



STEM CELL LABORATORY (STCL)



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Quality Control for BD FACSCalibur Flow Cytometer

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FLOW-GEN-014

QUALITY CONTROL FOR BD FACSCalibur™ FLOW CYTOMETER

1 PURPOSE

- 1.1 This procedure is to be used by Stem Cell Laboratory staff when using BD CaliBRITE™ beads and BD FACScomp™ software for the daily monitoring of BD FACSCalibur™ flow cytometer instrument performance, stability, and sensitivity operated in the Stem Cell Laboratory.

2 INTRODUCTION

- 2.1 The BD FACScomp™ software will automatically adjust the detector voltages and compensation for the cytometer and measures the separation between signal and noise for each parameter. This separation value is compared to an expected minimum separation value and a pass/fail message is displayed. Combined with daily instrument status checks, this process enables the user to monitor the performance of the instrument and determine if the instrument is ready for use. If the instrument fails to pass the quality control measures, it must be removed from service until the failure is resolved.

3 SCOPE AND RESPONSIBILITIES

- 3.1 This procedure is designed to be used by trained Stem Cell Laboratory staff on each day that flow cytometric testing is performed using the BD FACSCalibur Flow cytometers operated in the Stem Cell Laboratory. It is the responsibility of the STCL director, manager, and flow cytometry staff to ensure that the requirements of this procedure are successfully met.

4 DEFINITIONS/ACRONYMS

- | | | |
|-----|-------|---------------------------------------|
| 4.1 | BD | Becton Dickinson |
| 4.2 | MSDS | Material Safety Data Sheet |
| 4.3 | PE | Phycoerytherin |
| 4.4 | FITC | Fluorescein isothiocyanate |
| 4.5 | PerCP | Peridinin Chlorophyll Protein Complex |
| 4.6 | APC | Allophycocyanin |
| 4.7 | mL | Milliliter |
| 4.8 | ID | Identification |
| 4.9 | STCL | Stem Cell Laboratory |

5 MATERIALS

- 5.1 12x75 mm polystyrene test tubes
- 5.2 BD CaliBRITE™ 3 beads
- 5.3 BD CaliBRITE™ APC beads

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Durham, NC

- 5.4 BD FACSflow™ sheath fluid (keep ~25mls chilled at 2-8 degrees)
- 5.5 10% Bleach solution,
- 5.6 Household bleach, Clorox

6 EQUIPMENT

- 6.1 BD FACSComp™ software
- 6.2 BD FACSCalibur™ flow cytometer
- 6.3 12x75 mm polystyrene test tubes, BD Falcon
- 6.4 Vortex mixer

7 SAFETY

- 7.1 Review MSDS for BD CaliBRITE 3 and APC beads.
- 7.2 Use universal precautions and wear appropriate personal protective equipment (PPE) when working with biohazardous materials.

8 PROCEDURE

- 8.1 Turn on the FACSCalibur instrument to be used by pushing the green flipper switch on the lower right side of the instrument.
- 8.2 Turn on the workstation computer by pushing the ON button on the front panel of the computer terminal (just above the USB ports).
- 8.3 Look in the fluidics drawer located on the front left of the instrument. Check the sheath fluid level and the waste reservoir to determine if refill or empty is required.
- 8.4 In order to refill the sheath or empty the waste reservoir follow the instructions provided on pages 55-59 of the BD FACSCalibur instructions for Use (Part No. 643271 Rev. A, November 2007).
- 8.5 If the level is near or at the refill line drawn on the reservoir, the sheath reservoir should be taken to the open “In Use” cube of FACSFlow (sheath fluid) and filled with BD FACSFlow. **Do NOT fill past the designated fill line.**
- 8.6 The waste reservoir must be emptied in a sink designated for waste.
- 8.7 Add 400 milliliters of undiluted (Clorox) bleach to the emptied waste reservoir using the designated “Bleach” beaker at the waste sink area.
- 8.8 If the fluids are ok, make sure the black pressure switch located inside the fluid tank drawer between the tanks is closed (down as you face it) and that the sheath tank is pressurized. **NOTE: The black sleeve around the tank won’t move if the tank is pressurized.**
- 8.9 Fill a 12x75 mm polystyrene tube with about 2 mls of 10% bleach solution and place the tube on the instrument aspirator by swinging the tube holder arm to the right or left, placing the tube in position with the aspiration probe inside and seated at the top against the tube bal seal.

NOTE: If using an instrument with an automatic loader, this action will happen automatically when the tube rack is put in place and the loader cover is installed. A manual control pad located just to the right of the instrument allows the user to raise and lower the tube into place on the aspirator.

- 8.10 Place the instrument in Run mode High and allow the bleach solution to run about 5 minutes.
- 8.11 Follow this run using a tube with 2 mls of DI water for about 5 minutes.
- 8.12 At the end of this “clean” step, while the DI water tube is still on the cytometer, push the prime button.

NOTE: The prime action will take about 10 seconds after which time the machine automatically goes into STANDBY mode. Priming will help clear any air from the sample stream that might affect the movement of cells/beads through the flow cell.

- 8.13 Allow the instrument to warm up no less than 10 minutes (inclusive of the cleaning steps) prior to beginning FACSComp QC.
- 8.14 During the instrument warm up, label two 12 x 75 mm tubes 1 and 2.
- 8.15 To tube 1, add about 1.0 ml chilled sheath fluid.
- 8.16 To tube 2, add about 3 ml chilled sheath fluid.
- 8.17 Remove the current lot BD CaliBRITE™ 3 and APC beads from the refrigerator and mix bead vials by inversion back and forth.
- 8.18 Add 1 cloudy drop of unlabeled bead (white top vial) to each tube.
- 8.19 Add 1 cloudy drop of APC bead (aqua top vial) to each tube. Set tube 1 aside.
- 8.20 Add 1 cloudy drop of each of the remaining beads (PE red top, Fite green top, PerCP pink top) to tube 2.

NOTE: Once mixed, these two tube preparations may be split into 2 separate tubes to test performance of a second flow cytometer simultaneously.

- 8.21 Place tube 1 with tube 2 in order on the loader rack, if using automated mode. If using the manual mode, the tubes will be loaded manually and tubes 1 and 2 will be put on the sip when prompted.
- 8.22 Launch the FACSComp™ software from the “dock” on the desktop of the computer.
- 8.23 Go to the Cytometer menu and scroll to the STATUS menu. A window opens and the user can check the instrument status. Refer to the Maintenance log sheet in the instrument log book for acceptable limits and to record the following values:
 - Laser current
 - The sample voltage in Run HI mode with tube on the sip
 - The sample voltage in Run LO mode with tube on the sip
 - The sample voltage in Run with no tube on the sip.

NOTE: If any of the values are out of range or are drastically (>1) changed from the previous day's values, alert the flow cytometry supervisor, as this could indicate a problem with the fluidics or laser and may require instrument service.

- 8.24 If the above check is within acceptable limits, the user may proceed with instrument quality control testing.
- 8.25 Enter operator name on page 1 of FACSComp™ and mouse click Accept.
- 8.26 Proceed to page 2. Verify individual bead lot IDs, choose the appropriate setup option (i.e. Lyse/Wash, Lyse/No Wash, or both).

NOTE: Monday – Friday setup both options. On weekend days it may only be necessary to setup the Lyse/No Wash option.

- 8.27 If testing a new lot of beads; enter the new lot number and expiration date on the back of the Flow Cytometry Instrument QC Log.

NOTE: Typically a new lot is tested prior to the current lot so that the final saved instrument setup values will reflect the current in use lots. When the new lot is placed in use, record the date on this page in the designated space.

- 8.28 When target values need to be adjusted go to the FACSComp drop down menu and open EDIT TARGET VALUES.
- 8.29 Adjust the appropriate value and mouse click on OK.

NOTE: Each lot of APC Calibrite beads has its own target value assigned. The Calibrite3 Bead target values will only be changed when the current batch changes, which is infrequent. There will be a notification included in the box when this occurs.

- 8.30 With the instrument in RUN-HI mode, mouse click in the RUN box.
- 8.31 The run window appears with a START box at the bottom. Mouse click the START box to begin the automated FACSComp acquisition.
- 8.32 After the voltage settings are adjusted and time delay is checked, the software will prompt the user to load tube 2.

NOTE: This process is automated when using an instrument with a loader and rack carousel. See instructions for using the loader carousel on page 160 of the BD FACSCalibur Instructions for Use.

- 8.33 FACSComp™ will continue through completion of calibration and display the results.
- 8.34 If errors occur during the course of the run an alarm chime will sound to alert the user to the problem. Inform the flow supervisor if minor troubleshooting does not correct the underlying cause of the alarm.
- 8.35 Repeat these steps using the second setup option if required.
- 8.36 Enter the results on the appropriate FACSComp™ result log.
- 8.37 Print these results and place the hard copy in the instrument log book.

- 8.38 As needed, optimize the FACSCComp instrument settings. The threshold, FSC detector amplifier gains, and compensation settings will require optimization depending on the staining and acquisition procedures used.
- 8.39 If at any time during this process technical problems are encountered that minor troubleshooting will not correct or for which the technologist is unable to determine cause, the flow supervisor or lab manager should be notified so that corrective action may be undertaken.
- 8.40 Test samples must not be performed on an instrument that does not pass this quality control testing.

9 RELATED DOCUMENTS/FORMS

- 9.1 FLOW-FORM-006 BD FACSCalibur Flow Cytometer Maintenance Log
- 9.2 FLOW-FORM-007 Flow Cytometry Instrument QC Log

10 REFERENCES

- 10.1 BD CaliBRITE™ Bead product insert
- 10.2 BD FACSCalibur instructions for Use (Part No. 643271 Rev. A, November 2007)

11 REVISION HISTORY

| Revision No. | Author | Description of Change(s) |
|--------------|---------------|---|
| 05 | Melissa Reese | Removed reference to Activate bleach solution. Added comment in materials to refrigerate a small amount of FACSFlow. |

Signature Manifest**Document Number:** FLOW-GEN-014**Revision:** 05**Title:** Quality Control for BD FACSCalibur Flow Cytometer

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FLOW-GEN-014 Quality Control for BD FACSCalibur Flow Cytometer**Author**

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