UCMERCED

Stem Cell Instrumentation Foundry

SOP for BSL-2 samples on Aria3

Date Last Modified: 2/19/2020

Author: David Gravano

Table of Contents

1. Purpose:

This document provides 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 SE1 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.

All users performing a new BSL-2 sort must submit by email a Sort Biosafety Form found on the SCIF Website under "Policies and SOPs".

2. 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

3. PPE Requirements

- All room occupants during BSL-2 sorting must be wearing a barrier lab coat, gloves, and goggles
- All users must have a N-95 respirator available during certain parts of the procedure

4. Waste Handling

Solid waste:

- Empty tubes, tips, and other non-sharp items are to be placed in double bagged red biohazard container
- 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 ¾ 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!

5. Preparation for Sort

a. Clean any clutter from work surfaces and spray with 70% ethanol, including mouse and keyboard.

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

c. Turn biohazard vacuum (Buffalo Filter Whisper Unit) on and operate at 20%. Check vacuum reading. If vacuum is >2.4 inches of H2O notify the staff.

d. 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.

e. Wear gloves, barrier coat, N-95 respirator, and safety glasses before handling samples. The sliding door to the Aria3 room must be closed and remain closed throughout the sort.

f. 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. Note that respirator protection may otherwise be removed during the sorting process except during procedures as outlined above.

g. Have a spare nozzle available in case of a clog.

6. Procedure During Sort

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 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 70% ethanol or bleach sprayed Kimwipe to wipe outside of tubes.

7. Procedure During a Clog

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 by unchecking the “Stream” button in the stream camera window. This will shut off the stream, unload the sample, and close the aspirator door.

b. Open aspirator drawer by clicking the “Waste Drawer” button in the side stream camera window.

c. Increase the air evacuation rate on the AMS unit to 100%.

d. Wait 60 s. This procedure will clear aerosols from the sort chamber.

e. Close the waste drawer by toggling the switch as in step b.

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 DIH20 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.

<u>4. Decontamination Procedures:</u>

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!**