BD OneFlow™ PCD

10 tests per kit—Catalog No. 659913

IVD

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1. INTENDED USE

The BD OneFlow™ PCD (Plasma Cell Disorders) tube, when run in parallel with the BD OneFlow™ PCST (Plasma Cell Screening Tube), is intended for flow-cytometric immunophenotyping of normal and aberrant plasma cells in bone marrow as an aid in the diagnosis of multiple myeloma and other plasma cell disorders. The BD OneFlow PCD 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]

In alignment with the EuroFlow diagnostic algorithm, 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

* 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).

analyzed as a single large data file. They are markers that identify distinct cell populations in a particular cell lineage. Classification markers have been selected for their diagnostic utility in discriminating between cell types within a given lineage.

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. The BD OneFlow PCD tube contains a panel of fluorochrome-conjugated antibodies that identify normal and aberrant populations of plasma cells, and the data files generated are analyzed using BD FACSDiva™ software. Analysis of the dot plots allows for the identification of normal and abnormal cell populations.

4. REAGENT COMPOSITION

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

Table 1 BD OneFlow PCD antibody panel

Antibody	Fluorochrome	Clone	Isotype
CD38	FITC	HB7[3]	IgG_1, κ
CD28	PE	L293[4]	IgG_1, κ

Table 1 BD OneFlow PCD antibody panel

Antibody	Fluorochrome	Clone	Isotype
CD27	PerCP-Cy™5.5[a]	L128[5]	IgG_1, κ
CD19	PE-Cy™7[a]	SJ25-C1[3,6]	IgG_1, κ
CD117	APC	104D2[7]	IgG_1, κ
CD81	APC-H7	JS81[8]	IgG_1, κ
CD45	BD Horizon™ V450	2D1 (anti-HLe-1)[9,10]	IgG_1, κ
CD138	BD Horizon™ V500-C	MI15[11,12]	IgG_1, κ

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The antibodies in the BD OneFlow PCD tube were chosen for their ability to identify plasma cells.

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

CD27, CD28, CD117, and CD81 are classification markers used to identify aberrant plasma cell populations.

Refer to the article describing the EuroFlow antibody panels[1] for a full description of the utility of the antibodies chosen for the BD OneFlow PCD tube.

5. STORAGE AND HANDLING

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

CAUTION Ensure 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.

6. REAGENTS OR MATERIALS REQUIRED BUT NOT PROVIDED

– Installer DVD for the OneFlow PCD template (Catalog No. 659305)

  The OneFlow PCD template is provided on an installer DVD. The template contains two global worksheets: the BD OneFlow PCD Acquisition worksheet and the BD OneFlow PCD Analysis worksheet. You will have to order this the first time you order BD OneFlow PCD. The installer DVD also contains the OneFlow Setup template and templates for other BD OneFlow™ multicolor tubes.

  There are two application guides for the system, the *Instrument Setup Guide for BD OneFlow™ Assays*, and the *BD OneFlow™ Application Guide for Plasma Cell Disorders*. They are provided on separate DVDs along with the installer DVD.

– 15-mL conical polypropylene tubes
– Pasteur pipet
– Serological pipet
– Micropipettor with tips

- Vortex mixer
- Wash buffer (filtered PBS + 0.5% BSA + 0.1% sodium azide)
- Centrifuge
- BD FACSDiva™ CS&T IVD beads (Catalog No. 656046 or 656047)
- BD OneFlow™ Setup Beads (Catalog No. 658620)
- BD™ FC Beads 8-color kit for BD OneFlow™ Assays (BD FC Beads) (Catalog No. 658621)
- FIX & PERM®† Cell Fixation & Cell Permeabilization kit

7. INSTRUMENT

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

8. SPECIMENS

BD OneFlow PCD can be used for flow-cytometric immunophenotyping of BM aspirates collected in EDTA or heparin (for example, in BD Vacutainer® tubes). Stain the BM specimens within 24 hours of collection.‡ Store specimens at 15°C–27°C prior to staining.

NOTE 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]

† FIX & PERM® is a registered trademark of Nordic-MUbio BV.

‡ Samples stained within 24 hours of collection show concordance with the EuroFlow PCD reagent cocktail.

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.

9. PROCEDURE

Installing the OneFlow PCD template

The OneFlow PCD template has to be installed before you run the assay for the first time. Additional templates can be installed at the same time, as needed.

1. Insert the installer DVD and click the installer icon.

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

2. Follow the instructions in the dialog.

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

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

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

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

Setting up the cytometer

1. Use BD FACSDiva CS&T IVD beads (CS&T IVD beads) and BD FACSDiva software v8.0.1 or later, to define the baseline of the cytometer and to run a daily performance check of the cytometer. See the *BD FACSDiva™ CS&T IVD Beads* IFU and the *Instrument Setup Guide for BD OneFlow™ Assays* for more information.
2. Use BD OneFlow Setup beads, the OneFlow Setup template, lysed washed blood, and BD FACSDiva software v8.0.1 or later, to set photomultiplier tube (PMT) and scatter voltages monthly. See the *BD OneFlow™ Setup Beads* IFU and the *Instrument Setup Guide for BD OneFlow™ Assays* for more information.
3. Use BD FC Beads and BD FACSDiva software v8.0.1 or later, to set fluorescence compensation monthly. See the *BD™ FC Beads 8-color kit for BD OneFlow™ Assays* IFU and the *Instrument Setup Guide for BD OneFlow™ Assays* for more information.

Washing the specimen

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

1. Label a 15-mL conical tube with the specimen ID.
2. Invert the specimen in the collection tube 10 times to mix well.
3. Add 300 µL of the specimen with a cell count of up to 2.0×10^7 leucocytes per mL 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 540*g* for 5 minutes at 20°C–25°C.
7. Remove the supernatant without disturbing the cell pellet.
8. Vortex the tube until no cell aggregates remain before adding wash buffer.
9. Repeat steps 4–8 twice for a total of three washes.
10. Resuspend the cell pellet in 200 µL of wash buffer to give a final volume of approximately 300 µL.
11. Vortex vigorously 3–5 seconds to completely resuspend the cell pellet.

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

Staining the specimen

1. Make sure that the pouch is at 20°C–25°C before opening it.
2. For each patient specimen, remove a BD OneFlow PCD tube 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 PCD 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.
3. Vortex washed specimen 3–5 seconds to mix well.
4. Add 50 µL of wash buffer and 50 µL of washed specimen to the tube. Vortex vigorously 3–5 seconds to mix well.
5. Incubate for 30 minutes at 20°C–25°C in the dark.

6. Add 1.5 mL of wash buffer. Vortex vigorously 3–5 seconds to mix well.
7. Add an additional 1.5 mL of wash buffer. Vortex gently to mix.
8. Centrifuge at 540*g* for 5 minutes at 20°C–25°C.
9. Remove the supernatant without disturbing the cell pellet, leaving approximately 50 µL of residual liquid in the tube.
10. Vortex vigorously until the cell pellet is completely resuspended.
11. Add 100 µL of FIX & PERM Reagent A (fixation solution) to the 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.
12. Incubate for 15 minutes at 20°C–25°C in the dark.
13. Add 1.5 mL of wash buffer. Vortex vigorously 3–5 seconds to mix well.
14. Add an additional 1.5 mL of wash buffer. Vortex gently to mix.
15. Centrifuge at 540*g* for 5 minutes at 20°C–25°C.
16. Remove the supernatant without disturbing the cell pellet, leaving approximately 50 µL of residual liquid in the tube.
17. Vortex vigorously until the cell pellet is completely resuspended.

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

18. 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 19–21.

19. Add 100 µL of FIX & PERM Reagent B (permeabilization solution) to the tube.
20. Vortex vigorously 3–5 seconds to mix well.
21. Incubate for 15 minutes at 20°C–25°C in the dark.
22. Add 1.5 mL of wash buffer. Vortex vigorously 3–5 seconds to mix well.
23. Add an additional 1.5 mL of wash buffer. Vortex gently to mix.
24. Centrifuge at 540*g* for 5 minutes at 20°C–25°C.
25. Remove the supernatant without disturbing the cell pellet, leaving approximately 50 µL of residual liquid in the tube.
26. Add 200 µL of wash buffer to the tube. Vortex vigorously 3–5 seconds to completely resuspend the cell pellet.

    NOTE Acquire the sample within 1 hour of staining. Store the stained sample at 2°C–8°C in the dark until acquisition.

Setting up the experiment

1. From the menu bar, select **Edit > User Preferences,** then navigate to the **FCS** tab, and select **Export FCS after recording,** to automatically export the FCS files after acquisition. Click **OK.**
2. Confirm that the cytometer is in the 4-2H-2V BD default configuration.

3. From the menu bar, select **Experiment > New Experiment > Blank Experiment**. Click **OK**.

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

4. If prompted by the **CST Mismatch** dialog, select **Use CST Settings**.
5. Rename the experiment according to your laboratory practice.
6. In the **Browser**, right-click **Cytometer Settings > Link Setup** and select the appropriate compensation matrix calculated using BD FC Beads within the past 31 days. Click **Link**. See the *BD™ FC Beads 8-color kit for BD OneFlow™ Assays* IFU or the *Instrument Setup Guide for BD OneFlow™ Assays*.
7. If prompted by the **Cytometer Settings Mismatch** dialog, select **Overwrite**.
8. Right-click **Cytometer Settings > Unlink From** and select the previously linked compensation setup. Click **OK**.

   NOTE Unlinking the compensation setup allows updated application settings to be applied while retaining compensation values.

9. In the **Browser**, right-click **Cytometer Settings > Application Settings > Apply** and select the most recent application settings determined within the last 31 days using the BD OneFlow Setup beads. Click **Apply**.
10. A **Confirm** dialog opens. Select **Keep the compensation value**.
11. If prompted by the **Confirm Cytometer Changes** dialog, click **Yes** to overwrite the cytometer values for **FSC Area Scaling**.
12. From the menu bar, select **Experiment > New Specimen**.

    The **Panel Templates** dialog opens.

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

    NOTE If you want to acquire additional patient samples in the experiment, repeat steps 12–15 to add new specimens. Two **Confirm** dialogs will open asking if you want to create another PCD Acquisition worksheet or another PCD 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 PCD tube.

    NOTE If you cannot scan the barcode on the tube label, see Troubleshooting.

18. Manually add the appropriate information to the remaining keywords, as needed.

19. Click **OK** to close the **Experiment Layout**.

Acquiring the stained sample

1. 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 dot plot of the **BD OneFlow PCD Acquisition** worksheet to exclude debris, if needed.
5. Click **Record Data** in the **Acquisition Dashboard** and collect 100,000 total events.
6. Inspect the dot plots on the PCD acquisition worksheet, and adjust the gates as needed.

   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.

   NOTE See the *BD OneFlow™ Application Guide for Plasma Cell Disorders* for examples of the dot plots showing populations of normal cells in the PCD 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 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 PCD Analysis** worksheet tab.
4. Inspect the plots on page 1 of the PCD analysis worksheet, and adjust the gates as needed.

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

   The first three dot plots on 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 PCD 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.
5. Inspect the dot plots on page 2 of the PCD analysis worksheet.

   The dot plots on page 2 of the PCD 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 PCD 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 PCD analysis worksheet as a PDF.

   NOTE The 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 PCD analysis worksheet.

10. Analyze the next sample.

10. PERFORMANCE CHARACTERISTICS

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

Reproducibility

Two operators performed two separate runs per day over a period of eight days, alternating the runs on two BD FACSCanto II flow cytometers. 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 Table 2.

Table 2 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

**CD-Chex CD117™ is a trademark of Streck, Inc.

The overall reproducibility of Subset %CD38^{bright} comprises the first three components described above and donor-to-donor reproducibility. See Table 3.

Table 3 Reproducibility of Subset %CD38^{bright}

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

Repeatability

Two operators performed two separate runs per day over a period of eight days, alternating the runs on two BD FACSCanto II flow cytometers. 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 Table 4.

Table 4 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

Table 4 Repeatability of Subset %P

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 Table 5.

Table 5 Repeatability of Subset %CD38^{bright}

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

Agreement

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

††Sphero™ is a trademark of Spherotech, Inc.

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:

Overall % agreement = ((a+d)/(a+b+c+d))×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 Table 6.

Table 6 Agreement for identification of plasma cells as "Follow-up needed" or "No follow-up needed"

		Comparator method (Euroflow PCD cocktail)		
		Follow-up needed	No follow-up needed	Total
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

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 Table 7.

Table 7 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

11. LIMITATIONS

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

WARRANTY

Unless otherwise indicated in any applicable BD general conditions of sale for non-US customers, the following warranty applies to the purchase of these products.

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

TROUBLESHOOTING

Problem	Possible Cause	Solution
The resolution between debris and cells is poor.	Specimen was poorly lysed.	Repeat staining; vortex tubes until no cell aggregates remain before adding FIX & PERM Reagent A.
	Specimen is of poor quality.	Check cell viability.
	Instrument settings are inappropriate.	Follow proper instrument setup procedures. Optimize instrument settings as required.
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 PCD tube was exposed to light for too long.	Repeat staining with a new tube of BD OneFlow PCD.
	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.
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 PCD tube label cannot be scanned.	The barcode on the tube label has been compromised.	Scan the barcode on the BD OneFlow PCD 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.
Some of the keywords are missing from the statistics box in the analysis worksheet.	BD FACSDiva software did not import all of the keywords into the panel template.	1. Navigate to the analysis worksheet. 2. Right-click the statistics box and select **Edit Stats View**. 3. In the **Header** tab, select the **All** checkbox. 4. Click **OK**.

Problem	Possible Cause	Solution
The statement, **For in vitro diagnostic use**, does not appear in the footer of the analysis worksheet when it is printed.	The paper margins in the printer settings were changed.	1. From the BD FACSDiva software menu bar, select **File > Page Setup**. 2. Ensure that all of the margins are set to 2.54 cm or 1 inch, depending on your default standards. 3. Click **OK**.

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