Instrument Setup Guide for BD OneFlow™ Assays

IVD CE

23-16407-02
10/2019

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Regulatory information

BD FACSCanto II is a Class 1 Laser Product.

For In Vitro Diagnostic Use.

History

Revision	Date	Change made
23-16407-00	7/2014	Initial release
23-16407-01	4/2017	Added a step to overwrite cytometer values for FSC Area Scaling; updated New Zealand address
23-16407-02	10/2019	Updated the installer description and workflow. Updated the Australian and New Zealand addresses.

Contents

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BD OneFlow Assays

This chapter covers the following topics:

Overview of the BD OneFlow system

About the system

The BD OneFlow™ system provides a comprehensive set of reagents and protocols to reproducibly set up the cytometer and stain patient specimens. The consistent instrument setup and sample staining enable you to acquire and analyze patient specimens for immunophenotyping of normal and aberrant cell populations in a manner compatible with that prescribed by the EuroFlow™ Consortium.

Materials needed

In addition to the relevant multicolor tube, the BD OneFlow system requires the following BD products:

- BD FACSDiva™ CS&T IVD beads (CS&T IVD beads)
  - Catalog No. 656046 or 656047
- BD OneFlow™ Setup beads
  - Catalog No. 658620

  The BD OneFlow Setup beads come with two median fluorescence intensity (MFI) target range cards, which contain lot-specific MFI target ranges. They are clearly marked as being for monthly use or for daily use (see below).
- BD® FC Beads 8-color kit for BD OneFlow™ Assays (BD FC beads)
  - Catalog No. 658621
- Installer with OneFlow templates
  - OneFlow Setup template
  - OneFlow template for the appropriate multicolor tube
- BD FACS™ lysing solution
  - Catalog No. 349202
- BD FACSCanto™ II flow cytometer with a 3-laser, 8-color, 4-2H-2V BD default optical configuration, running BD FACSDiva software v.8.0.1, or later.

In addition, you will need a specimen of lysed washed blood (LWB).

Cytometer QC

CS&T IVD beads are used within the CST module to set the baseline for the cytometer. After the cytometer baseline is established, CS&T IVD beads are used to perform a daily performance QC for the cytometer.

Differences in day-to-day cytometer performance are measured and corrected using the beads.

The day-to-day performance of the cytometer can be tracked in the CST module.

Setting detector voltages

BD OneFlow Setup beads are used to set photomultiplier tube voltages (PMTVs) to target MFI (TMFI) values for each of the fluorochromes. The EuroFlow Consortium has determined a set of MFI target ranges for the system.[1] Use the BD OneFlow™ TMFI Setup worksheet (TMFI Setup worksheet) to set the PMTVs to their MFI target ranges. The lot-specific MFI target ranges are provided on two MFI target range cards included in the kit. Use the monthly MFI target range card when you set the PMTVs to their MFI target ranges.

After setting the PMTVs to their MFI target ranges, normal lysed washed peripheral blood is used to set forward and side scatter voltages (FSC and SSC, respectively). Use the BD OneFlow™ Scatter Setup worksheet (Scatter Setup worksheet) to set the FSC and SSC voltages.

Application settings

All of the detector voltages are saved as application settings. Application settings are expected to be stable for one month. The PMTVs are expected to remain within a range of +/-15% of the initial target MFI values during that time if the instrument is maintained properly. Cytometer performance is checked on a daily basis in the CST module. The saved application settings are automatically updated to align with the cytometer performance

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

and can be applied each day. This simplifies daily cytometer setup since PMTVs and scatter voltages do not have to be set every day. However, we recommend that you confirm that the PMTVs fall within the acceptable range on a daily basis. Use the daily MFI target range card when you do this.

In addition to being determined once a month, application settings must be renewed whenever a new baseline is defined using CS&T IVD beads, for example, when the baseline expires or after performing cytometer service or maintenance. See the *BD FACSDiva™ CS&T IVD Beads* instructions for use (IFU) for more information. You will also have to create new application settings if the PMTVs fall outside of the acceptable range of +/- 15%. Use the monthly MFI target range card when you reset the PMTVs to their MFI target ranges.

Compensation

FC beads enable BD FACSDiva software to calculate spillover values (SOVs) for a fluorescence compensation matrix. A new SOV matrix must be calculated each time application settings are created. Day-to-day differences in cytometer performance are measured and corrected using CS&T IVD beads, resulting in slight differences in the PMTVs. Because the SOV matrix for fluorescence compensation is calculated for a particular set of PMTVs and scatter voltages, you must first link and then unlink from the compensation settings before you apply the saved application settings. When prompted, you keep the compensation value.

Specimen acquisition and analysis

The BD OneFlow™ multicolor tubes are used to stain patient specimens. The stained specimens are acquired on the cytometer and then analyzed to identify normal and aberrant populations of hematopoietic cells. Use the template designated for the tube you are using.

Workflows for the BD OneFlow setup

The BD OneFlow assays comprise several discrete workflows. The steps for each workflow and the reagents and materials you will need are summarized in the following tables.

Initial setup

Task	Reagent or Material	Template needed	Outcome
Defining a baseline	CS&T IVD beads BD FACSFlow	None	Cytometer baseline is set
Running a performance check	CS&T IVD beads BD FACSFlow	None	Daily QC for cytometer performance
Installing the BD OneFlow panel templates	BD OneFlow installer for the template of the multicolor tube you are using	None	The panel templates are installed in the proper folder
Setting up automatic export of FCS files	None	None	FCS files will be exported after being acquired
Adjusting fluorescent PMT voltages	BD OneFlow Setup beads BD FACSFlow Monthly MFI target range card	OneFlow Setup template (TMFI Setup worksheet)	Application settings are created

Task	Reagent or Material	Template needed	Outcome
Adjusting the FSC and SSC voltages	Lysed washed normal peripheral blood (LWB) Wash buffer	OneFlow Setup template (Scatter Setup worksheet)	Application settings are created
Calculating compensation	BD FC beads BD FC beads dilution buffer	None	SOV matrix is calculated

Monthly setup

Task	Reagent or Material	Template needed	Outcome
Running a performance check	CS&T IVD beads BD FACSFlow	None	Daily QC for cytometer performance
Adjusting fluorescent PMT voltages	BD OneFlow Setup beads BD FACSFlow Monthly MFI target range card	TMFI Setup worksheet	Application settings are created
Adjusting the FSC and SSC voltages	Lysed washed normal peripheral blood (LWB) Wash buffer	Scatter Setup worksheet	Application settings are created
Calculating compensation	BD FC beads BD FC beads dilution buffer	None	SOV matrix is calculated

Daily setup

Task	Reagents or Materials	Template needed	Outcome
Running a performance check	CS&T IVD beads BD FACSFlow	None	Daily QC for cytometer performance
Confirming PMT voltages	BD OneFlow Setup beads BD FACSFlow Daily MFI target range card	TMFI Setup worksheet	PMTVs are confirmed to be within the acceptable range

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Initial setup

This chapter covers the following topics:

Defining a baseline

Administrator task

The cytometer baseline has to be defined at initial setup, whenever the baseline expires, and whenever you perform cytometer maintenance or service. The system administrator defines the baseline in the CST module.

See the *BD FACSDiva™ CS&T IVD Beads* IFU for more information.

Running a performance check

The performance check has to be run whenever you define a new baseline. It is also run on a daily basis to measure any changes in the cytometer's performance, and correct them.

Setting up the cytometer

1. Turn on the cytometer.
2. Check that all fluid levels are appropriate.
3. Open BD FACSDiva software v.8.0.1, or later.
4. Perform fluidics startup.

   From the menu bar, select **Cytometer** > **Fluidics Startup**. Click **OK** when prompted.
5. Allow the cytometer to warm up for at least 15 minutes.

Preparing CS&T IVD beads

1. Label a 12 x 75 mm polystyrene tube *CST*.
2. Mix the CS&T IVD beads vial by gentle inversion.
3. Add to the labeled tube:
   a. 0.35 mL of BD FACSFlow
   b. 1 drop of CS&T IVD beads

4. Vortex the tube gently.

   Note: Store tube for up to 8 hours at 2°C–25°C in the dark if not acquiring immediately.

Running the daily performance check

1. From the menu bar, select **Cytometer > CST**.

   The BD® Cytometer Setup and Tracking (CST) module opens.

2. Navigate to the **Setup** tab of the CST module and do the following:

   a. Confirm that the default 4-2H-2V cytometer configuration is selected.

   b. Navigate to the **Setup Control** window and do the following:

   - Select **Check Performance** from the **Characterize** menu.
   - If the cytometer is equipped with a loader, select the **Load Tube Manually** checkbox.
   - Verify that the bead lot ID selected matches your current lot of CS&T IVD beads. If not, select the correct CS&T IVD bead lot ID from the **Lot ID** drop-down menu.
   - Gently vortex the diluted CS&T IVD beads and install the tube on the cytometer.
   - Click **Run**. Click **OK** to confirm that the tube has been loaded.

     The **Checking Cytometer Performance** window opens. Once the performance check is complete, a dialog opens prompting you to remove the tube. A second dialog opens when the performance check has completed.

   c. To view the Cytometer Performance Report, click **View Report**. To print it, select **File > Print**.

      You can verify that Cytometer Performance passed in the System Summary view.

Note: If the results did not pass, re-run the performance check. Perform additional troubleshooting, as needed. See the *BD FACSDiva™ CS&T IVD Beads* IFU for additional information.

d. Click the close box to exit the CST module and return to the BD FACSDiva workspace.

e. If prompted by the **CST Mismatch** window, select **Use CST Settings**.

Installing the BD OneFlow panel templates

About panel templates

We provide the BD panel template you will need to set the PMT voltages and scatter voltages for the BD OneFlow application settings. We also provide tube-specific templates you will need for acquiring the stained patient samples. Before you use the BD OneFlow assays you will have to install the relevant panel templates. They are provided on an installer. You should also confirm that the cytometer is running BD FACSDiva software v.8.0.1, or later.

Installing the templates

1. Insert the installer.
2. Click the installer icon.
3. A welcome dialog opens. Click **Next**.
4. Select the **Agree to Terms** button when prompted.
5. Select **Next** or **Cancel**, as appropriate.

6. Select the checkboxes next to the templates that you want to install. Select **Next** or **Cancel**.

   Alternatively you can select the **Select All** checkbox.

   You will need the following templates:

   - OneFlow Setup template
   - OneFlow template for the multicolor tube you are using

   A dialog opens, informing you where the templates will be copied and pasted.

7. Select **Next** or **Cancel**.

8. Select **Install** or **Cancel**.

9. Click **Finish** to close the dialog.

   A dialog opens, informing you that the installation was successful.

10. Click **OK** to close the dialog.

11. The installer ReadMe file opens. Click the close box when you have finished reading it.

12. Remove the installer.

13. Navigate to D:\BDExport\Templates\Panel\BD Panels to verify that the templates were installed correctly.

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

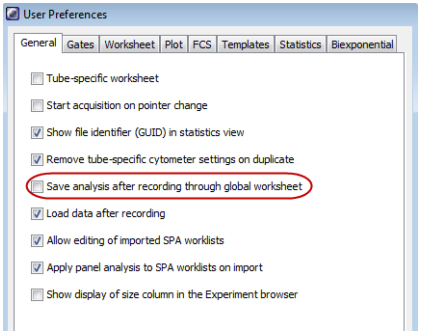
Setting up automatic export of FCS files

Recommended

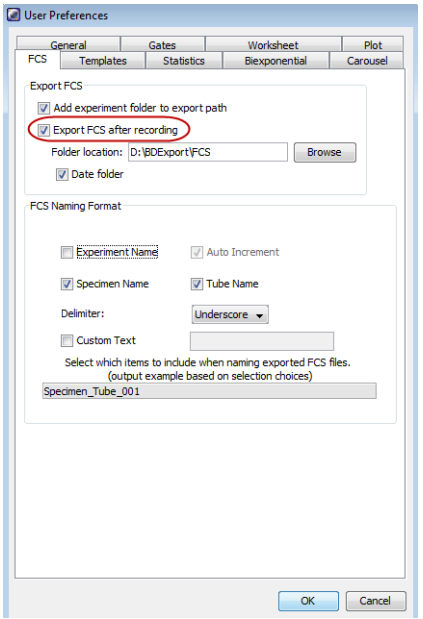
We recommend that you set up the user preferences so that the FCS files are automatically exported after they are acquired. This has to be done for each user.

Editing the User Preferences

1. From the menu bar, select **Edit > User Preferences**.
2. Navigate to the **General** tab and clear the **Save analysis after recording through global worksheet** checkbox.



3. Navigate to the **FCS** tab and select **Export FCS after recording**.



4. Click **OK**.

Preparing the setup reagents

Before you begin

1. Remove the following BD FC beads pouches from the refrigerator:
   - FITC
   - PE
   - PerCP-Cy™5.5
   - PE-Cy™7
   - APC
   - APC-H7

- V450
- V500-C

2. Allow the bead pouches to reach 18°C–25°C before opening each pouch.

Preparing lysed washed blood

You will use a lysed washed blood (LWB) specimen to adjust FSC and SSC voltages.

Warning All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection 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.

1. Label a 12 × 75 mm polystyrene tube *LWB*.
2. Add 100 µL of whole blood from a normal donor to the tube.
3. Add 2 mL of 1X BD FACS lysing solution.
4. Vortex 3–5 seconds to mix well.
5. Incubate for 10 minutes at 18°C–25°C.
6. Centrifuge at 540*g* for 5 minutes at 20°C–25°C.
7. Remove the supernatant without disturbing the cell pellet.

   Note: Leave approximately 50 µL of residual liquid in the tube.

8. Vortex 3–5 seconds to resuspend the cell pellet.
9. Add 2 mL of wash buffer (filtered PBS + 0.5% BSA + 0.1% sodium azide).
10. Vortex 3–5 seconds to mix well.
11. Centrifuge at 540*g* for 5 minutes at 20°C–25°C.

12. Remove the supernatant without disturbing the cell pellet.

    Note: Leave approximately 50 µL of residual liquid in the tube.

13. Vortex 3–5 seconds to resuspend the cell pellet.
14. Add 250 µL of wash buffer.
15. Vortex 3–5 seconds to mix well.
16. Save the LWB specimen to adjust FSC and SSC voltages.
17. Store at 2°C–25°C until acquisition.

Preparing BD OneFlow setup beads

1. Label a 12 × 75 mm polystyrene tube *Setup beads*.
2. Thoroughly mix the BD OneFlow Setup beads vial.
3. Add 2 drops of beads to 700 µL of BD FACSFlow. Protect from light.

   Note: Verify that the lot number on the monthly MFI target range card matches the lot number on the vial of BD OneFlow Setup beads that you are using.

4. Return the vial of BD OneFlow Setup beads to 2°C–8°C storage.
5. Proceed to acquisition or store tubes appropriately until acquired.

   Note: You will need the monthly MFI target range card at the cytometer.

   Note: If not acquiring immediately, store the diluted beads, protected from light, for up to:

   - 1 hour at 18°C–25°C
   - 8 hours at 2°C–8°C

Preparing BD FC beads

1. Open a pouch of BD FC beads, remove one tube, and place it in a rack, protected from light.
2. Reseal the pouch immediately and return it to 2°C–8°C storage as soon as possible.

   Note: Ensure the pouch is completely resealed after removing a tube. The reagent is very sensitive to moisture. Do not remove the desiccant pack from the pouch. Protect the bead tubes from light before and after reconstitution. Some of the dyes used to manufacture the beads are very light sensitive. Fluorescence SOVs can change if the beads are exposed to light.
3. Repeat steps 1 and 2 for the remaining fluorochrome tubes.
4. Add 0.5 mL of BD FC beads dilution buffer to each tube.

   Note: Use only the bead dilution buffer included with the kit. Use of other diluents could result in incorrect SOVs.
5. Vortex the tubes vigorously for 3–5 seconds.
6. Protect tubes from light.

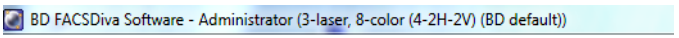
   Note: If not acquiring immediately, store the rehydrated bead tubes, protected from light, for up to:

   - 1 hour if stored at 18°C–25°C
   - 4 hours if stored at 2°C–8°C

Setting up the cytometer

Before you begin

1. In BD FACSDiva software v.8.0.1, or later, ensure that cytometer warmup is complete, fluidics startup has been performed, and that the cytometer is in the default 4-2H-2V configuration.



2. Verify that the performance check was completed and passed for the default 4-2H-2V configuration using CS&T IVD beads on that day.

Creating the setup experiment

1. Create a new experiment.

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

      Note: Alternatively, create a new experiment using the **Experiment** icon in the **Browser**.

   b. If prompted by the **CST Mismatch** window, select **Use CST Settings**.

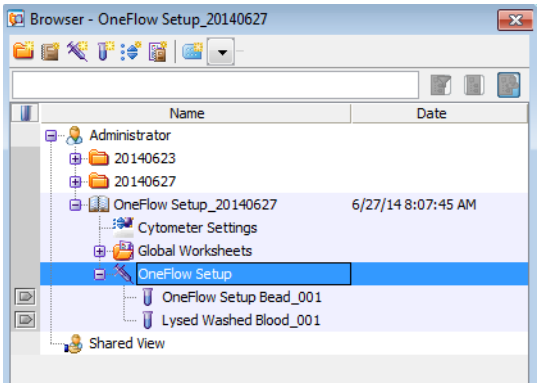
   c. Rename the experiment, for example, as *OneFlow Setup_today's date*.

2. Import the OneFlow Setup template.

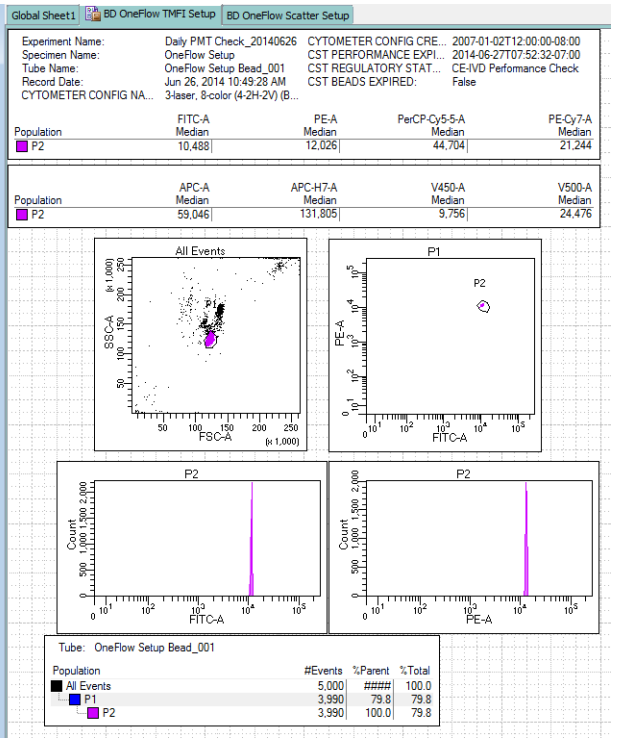
   a. From the menu bar, select **Experiment > New Specimen.**

   b. The **Panel Templates** dialog opens. Navigate to the **BD Panels** tab and select the OneFlow Setup template. Click **OK**.

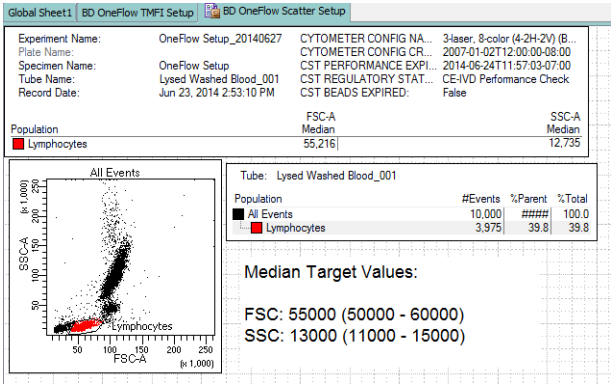
   c. In the **Browser**, expand the OneFlow Setup specimen to see the OneFlow Setup Bead tube and the Lysed Washed Blood tube.



Note: The OneFlow Setup template comprises two global worksheets. The TMFI Setup worksheet is shown below.

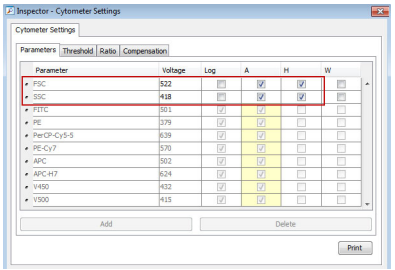


The Scatter Setup worksheet is shown below.



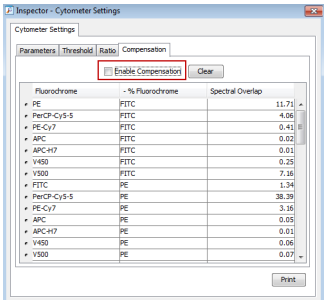
3. In the **Browser**, click **Cytometer Settings**.

4. In the **Inspector**, navigate to the **Parameters** tab and ensure that FSC-A, FSC-H, SSC-A, and SSC-H, as well as "A" for all fluorescent parameters, are selected.



Note: If necessary, activate a parameter by selecting the checkbox next to it.

5. In the **Inspector**, navigate to the **Compensation** tab and deselect the **Enable Compensation** checkbox.



Adjusting fluorescent PMT voltages

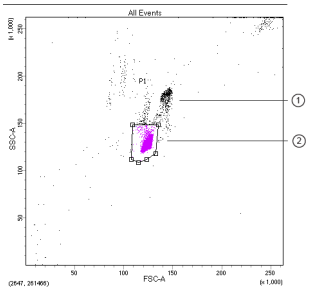
Adjusting PMTVs to achieve target MFI values

1. In the **Browser**, set the current tube pointer to the OneFlow Setup Bead tube.
2. In the **Acquisition Dashboard**, set **Events To Record** to 5,000.
3. Vortex the tube of diluted BD OneFlow Setup beads for 3–5 seconds.
4. Install the tube on the cytometer.
5. In the **Acquisition Dashboard**, adjust the flow rate to **Low**, and click **Acquire Data**.

   Note: It may take 10–15 seconds until events begin to appear.

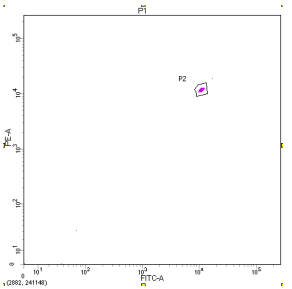
6. In the FSC-A vs SSC-A dot plot, adjust the **P1** gate to include only the singlet bead population (no aggregates).

   Note: Enlarge the FSC-A vs SSC-A dot plot to see the singlet bead population more clearly.

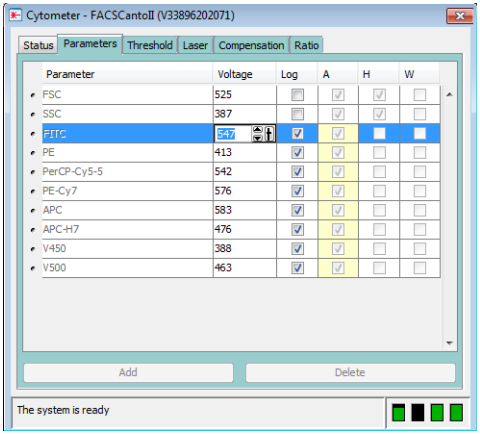


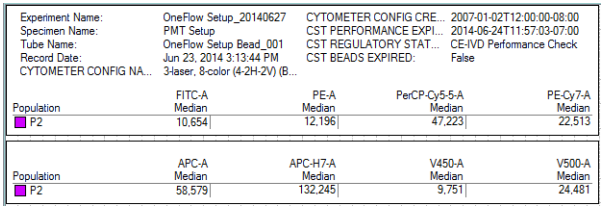
No.	Description
1	Aggregates
2	Singlets

7. In the FITC-A vs PE-A dot plot, adjust the **P2** gate to include only the singlet bead population.



8. In the **Cytometer** window, select the **Parameters** tab and adjust the voltages for FITC, PE, PerCP-Cy5.5, PE-Cy7, APC, APC-H7, V450, and V500 so that the MFI of the bead population in the **P2** gate from the statistics box falls within the corresponding range on the monthly MFI target range card.





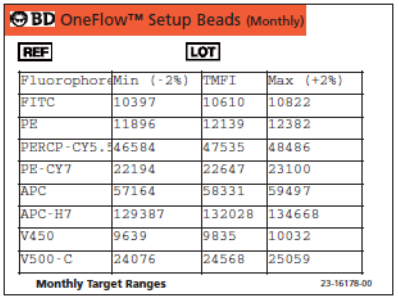
Experiment Name:	OneFlow Setup_20140627	CYTOMETER CONFIG CRE...	2007-01-02T12:00:00-08:00
Specimen Name:	PMT Setup	CST PERFORMANCE EXPI...	2014-06-24T11:57:03-07:00
Tube Name:	OneFlow Setup Bead_001	CST REGULATORY STAT...	CE-IVD Performance Check
Record Date:	Jun 23, 2014 3:13:44 PM	CST BEADS EXPIRED:	False
CYTOMETER CONFIG NA...	3-laser, 8-color (4-2H-2V) (B...		

Population	FITC-A Median	PE-A Median	PerCP-Cy5-5-A Median	PE-Cy7-A Median
P2	10,654	12,196	47,223	22,513

Population	APC-A Median	APC-H7-A Median	V450-A Median	V500-A Median
P2	58,579	132,245	9,751	24,481

Note: You should adjust the PMTVs so that they are as close as possible to the TMFI value in the center column of the monthly MFI target range card. MFI target values are lot-specific. Ensure that the lot number on the monthly MFI target range card is the same as the lot number on the vial of

BD OneFlow Setup beads that you are using. The numbers in the image are examples and are not to be used.


BD OneFlow™ Setup Beads (Monthly)

REF LOT

Fluorophore	Min (-2%)	TMFI	Max (+2%)
FITC	10397	10610	10822
PE	11896	12139	12382
PERCP-CY5.5	46584	47535	48486
PE-CY7	22194	22647	23100
APC	57164	58331	59497
APC-H7	129387	132028	134668
V450	9639	9835	10032
V500-C	24076	24568	25059

Monthly Target Ranges 23-16178-00

9. If needed, increase the size of the **P2** gate to ensure that the singlet bead population remains within the gate while adjusting the PMTVs.
10. Click **Record Data** in the **Acquisition Dashboard**.
11. Verify that the values are within the ranges on the monthly MFI target range card.

Adjusting the FSC and SSC voltages

Adjusting scatter voltages for cells

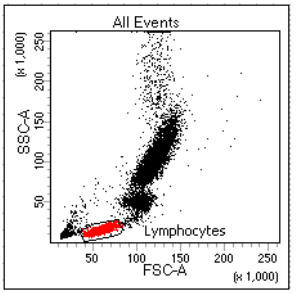
Use the normal LWB sample that you prepared for this procedure.

1. Acquire the *LWB* tube.
   a. In the **Browser**, set the current tube pointer to the tube Lysed Washed Blood.
   b. In the **Acquisition Dashboard**, confirm that **Events To Record** is set to 10,000 total events.
   c. Vortex the tube for 3–5 seconds.
   d. Install the tube on the cytometer.
   e. In the **Acquisition Dashboard**, confirm that the flow rate is set to **Low**, and click **Acquire Data**.

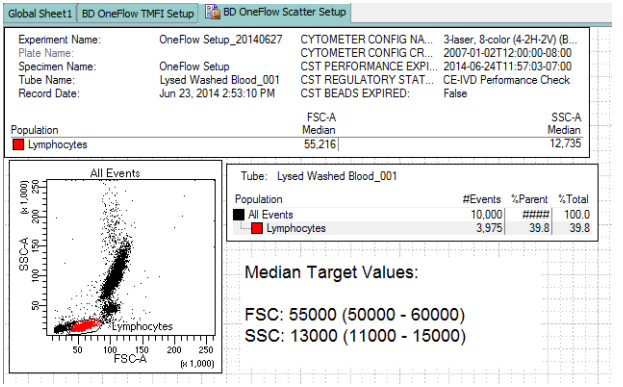
2. In the **Cytometer** window, select the **Parameters** tab and lower the voltages for FSC and SSC so that the lymphocyte population is on scale.

3. In the **Cytometer** window, navigate to the **Threshold** tab and set the FSC threshold to 10,000.

4. Adjust the lymphocyte gate to encompass the entire lymphocyte population in the FSC vs SSC dot plot.



5. Adjust FSC and SSC voltages to place the lymphocyte population within the FSC-A and SSC-A target ranges given on the Scatter Setup worksheet.



Note: If necessary, click **Cytometer Settings** in the **Browser** to display voltages in the **Inspector** window.

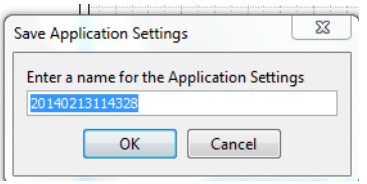
6. If needed, re-adjust the lymphocyte gate.

7. Click **Record Data** in the **Acquisition Dashboard**.
8. Verify that the values are within the target ranges on the Scatter Setup worksheet.

Saving application settings

1. In the **Browser**, right-click **Cytometer Settings**.
2. From the drop-down menu, select **Application Settings > Save**.
3. Click **OK**.

   Note: Save the application settings using the default name. Do not rename the application settings.



   Note: The saved voltages should reflect those established with the BD OneFlow Setup beads and lysed washed blood in the above steps.

4. When prompted, click **Yes** to maintain the modified threshold values.

Calculating compensation

About compensation

The emission spectra of the fluorochromes exhibit spectral overlap. BD FC beads enable BD FACSDiva software to calculate spillover values (SOVs) for a fluorescence compensation matrix to correct for the spectral overlap in other detector channels.

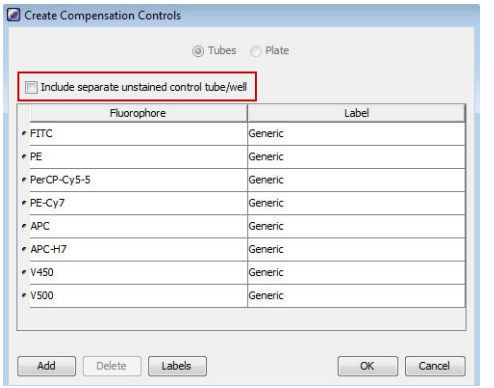
Calculating compensation

1. Create compensation controls.
   a. From the menu bar, select **Experiment > Compensation Setup > Create Compensation Controls**.

      The **Create Compensation Controls** dialog opens.

   b. Clear the **Include separate unstained control tube/well** checkbox.

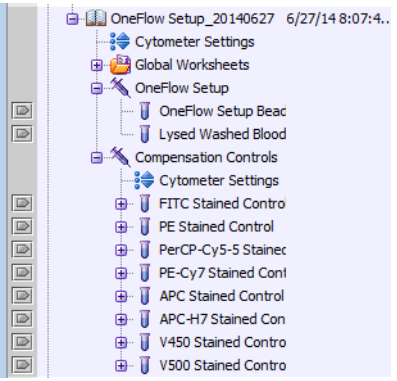
   c. Create generic (not label-specific) compensation controls for FITC, PE, PerCP-Cy5.5, PE-Cy7, APC, APC-H7, V450, and V500.



   d. Click **OK**.

2. Acquire the compensation controls.
   a. In the **Browser**, expand the **Compensation Controls** specimen.

b. Set the current tube pointer to the **FITC Stained Control** tube.



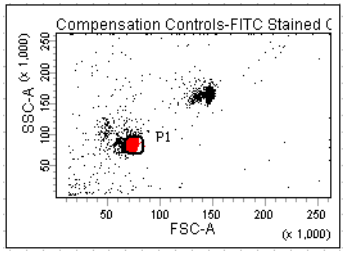
c. In the **Acquisition Dashboard**, confirm that **Events To Record** is set to 5,000 total events.

d. In the **Acquisition Dashboard**, adjust the flow rate to **Medium**.

e. Vortex the BD FC beads FITC tube for 3–5 seconds to mix well.

f. Install the tube on the cytometer. Click **Acquire Data.**

g. Click the **P1** gate in the FSC-A vs SSC-A dot plot and adjust the gate to fully encompass the singlet bead population.



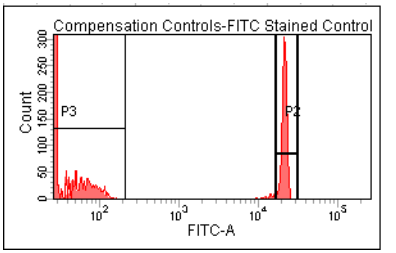
Note: If the singlet bead population is not resolved, increase the FSC voltage until the singlet bead population is discernible.

h. Right-click the **P1** gate border and select **Apply to all compensation controls**.

i. Click **Record Data** in the **Acquisition Dashboard**.

j. Verify that the **P2** interval gate encompasses the FITC-positive population.

k. Add a **P3** interval gate to the histogram and ensure that it encompasses the negative population. Verify that the left side of the **P3** interval gate starts at the y-axis.



l. Set the current tube pointer to the next tube.

m. Repeat steps 2e–2l for the remaining BD FC beads fluorochrome tubes.

Note: Acquire the compensation tubes in the order listed in the **Browser**.

3. Calculate compensation.

a. From the menu bar, select **Experiment > Compensation Setup > Calculate Compensation**.

b. Name the compensation matrix, for example, with OneFlow appended to the default name.

Note: This will help you distinguish this compensation matrix from compensation matrices created for other purposes.

c. Select **Link and Save**.

4. Print the compensation matrix.
    a. In the **Browser**, click **Cytometer Settings**.
    b. In the **Inspector**, navigate to the **Compensation** tab and click **Print**.

Next step

The cytometer is ready to acquire stained patient samples. See the BD OneFlow Application Guide or the IFU for the multicolor tube that you are using.

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3

Monthly setup

This chapter covers the following topics:

Running a performance check

1. Turn on the cytometer.
2. Check that all fluid levels are appropriate.
3. Open BD FACSDiva software v.8.0.1, or later.
4. Perform fluidics startup.

   From the menu bar, select **Cytometer > Fluidics Startup**. Click **OK** when prompted.
5. Allow the cytometer to warm up for at least 15 minutes.
6. Run the CS&T performance check. See Running a performance check (page 14) for the procedure.

Creating application settings

Prepare the setup reagents, LWB and BD FC beads, as described in Preparing the setup reagents (page 19).

Prepare the BD OneFlow Setup beads as follows:

1. Label a 12 × 75 mm polystyrene tube *Setup Beads*.
2. Thoroughly mix the BD OneFlow Setup beads vial.
3. Add 1 drop of beads to 350 µL of BD FACSFlow. Protect from light.
4. Proceed to acquisition or store tubes appropriately until acquired.

   Note: If not acquiring immediately, store the diluted beads, protected from light, for up to:

   - 1 hour at 18°C–25°C
   - 8 hours at 2°C–8°C

5. Adjust the PMTVs as described in Adjusting fluorescent PMT voltages (page 26).
6. Adjust the FSC and SSC voltages as described in Adjusting the FSC and SSC voltages (page 29).
7. Save the application settings as described in Saving application settings (page 31)

Calculating compensation

Calculate the SOV matrix for fluorescence compensation whenever you create new application settings. See Calculating compensation (page 32) for the procedure.

Next step

The cytometer is ready to acquire stained patient samples. See the BD OneFlow Application Guide or the IFU for the multicolor tube that you are using.

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4

Daily setup

This chapter covers the following topics:

Running a performance check

1. Turn on the cytometer.
2. Check that all fluid levels are appropriate.
3. Open BD FACSDiva software v.8.0.1, or later.
4. Perform fluidics startup.

   From the menu bar, select **Cytometer > Fluidics Startup**. Click **OK** when prompted.
5. Allow the cytometer to warm up for at least 15 minutes.
6. Run the CS&T performance check. See Running a performance check (page 14) for the procedure.

Confirming PMT voltages

Recommended

Cytometer performance is checked on a daily basis in the CST module. The saved application settings are automatically updated to align with the cytometer performance and can be applied each day. You can check that the updated PMT voltages in the BD OneFlow application settings still fall within the target ranges (+/-15% of the target values set monthly). Use the daily MFI target range card to do this.

Confirming PMTVs

1. Prepare the BD OneFlow Setup beads as described in Creating application settings (page 38).

   Note: Verify that the lot number on the daily MFI target range card matches the lot number on the vial of BD OneFlow Setup beads that you are using.

2. Create a new experiment.

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

      Note: Alternatively, create a new experiment using the **Experiment** icon in the **Browser**.

   b. If prompted by the **CST Mismatch** window, select **Use CST Settings**.

   c. Rename the experiment, for example, as *Daily PMT check_today's date*.

3. Link compensation.

   a. In the **Browser**, right-click **Cytometer Settings.**

   b. From the drop-down menu, select **Link Setup**.

   c. Select the appropriate compensation matrix created using BD FC beads within the past 31 days.

   d. If prompted by the **Cytometer Settings Mismatch** window, select **Overwrite**.

4. Unlink compensation.

   a. In the **Browser**, right-click **Cytometer Settings**.

   b. From the drop-down menu, select **Unlink From** the previously linked compensation setup.

   c. A **Confirm** dialog opens. Click **OK** to unlink from the previously linked compensation setup.

5. Apply application settings.

   a. In the **Browser**, right-click **Cytometer Settings**.

   b. From the drop-down menu, select **Application Settings > Apply**.

   c. Select the most recent Application Settings created within the past 31 days using the BD OneFlow Setup beads. Click **Apply**.

d. When prompted by the **Confirm** dialog, select **Keep the compensation value**.

e. If prompted by the **Confirm Cytometer Changes** dialog, click **Yes** to overwrite the cytometer values for **FSC Area Scaling**.

6. Import the OneFlow Setup template.

   a. From the menu bar, select **Experiment > New Specimen**

   b. The **Panel Templates** dialog opens. Navigate to the **BD Panels** tab and select the OneFlow Setup template. Click **OK**.

   c. In the **Browser**, expand the specimen to see the OneFlow Setup Bead tube and the Lysed Washed Blood tube.

      Note: You will not use the Lysed Washed Blood tube when confirming the PMTVs.

7. In the **Browser**, click **Application Settings**.

8. In the **Inspector**, navigate to the **Parameters** tab and ensure that FSC-A, FSC-H, SSC-A, and SSC-H, as well as "A" for all fluorescent parameters, are selected.

   Note: If necessary, activate a parameter by selecting the checkbox next to it.

9. In the **Inspector**, navigate to the **Compensation** tab and deselect the **Enable Compensation** checkbox.

10. Gently vortex the tube of diluted BD OneFlow Setup beads.

11. Set the current tube pointer to the OneFlow Setup Bead tube.

12. Install the tube on the cytometer.

13. In the **Acquisition Dashboard**, adjust the flow rate to **Low**. Click **Acquire Data**.

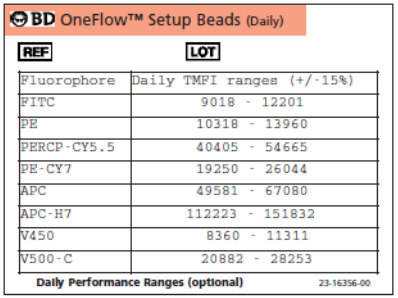
    Note: It may take 10–15 seconds before events start to appear.

14. In the TMFI Setup worksheet, adjust the **P1** gate in the FSC-A vs SSC-A dot plot to include only the singlet bead population.

Note: Enlarge the FSC-A vs SSC-A dot plot to see the singlet bead population more clearly.

Note: If necessary, increase FSC and SSC voltages to see the bead population.

15. In the TMFI Setup worksheet, adjust the **P2** gate in the FITC-A vs PE-A dot plot to include only the singlet bead population.
16. In the **Acquisition Dashboard**, set **Events to Record** to 5,000. Click **Record Data**.
17. Confirm that the MFI of the bead population in the **P2** gate falls within the target ranges found on the BD OneFlow Setup beads daily MFI target range card.



BD OneFlow™ Setup Beads (Daily)

REF LOT

Fluorophore	Daily TMFI ranges (+/-15%)
FITC	9018 - 12201
PE	10318 - 13960
PERCP-CY5.5	40405 - 54665
PE-CY7	19250 - 26044
APC	49581 - 67080
APC-H7	112223 - 151832
V450	8360 - 11311
V500-C	20882 - 28253

Daily Performance Ranges (optional) 23-16356-00

Note: MFI target values are lot-specific. Ensure that the lot number on the daily MFI target range card is the same as the lot number on the vial of BD OneFlow Setup beads that you are using. The numbers in the image are examples and are not to be used.

Next step

If the MFIs for all of the fluorochromes are within the target ranges, then the cytometer is ready to acquire stained patient samples. See the BD OneFlow Application Guide or the IFU for the multicolor tube that you are using.

Note: If the MFI for any fluorochrome is not within the target range, troubleshoot the cytometer before continuing. See the

Troubleshooting section, Failure to achieve target MFIs (page 51) for more information.

5

Troubleshooting

This chapter covers the following topics:

Problems running the performance check using CS&T IVD beads

Problem	Possible causes	Solution
No beads were detected.	Beads not mixed prior to diluting, beads too dilute, debris in the beads suspension, incorrect beads used.	Vortex the beads vial, prepare a fresh suspension of beads, and re-run the performance check.
	There are air bubbles in the flow cell or sheath filter.	Check the fluidics for bubbles and debris. See the cytometer IFU for more information.
	There are clogs in the sample line.	Check the fluidics for clogs and debris. See the cytometer IFU for more information.
	Backpressure in the waste lines is too high.	Check the waste tank vent for obstructions. See the cytometer IFU for more information.
	There is high scatter noise (FSC or SSC).	Perform monthly maintenance. See the cytometer IFU for more information. Call BD Biosciences.
Performance check was completed with warnings.	There was a relative change in the performance of the cytometer.	Review the Cytometer Performance Report to determine whether the specific warnings impact the experiment, then continue.
		Prepare a fresh suspension of beads and re-run the performance check.
		Perform monthly cleaning procedure. See the cytometer IFU for more information.

Problem	Possible causes	Solution
Performance check failed.	The rCV ratio of dim to mid beads is less than 1.5.	Prepare a fresh suspension of beads and re-run the performance check.
		Perform monthly cleaning procedure. See the cytometer IFU for more information.
	Value(s) for any of the measurements used to check the cytometer performance are out of specifications.	Prepare a fresh suspension of beads and re-run the performance check.
		Perform monthly cleaning procedure. See the cytometer IFU for more information. Call BD Biosciences.

Problems using the BD OneFlow Setup beads

Problem	Possible Causes	Solution
No beads were detected.	Beads were not mixed prior to diluting, beads were too dilute, debris was in the beads suspension, incorrect beads were used.	Vortex the beads vial, prepare a fresh suspension of beads, and re-run the performance check.
	There are air bubbles in the flow cell or sheath filter.	Check the fluidics for bubbles and debris. See the cytometer IFU for more information.
	There are clogs in the sample line.	Check the fluidics for clogs and debris. See the cytometer IFU for more information.
	Backpressure in the waste lines is too high.	Check the waste tank vent for obstructions. See the cytometer IFU for more information.
	There is high scatter noise (FSC or SSC).	Perform monthly maintenance. See the cytometer IFU for more information. Call BD Biosciences.
	FSC threshold is set too high.	Lower the FSC threshold.
	FSC and SSC PMTVs are not optimized.	Optimize FSC and SSC PMTVs.

Problem	Possible Causes	Solution
Could not achieve target MFIs.	The cytometer requires cleaning.	Clean the cytometer. See Failure to achieve target MFIs (page 51).
	Compensation settings might be applied.	Deselect **Enable Compensation** in the **Inspector**.
Some of the dot plots are grayed out.	FSC-H and SSC-H were not selected when the application settings were created.	Check whether the FSC-H and SSC-H are selected on the **Parameters** tab of the **Inspector**.

Failure to achieve target MFIs

1. From the menu bar, select **Cytometer** > **Cleaning Modes** > **Clean Flow Cell**.
   a. Clean the flow cell using FACSClean.
   b. Clean the flow cell using deionized water.
2. From the menu bar, select **Cytometer** > **Cleaning Modes** > **Degas Flow Cell** to drain and refill the flow cell.
3. Prepare a fresh dilution of the BD OneFlow Setup beads and repeat Creating application settings (page 38) or Confirming PMT voltages (page 42), as appropriate.
4. If MFI target values are reached, troubleshooting is complete.
5. If MFI target values are not reached, visually inspect the flow cell for bubbles.
6. If the MFI target values for only the violet or red laser detectors are off, the laser delay may require adjusting. Call BD Biosciences.

Problems setting up the scatter (FSC and SSC) voltages

Problem	Possible causes	Solution
No cells were detected in the lysed, washed blood sample.	Cell concentration in prepared samples is too low.	Prepare a new sample.
	There are air bubbles in the flow cell or sheath filter.	Check the fluidics for bubbles and debris. See the cytometer IFU for more information.
	There are clogs in the sample line.	Check the fluidics for clogs and debris. See the cytometer IFU for more information.
	Backpressure in the waste lines is too high.	Check the waste tank vent for obstructions. See the cytometer IFU for more information.
	There is high scatter noise (FSC or SSC).	Perform monthly maintenance. See the cytometer IFU for more information. Call BD Biosciences.
	FSC threshold is set too high.	Optimize FSC and SSC PMTVs

Problems using BD FC beads

Problem	Possible causes	Solution
Calculation of compensation was not successful.	Gates are not properly adjusted.	Adjust the gates to include the appropriate bead populations and then recalculate compensation.
	BD FC Beads are expired.	Prepare new bead tubes from a current lot, then recalculate compensation.
	Rehydrated bead tubes are exposed to light or used beyond the stability period.	Prepare new bead tubes, then recalculate compensation.
	There is a problem with the cytometer fluidics.	Check cytometer fluidics for bubbles or debris. See cytometer IFU for more information.

Problem	Possible causes	Solution
No beads were detected.	Pouch was not resealed properly.	Open a new pouch, or use tubes from a pouch that was resealed properly.
	There are air bubbles in the flow cell or sheath filter.	Check the fluidics for bubbles and debris. See the cytometer IFU for more information.
	There are clogs in the sample line.	Check the fluidics for clogs and debris. See the cytometer IFU for more information.
	Backpressure in the waste lines is too high.	Check the waste tank vent for obstructions. See the cytometer IFU for more information.
	There is high scatter noise (FSC or SSC).	Perform monthly maintenance. See the cytometer IFU for more information. Call BD Biosciences.
	FSC threshold is set too high.	Lower the FSC threshold.
	FSC and SSC PMTVs are not optimized.	Optimize FSC and SSC PMTVs.

Templates did not import correctly

You may observe that templates do not import correctly. For example, there might not be dot plots in the global worksheet, or the plots from the wrong worksheet appear when you import a panel template.

If you suspect that the templates did not import correctly:

1. Close the current experiment.
2. Create a new experiment.
3. Repeat any portions of the workflow that you performed in the first experiment prior to the template failing to import correctly.
4. Report the issue to BD Biosciences.

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