

**SCIF SOP for BSL-2 samples on Aria3**

Date Last Modified: July 2, 2015

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**Overview of BSL-2+ Sorting Area and Procedures:**

This document aims to provide a description of the facility, instrumentation, and safety procedures for use of the BSL-2+ SCIF sorting area. Cell sorters that produce a jet-in-air stream pose an elevated biosafety risk due to the aerosols generated, particularly during a clog of the nozzle. In an effort to comply with safety guidelines put out by the International Society for Advancement of Cytometry, the Stem Cell Instrumentation Foundry (SCIF) at UC Merced has developed this safety manual in conjunction with UC Merced EH&S. Failure to adhere to the safety guidelines in this manual may result in increased risk to pathogen exposure.

This SOP is derived from the following publication on Sort Biosafety Standards:

Holmes KL, Fontes B, Hogarth P, et al. International Society for the Advancement of Cytometry Cell Sorter Biosafety Standards. *Cytometry Part A : the journal of the International Society for Analytical Cytology*. 2014;85(5):434-453. doi:10.1002/cyto.a.22454.

The Aria3, located in SE2 room 153, is the only sorter in the SCIF capable of sorting BSL-2 samples. It has an aerosol management system to mitigate the operator's risk of exposure to potential pathogens in samples.

**EH&S Requirements:**

Through UC Merced EH&S the user must complete the following trainings in addition to general lab safety training:

- Biosafety
- Bloodborne Pathogen
- Users running BSL-2 samples on the Aria3 must submit a copy of their Biological Use Authorization (BUA) to SCIF prior to running samples

Aerosol Management Option: The Aria3 is equipped with an Aerosol Management Option (AMO) pictured below, designed to rapidly evacuate aerosolized particles from the instrument.

[https://www.bdbiosciences.com/documents/BD\\_FACSAria\\_System\\_Family\\_AMO.pdf](https://www.bdbiosciences.com/documents/BD_FACSAria_System_Family_AMO.pdf)



### **Solid waste:**

- Empty tubes and other non-sharp items are to be placed in double bagged red biohazard container
- Empty tips and any other sharps are to be placed in the red sharps container

### **Liquid waste:**

- The waste collection tank must be filled with 1L of 100% bleach prior to running the cytometer. Tank should be emptied once it reaches approximately  $\frac{3}{4}$  full. Empty by pouring waste down the sink.
- Any other liquid waste such as unrun sample should be placed in 10% final bleach solution for 30 minutes prior to dumping down the sink.
- No liquid waste should be disposed of in the red biohazard containers or in the trash!

## **BSL2 with Enhanced Precautions SOP–FACS Aria**

### **1. Preparation before the sort:**

a. If not using a sealed keyboard and mouse, cover keyboard, mouse and other instrument control surfaces with plastic wrap; clear surfaces of clutter, use absorbent pads for samples.

b. Using a damp paper towel(s), wipe up dried bleach residue from instrument areas, paying particular attention to the sample uptake area, O-rings, charge plates, and the side stream viewing window. Warning: Failure to remove salt residue from the sample uptake system may cause the pressurized seal to fail and release potential aerosols!

c. Prepare sort collection chamber as necessary. Install the correct collection tube holder. Close sort collection chamber door.

d. If the Aria is contained within a Biosafety Cabinet (BSC), turn the BSC blower fan on and turn the evacuation vacuum on low.

e. If not using a BSC, turn biohazard vacuum (Buffalo Filter Whisper Unit) on and operate at 20%. Check vacuum reading. If vacuum is  $>2.4$  inches of H<sub>2</sub>O, change HEPA filter. Note: HEPA filter must be changed every 6 months, regardless of vacuum reading.

f. Procedure for changing HEPA filter on AMS unit:

While wearing gloves, barrier lab coat with disposable apron, N-95 rated face mask (respirator) or PAPR and goggles/safety glasses, place the Buffalo unit HEPA filter inside an orange biohazard plastic bag. Disconnect hose from the Aria and also place within the bag. Seal the bag and place within a Medical Pathological Waste container for disposal according to Institutional guidelines. Install a new HEPA filter and hose.

g. Make sure sheath tank is filled and standard waste tank contains enough bleach to give a final 10% (1:10 dilution of household bleach) solution when filled. Fill a spray bottle with a freshly made 10% (1:10 dilution) bleach solution for work area decontamination.

h. Wear gloves, lab coat, N-95 rated face mask (respirator) or PAPR and goggles/safety glasses (or N-95 mask with face shield) before handling samples. Lab door must be closed and investigators are to remain outside of the lab until data files of the experimental controls and samples have been collected and tubes are no longer being manipulated. Note that investigators may remain in the lab if wearing N-95 or better respiratory protection, but must have been fit-tested and cleared by Occupational Medicine.

i. Respirators must remain on during all procedures associated with sample manipulation, including sample tube cap removal and loading of sample on instrument, or when removing collection tubes or other procedures where the sort or collection chamber is opened as outlined below in Section 3. Note that respirator protection may otherwise be removed during the sorting process except during procedures as outlined above.

j. Area within a Class II BSC: Operator must wear gloves and closed front lab coat and goggles/safety glasses. N-95 rated face mask (respirator) or PAPR must be worn during instrument/sample manipulation. Lab door may remain open, but notification of a potential biohazard must be posted outside the lab entrance. Investigators may remain in the room during sorting operations and respirators are not required for personnel other than the operator.

k. Have a spare nozzle, with new O-ring installed (or spare integrated nozzle), available in case of a clog.

## 2. Procedures during sorting/analysis:

a. Filter samples before sort to avoid clogs.

b. Fill sample tube with as much sample as possible to minimize loading and unloading sample. DO NOT fill higher than 1/4 inch from the top of the tube.

c. Make sure the “Sweet Spot” is enabled.

d. Close sort collection chamber door before starting sample.

e. When changing collection tubes:

i. Stop the sample flow and close the aspirator drawer by clicking the ‘Acquire’ button.

ii. Wait at least 60 s before opening sort collection chamber door.

iii. When removing collection tubes, be aware that the outside of the tube is potentially contaminated, use alcohol swab or bleach to wipe outside of tubes.

## 3. Procedures in the event of a nozzle obstruction:

If during the sort the stream is deflected (due in part to a clogged nozzle), the sort is designed to stop automatically and block the sort tubes. The sort will not restart until the operator has cleared the clog. In the event of a nozzle clog, DO NOT open sort collection chamber door or sort block door before following this procedure:

- a. If the system has not already shut down automatically, turn off the stream using the button labeled with a “\_” on the Breakoff window. This will shut off the stream, unload the sample and close the aspirator door.
- b. Open aspirator drawer using software controls.
- c. Increase the air evacuation rate on the AMS unit to 100%, or if using a BSC, push the high evacuation button (low button must also remain on).
- d. Wait at least 60 s. This procedure will clear aerosols from the sort chamber. (Note that this step assumes that a modification to tube holder(s) (universal top component on Aria II) involving the drilling of three holes and the sort chamber door involving the drilling of 1 hole with attachment of 0.22 mm filter, has been previously performed. see Ref. 3).
- e. Close the aspirator drawer.
- f. With the sort block chamber door, aspirator drawer and collection chamber door all closed, turn the stream on and off several times or perform the “Clean flow Cell” procedure with DIH<sub>2</sub>O followed by turning the stream on to see if the clog will clear itself.
- g. Turn stream off.
- h. Open the aspirator drawer and evacuate for at least 60s before closing the aspirator drawer again.
- i. The sort block chamber door and sort collection chamber door can now be opened.
- j. If it is necessary to change nozzles, remove nozzle and O-ring and place in tube with 10% (1:10 dilution) bleach for 30 min. Thoroughly rinse nozzle in water and let air-dry. Discard O-ring if not using nozzles with integrated O-rings. Spare integrated nozzle or spare nozzle with O-ring may be installed while obstructed nozzle is soaking in bleach.
- k. With stream turned off, open the sort block chamber door and dry plates and surfaces as needed.
- l. When removing collection tubes, be aware that the outside of the tube is potentially contaminated, use alcohol swab or bleach to wipe outside of tubes.
- m. Set AMS unit to 20% vacuum or if enclosed within a BSC, toggle the high evacuation button off.
- n. Make sure that all chamber doors are closed and restart the stream.

#### 4. Decontamination Procedures:

- a. Disengage “Sweet Spot” and turn the stream off.
- b. Disinfect sample lines using a freshly made 10% bleach solution as follows:

- i. Fill a tube with a volume of 10% bleach equal to or greater than the volume of sample that was sorted and place on the sample stage.
  - ii. Select from the menu—Instrument>Cleaning Modes>Clean Flow Cell. Perform this step three times or until a bleach drop is visible in the stream camera view.
  - iii. Wait 30 or more minutes with 10% bleach in flow cell.
  - iv. Fill a tube with DI water, Select from the menu— Instrument>Cleaning Modes>Clean Flow Cell.
  - v. Fill a tube with 70% ETOH, Select from the menu—Instrument>Cleaning Modes>Clean Flow Cell. Perform this step three times or until an ETOH drop is visible in the stream camera view. Shutdown instrument.
- c. Clean all surfaces around optical bench, sort block chamber and charge plates, sort collection chamber, sample introduction area and sample tube holder(s) with a prepackaged 10% bleach towel and/or 10% (1:10 dilution) bleach from a spray bottle. Clean keyboard cover, remove any plastic wrap and discard in Medical Pathological Waste.
- d. When leaving the lab:
- i. Make sure all samples are capped.
  - ii. Remove gloves, respirator, and lab coat (remember outside of gloves are contaminated!).
  - iii. **WASH HANDS!**