CST Performance Assessment Beads

- CSTs should be run at least once a day (This is your only Instrument control unless you provide a sample control of your own).
- CSTs should be run for each nozzle on to be used on the sorter that day.

Procedure:

1. Prepare CSTs: Add 1 drop for 500μL PBS. (CST beads are in a black tube with a black lid, stored at 4°C)
2. After initial Analyzer prep (See appropriate SOP), find the Cytometer>CST Tab.
    a. For sorters: Mare sure you are in correct configuration and stream is stable without sweet spot on.
3. FACS diva will temporarily disconnect and initiate the CST program. Wait 1 minute for CST to connect. It sometimes pops up behind FACS diva so minimize the screen to see fully.
4. Find the tab in the right corner and click check performance. Install your tube of CST beads.
    a. LSRs: If running on LSR, **make sure fluids is set to RUN and LOW**
    b. Arias: The sorters will automatically load the tube once run is selected.
5. Allow the program to run beads. It should take approximately 2-5minutes.
6. Check the report and make sure the Robust Confidence Values (Robust CVs) are not too high. Instruments will often pass with warnings and that is ok.
7. Scroll to the bottom and check that the laser delays are not too different from the day before. If they are, it may indicate a bad run and you may have to clean the machine with bleach and water and rerun.

What to do if CSTs fail:

1. Clean the machine with 10min intervals of 10% bleach, 70% ethanol and water and retry CSTs. For LSRs, try to prime after cleaning to alleviate any air bubbles.
2. Prepare new beads. Beads kept at room temp or in the light will lose red signal rapidly.
3. Make sure the correct lot is highlighted on the CSTs that you are using (CST lots are located on the side of the bottle).
4. Assess the problem: If the CVs are tolerably high in your detection channels or if your channels of interest pass CST, the machine is likely ok to use. You should still contact the people below to let them know there were problems so BD can be contacted in the event a laser alignment is needed.

Adding Bead Lots to CST:

1. Download CST Bead lots from here : http://www.bdbiosciences.com/us/instruments/research/software/flow-cytometry-acquisition/bd-facsdiva-software/m/111112/resourcestools/beadlotfiles
2. Place file on each BD instrument computer in the CST bead lot folder on the desktop.
3. In CST mode, find the tools>bead lots
4. Click import and find the bead lot you added to each computer.
5. Once it is imported exit from the window and run a baseline for the new CST lot. Once completed, run a performance check.

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Operating the BD Aerosol Management System

The BD aerosol management option (AMO) is a device that promotes the containment of aerosols by evacuating the sort collection chamber in the BD FACSAria II/III cell sorters. The option uses an attached vacuum source to rapidly evacuate aerosolized particles through an ultra-low penetrating air filter during routine sorting or analysis. The AMO must be used at all times with all biological specimens rated BSL2 on the BD FACSAria systems in the PAVIR FACS Facility.

Procedure:

1. Install the splash shield (if sorting into plates) or the tube holder (if sorting into tubes) below the aspirator drawer.
2. Ensure that an air filter is installed in the sort collection chamber door.
3. Close the sort collection chamber door. NOTE: The sort collection chamber door must be closed for the evacuator to generate negative pressure in the chamber.
4. Switch on the main power on the back of the **Evacuator**.
5. Press the up or down arrow button to set the suction control rate to 20%. NOTE: Do not set the suction control rate above 20% for sorting. Higher rates could affect the stability of the side streams.
6. Verify that the filter flow gauge reads less than 2.4 inches of H_2O.
7. Set up the FACSAria for sorting your cells.
8. Sort your cells, ensuring that the sort collection chamber door and the sample injection chamber door remain closed while cells are being sorted.
9. When sorting is complete, stop the sample flow into the sort collection chamber. (***Click the SORT button to stop the sort, then click the UNLOAD button to unload the sample tube***).
10. Press the up/arrow button on the **Evacuator** repeatedly to increase the suction to 100%.
11. **Wait at least 2 minutes before opening the sort chamber doors** to allow potentially hazardous aerosols to evacuate.
12. Open the sort collection chamber door.
13. **Remember to decrease the suction control rate to 20%** on the **Evacuator** when you return to sorting samples.
14. Turn off the **Evacuator** after you have finished running biological specimens by placing the instrument in standby by pressing the POWER button on the membrane panel of the evacuator and switching off the main power on the back of the evacuator.

Nozzle Swap SOP (Transitioning from one diameter to another)

1. Shut of the Stream and dry off the nozzle area
2. Replace the nozzle with a nozzle with the correct diameter for you sort.
3. Change the stream pressure to accommodate your new nozzle
    a. **Sort menu/ Sort setup/ chose the correct nozzle size**
4. Change the **Cytometer Configurations**
    a. **Cytometer Menu/ View Configurations** (Choose the correct settings; Click Set Configurations)
    b. Close the View Configurations window and be patient while the configuration lock in.
5. Upon prompting, select "**Use CST settings"**
6. Turn on the Stream and wait 1-2 minutes for the stream to stabilize.
    a. Optimal Stream images and approximate nozzle settings are posted on the instruments
7. Run the CST settings for the new nozzle as per the CST SOP on the opposite side
8. Continue with the Aria Setup SOP's AccuDrop Setup.