YALE-NEW HAVEN HOSPITAL	TITLE: Facscanto II Instrument Set-up		DEPT OF LAB MEDICINE – Flow Cytometry Policy and Procedure Manual DOCUMENT # FLW-17 Page 1 of 5
WRITTEN BY: **Denise Scinto**	**EFFECTIVE DATE:** **July 1, 2009**	**REVISION:** **Aug, 2012 by Teodorico Lee**	**SUPERCEDES:**

Principle:

After instrument Startup each day, the Facscanto II operator will run both the BD Facs 7-color beads using the Facscanto Clinical Software as well as the CS & T beads using the FacsDiva Software to ensure automated setup and instrument QC. During setup, detector voltages are adjusted to place setup beads at defined target values, sensitivity values are measured, and spectral overlap values are calculated and applied to compensate data for fluorescence spillover. The Levey-Jennings feature in both the Canto and Diva software can automatically track cytometer setup values over time. These values can be used to monitor cytometer performance over time and see shifts or trends in parameters as they occur. Setup should be run every 24 hours. The software tracks the time between setups and displays it in the status window. Any Setup age >24 hours will appear in red. A successful setup resets the timer. This Procedure also monitors **optical alignment** and Instrument reproducibility. It ensures acceptable and **constant laser current** using software displays and alerts when laser current exceeds acceptable limits.

Materials and Reagent:

1. Facs 7 Color Bead Setup Beads: Stored at 2-8° C until kit expiration.
2. CS&T Beads: Stored at 2-8° C until bead expiration.
3. Facsflow Sheath Diluent.
4. 12 x 75 mm test tubes
5. (1) BD Facsloader Carousel
6. Pipet: 500 uL
7. Vortex

Procedure: BD Facs 7-color Bead Setup

1. Remove (1) foil pack of BD 7 color beads from kit.
2. Add BD Facs 7-color setup bead diluent up to the fill line marked on the tube.
3. Vortex gently.
4. Open BD Facscanto Clinical Software and select Cytometer>Setup>Standard Setup to open the Cytometer Setup Wizard.
5. Select the current bead lot from the Lot ID Menu.
6. Check lot ID, Targets, and spectral overlap factors in the setup lot information window against the BD Facs 7-color setup bead label.
7. If necessary, enter new values manually or with barcode reader.
8. Barcode reader method:

- Click Scan barcodes in the Setup Lot Info window of the Wizard.
- The Scan barcode dialog appears.
- Locate the barcode on the Setup Bead Label.
- Hold and aim the barcode reader at the center of the barcode.
- Press and hold the trigger until you hear a beep.
- Check software entries for accuracy.

9. Manual method:
    - Click New Lot ID in the Setup Lot Info window of the Wizard.
    - Select the bead product, enter lot ID and the exp.date from the label
    - Click OK.
    - Enter the target values of the bead lot.
    - Click the spectral overlap tab and enter spectral overlap factors for the bead lot.
    - Check all values for accuracy, and click next to proceed to loading bead tubes.
10. Vortex and Place the bead tube uncapped in position 1 of a carousel.
11. Prepare the cytometer for automatic loading. Move tube guide forward and place Aspirator bar horizontal.
12. Install the carousel onto the Loader (push all the way back) and close the doors shut tightly.
13. Clear the "Run Setup in Manual Mode" checkbox and click Next in the cytometer setup Wizard.
14. The software adjusts cytometer settings to place the beads on scale. The operator Will notice that there are (2) populations of cells and the beads will move to the baseline and back on scale during the process.
15. If set up is successful, click Finish to save results. View setup results/report. The report contains cytometer QC and Pass/Fail information. Print the report from this view.
16. If Setup is unsuccessful or values are out of range, do not click Finish. You can Cancel and Run Setup again. Refer to troubleshooting guide if Setup continues to Fail. Record out of Range findings on Instrument Problem Log and alert Supervisor.
17. Successful reports are automatically saved in C:\\ProgramFiles\BDFacscanto Software\SetupReports folder. Access this folder to reprint reports.
18. Review Levey Jennings Reports weekly to monitor setup data for trends.
19. From the main window, select the Levey-Jennings tab. An explanation Point (!) on the tab indicates an out-of–range value on a report.
20. Parameters outside the limits are shown with a red X in the affected plot.
21. To add Comments to the report, click Comments and enter text. Click OK and Print report.
22. Levey-Jennings Reports for 7-Color Bead Data is Printed and Reviewed Monthly by the Flow Cytometry Supervisor.

Procedure: Cytometer Setup and Tracking (CS&T) Bead Setup

1. Prepare CS&T beads by adding 2 drops of well mixed beads to 500 uL of FacsFlow Sheath fluid in a 12 x 75 mm tube. Vortex tube gently.

2. Open Facsdiva Software and select Cytometer CST. The cytometer will Disconnect from the Facsdiva interface and connects to CS&T.
3. The BD CS&T workspace appears. Verify that the cytometer configuraton Is set to 2 laser 6-color (4-2) APC-H7. Choose Cytometer>View Configurations to open window and view cytometer details.
4. Verify that the Load Tube Manually checkbox is cleared.
5. Verify that the information under Setup Beads matches the information on the cytometer Setup and Tracking beads. If needed, select the correct Lot ID from the list.
6. Click Run, and install a carousel with the bead tube in Position 1.
7. Click OK to confirm the carousel is installed. Plots are displayed in the CS&T Workspace and the performance check is run. This takes 5 minutes.
8. When performance check is complete, a dialog appears. Remove carousel from the Loader.
9. Setup completed successfully will appear. Click View Report.
10. Select File and Print to print a copy of the report.
11. Verify that the performance has passed. In the Setup Tab, there should be a green check box displayed and a Passed next to it.
12. Refer to the BD Cytometer Setup and Tracking Guide if any parameter did not pass, and/or rerun CS&T beads. Record results that did not pass onto Instrument Problem Log and alert Supervisor of findings.
13. Performance Check settings are automatically entered into Levey-Jennings plots for performance tracking. Weekly Review by Supervisor is required to monitor shifts and trends. Reports are printed Monthly and signed by supervisor for Review.
14. Select File>Exit to close the CS&T workspace and reconnect to the BD FAcsDiva interface.
15. If the CST Mismatch dialog is displayed select "Don't show this message" and click use CST settings.

Baseline Cytometer Performance:

1. Whenever a new lot of BD CS&T beads are used, or a new cytometer Configuration is created, a new baseline must be defined. Algorithms within BD Facsdiva software differentiate fluorescent signals from the cyotmeter Setup and Tracking beads to calculate and report measurements.
2. Baseline CS&T can only be performed by the Administrator assigned to the Canto instrument.
3. Custom Configurations can only be created by the Administrator.

References:

BD Facscanto II Instruction Manual
BD Biosciences, San Jose CA
July 2007

BD Facscanto II Training Manual
BD Biosciences, San Jose CA
January 2009

BD Cytometer Setup and Tracking Application Guide
BD Biosciences, San Jose CA
June 2007

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