Instrument Restart after Storage Quick Reference Guide

Overview

This guide contains instructions for restarting the system after an extended shutdown period for the following BD flow cytometers.

- BD Accuri™ C6, C6 Plus and BD FACSVia™ Cytometers
- BD FACSAria™ Family of Cell Sorters
- BD FACSCalibur™ Cytometers
- BD FACSCanto™ Family of Cytometers
- BD FACSLyric™ and BD FACSVerse™ Cytometers
- BD FACS™ Lyse Wash Assistant Systems
- BD FACSMelody™ Cell Sorters
- BD FACS™ Sample Prep Assistant Systems
- BD Influx™ and BD FACSJazz™ Cell Sorters
- BD LSRFortessa™, BD FACSCelesta™ and BD FACSymphony™ Family of Cytometers

General Recommendations

Perform these procedures when starting up your instrument after an extended period. For any system, we recommend

- Check all fluidic tanks for contamination and replace all cubitainers as applicable.
- Replace all fluid filters as applicable to your instrument.
- Where noted, use filters that are dedicated for the specific fluid.
- Perform an additional clean or system flush before running Quality Control (QC) on your instrument.
- Use sterile deionized water (DI), not distilled.
- Reconnect all fluidic lines that have been disconnected. Check electronic connections.
- Clean the external surfaces of the instrument and workstation with a clean lint-free cloth or disposable wipe.
- Dispose of all liquid waste according to your standard biohazard protocols.

BD Accuri C6, C6 Plus and BD FACSVia Cytometers

1. Clean and rinse the fluid bottles. Replace the fluid bottle filters and the in-line sheath filter.
   Note: We also recommend replacing the peristaltic pump tubing every 2 months, which could include an extended storage.
2. Fill the fluid bottle filters with the respective fresh fluid: sheath, BD FACS™ Clean Solution and detergent.
3. Add 200 mL of undiluted bleach to the waste bottle.
4. Turn on the cytometer and perform the Start up cycle, (approximately 15 minutes to complete).
5. Turn off the cytometer to automatically run the Clean Fluidics cycle, (approximately 13 minutes to complete).
6. Repeat steps 4 and 5 two more times, allowing the fluidics cycles to complete before cycling the power.

For more information, see the Startup and shutdown chapter in the *BD Accuri C6 Plus System User Guide* or the *BD FACSVia Instructions for Use*. Procedure: **Startup workflow, Filling the fluid bottles, Emptying the waste bottle, Starting up the system, Shutting down the system.**

For more information, see the Maintenance chapter in the *BD Accuri C6 Plus System User Guide* or the *BD FACSVia Instructions for Use*. Procedure: **Cleaning the fluid bottles, Replacing the fluid bottle filters, Replacing the in-line sheath filter, Replacing the peristaltic pump tubing**

BD FACSAria II, III, Fusion and BD FACSymphony S6 Cell Sorters

1. Refill each 5-L tank with the appropriate fresh solution, using fresh 10% bleach, 70% ethanol, and sterile DI water. Empty the waste, if needed.

2. Turn on the workstation and the cytometer main power. Wait for lasers to warm up. Start BD FACSDiva™ Software:
   - Select **Cytometer > Fluidics Startup** and follow the on-screen prompts.
   - Click **OK** to complete the process.

For more information, see the Running Samples chapter in the *BD FACSAria II, III, BD FACSAria Fusion* or *BD FACSymphony S6 User Guide*.
Procedure: **Cytometer Startup**

BD FACSCalibur Systems

1. Perform the Startup procedure.
   - Remove the sheath reservoir, rinse and fill with fresh sheath fluid to 75% capacity.
   - Remove the waste reservoir, rinse and add 400 mL of undiluted bleach.

2. Install a new sheath filter, (perform the Changing the Sheath Filter procedure)
   - Install the reservoirs into the drawer, reconnect the tubing to their color-coated fittings and reconnect level sensor lines.
   - Pressurize the sheath tank and check the sheath filter and lines for air bubbles.

3. Prime the fluidics to remove bubbles from the flow cell. Repeat until no bubbles are observed in the flow cell.

For more information, see the Starting Up chapter in the *BD FACSCalibur Instructions for Use (IFU).*
Procedure: **Filling the Sheath Reservoir, Priming the Fluidics, Emptying the Waste Reservoir.**

For more information, see the Maintenance chapter in the *BD FACSCalibur Instructions for Use (IFU).*
Procedure: **Changing the Sheath Filter.**

BD HTS option

4. If you have the BD HTS option and fluidics are filled with DI water.
   - Empty and rinse the fluidic tanks. Reconnect all fluidic lines.
   - Initialize and prime the fluidics by selecting **Sampler > Prime,** wait approximately one minute and then repeat.

For more information, see the Introduction chapter in the *BD High Throughput Sampler User's Guide.*
Procedure: **Cytometer Connections**

For more information, see the Running Samples chapter in the *BD High Throughput Sampler User's Guide.*
Procedure: **Acquiring Data**

BD FACSFlow™ Supply System

5. If you have the BD FACSFlow Supply System, install a new 20-L sheath cubitainer and replace the waste cubitainer.
   - Reconnect the probes to the containers and power on the system.
   - Press and hold the Prime button on the BD FACSFlow Supply System until there are no bubbles and the sheath plenum tank is about an inch full.
   - Observe that the pump refills the sheath plenum tank as sheath is used.

For more information, see the System Overview chapter in the *BD FACSFlow Supply System* for a description of each component.

BD FACSCanto II and 10-Color Configuration Systems

1. Fill tanks with fresh fluids and empty the waste.

2. Turn on the power to the cytometer, start up the computer, launch the BD FACSuite™ Software and log in. Fluidics Startup should start automatically. If not, select **Cytometer > Fluidics Startup**.

3. Check for air bubbles in the flow cell.
   Select **Cytometer > Cleaning Modes > De-gas Flow Cell**. Repeat if needed.

4. Perform a long clean.
   Select **Cytometer > Cleaning Modes > Long Clean**.

5. We recommend repeating the fluidics startup by selecting **Cytometer > Fluidics Startup**.

For more information, see the Starting Up chapter in the *BD FACSCanto II* or *BD FACSCanto 10-color Instructions for Use.*
Procedure: **Starting Up**

BD FACSLyric and BD FACSVerse Systems

1. Refill the tanks with fresh solutions and empty the waste.

2. Replace the sheath filter before you begin.

3. Start up the system.
   - Turn on the power to the system and wait 20 minutes for the system to warm up.
   - Start BD FACSuite™ Software and log in with your username and password.
   - Verify the cytometer is connected and check the fluid levels.

4. To remove air bubbles from the sheath filter, select **Cytometer > Fluidics > Purge Sheath Filter**. Run the command twice.

5. Select **Cytometer > Monthly Clean** to start the monthly clean procedure.
   - Follow the instructions in the **Monthly Clean** dialog.

BD FACSLyric and BD FACSVerse Systems - continued

5. When the process is complete, remove the bypass assembly and re-install the sheath filter.

   Remove air bubbles from the sheath filter that might have formed during the process. Select **Cytometer > Fluidics > Purge Sheath Filte**r. Run the command twice.

   For more information, see the System Startup and Shutdown chapter in the *BD FACSLyric* or *BD FACSVerse Reference System*.
   Procedure: **Performing system startup**

   For more information, see the Maintenance chapter in the *BD FACSLyric* or *BD FACSVerse Reference System*.
   Procedure: **Performing the monthly clean procedure**

   For more information, see the Maintenance chapter in the *BD FACSLyric* or *BD FACSVerse Reference System*.
   Procedure: **Replacing the sheath filters**

BD FACS Lyse Wash Assistant

1. Empty and fluid from the tanks, then clean and rinse with water.

2. Fill and prepare the tanks.
   - Add appropriate solutions to each tank
   - Reconnect fluid lines with the corresponding color-coded connectors into their matching ports.

3. Perform a run to check for instrument operation and to fill the lines
   - Install 5 tubes of DI water into positions 1–5 of the carousel and close the reagent door.
   - Select the **Wash Only** protocol**.**
   - Remove rack and continue with running samples.

   For more information, see the Getting Started chapter in the *BD FACS Lyse Wash Assistant User Guide.*
   Procedure: **Filling the tanks, Installing and connecting the tanks, Priming the fluid lines**

   For more information, see the Processing Samples chapter in the *BD FACS Lyse Wash Assistant User Guide.*
   Procedure: **Processing samples**

BD FACSMelody Cell Sorters

1. Perform an extended fluidics startup:
   - Ensure that the air supply or compressor is turned on, the waste tank is empty and the sheath tank is filled to the weld line.
   - Power on the instrument and computer.
   - Start BD FACSChorus™ Software and log in.
   - Select **Run Extended Fluidics Startup** and follow the prompts.
2. Restart the stream by loading a tube and clicking **Start Stream** in the Stream View window.
3. On the Cytometer page, select **Sample Line Backflush** and then click **Start**.

For more information, see the System startup and shutdown chapter in the *BD FACSMelody User Guide*.
Procedure: **System Startup**

For more information, see the Maintenance chapter in the *BD FACSMelody User Guide*.
Procedure: **Changing the fluid filter**, **Backflushing the Sample Line**

BD FACS Sample Prep Assistant (SPA) II and III Systems

1. Empty and fluid from the tanks, then clean and rinse with water.
2. Fill and prepare the tanks.
   - Add appropriate solutions to each tank including bleach and antifoam solution to the waste tank.
   - Reconnect fluid lines and the sensor connectors to the fluidics tower.
3. Power on the instrument and then the workstation.
   - Start the SPA software to initialize and prime.
   - Select **Instrument > Prime**.
4. Select **Instrument > Prime** from the main menu in the **Prep Worklist** window.

   Select **DI WATER** from the **Fluid Type** menu and click **Yes**.
   - Repeat the DI water prime 4 times.
   - Select **LYSE** for the **Fluid Type** and repeat 4 times.
   - Select **FLOW** for the **Fluid Type** and repeat 4 times.
   - Select **DI WATER** for the **Fluid Type** menu and repeat 4 times
   - If air bubbles are visible in the lines, prime all fluid lines again as described above.
   - Inspect connections and fittings for any fluid leakage.

BD FACS Sample Prep Assistant (SPA) II and III Systems - continued

5. Follow the **Probe Replacement Guidelines** in the User's Guide and replace the probe if advised

6. Perform a short sample processing run to check instrument operation.

For more information, see the Instrument Requirements, Installation, and Startup chapter in the *BD FACS Sample Prep Assistant III User Guide.*
Procedures: **Instrument Startup, Bulk Fluid Preparation, Flow Container and Waste Tank**

For more information, see the Sample Preparation chapter in the *BD FACS Sample Prep Assistant III User Guide.*
Procedures: **Daily Startup, Setting Up for a Processing Run, Sample Processing**

For more information, see the Shutdown and Maintenance chapter in the *BD FACS Sample Prep Assistant III User Guide.*
Procedures: **Prime the Lines with Water**

For more information, see the Sample Preparation chapter in the *BD FACS Sample Prep Assistant III User Guide.*
Procedures: **Probe Replacement Guidelines, Replacing the Probe**

BD Influx and BD FACSJazz Systems

NOTE: Perform the **Dry Startup workflow** in the user's guide.

1. Power up the system.

2. Prepare the fluidic tanks
   - Fill the sheath tank with up to 7 L of sheath fluid. Do not fill past the weld line.
   - Empty the waste tank and add enough bleach to achieve a 10% concentration.
   - Verify the waste tank pressure gauge reads between 5" Hg and 10" Hg.
   - Verify the sheath tank pressure is between 27 and 29 psi.

3. Flush the system.
   - Verify the nozzle and nut are removed and place the flush bucket under the nozzle.
   - In the **Pressure Console** pane, click **Rinse** and run for at least 30 seconds.

4. Clean the nozzle tip by sonicating for 1 to 2 minutes and flush with the syringe.

5. Backflush the sample line.

For more information, see the System startup chapter in the *BD Influx* or the *BD FACSJazz User Guide.*
Procedures: **Startup Workflow – Dry startup workflow, Powering up the system, Flushing the system, Cleaning the nozzle tip, Backflushing the sample line, Removing bubbles for the nozzle tip, Introducing a sample into the system**

BD LSRFortessa, BD LSRFortessa X-20, BD FACSCelesta and BD FACSymphony Systems

1. If tanks contain fluid, empty, clean and rinse with water.

2. Power on the workstation and then the instrument.

3. Prepare the sheath and waste container.
   - Reconnect the fluidic lines and bypass the sheath filter.
   - Fill the sheath tank with approximately 2 liters of BD FACSRinse Solution.

4. Perform this modified system flush:
   - Run a tube of BD FACSRinse Solution on high for 30 minutes.
   - Empty the sheath tank and rinse out with DI water.
   - Fill the sheath tank with sheath fluid and install a new sheath filter.
   - Vent any bubbles from the sheath filter

5. Perform this modified priming of the fluidics:
   - Press the **PRIME** fluid control button to force the fluid out of the flow cell and into the waste container.
   - The **STANDBY** button turns amber after completion. Install a tube of DI water and run for 10 seconds.
   - Press the **PRIME** button again and then immediately push the **RUN** button. Repeat this step ten times back and forth between the **PRIME** and **RUN** buttons.

For more information, see the Cytometer setup chapter in the *BD LSRFortessa*, *BD LSRFortessa X-20, BD FACSCelesta,* or the *BD FACSymphony User Guide.*
Procedure: **Flushing the System.**

BD LSRFortessa, BD LSRFortessa X-20, BD FACSCelesta and BD FACSymphony Systems

BD HTS option

1. If you have the BD HTS option and fluidics are filled with DI water.
   - Reconnect all of the fluid lines.
   - Initialize and prime the fluidics by selecting **HTS > Prime**.

2. If the unit was not shutdown in DI water,
   - Initialize the unit and prime with a beaker of warm (not hot) DI water using the purge tool. See page the *BD HTS Customer Care kit User's guide*. If no errors occur during the prime, let the unit sit in DI water for several hours.
   - If a pump error occurs, lubricate the outer edge of the plunger with a tiny amount of lubricant. Use only enough lubricant to shine the edge of the plunger surface. Make sure there is no lubricant on the top of the plunger.
   - Prime again with the sheath line connected to the instrument.
   - Run the daily QC.

For more information, see the Running Samples chapter in the *BD High Throughput Sampler User Guide.*
Procedure: **Starting Up**

For more information, see the Introduction chapter in the *BD High Throughput Sampler User Guide.*
Procedure: **Cytometer Connections.**

For more information, see the Introduction chapter in the *BD HTS Customer Care kit User's guide.*

BD FACSFlow Supply System

3. If you have the BD FACSFlow Supply System, install a new 20-L sheath cubitainer and replace the waste cubitainer.
   - Reconnect the probes to the containers.
   - Press and hold the prime button on the BD FACSFlow Supply System until there are no bubbles and the sheath plenum tank is about an inch full.
   - Observe that the pump refills the sheath plenum tank as sheath is used.
   - Proceed with the appropriate system flush based on your instrument.

For more information, see the System Overview chapter in the *BD FACSFlow Supply System* for a description of each component.

For more information, see the Maintenance chapter in the *BD FACSFlow Supply System.*
Procedure: **Replacing the sheath and waste cubitainer, Flushing the system**

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