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| Analyzing the Performance Setup on the BD FACSCanto II |
| **Purpose** | The 7-color setup is intended to validate the performance of the lasers and detectors of the FACSCanto II cytometer in FACSCanto clinical software mode, used for single platform analysis of MultiTEST samples.CS&T (Cytometer Setup and Tracking) is intended to validate cytometer performance in FACSDiva software mode, used for analyzing leukemia/lymphoma panels and dual platform immune status panels.Daily performance checks verify cytometer function and allow monitoring of instrument trends over time. |
| **Policy Statements** | Applies to Becton Dickinson FACSCanto II Flow Cytometer and technologist analyzing flow cytometry specimens. |
| **Principle and Clinical Significance** | 7-Color Setup beads are used to run daily performance checks for lyse/no wash single platform analysis in FACSCanto software mode. Fluorophore labeled beads are used by the software to adjust voltages, correct for spectral overlap (compensation), and monitor instrument performance.CS&T beads are used to run performance checks in FACSDiva software mode. Bright, mid and dim fluorophore labeled beads are used by the CS&T program to measure variation from the defined baseline. Laser delays, PMT voltages and area scaling factors are adjusted accordingly.  |
| **Materials** |

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| **Supplies** | **Equipment** |
| BD FACS 7 – Color Setup Bead KitBD CS&T Setup Beads12 x 75 polystyrene Falcon tubesPhosphate buffered saline (PBS) | BD FACSCanto II Flow Cytometer |

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|  | **FACSCanto 7-Color Setup:** |
| **Procedure** | **Step** | Action | **Related Document** |
|  | 1 | Prepare BD FACS 7 – Color set up beads. • Open foil pouch • Add BD FACS setup bead diluent to fill line marked on tube • Vortex gentlyNote: After resuspension in BD FACS setup bead diluent, a bead tube from the BD FACS 7- color setup beads is stable for 8 hours at 2°-8° C or 1 hour at 20°to 25°C. Keep the resuspended bead tube protected from light. |  |
|  | 2 | In FACSCanto software, select Cytometer > Setup > Standard Setup. The Cytometry Setup Wizard appears. |  |
|  | 3 | Place tube in position 1 of the carousel. Alternatively, if running manually, install the tube on the SIT and check the “Load Tube Manually” box. |  |
|  | 4 | Select the current bead lot from the Lot ID and click Next. Lot ID’s can be found on the foil pouch, box or sticker.  |  |
|  | 5 | Performance check will run. When complete, review setup report. If the setup report indicates that any parameters have failed, select Cancel or Back. Click Yes in the Confirm Cancel dialog to discard the current results and revert the cytometer settings to the previous values. Troubleshooting strategies for possible errors during cytometer setup can be found in the BD FACSCanto II Clinical Users Guide or Software Help. After troubleshooting, rerun the cytometer setup. |  |
|  | 6 | Review Levey-Jennings plot under Levey-Jennings tab. Plots are printed at the end of each month and filed with the month’s setup result printouts. |  |
|  | **FACSDiva CS&T Setup Beads:** |
|  | **Step** | Action | **Related Document** |
|  | 1 | In FACSDiva software, select Cytometer > CST. The Cytometer Setup and Tracking program will open. |  |
|  | 2 | Prepare CS&T beads by adding 1 drop of well mixed beads to a Falcon tube. Dilute with 350 µl PBS and vortex to mix.Note: Diluted beads are stable 24 hours at 2°-8° C or 8 hours at 15°- 20° C. Store protected from light.  |  |
|  | 3 | In the Setup Control window, use the drop down box in the Characterize menu to select Check Performance. Verify the bead lot ID. |  |
|  | 4 | Load the bead tube in position 1 of the auto loader carousel. Alternatively, install the tube on the SIT and check “Load Tube Manually.” Click Run. |  |
|  | 5 | Review performance results when run is finished. See BD Cytometer Setup and Tracking Application Guide for troubleshooting information. Rerun performance check after troubleshooting to obtain a valid setup. Note: Diva software does NOT prevent users from running experiments with a failed performance check.  |  |
|  | 6 | Review Levey-Jennings plot. Plots are printed at the end of each month and filed with the month’s setup result printouts.  |  |
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| **Procedure Notes** | 2 | Al Quigley | 03/31/2013 | Reformatted for CMS Web |
|  | Amanda McCaustland |  | Added CS&T, removed archiving |

 | Changing 7-Color Setup Bead Lots:* In the Cytometer Setup Wizard, click New Lot ID. Enter the targets and spectral overlap factors from the package insert. Click Next.

Changing CS&T Bead Lots:* Import bead data for the new lot from bdbiosciences.com. Save the .bls file to the Flow Lab folder on the G drive.
* In the CS&T workspace, select Tools > Bead Lots. Click "Import" and select the .bls file for the new lot. Click "Open" to import the bead lot into CS&T.
* Before the old lot of beads is used up, the new lot will need to be normalized to the old lot. Select "Reset Target Values" in CS&T and verify the Lot IDs for both the old and new lot. Prepare bead tubes for both lots according to the CS&T Research Beads technical data sheet and follow prompts to run.
* If target values are not reset before the old bead lot is used up, run a new baseline.

Running a Baseline:* In the CS&T workspace, select "Define Baseline."
* Prepare beads according to the CS&T Research Beads technical data sheet and follow prompts to run.

BD FACSCanto II Flow Cytometer-Training Manual 23-105300-00 Rev. A.BD Cytometer Setup and Tracking Application Guide 23-15134-00 |
| **References** |
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| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1 | Jim Berger | 10/01/2010 | Initial Version |
| 2 | Al Quigley | 03/31/2013 | Reformatted for CMS Web |
| 3 | Amanda McCaustland | 08/28/20 | Added CS&T, removed outdated archiving instructions, added lot change instructions |