjusting the Leukocytes and Lymphocytes gates.

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

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

The dot plots on page 3 of the laboratory report are used to analyze the plasma cells in the sample. Dot plots and gates are provided to identify CD38⁺ cells, and Plasma cells. The remaining dot plots characterize the plasma cells in the sample.

- Inspect page 4 of the laboratory report.

Page 4 of the laboratory report includes lot and expiration dates for BD® CS&T Beads and the BD OneFlow™ reagent, reference settings, tube settings, and cytometer configuration.

6. (Optional) Select the **Physician Report** tab to view the report.
The BD OneFlow™ PCD Physician Report contains a high level summary of the assay results.
7. (Optional) Select the **Supplemental Report** tab to add additional dot plots to further analyze the sample.
See the *BD OneFlow™ Application Guide for Plasma Cell Disorders for BD FACSLyric™ Flow Cytometers* for more information.

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

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

8. Select the **Laboratory Report** tab.
9. Click **E-sign**.
The **E-Signature** dialog opens.
10. Select a user ID.
11. Type your password.
12. (Optional) Enter any comments.
13. Click **Sign**.
The signer's user ID, date and time, and comments are added to the E-signature box in all three reports.
14. Click **Approve**.
The Laboratory and Physicians Reports are automatically exported to C:\BD Export Clinical. If needed, manually export the Supplemental Report.
See the *BD FACSLyric™ System Instructions For Use* for more information and export options.

Analyzing the Data Using BD FACSDiva™ Software

To analyze a sample stained using BD OneFlow™ PCST:

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™ PCST 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 might 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 the FSC and SSC singlets. Debris and doublets are excluded by adjusting the gates.

The CD38⁺ cells are identified in the CD38 FITC-A vs CD45 V450-A dot plot, and then plasma cells are identified in the CD38 FITC-A vs CD138 V500-A dot plot. The plasma cells are subsequently characterized by gating on the cells expressing cyIgk and cyIgλ. These three dot plots are repeated at the top of page 2 of the PCST analysis worksheet for reference. The CD38 FITC-A vs SSC-A dot plot is included for informational purposes to allow for the visualization of CD38^{bright} cells.

B cells are identified in the CD19 PE-Cy7-A vs SSC-A dot plot and then characterized in the cyIgK APC-A vs cyIgL APC-H7-A dot plot.

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

5. Inspect the dot plots on page 2 of the analysis worksheet.

The cyIgk⁺ plasma cells and cyIgλ⁺ plasma cells are further characterized according to the levels of CD19, CD45, CD56, and β2-Microglobulin expression.

6. Examine the results in the statistics box on page 3 of the analysis worksheet.

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

7. Perform further analyses as needed.

NOTE The gates provided in the dot plots of the analysis worksheet are for normal populations of cells. If your analysis shows cell populations which fall outside of the provided gates, they might represent aberrant cell populations, and will require further analysis.

8. Save the BD OneFlow™ PCST analysis worksheet as a PDF.

NOTE The BD OneFlow™ PCST 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.

9. (Optional) Click **Print** to print the analysis worksheet as a PDF.
10. Analyze the next sample.

To analyze a sample stained using BD OneFlow™ PCD:

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™ PCD Analysis** worksheet tab.
4. Inspect the 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 the FSC and SSC singlets. Debris and doublets are excluded by adjusting the gates.

The CD38⁺ cells are identified in the CD38 FITC-A vs CD45 V450-A dot plot, and then plasma cells are identified in the CD38 FITC-A vs CD138 V500-A dot plot. These two dot plots are repeated at the top of page 2 of the analysis worksheet for reference. The CD38 FITC-A vs SSC-A dot plot is included for informational purposes to allow for the visualization of CD38^{bright} cells.

B cells are identified in the CD19 PE-Cy7-A vs SSC-A dot plot.

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

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5. Inspect the dot plots on page 2 of the analysis worksheet.
The dot plots on page 2 of the analysis worksheet include markers that can help characterize the plasma cells as being normal or aberrant.
 6. Examine the results in the statistics box on page 3 of the analysis worksheet.
Confirm that all of the keywords are present in the statistics box. If any of the keywords are missing, see Troubleshooting.
 7. Perform further analyses as needed.
NOTE The gates provided in the dot plots of the PCD analysis worksheet are for normal populations of cells. If your analysis shows cell populations which fall outside of the provided gates, they might represent aberrant cell populations, and will require further analysis.
 8. Save the BD OneFlow™ PCD analysis worksheet as a PDF.
NOTE The BD OneFlow™ PCD 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.
 9. (Optional) Click **Print** to print the analysis worksheet.
 10. 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 Plasma Cell Disorders for BD FACSLyric™ Flow Cytometers*

For BD FACSCanto™ II flow cytometers:

- *BD OneFlow™ Application Guide for Plasma Cell Disorders*

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 the BD OneFlow™ Plasma Cell Disorders Panel 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.
- The BD OneFlow™ Plasma Cell Disorders Panel 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™ PCST and BD OneFlow™ PCD. The stability of EDTA- or heparin-anticoagulated 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 28 hours post collection and stained samples were tested up to 80 minutes 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 (BD OneFlow™ PCST)

Method comparison, BD FACSLyric™ vs BD FACSCanto™ II flow cytometer (BD OneFlow™ PCST)

A method comparison study between the BD OneFlow™ system on the BD FACSLyric™ flow cytometer (Investigational Method) and the BD OneFlow™ system on the BD FACSCanto™ II flow cytometer (Comparator Method) was performed at 4 clinical sites. The BD OneFlow™ system on BD FACSLyric™ comprises BD[®] CS&T Beads, BD[®] FC Beads 7-Color Kit, BD[®] FC Beads 5-Color Kit, and BD OneFlow™ PCST 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™ PCST 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™ PCST acquired on a BD FACSCanto™ II flow cytometer (4-2H-2V) using BD FACSDiva™ software v8.0.2 and the BD OneFlow™ PCST template. A total of 80 evaluable BM specimens were enrolled in the study. Specimens were collected in the anticoagulants shown. See the following table.

Table 3 Anticoagulants used to collect specimens

Specimen type	Anticoagulant	
	EDTA	Heparin
Abnormal plasma cells	41	1
Normal polyclonal plasma cells	35	3
Total	76	4

For all enrolled specimens, the first wash step was started within 24 hours of collection. All stained samples were acquired within 45 minutes of final resuspension. For all enrolled specimens, the number of plasma cell events and percentages were within the ranges shown. See the following table.

Table 4 Ranges of plasma cell events and percentages

Plasma cells	Range
Number of events	12–38,065
% of SSC Singlets	0–42.4%

Specimens were identified as “Aberrant plasma cells” or “Normal polyclonal plasma cells” using the two systems, and compared.

Agreement was calculated as follows:

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

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

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

wherein,

a = number of samples identified as “Aberrant plasma cells” for both systems,

b = number of samples identified as “Aberrant plasma cells” on the BD FACSLyric™ flow cytometer but “Normal polyclonal plasma cells” on the BD FACSCanto™ II flow cytometer,

c = number of samples identified as “Normal polyclonal plasma cells” on the BD FACSLyric™ flow cytometer but “Aberrant plasma cells” on the BD FACSCanto™ II flow cytometer,

d = number of samples identified as “Normal polyclonal plasma cells” for both systems.

The results for the identification of specimens as “Aberrant plasma cells” or “Normal polyclonal plasma cells” were tabulated. See the following table.

Table 5 Agreement for specimens identified as “Aberrant plasma cells” or “Normal polyclonal plasma cells”

		Comparator method (BD FACSCanto™ II)		
		Aberrant plasma cells	Normal polyclonal plasma cells	Total
Investigational method (BD FACSLyric™)	Aberrant plasma cells	42	0	42
	Normal polyclonal plasma cells	0	38	38
Total		42	38	80

Overall % agreement is 100%. The lower 95% confidence limit is 96.32%.

Positive agreement for “Aberrant plasma cells” is 100%. Negative agreement for “Normal polyclonal plasma cells” is 100%.

Equivalency (BD FACSLyric™ flow cytometer)

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

The mean bias for each cell population being a percentage of the indicated population on the BD FACSLyric™ flow cytometer versus the BD FACSCanto™ II flow cytometer was calculated for all evaluable specimens. See the following table.

Table 6 Summary of mean bias for subset percentages

Population	No. of specimens ^a	Mean bias	Lower 95% CL of mean bias	Upper 95% CL of mean bias
Plasma cells (%SSC singlets)	80	-0.04	-0.14	0.06
cyIgKappa (%Plasma cells)	80	-2.03	-3.37	-0.69
cyIgLambda (%Plasma cells)	80	-0.55	-1.71	0.62

a. Some specimens might have been excluded to a low number of events acquired.

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 (BD OneFlow™ PCST)

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™ PCST. 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 7 BD FACS™ Universal Loader vs manual acquisition for BD OneFlow™ PCST

Cell Population	N	Mean		Absolute % bias (95% CI)
		Loader	Manual	
CD38 ⁺ cells (%SSC singlets)	20	6.20	6.30	-0.10 (-0.33, 0.13)
Plasma cells (%SSC singlets)	20	6.50	7.00	-0.50 (-1.10, 0.11)
cyIgk ⁺ Plasma cells (%Plasma cells)	20	54.11	54.29	-0.18 (-1.37, 1.00)
cyIgl ⁺ Plasma cells (%Plasma cells)	20	24.73	24.37	0.36 (-0.47, 1.18)
B cells (%SSC singlets)	20	3.07	3.02	0.05 (-0.03, 0.12)

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™ PCST showed 100% agreement.

Precision (within-site, control material) (BD OneFlow™ PCST)

A 21-day single-site precision study was performed to assess the within-site precision (repeatability and reproducibility) of BD OneFlow™ PCST using control material. Estimates of precision were determined across three BD FACSLytic™ flow cytometers and a minimum of three operators by acquiring CD-Chex CD103 Plus® or BD Multi-Check™ Control supplemented with CD-Chex CD117® Plus, stained in duplicate by each operator using three lots of BD OneFlow™ PCST. 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 8 Summary of within-site precision of subset percentages for BD OneFlow™ PCST

Population (% of positive)	Mean	Repeatability		Reproducibility		Total precision	
		SD	%CV	SD	%CV	Upper SD	Upper %CV
CD38 ⁺	41.84	0.66	1.58	0.97	0.02	1.73	4.14
CD138 ⁺	99.83	0.03	0.03	0.06	0.00	0.09	0.09
CD45 ⁺	33.22	0.48	1.43	1.12	0.03	1.40	4.21
B cells	14.03	0.24	1.69	0.35	0.03	0.66	4.73
CD56 ⁺	4.59	0.30	6.45	0.33	0.07	0.63	13.76
Igκ ⁺	59.33	1.21	2.04	1.86	0.03	3.53	5.94
Igλ ⁺	40.27	1.16	2.89	1.86	0.05	3.61	8.97
b2M ⁺	97.46	0.41	0.42	0.65	0.01	1.09	1.12

Precision (multi-site, control material) (BD OneFlow™ PCST)

Multi-site precision was evaluated across three sites using one lot of BD OneFlow™ PCST to stain three replicates of CD-Chex CD103 Plus[®] or BD Multi-Check™ Control supplemented with CD-Chex CD117[®] Plus. Two runs per day were acquired on a BD FACSLyric™ flow cytometer by one operator per site over a period of 5 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, 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 9 Summary of multi-site precision of subset percentages for BD OneFlow™ PCST

Population (% of positive)	Mean	Repeatability		Reproducibility		Total precision	
		SD	%CV	SD	%CV	Upper SD	Upper %CV
CD38 ⁺	41.88	0.57	1.37	1.15	0.03	2.30	5.50
CD138 ⁺	99.78	0.06	0.06	0.06	0.00	0.12	0.12
CD45 ⁺	33.12	0.56	1.68	1.27	0.04	1.89	5.69
B cells	14.31	0.30	2.13	0.20	0.01	0.44	3.05
CD56 ⁺	4.84	0.27	5.63	0.18	0.04	0.38	7.79
Igκ ⁺	57.86	1.62	2.80	1.33	0.02	2.48	4.29
Igλ ⁺	41.77	1.56	3.73	1.06	0.03	2.19	5.25
b2M ⁺	97.00	0.33	0.34	0.58	0.01	1.02	1.05

Detection capability (BD OneFlow™ PCST)

The detection capability of BD OneFlow™ PCST was assessed. An abnormal specimen was added to a hematologically normal bone marrow specimen such that the abnormal population was at 0%, 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™ PCST were evaluated in the study.

Table 10 Detection capability of BD OneFlow™ PCST

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 heterogeneous CD38 ⁺ CD138 ⁺ IgK ⁺	0.000	20	0	0.004 (0.003, 0.004)	N/A ^c	N/A
	0.050	20	20	0.038 (0.033, 0.042)	0.0093 (0.0174)	100 (88.1)
	0.100	20	20	0.081 (0.075, 0.087)	0.0127 (0.0238)	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 0.1% of all events is recommended for BD OneFlow™ PCST.

BD FACSLyric™ Flow Cytometer (BD OneFlow™ PCD)

Method comparison, BD FACSLyric™ vs BD FACSCanto™ II flow cytometer (BD OneFlow™ PCD)

A method comparison study between the BD OneFlow™ system on the BD FACSLyric™ flow cytometer (Investigational Method) and the BD OneFlow™ system on the BD FACSCanto™ II flow cytometer (Comparator Method) was performed at 4 clinical sites. The BD OneFlow™ system on BD FACSLyric™ comprises BD[®] CS&T Beads, BD[®] FC Beads 7-Color Kit, BD[®] FC Beads 5-Color Kit, and BD OneFlow™ PCD 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™ PCD 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™ PCD acquired on a BD FACSCanto™ II flow cytometer (4-2H-2V) using BD FACSDiva™ software v8.0.2 and the BD OneFlow™ PCD template. A total of 78 evaluable BM specimens were enrolled in the study. Specimens were collected in the anticoagulants shown. See the following table.

Table 11 Anticoagulants used to collect specimens

Specimen type	Anticoagulant	
	EDTA	Heparin
Presence of aberrant plasma cells	41	1
Absence of aberrant plasma cells	33	3
Total	74	4

For all enrolled specimens, the first wash step was started within 24 hours of collection. All stained samples were acquired within 45 minutes of final resuspension. For all enrolled specimens, the number of plasma cell events and percentages were within the ranges shown. See the following table.

Table 12 Ranges of plasma cell events and percentages

Plasma cells	Range
Number of events	19–38,065
% of SSC Singlets	0–41.9%

All evaluable specimens were characterized as containing aberrant plasma cells (“Presence of aberrant plasma cells”) or lacking aberrant plasma cells (“Absence of aberrant plasma cells”) using the two systems, and compared.

Agreement was calculated as follows:

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

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

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

wherein,

a = number of samples characterized as “Presence of aberrant plasma cells” for both systems,

b = number of samples characterized as “Presence of aberrant plasma cells” on the BD FACSLyric™ flow cytometer but characterized as “Absence of aberrant plasma cells” on the BD FACSCanto™ II flow cytometer,

c = number of samples characterized as “Absence of aberrant plasma cells” on the BD FACSLyric™ flow cytometer but characterized as “Presence of aberrant plasma cells” on the BD FACSCanto™ II flow cytometer,

d = number of samples characterized as “Absence of aberrant plasma cells” for both systems.

The results for the identification of specimens as “Presence of aberrant plasma cells” versus “Absence of aberrant plasma cells” were tabulated. See the following table.

Table 13 Agreement for identification of specimens as “Presence of aberrant plasma cells” or “Absence of aberrant plasma cells”

		Comparator method (BD FACSCanto™ II)		Total
		Presence of aberrant plasma cells	Absence of aberrant plasma cells	
Investigational method (BD FACSLyric™)	Presence of aberrant plasma cells	42	0	42
	Absence of aberrant plasma cells	0	36	36
	Total	42	36	78

Overall % agreement is 100%. The lower 95% confidence limit is 96.23%.

The positive agreement for the presence of aberrant plasma cells is 100%. The negative agreement for the absence of aberrant plasma cells is 100%.

Equivalency (BD OneFlow™ PCD)

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

The mean bias for the plasma cell population being a percentage of the SSC singlets on the BD FACSLyric™ flow cytometer versus the BD FACSCanto™ II flow cytometer was calculated for all evaluable specimens. See the following table.

Table 14 Summary of mean bias for subset percentages

Population	No. of specimens ^a	Mean bias	Lower 95% CL of mean bias	Upper 95% CL of mean bias
Plasma cells (%SSC singlets)	78	0.19	-0.1	0.48

a. Some specimens might have been excluded due to a low number of events acquired.

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 (BD OneFlow™ PCD)

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™ PCD. 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 15 BD FACS™ Universal Loader vs manual acquisition for BD OneFlow™ PCD

Population	N	Mean		Absolute % bias (95% CI)
		Loader	Manual	
CD38+ cells (%SSC singlets)	20	6.14	6.06	0.08 (-0.10, 0.25)
Plasma cells (%SSC singlets)	20	5.10	5.28	-0.18 (-0.51, 0.15)
B cells (%SSC singlets)	20	2.84	2.87	-0.03 (-0.14, 0.08)

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™ PCD showed 100% agreement.

Precision (within-site, control material) (BD OneFlow™ PCD)

A 21-day single-site precision study was performed to assess the within-site precision (repeatability and reproducibility) of BD OneFlow™ PCD using control material. Estimates of precision were determined across three BD FACSLyric™ flow cytometers and a minimum of three operators by acquiring CD-Chex CD103 Plus® or BD Multi-Check™ Control supplemented with CD-Chex CD117® Plus, stained in duplicate by each operator using three lots of BD OneFlow™ PCD. 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 792 samples were analyzed for most populations. A total of 828 samples were analyzed for CD38⁺ and CD38⁺CD138⁺ cells. 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 16 Summary of within-site precision of subset percentages for BD OneFlow™ PCD

Population (% of positive)	Mean	Repeatability		Reproducibility		Total precision	
		SD	%CV	SD	%CV	Upper SD	Upper %CV
CD27 ⁺	20.03	0.38	1.89	0.59	0.03	0.81	4.03
CD19 ⁺	13.98	0.25	1.76	0.30	0.02	0.63	4.50
CD117 ⁺	4.55	0.13	2.92	0.56	0.12	0.69	15.13
CD81 ⁺	98.35	0.21	0.21	1.02	0.01	3.02	3.07
CD28 ⁺	63.32	0.40	0.64	1.64	0.03	5.20	8.21
CD45 ⁺	31.56	0.56	1.79	0.99	0.03	1.78	5.63
CD38 ⁺	98.96	0.22	0.22	0.77	0.01	1.23	1.25
CD38 ⁺ CD138 ⁺	99.28	0.15	0.15	0.21	0.00	0.29	0.29

Precision (multi-site, control material) (BD OneFlow™ PCD)

Multi-site precision was evaluated across three sites using one lot of BD OneFlow™ PCD to stain three replicates of CD-Chex CD103 Plus[®] or BD Multi-Check™ Control supplemented with CD-Chex CD117[®] Plus. Two runs per day were acquired on a BD FACSLyric™ flow cytometer by one operator per site over a period of 5 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, and between sites) were estimated. A total of 90 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 17 Summary of multi-site precision of subset percentages for BD OneFlow™ PCD

Population (% of positive)	Mean	Repeatability		Reproducibility		Total precision	
		SD	%CV	SD	%CV	Upper SD	Upper %CV
CD27 ⁺	19.85	0.36	1.79	0.53	0.03	0.86	4.33
CD19 ⁺	14.02	0.27	1.90	0.15	0.01	0.36	2.56
CD117 ⁺	5.12	0.15	2.90	0.69	0.13	1.94	37.95
CD81 ⁺	99.08	0.10	0.10	0.56	0.01	1.92	1.94
CD28 ⁺	64.33	0.41	0.64	0.83	0.01	1.64	2.56
CD45 ⁺	29.16	0.53	1.80	1.00	0.03	1.40	4.80
CD38 ⁺	98.76	0.19	0.19	0.69	0.01	1.58	1.60
CD38 ⁺ CD138 ⁺	99.36	0.08	0.08	0.16	0.00	0.38	0.38

Detection capability (BD OneFlow™ PCD)

The detection capability of BD OneFlow™ PCD was assessed. An abnormal specimen was added to a hematologically normal bone marrow specimen such that the abnormal population was at 0%, 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™ PCD were evaluated in the study.

Table 18 Detection capability of BD OneFlow™ PCD

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 heterogeneous CD38 ⁺ CD138 ⁺ CD19 ⁻ CD117 heterogeneous CD81 ⁻	0.000	20	0	0.002 (0.000, 0.001)	N/A ^c	N/A
	0.050	20	20	0.045 (0.040, 0.051)	0.0120 (0.0225)	100 (88.1)
	0.100	20	20	0.073 (0.066, 0.081)	0.0163 (0.0306)	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 0.1% of all events is recommended for BD OneFlow™ PCD.

BD FACSCanto™ II Flow Cytometer (BD OneFlow™ PCST)

Method comparison, BD FACSCanto™ II flow cytometer vs EuroFlow system (BD OneFlow™ PCST)

A side-by-side comparison study between the BD OneFlow™ PCST system on the BD FACSCanto™ II flow cytometer and the EuroFlow PCST system on the BD FACSCanto™ II flow cytometer was performed. 48 BM samples were collected at 2 external clinical sites. The BD OneFlow™ PCST system comprises BD OneFlow™ Setup Beads, BD[®] FC Beads for compensation, and the BD OneFlow™ PCST reagent. The EuroFlow PCST reference system comprises Sphero™ Rainbow calibration particles (8 peaks), single color stained cells plus BD[®] CompBead particles for compensation, and the EuroFlow PCST reagent cocktail. 48 BM samples were identified as being “Follow-up needed” or “No follow-up needed” using the two systems, and compared. Samples identified as “Follow-up needed” were further classified as belonging to the plasma cell lineage or another lineage.

Agreement was calculated as follows:

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

wherein,

a = number of samples “Follow-up needed” for both systems,

b = number of samples “Follow-up needed” for the BD OneFlow™ system but “No follow-up needed” for the EuroFlow system,

c = number of samples “No follow-up needed” for the BD OneFlow™ system but “Follow-up needed” for the Euroflow system, and

d = number of samples “No follow-up needed” for both systems.

The results for the identification of cells as being “Follow-up needed” or “No follow-up needed” were tabulated. See the following table.

Table 19 Agreement for cells being “Follow-up Needed” or “No follow-up needed”

		Comparator method (Euroflow PCST cocktail)		Total
		Follow-up needed	No follow-up needed	
Investigational method (BD OneFlow™ PCST)	Follow-up needed	29	0	29
	No follow-up needed	0	19	19
Total		29	19	48

Overall % agreement is 100%.

Results calculated based on 95% lower confidence interval are 93.9% agreement.

The results for samples requiring follow-up for the plasma cell lineage or not requiring follow-up for the plasma cell lineage were tabulated. See the following table.

Table 20 Agreement for samples requiring follow-up for the plasma cell lineage or not requiring follow-up for the plasma cell lineage

		Comparator method (Euroflow PCST cocktail)		Total
		Follow-up needed	No follow-up needed	
Investigational method (BD OneFlow™ PCST)	Follow-up needed	22	0	22
	No follow-up needed	0	26	26
Total		22	26	48

Overall % agreement is 100%.

Results calculated based on 95% lower confidence interval are 93.9% agreement.

The results for samples requiring follow-up for a non-plasma cell lineage (other lineage) or not requiring follow-up for a non-plasma cell lineage (other lineage) were tabulated. See the following table.

Table 21 Agreement for samples requiring follow-up for a non-plasma cell lineage (other lineage) or not requiring follow-up for a non-plasma cell lineage (other lineage)

		Comparator method (Euroflow PCST cocktail)		Total
		Follow-up needed	No follow-up needed	
Investigational method (BD OneFlow™ PCST)	Follow-up needed	7	0	7
	No follow-up needed	0	41	41
Total		7	41	48

Overall % agreement is 100%.

Results calculated based on 95% lower confidence interval are 93.9% agreement.

Equivalency (BD FACSCanto™ II flow cytometer)

Bone marrow specimens collected at 2 external clinical laboratories were obtained from patients with plasma cell disorders, with other hematological disorders, or with no hematological abnormalities. Specimens were analyzed side-by-side using the BD OneFlow™ PCST system and the EuroFlow PCST system described previously. Plasma cells (CD45⁺, CD38⁺, CD138⁺) were identified as being a percentage of the SSC singlets. cyIgκ⁺ cells (CD45⁺, CD38⁺, CD138⁺, cyIgκ⁺, cyIgλ⁻) and cyIgλ⁺ cells (CD45⁺, CD38⁺, CD138⁺, cyIgλ⁺, cyIgκ⁻) were identified as being a percentage of plasma cells. Deming regression statistics indicate that the results obtained using the two systems are substantially equivalent. See the following table.

Table 22 Equivalency of the BD OneFlow™ system to the EuroFlow system

Marker	Sample size	Intercept	Slope	Lower 95% CL ^a of slope	Upper 95% CL of slope
Plasma cells (%SSC)	48	-0.21	0.95	0.70	1.06
cyIgκ ⁺ cells (%plasma cells)	46	5.85	0.97	0.85	1.04
cyIgλ ⁺ cells (%plasma cells)	46	3.79	0.95	0.89	1.01

a. CL = Confidence Limit

Precision (reproducibility) (BD OneFlow™ PCST)

Two operators performed two separate runs per day over a period of eight days, alternating the runs on two BD FACSCanto™ II flow cytometers. The reproducibility of CD38, CD56, β2-Microglobulin, CD19, Anti-Kappa, Anti-Lambda, and CD45 was assessed using BD Multi-Check™ Control. The reproducibility of CD138 was assessed using BM. For each run, duplicate samples of the appropriate control (BD Multi-Check™ Control or BM) were stained using three lots of BD OneFlow™ PCST by each operator, acquired using the BD OneFlow™ PCST Acquisition worksheet, and analyzed using BD FACSDiva™ software. Cell populations staining positively for CD38, CD56, β2-Microglobulin, CD19, Anti-Kappa, Anti-Lambda, and CD45 were identified as being a percentage of the parent population (Subset %P). CD138⁺ cells were identified as being a percentage of the CD38^{bright} plasma cells (Subset %CD38^{bright}) in BM. The overall reproducibility of Subset %P was calculated for each of the antibodies. The overall reproducibility comprises four components: operator/instrument-to-operator/instrument, lot-to-lot, run-to-run, and day-to-day reproducibility. See the following table.

Table 23 Reproducibility of Subset %P

Population	DF ^a	SD ^b	%CV ^c	UCL ^d
CD56 ⁺	95	1.45	11.2	12.56
β2-Microglobulin ⁺	95	0.15	0.15	0.17
CD19 ⁺	95	0.49	3.27	3.66
cyIgκ ⁺	95	0.97	1.73	1.94
cyIgλ ⁺	95	0.73	1.86	2.08
CD45 ⁺	95	1.14	1.19	1.33
CD38 ⁺	95	1.23	11.81	13.24

a. DF = Degrees of Freedom
b. SD = Standard Deviation
c. %CV = % Coefficient of variation
d. UCL = Upper confidence limit of the 95% confidence interval

The overall reproducibility of Subset %CD38^{bright} comprises operator/instrument-to-operator/instrument, lot-to-lot, run-to-run, and donor-to-donor reproducibility. See the following table.

Table 24 Reproducibility of Subset %CD38^{bright}

Population	DF	SD	%CV	UCL
CD138 ⁺	88	7.53	13.58	15.30

Precision (repeatability) (BD OneFlow™ PCST)

Two operators performed two separate runs per day over a period of eight days, alternating the runs on two BD FACSCanto™ II flow cytometers. The repeatability of CD38, CD56, β2-Microglobulin, CD19, Anti-Kappa, Anti-Lambda, and CD45 was assessed using BD Multi-Check™ Control. The repeatability of CD138 was assessed using BM. For each run, duplicate samples of the appropriate control (BD Multi-Check™ Control or BM) were stained using three lots of BD OneFlow™ PCST by each operator, acquired using the BD OneFlow™ PCST Acquisition worksheet, and analyzed using BD FACSDiva™ software. Cell populations staining positively for CD38, CD56, β2-Microglobulin, CD19, Anti-Kappa, Anti-Lambda, and CD45 were identified as being a percentage of the parent population (Subset %P). CD138⁺ cells were identified as being a percentage of the CD38^{bright} plasma cells (Subset %CD38^{bright}) in BM. The intra-assay precision (tube-to-tube repeatability) of Subset %P was calculated for each of the antibodies. See the following table.

Table 25 Repeatability of Subset %P

Population	DF	SD	%CV	UCL
CD56 ⁺	96	0.33	2.56	2.87
β2-Microglobulin ⁺	96	0.08	0.08	0.09
CD19 ⁺	96	0.28	1.84	2.05
cyIgκ ⁺	96	0.87	1.55	1.74
cyIgλ ⁺	96	0.82	2.08	2.33
CD45 ⁺	96	0.26	0.27	0.30
CD38 ⁺	96	0.24	2.34	2.62

The tube-to-tube repeatability of Subset %CD38^{bright} for CD138 was calculated. See the following table.

Table 26 Repeatability of Subset %CD38^{bright}

Population	DF	SD	%CV	UCL
CD138 ⁺	96	2.85	5.13	5.75

BD FACSCanto™ II Flow Cytometer (BD OneFlow™ PCD)

Method comparison, BD FACSCanto™ II flow cytometer vs EuroFlow system (BD OneFlow™ PCD)

A side-by-side comparison study between the BD OneFlow™ PCD system on the BD FACSCanto™ II flow cytometer and the EuroFlow PCD system on the BD FACSCanto™ II flow cytometer was performed. 48 BM samples were collected at 2 external clinical sites from patients with plasma cell disorders, other leukemia or lymphoma disorders, or no disorder. The BD OneFlow™ PCD system comprises BD OneFlow™ Setup Beads, BD[®] FC Beads for compensation, and the BD OneFlow™ PCD reagent. The EuroFlow PCD reference system

comprises Sphero™ Rainbow calibration particles (8 peaks), single color stained cells for compensation, and the EuroFlow PCD reagent cocktail. The plasma cell population from 48 BM samples was identified as being “Follow-up needed” or “No follow-up needed” using the two systems, and compared.

Agreement was calculated as follows:

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

wherein,

a = number of samples “Follow-up needed” for both systems,

b = number of samples “Follow-up needed” for the BD OneFlow™ system but “No follow-up needed” for the EuroFlow system,

c = number of samples “No follow-up needed” for the BD OneFlow™ system but “Follow-up needed” for the EuroFlow system, and

d = number of samples “No follow-up needed” for both systems.

The results for the identification of plasma cells as being “Follow-up Needed” or “No follow-up needed” were tabulated. See the following table.

Table 27 Agreement for identification of plasma cells as “Follow-up needed” or “No follow-up needed”

		Comparator method (Euroflow PCD cocktail)		Total
		Follow-up needed	No follow-up needed	
Investigational method (BD OneFlow™ PCD)	Follow-up needed	22	0	22
	No follow-up needed	0	26	26
	Total	22	26	48

Overall % agreement is 100%.

Results calculated based on 95% lower confidence interval are 93.9% agreement.

Equivalency (BD FACSCanto™ II flow cytometer)

Bone marrow specimens collected at 2 external clinical laboratories were obtained from patients with plasma cell disorders, with other hematological disorders, or with no hematological abnormalities. Specimens were analyzed side-by-side using the BD OneFlow™ PCD system and the EuroFlow PCD system described previously. Plasma cells (CD45⁺, CD38⁺, CD138⁺) were identified as being a percentage of the SSC singlets. Deming regression statistics indicate that the results obtained using the two systems are substantially equivalent. See the following table.

Table 28 Equivalency of the BD OneFlow™ system to the EuroFlow system

Marker	Sample size	Intercept	Slope	Lower 95% CL ^a of slope	Upper 95% CL of slope
Plasma cells (%SSC)	48	0.26	0.92	0.83	1.08

a. CL = Confidence Limit

Precision (reproducibility) (BD OneFlow™ PCD)

Two operators performed two separate runs per day over a period of eight days, alternating the runs on two BD FACSCanto™ II flow cytometers. The reproducibility of CD38, CD28, CD27, CD19, CD117, CD81, and CD45 was assessed using BD Multi-Check™ Control supplemented with CD-Chex CD117 Plus. The reproducibility of CD138 was assessed using BM. For each run, duplicate samples of the appropriate control (BD Multi-Check™ Control with CD-Chex CD117 Plus, or BM) were stained using three lots of BD OneFlow™ PCD by each operator, acquired using the BD OneFlow™ PCD Acquisition worksheet, and

analyzed using BD FACSDiva™ software. Cell populations staining positively for CD38, CD28, CD27, CD19, CD117, CD81, and CD45 were identified as being a percentage of the parent population (Subset %P). CD138⁺ cells were identified as being a percentage of the CD38^{bright} plasma cells (Subset %CD38^{bright}) in BM. The overall reproducibility of Subset %P was calculated for each of the antibodies. The overall reproducibility comprises four components: operator/instrument-to-operator/instrument, lot-to-lot, run-to-run, and day-to-day reproducibility. See the following table.

Table 29 Reproducibility of Subset %P

Population	DF ^a	SD ^b	%CV ^c	UCL ^d
CD28 ⁺	95	0.53	0.71	0.80
CD27 ⁺	95	0.72	1.0	1.12
CD19 ⁺	95	0.18	1.21	1.36
CD81 ⁺	95	1.46	1.53	1.71
CD117 ⁺	95	0.32	5.51	6.18
CD45 ⁺	95	0.50	0.52	0.59
CD38 ⁺	95	2.34	6.07	6.8

a. DF = Degrees of Freedom
b. SD = Standard Deviation
c. %CV = % Coefficient of variation
d. UCL = Upper confidence limit of the 95% confidence interval

The overall reproducibility of Subset %CD38^{bright} comprises operator/instrument-to-operator/instrument, lot-to-lot, run-to-run, and donor-to-donor reproducibility. See the following table.

Table 30 Reproducibility of Subset %CD38^{bright}

Population	DF	SD	%CV	UCL
CD138 ⁺	88	8.20	16.22	18.29

Precision (repeatability) (BD OneFlow™ PCD)

Two operators performed two separate runs per day over a period of eight days, alternating the runs on two BD FACSCanto™ II flow cytometers. The repeatability of CD38, CD28, CD27, CD19, CD117, CD81, and CD45 was assessed using BD Multi-Check™ Control supplemented with CD-Chex CD117 Plus. The repeatability of CD138 was assessed using BM. For each run, duplicate samples of the appropriate control (BD Multi-Check™ Control with CD-Chex CD117 Plus, or BM) were stained using three lots of BD OneFlow™ PCD by each operator, acquired using the BD OneFlow™ PCD Acquisition worksheet, and analyzed using BD FACSDiva™ software. Cell populations staining positively for CD38, CD28, CD27, CD19, CD117, CD81, and CD45 were identified as being a percentage of the parent population (Subset %P). CD138⁺ cells were identified as being a percentage of the CD38^{bright} plasma cells (Subset %CD38^{bright}) in BM. The intra-assay precision (tube-to-tube repeatability) of Subset %P was calculated for each of the antibodies. See the following table.

Table 31 Repeatability of Subset %P

Population	DF	SD	%CV	UCL
CD28 ⁺	96	0.37	0.50	0.56
CD27 ⁺	96	0.35	0.48	0.54

Population	DF	SD	%CV	UCL
CD19 ⁺	96	0.25	1.71	1.91
CD81 ⁺	96	0.37	0.39	0.44
CD117 ⁺	96	0.10	1.77	1.99
CD45 ⁺	96	0.36	0.37	0.42
CD38 ⁺	96	0.77	2.01	2.25

The tube-to-tube repeatability of Subset %CD38^{bright} for CD138 was calculated. See the following table.

Table 32 Repeatability of Subset %CD38^{bright}

Population	DF	SD	%CV	UCL
CD138 ⁺	96	2.69	5.32	5.95

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 (Igκ and Igλ) 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.
	Washed specimen was not stained within 30 minutes of the last wash.	Repeat staining with a freshly prepared specimen.
	The BD OneFlow [™] tube was exposed to light for too long.	Repeat staining with a new tube.
	Cells were not acquired within 1 hour of staining.	Repeat staining with fresh specimen. Acquire promptly.
Few or no cells	Cell concentration was too	Resuspend fresh specimen at a higher concentration. Repeat staining

Problem	Possible Cause	Solution
are recorded.	low.	and acquisition.
	Cytometer is malfunctioning.	Troubleshoot the instrument. See the cytometer instructions for use for more information.

Problems using the reagents on BD FACSLyric™ flow cytometers:

Problem	Possible Cause	Solution
Not enough cells of interest are acquired.	Cell concentration was too low.	Resuspend fresh specimen at a higher concentration. Repeat staining and acquisition.
	The default setting of 100,000 events acquired is too low.	Change the number of events acquired. Repeat staining and acquisition. See the <i>BD OneFlow™ Application Guide for Plasma Cell Disorders for BD FACSLyric™ Flow Cytometers</i> .
The FSC-A vs SSC-A dot plot is abnormal.	Cytometer needs adjusting.	Contact BD Biosciences.
The csv file and report are not exported automatically.	The reagent lot number and expiration date were not added to the Library.	<ol style="list-style-type: none"> 1. Add the reagent lot number and expiration date to the Library. 2. Export the csv file and the report PDF manually. See the <i>BD FACSLyric™ System Instructions For Use</i>.

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

Problem	Possible Cause	Solution
The resolution between debris and lymphocytes is poor.	Instrument settings are inappropriate.	Follow proper instrument setup procedures. See the <i>Instrument Setup Guide for BD OneFlow™ Assays</i> .
Some of the dot plots are dimmed.	FSC-H and SSC-H were not selected when the application settings were created.	Check that FSC-H and SSC-H are selected on the Parameters tab of the Inspector .
The barcode on the BD OneFlow™ tube label cannot be scanned.	The barcode on the tube label has been compromised.	Scan the barcode on the BD OneFlow™ pouch label into the Product ID keyword field in the Experiment Layout . Next, manually enter a semicolon (;) followed by the six-digit tube-specific ID, found adjacent to the barcode on the tube label, after the last digit of the barcode.
The dot plots on the worksheets are missing or the dot plots do not have gates.	The template did not import correctly.	<ol style="list-style-type: none"> 1. Close the current experiment. 2. Create a new experiment. 3. Re-import the BD OneFlow™ template.
Some of the keywords are missing from the statistics	BD FACSDiva™ software did not	<ol style="list-style-type: none"> 1. In the Browser, set the current tube pointer to the tube that you are analyzing. 2. Navigate to the analysis worksheet.

Problem	Possible Cause	Solution
box in the analysis worksheet.	import all of the keywords into the panel template.	<ol style="list-style-type: none"> 3. Right-click the statistics box and select Edit Stats View. 4. In the Header tab, select the All checkbox. 5. Click OK.
The statement, For in vitro diagnostic use , does not appear in the footer of the analysis worksheet when it is printed.	The paper margins in the printer settings were changed.	<ol style="list-style-type: none"> 1. From the BD FACSDiva™ software menu bar, select File > Page Setup. 2. Ensure that all of the margins are set to 2.54 cm or 1 inch, depending on your default standards. 3. Click OK.

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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.

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HISTORY

Revision	Date	Changes made
23-16816-01	2020-05	Revised to support using the product on BD FACSLytic™ flow cytometers.
23-16816-02	2021-01	Revised to support using the product with the BD FACS™ Universal Loader.
23-16816(03)	2023-01	Updated to meet requirements of Regulation (EU) 2017/746. Merged BD OneFlow™ PCST and BD OneFlow™ PCD IFUs to support the panel.

SYMBOLS GLOSSARY [L006715(06) 2021-08]

Some symbols listed below may not apply to this product.

US Customers only: For symbol glossary, refer to bd.com/symbols-glossary

Symbol	Meaning
	Manufacturer
	Authorized representative in the European Community
	Authorized 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 <i>instructions for use</i>
	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 <i>instructions for use</i> or consult electronic <i>instructions for use</i>
	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

Symbol	Meaning
	Patient number
	This way up
	Do not stack
	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
	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

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