

 **BD OneFlow™ PCST**

10 tests per kit—Catalog No. 659912

23-16816-02
1/2021



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1. INTENDED USE

The BD OneFlow™ PCST (Plasma Cell Screening Tube) is intended for flow-cytometric immunophenotyping of normal polyclonal and aberrant plasma cell populations in bone marrow as an aid in the diagnosis of hematological disorders. The BD OneFlow™ PCST tube is designed for use with a suitably equipped BD flow cytometer and software designated for in vitro diagnostic use.

2. SUMMARY AND EXPLANATION

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

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

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

populations in a particular cell lineage. Classification markers have been selected for their diagnostic utility in discriminating between cell types within a given lineage and in classifying the abnormal cell type in the sample.

3. PRINCIPLES OF THE PROCEDURE

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

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

4. REAGENT

Reagent Composition

BD OneFlow™ PCST consists of two single-use tubes containing fluorochrome-conjugated antibodies in an optimized dried formulation. BD OneFlow™ PCST (S) contains antibodies that recognize markers on the surface of cells, and BD OneFlow™ PCST (C) contains antibodies that recognize Igκ and Igλ in the cytoplasm of cells after fixing and permeabilizing them. See Table 1.

Table 1 BD OneFlow™ PCST antibody panel

Antibody	Tube	Fluorochrome	Clone	Isotype
CD38	S	FITC	HB7 ³	IgG ₁ , κ
CD56	S	PE	MY31 (Leu-19) ^{4,5}	IgG ₁ , κ
β2-Microglobulin	S	PerCP-Cy™5.5 ^a	TÜ996	IgM, κ
CD19	S	PE-Cy™7 ^a	SJ25-C1 ^{3,7}	IgG ₁ , κ
Anti-Kappa	C	APC	TB28-2 ⁸	IgG ₁ , κ
Anti-Lambda	C	APC-H7	1-155-2 ⁸	IgG ₁ , κ
CD45	S	V450 ^b	2D1 (anti-HLe-1) ^{9,10}	IgG ₁ , κ
CD138	S	V500-C ^b	MI15 ^{11,12}	IgG ₁ , κ

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

The antibodies in the BD OneFlow™ PCST tube were chosen for their ability to identify and characterize plasma cells.

CD38, CD138, CD45, and CD19 are backbone markers used to identify plasma cells.

CD56 and β2-Microglobulin are classification markers used to identify aberrant plasma cell populations.


Anti-Kappa and Anti-Lambda are used to assess the clonality of the plasma cells.

CD19, Anti-Kappa, and Anti-Lambda are also used to identify and characterize mature B cells.

Refer to the article describing the EuroFlow antibody panels¹ for a full description of the utility of the antibodies chosen for the BD OneFlow™ PCST tube.

Precautions

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

	Warning
	H317: May cause an allergic skin reaction. H412: Harmful to aquatic life with long lasting effects.
Prevention	P261: Avoid breathing dust/fume/gas/mist/vapors/spray. P272: Contaminated work clothing should not be allowed out of the workplace. P280: Wear protective gloves/protective clothing/eye protection/face protection. P273: Avoid release to the environment.
Response	P302+P352: IF ON SKIN: Wash with plenty of water/... P333+P313: If skin irritation or rash occurs: Get medical advice/attention. P321: Specific treatment (see Safety Data Sheet). P363: Wash contaminated clothing before reuse.
Disposal	P501: Dispose of contents/container to an appropriate treatment and disposal facility in accordance with applicable laws and regulations, and product characteristics at time of disposal.

Storage and Handling

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

CAUTION Ensure 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.

5. INSTRUMENTS

BD OneFlow™ PCST is for use on the following BD instruments.

Table 2 Recommended BD instruments

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

a. 8-color (4-Blue 2-Red 2-Violet), 10-color (4-Blue 3-Red 3-Violet), or 12-color (4-Blue 3-Red 5-Violet)

b. 3-laser, 8-color, 4-2H-2V BD default (4-2H-2V) optical configuration

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

6. SPECIMENS

BD OneFlow™ PCST can be used for flow-cytometric immunophenotyping of BM aspirates collected in EDTA or heparin¹³⁻¹⁹ (for example, in BD Vacutainer® blood collection tubes). Store specimens at 15°C–27°C prior to staining. Each type of specimen can have different storage conditions and limitations that should be considered prior to collection and analysis.^{13,14,17}

Specimens should be processed immediately after collection, or up to 24 hours after collection if stored at room temperature.^{15,16,18,19} If a longer period of time is needed, each laboratory should validate that specimens processed and stored according to their procedures produce equivalent results to specimens processed immediately after collection.

Specimens with large numbers of nonviable cells can give erroneous results due to selective loss of populations and to increased nonspecific binding of antibodies to nonviable cells. Viability of specimens should be assessed and a cutoff value established. A cutoff value of at least 80% viable cells has been suggested.¹³

Samples should be acquired within 60 minutes if kept at room temperature, protected from light. If a longer period of time is needed, each laboratory should validate that stained specimens acquired after being held under their storage conditions produce equivalent results to specimens acquired immediately after staining. Protect stained specimens from light until they are acquired.

WARNING All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection^{14,15} and dispose of with proper precautions in

accordance with federal, state, and local regulations. Never pipette by mouth. Wear suitable protective clothing, eyewear, and gloves.

7. REAGENTS AND MATERIALS

Reagents Provided

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

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

Reagents and Materials Required but Not Provided

For BD FACSLyric™ flow cytometers:

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

An installer is required for the BD OneFlow™ PCST 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™ PCST assay, you will have to order the installer for BD OneFlow™ Assays the first time you order BD OneFlow™ PCST. The installer also contains assays for other *BD OneFlow™* reagents.

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

For BD FACSCanto™ II flow cytometers:

- BD OneFlow™ Assay Templates Installer (Catalog No. 659305)
An installer is required for the BD OneFlow™ PCST template. The template contains two global worksheets: the BD OneFlow™ PCST Acquisition worksheet and the BD OneFlow™ PCST Analysis worksheet. Unless you already have the current BD OneFlow™ PCST template, you will have to order the installer the first time you order BD OneFlow™ PCST. The installer also contains the OneFlow Setup template and templates for other BD OneFlow™ reagents.

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

- 15-mL conical polypropylene tubes
- Pasteur pipet
- Serological pipet
- Micropipettor with tips
- Vortex mixer
- Centrifuge
- Wash buffer (filtered PBS + 0.5% BSA + 0.09% or 0.1% sodium azide)
- FIX & PERM® Cell Fixation & Cell Permeabilization kit

For BD FACSLyric™ flow cytometers:

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

For BD FACSCanto™ II flow cytometers:

- BD FACSDiva™ CS&T IVD Beads (Catalog No. 656046 or 656047)
- BD OneFlow™ Setup Beads (Catalog No. 658620)
- BD® FC Beads 8-Color Kit for BD OneFlow™ Assays (Catalog No. 658621)

8. PROCEDURE

Installing the BD OneFlow™ PCST Assay or Template

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

To install the BD OneFlow™ assay in BD FACSuite™ Clinical application:

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

1. Insert the installer and click the installer icon.

The InstallShield Wizard for BD OneFlow™ Assays opens.

2. Click **Next**.

The license agreement opens.

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

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

The **Custom Setup** dialog opens.

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

- Click **Install**.

The assays will be installed in the Library.

- Click **Finish**.

The InstallShield Wizard closes.

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

The ReadMe file opens.

- Click the close box when finished reading it.

- Remove the installer.

To install the BD 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.

1. 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.

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

Setting up the Cytometer

For BD FACSLyric™ flow cytometers:

1. Use BD® CS&T Beads and BD FACSuite™ Clinical application v1.4 or later, to perform Characterization QC (CQC) every 6 months or as needed, perform daily Performance QC (PQC), and perform daily assay and tube settings setup. For assay and tube settings setup, select the **Run Setup** and **Generate Reports** checkboxes.
2. Use the BD® FC Beads 7-Color Kit, BD® FC Beads 5-Color Kit, and BD FACSuite™ Clinical application v1.4 or later, to update reference settings every 60 days.

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

For BD FACSCanto™ II flow cytometers:

1. Use BD FACSDiva™ CS&T IVD Beads (CS&T IVD beads) and BD FACSDiva™ software v8.0.1 or later, to define the baseline of

the cytometer and to run a daily performance check of the cytometer.

2. Use BD OneFlow™ Setup Beads, lysed washed blood, and BD FACSDiva™ software v8.0.1 or later, to set photomultiplier tube (PMT) and scatter voltages monthly.
3. Use BD® FC Beads and BD FACSDiva™ software v8.0.1 or later, to set fluorescence compensation monthly.
4. We recommend that you confirm that the PMT voltages (PMTVs) are still within their daily target ranges.

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

Processing the Specimen

Washing the specimen

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

1. Label a 15-mL conical tube with the specimen ID.
2. Invert the specimen in the collection tube 10 times to mix well.
3. Add 300 µL of the specimen to the labeled conical tube.
4. Add 10 mL of wash buffer (filtered PBS + 0.5% BSA + 0.1% sodium azide).
5. Invert the tube 3–5 times to mix well.
6. Centrifuge at 540g for 5 minutes at 20°C–25°C.
7. Remove the supernatant without disturbing the cell pellet.
8. Vortex the tube until no cell aggregates remain before adding wash buffer.
9. Repeat steps 4–8 twice for a total of three washes.

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10. Resuspend the cell pellet in 200 μ L of wash buffer to give a final volume of approximately 300 μ L.
 11. Vortex vigorously 3–5 seconds to completely resuspend the cell pellet.
NOTE Start staining the specimen using the BD OneFlow™ PCST tube within 30 minutes of the last wash. Store the washed specimen at 20°C–25°C until you stain it.

Staining the specimen

12. 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.
13. For each patient specimen, remove a BD OneFlow™ PCST (S) tube from its pouch and reseal the pouch immediately. Do not remove the BD OneFlow™ PCST (C) tube at this time.
14. Place the tubes in a rack, protected from light.
Start staining the specimen within one hour of removing a tube from the pouch.
15. 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.
16. Write the patient ID on the BD OneFlow™ PCST (S) 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.
17. Vortex washed specimen 3–5 seconds to mix well.

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18. Add 50 μ L of wash buffer and 50 μ L of washed specimen to the tube. Vortex vigorously 3–5 seconds to mix well.
 19. Incubate for 30 minutes at 20°C–25°C in the dark.
 20. Add 1.5 mL of wash buffer. Vortex vigorously 3–5 seconds to mix well.
 21. Add an additional 1.5 mL of wash buffer. Vortex gently to mix.
 22. Centrifuge at 540g for 5 minutes at 20°C–25°C.
 23. Remove the supernatant without disturbing the cell pellet, leaving approximately 50 μ L of residual liquid in the tube.
 24. Vortex vigorously until the cell pellet is completely resuspended.
 25. Add 100 μ L of FIX & PERM Reagent A (fixation solution) to the tube. Vortex vigorously 3–5 seconds to mix well.
 26. Incubate for 15 minutes at 20°C–25°C in the dark.
 27. Add 1.5 mL of wash buffer. Vortex vigorously 3–5 seconds to mix well.
 28. Add an additional 1.5 mL of wash buffer. Vortex gently to mix.
 29. Centrifuge at 540g for 5 minutes at 20°C–25°C.
 30. Remove the supernatant without disturbing the cell pellet, leaving approximately 50 μ L of residual liquid in the tube.
 31. Vortex vigorously until the cell pellet is completely resuspended.

NOTE If you are unable to obtain a single-cell suspension, see Troubleshooting.

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32. 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 21–23.

33. 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.

NOTE Write the patient ID on the BD OneFlow™ PCST (C) tube label within the area provided. 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.

34. Add 100 μL of FIX & PERM Reagent B (permeabilization solution) to the BD OneFlow™ PCST (C) tube.
35. Transfer 100 μL 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.
36. Vortex the BD OneFlow™ PCST (C) tube vigorously 3–5 seconds to mix well.
37. Incubate for 15 minutes at 20°C–25°C in the dark.
38. Add 1.5 mL of wash buffer. Vortex vigorously 3–5 seconds to mix well.
39. Add an additional 1.5 mL of wash buffer. Vortex gently to mix.
40. Centrifuge at 540g for 5 minutes at 20°C–25°C.

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41. Remove the supernatant without disturbing the cell pellet, leaving approximately 50 μ L of residual liquid in the tube.
 42. Add 200 μ L of wash buffer to the tube. Vortex vigorously 3–5 seconds to completely resuspend the cell pellet.

NOTE Samples should be acquired within 60 minutes if kept at room temperature, protected from light. If a longer period of time is needed, each laboratory should validate that stained specimens acquired after being held under their storage conditions produce equivalent results to specimens acquired immediately after staining. Protect stained specimens from light until they are acquired.

Setting up the Assay (BD FACSLyric™ Flow Cytometer)

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

1. From the BD FACSuite™ Clinical application navigation bar, click the Library icon.
The Library workspace opens.
2. Expand the **Beads and Reagents** menu and select **Reagents**.
3. Select OneFlow PCST from the **Product Name** list.
The **OneFlow PCST** pane opens at the bottom of the page.
4. Click **Add Lot**.
The **Add New Lot** dialog opens.

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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™ PCST (S) and BD OneFlow™ PCST (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** or **Universal Loader** from the **Loading Option** menu.
6. If using the Loader, select **30 Tube Rack** or **40 Tube Rack** from the **Carrier Type** menu.

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

Setting up the Experiment (BD FACSCanto™ II Flow Cytometer)

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

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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 PCST template.
NOTE Make sure that you select the template for the tube you are acquiring.
 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™ PCST Acquisition worksheet or another BD OneFlow™ PCST Analysis worksheet. Click **Cancel** in each dialog.

- From the menu bar, select **Experiment > Experiment Layout** and navigate to the **Keywords** tab.
- Highlight the **Product ID** keyword for the appropriate tube, and scan the barcode on the BD OneFlow™ PCST (C) tube label.
NOTE If you cannot scan the barcode on the tube label, see **Troubleshooting**.
- Manually add the appropriate information to the remaining keywords, as needed.
- 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 BD OneFlow™ PCST 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 FACST™ 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™ PCST Acquisition sheet opens. The acquisition sheet contains dot plots and gates to identify Leukocytes, Lymphocytes, B cells, T cells, NK cells, and their relevant subpopulations.

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4. Examine each dot plot on the acquisition sheet.

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

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

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

To acquire the sample using BD OneFlow™ PCST 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™ PCST Acquisition sheet opens. The acquisition sheet contains dot plots and gates to identify Cells, CD38⁺ cells, and B cells.

4. Examine each dot plot on the acquisition sheet.

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

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

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

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

For BD FACSCanto™ II flow cytometers:

1. Vortex the cells 3–5 seconds at low speed immediately before acquiring the tube on the flow cytometer.
2. In the **Browser**, expand the appropriate specimen and set the current tube pointer to that tube.
3. Install the tube on the cytometer. Adjust the flow rate to **Medium** in the **Acquisition Dashboard**. Click **Acquire Data**.
4. Verify that the population is on scale and adjust the gate in the first plot of the **BD OneFlow™ PCST Acquisition** worksheet to exclude debris, if needed.
5. Click **Record Data** in the **Acquisition Dashboard** to collect total events.

NOTE The template will automatically collect 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.

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6. 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 CD38 FITC-A vs CD45 V450-A dot plot is used to identify CD38⁺ cells.

The CD19 PE-Cy7-A vs SSC-A dot plot is used to identify B cells.

The dot plots for the remaining markers 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.

Examine the cyIgK APC-A vs cyIgL APC-H7-A dot plots to assess the clonality of the CD38⁺ cells and the B cells.

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

7. Acquire the next sample.
8. 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™ PCST assay v1.0 can be opened in BD OneFlow™ PCST assay v1.1.

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.

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.

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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.

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.

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9. Click **E-sign**.

The **E-Signature** dialog opens.

10. Select a user ID.
11. Type your password.
12. (Optional) Enter any comments.
13. Click **Sign**.

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

14. Click **Approve**.

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

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

Analyzing the Data Using BD FACSDiva™ Software

1. From the menu bar, select **File > Import > Experiments**.
2. Select the experiment that you want to analyze. Click **Import**.

The experiment with the associated acquisition and analysis worksheets opens.

3. Select the **BD OneFlow™ 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 cyIgκ 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 cyIgκ⁺ 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.

9. LIMITATIONS

- Use of therapeutic monoclonal antibodies in patient treatment can interfere with recognition of target antigens by this reagent. This should be considered when analyzing samples from patients treated in this fashion. BD Biosciences has not characterized the effect of the presence of therapeutic antibodies on the performance of this reagent.
- Diagnostic evaluation of hematologic disorders using this reagent should be performed in the context of a thorough immunophenotypic analysis including other relevant markers.
- Use of BD OneFlow™ PCST 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.

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- BD OneFlow™ PCST has not been tested on specimens from patients with minimal residual disease (MRD).
 - Avoid using potentially compromised specimens, including clotted, hemolyzed, frozen, or refrigerated specimens.

10. PERFORMANCE CHARACTERISTICS

BD FACSLyric™ Flow Cytometer

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

Reproducibility and repeatability (BD FACSLyric™ flow cytometer)

A 5-day study was performed at one site to assess the reproducibility and repeatability of BD OneFlow™ PCST using control material. Two sources of control material were used, BD Multi-Check™ Control and CD-Chex CD103 Plus®, in order to assess all of the markers recognized by the antibodies in BD OneFlow™ PCST. Estimates of precision were determined across three BD FACSLyric™ flow cytometers and three operators by acquiring the each of the control materials 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 5 tested days.

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

Table 3 Reproducibility of subset percentages

Subset	Mean	SD	Upper 95% CL of SD	%CV	Upper 95% CL of %CV
CD56+ cells (% Lymphocytes)	11.92	0.84	0.95	7.02	7.89
CD19 (% SSC Singlets)	4.56	0.08	0.09	1.78	2
IgK (% B cells)	58.14	1.39	1.59	2.39	2.69
IgL (% B cells)	39.1	1.29	1.47	3.3	3.71

Table 4 Repeatability of subset percentages

Subset	Mean	SD	Upper 95% CL of SD	%CV	Upper 95% CL of %CV
CD56+ cells (% Lymphocytes)	11.92	0.73	0.83	6.11	6.87
CD19 (% SSC Singlets)	4.56	0.16	0.19	3.58	4.02
IgK (% B cells)	58.14	2.32	2.65	3.98	4.48
IgL (% B cells)	39.1	2.32	2.65	5.93	6.67

Method comparison (BD FACSLyric™ flow cytometer)

A method comparison study between the BD OneFlow™ system on the BD FACSLyric™ flow cytometer (Investigational Method) and the BD OneFlow™ system on the BD FACSCanto™ II flow cytometer (Comparator Method) was performed at 4 clinical sites. The BD OneFlow™ system on BD FACSLyric™ comprises BD® CS&T Beads, BD® FC Beads 7-Color Kit, BD® FC Beads 5-Color Kit, and BD OneFlow™ 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. 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 Table 5.

Table 5 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 Table 6.

Table 6 Ranges of plasma cell events and percentages

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

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

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 Table 7.

Table 7 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 Table 8.

Table 8 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.

BD FACSCanto™ II Flow Cytometer

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

Reproducibility (BD FACSCanto™ II flow cytometer)

Two operators performed two separate runs per day over a period of eight days, alternating the runs on two BD FACSCanto™ II flow cytometers. The reproducibility of CD38, CD56, β 2-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, β 2-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 Table 9.

Table 9 Reproducibility of Subset %P

Population	DF ^a	SD ^b	%CV ^c	UCL ^d
CD56 ⁺	95	1.45	11.2	12.56
β 2-Microglobulin ⁺	95	0.15	0.15	0.17
CD19 ⁺	95	0.49	3.27	3.66
cyIg κ ⁺	95	0.97	1.73	1.94
cyIg λ ⁺	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 the first three components described above and donor-to-donor reproducibility. See Table 10.

Table 10 Reproducibility of Subset %CD38^{bright}

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

Repeatability (BD FACSCanto™ II flow cytometer)

Two operators performed two separate runs per day over a period of eight days, alternating the runs on two BD FACSCanto™ II flow cytometers. 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 Table 11.

Table 11 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 Table 12.

Table 12 Repeatability of Subset %CD38^{bright}

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

Agreement (BD FACSCanto™ II flow cytometer)

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 Table 13.

Table 13 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 Table 14.

Table 14 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 Table 15.

Table 15 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	

Table 15 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)

Investigational method (BD OneFlow™ PCST)		Comparator method (Euroflow PCST cocktail)		
	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 Table 16.

Table 16 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

Table 16 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
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

11. 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.
	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.

Problem	Possible Cause	Solution
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™ PCST tube was exposed to light for too long.	Repeat staining with a new tube of BD OneFlow™ PCST.
	Cells were not acquired within 1 hour of staining.	Repeat staining with fresh specimen. Acquire 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 BD OneFlow PCST 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.

Problem	Possible Cause	Solution
The csv file and report are not exported automatically.	The reagent lot number and expiration date were not added to the Library.	<ol style="list-style-type: none"> 1. Add the reagent lot number and expiration date to the Library. 2. Export the csv file and the report PDF manually. See the <i>BD FACSLyric™ Clinical System Instructions For Use</i>.

Problems using BD OneFlow™ PCST on BD FACSCanto™ II flow cytometers:

Problem	Possible Cause	Solution
The resolution between debris and cells 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™ PCST (C) tube label cannot be scanned.	The barcode on the tube label has been compromised.	Scan the barcode on the BD OneFlow™ PCST (C) 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 OneFlow™ PCST 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.	<ol style="list-style-type: none"> 1. In the Browser, set the current tube pointer to the tube that you are analyzing. 2. Navigate to the analysis worksheet. 3. Right-click the statistics box and select Edit Stats View. 4. In the Header tab, select the All checkbox. 5. Click OK.
The statement, For in vitro diagnostic use , does not appear in the footer of the analysis worksheet when it is printed.	The paper margins in the printer settings were changed.	<ol style="list-style-type: none"> 1. From the BD FACSDiva™ software menu bar, select File > Page Setup. 2. Ensure that all of the margins are set to 2.54 cm or 1 inch, depending on your default standards. 3. Click OK.

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HISTORY

Revision	Date	Change made
23-16816-00	2/2016	Initial release
23-16816-01	5/2020	Revised to support using the product on BD FACSLyric flow cytometers.
23-16816-02	1/2021	Revised to support using the product with the BD FACSTM Universal Loader.

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