

BD FACS Aria Fusion SOP

General Information:

The BD FACS Aria Fusion Cell Sorter is located on the 14th floor of MaRS West Tower, room 1445. This space is approved for work at BSL2. The main lab doors are marked with signage indicating it is a BSL2 working environment and is only accessible to by authorized personnel via electronic door locks. Room 1445 has signage indicating that only authorized personnel may enter.

Sorters generate droplets on the order of 80 μ M during controlled operation, but when clogs occur, satellites and other aerosols can be caught up on air currents and quickly dispersed randomly throughout a room when no aerosol containment measures are in place. For this reason, all unfixed human specimens and cell cultures are treated as infectious in the lab. The FACS Aria Fusion has been placed inside a Class II, A2 biological safety cabinet to protect operators from potential harm, while also serving to maintain sample sterility.

The intent of this standard operating procedure (SOP) is to outline the Biosafety plan for the preparation, transport and processing of samples for cell sorting and is a supplement to the general laboratory safety procedures outlined by [UofT's biosafety manual](https://ehs.utoronto.ca/our-services/biosafety/biosafety-manual/) (<https://ehs.utoronto.ca/our-services/biosafety/biosafety-manual/>) and [Canadian Biosafety Standard Guidelines](https://www.canada.ca/en/public-health/services/canadian-biosafety-standards-guidelines.html) (<https://www.canada.ca/en/public-health/services/canadian-biosafety-standards-guidelines.html>).

Cell Sorter Access:

All users must have completed the following EHS training modules prior to gaining access to room 1445:

EHS603 (Blood Borne Pathogens)

EHS101 (WHMIS and Lab Safety)

EHS601 (Laboratory Biosafety)

EHS112 if applicable (WHMIS Refresher)

EHS602 if applicable (Biosafety Refresher)

All users must also be fully trained on the operation of the Aria Fusion before using the machine. Once training and user proficiency have been verified, users will be setup with a unique username and password to allow access. Users are prohibited from sharing passwords with anyone. If a user is found to have shared their password, their access will be revoked.

Users must also update their MOU to include the Aria Fusion cell sorter.

Safety:

Samples may be of BSL1 or 2 therefore is it best to treat all samples as if they are infectious for which proper safety measures must be followed.

Personal Protective Equipment:

- A lab coat must be worn by all personnel when using the sorter.
- Gloves are required at all times
 - If working with encephalomyocarditis virus infected samples or human samples, double gloves are required when handling the samples.
 - *All cuts and abrasions should be covered with water proof bandages prior to donning gloves.*
 - Gloves are to be removed BEFORE touching/opening the door to room 1445. If users are found to be keeping their gloves on while touching/opening the door, their access to the sorter will be revoked.
- Attention should be paid to ensure bare skin at the wrists is covered. Gloves with longer than standard wrists, or protective disposable sleeves, are recommended
- Do not touch items outside of the hood (e.g., incubators, microscopes, drawers) with the outer pair of gloves when working with encephalomyocarditis virus infected samples or human samples. Remove your outer pair of gloves first.

Instrument Set-Up, Operations and Maintenance:

Quality Control and Maintenance:

The FACS Aria Fusion sorter is maintained under a service contract by BD Biosciences. All manufacturer recommended quality control procedures (as outlined in the instrument manuals) will be followed by trained operators.

No modifications to the instruments are to be made without manufacturer involvement.

Users will be trained carefully in the proper instrument operation and relevant safety procedures according to the manufacturer specifications. These personnel will be required to update their biosafety training regularly to ensure compliance with university policy.

The user will handle the instrument start-up, quality control, pre-sort sample line decontamination and stream calibration prior to beginning any experiment.

Sample Handling and Transport:

- All samples should be prepared prior to bringing them to the cell sorter i.e. washed in saline, filtered, and placed on ice.
- Users are responsible for proper sample preparation prior to sorting.

- When samples of risk group 2+ classification are brought to room 1445 for sorting they must be transported within leak-proof, impact-resistant containers. Surface decontamination of the containers prior to removal from their original containment zone is required. The container must also be labelled appropriately to assist in identifying its contents to aid in eliciting the correct spill response procedures should an incident occur.
- When the user arrives at room 1445, all sample handling will be conducted inside the BSC.
- All samples must be opened inside the BSC and sealed before leaving the BSC.
- If samples require mixing, ensure the tube cap is tightly closed and the outside of the tube has been disinfected before placing on a vortex, otherwise use a pipettor with attached filter tip to mix the sample, by gentle pipetting inside the BSC.
- Samples are generally kept on ice to lower metabolic activity and ice buckets will be kept on the bench outside of the BSC due physical constraints within the BSCs.
- If centrifugation of the samples is necessary, centrifuge tubes should be prepared and sealed in the biosafety cabinet. Biosafety-approved tube holders and buckets should be securely closed, wiped down with 70% ethanol and then transported to the centrifuge. After the spin, these tube-containing buckets must be unloaded inside the biosafety cabinet.
- When a sort is ongoing, the BSC doors must be closed and the fan turned on. The hood must be allowed to equilibrate for 2 minutes before loading samples.
- The use of filter-plugged pipette tips is recommended for working with all sample types.

Post-Sort Procedures:

- Clean the working areas in and around the cytometer and BSC with either 70% ethanol or PREempt wipes (0.5% w/w H₂O₂). Each user is responsible for providing their own cleaning consumables.
- Clean the instrument by running FACS Clean, FACS Rinse and ddH₂O for 5-10 minutes each.
- All work surfaces (desktop, computer keyboard and mouse) should be cleaned with 70% ethanol or virucidal wipes when finished.
- Handwashing after sorting or handling potentially infected samples is required and in general when exiting the lab.

Instrument Decontamination:

Instruments should be properly decontaminated after each experiment with the following procedures.

Inter- experiment Decontamination: Run FACS Clean (beach), BD FACS Rinse™ (detergent solution) and DI water for at least 5 minutes each.

End of Day Shut-down/Decontamination:

To be performed by the last user of the day. Following a FACS Clean, FACS Rinse, DI water cleaning, the Aria Fusion may be shut-down by turning off the stream, inserting the closed loop nozzle and performing the “clean flow cell” action. Disinfect the nozzle with 70% Ethanol for 10 minutes, rinse with DI water, sonicate for 1 – 2 minutes and then store dry.

Handling of Clogs and While Sorting:

- Stop the stream if it has not already shut off.
- Don **two** pairs of gloves and an N-95 mask, in addition to wearing a lab coat, ensuring no skin is exposed between the hands and end of the sleeves
 - o If any open wounds are present, first cover with waterproof bandages.
- Open the BSC (Aria) to flip the flow cell access door up and then close the BSC to re-establish containment (wait 5 minutes)
- Remove the sample collection tube device and close the collection tubes.
- Open the Flow Cell access door and allow aerosols to be evacuated out of the chamber via the BSC for 10 minutes.
- Clean all accessible areas with the appropriate agent for the pathogen in use (usually **6% Virox solution, or 70% ethanol**)
- Open the hood to remove the nozzle and disinfect with 70% ethanol for 10 minutes and then sonicate for 1 minute with DI water.
- Disinfect wet interior surfaces with the appropriate neutralizing agent.
- Discard all waste materials and extra PPE in the yellow (level 2) biohazard bins.

Spill Clean-Up Procedures

Inside the BSC:

- Allow aerosols to settle
- Cover the spill with absorbent material and disinfect with 1% HOCl or a 6% virox solution
- Wait 30 minutes to ensure adequate decontamination
- Wipe up spill material and dispose in the yellow biohazard containers.

Outside the BSC:

- Evacuate room 1445 and wait 20-30 minutes for aerosols to settle down. Place sign on door to prevent people from entering.
- Locate the spill kit located under the handwashing sink
- Don protective clothing: gloves, lab coat, goggle, mask
- Cover the spill with absorbent material like paper towel.
- Decontaminate the spill by gently pouring bleach around the spill, but not directly on top.
 - o Bleach must be diluted to 1% HOCl (final concentration)
 - o Work from the edge of the spill inwards
 - o Wait 30 minutes to ensure adequate decontamination
 - o Wipe up spill material and dispose in the yellow biohazard containers.
- Decontaminate all exposed surfaces with diluted bleach (final concentration of 1% hypochlorite).
- Wash hands thoroughly with soap and water immediately after clean-up is complete.

Waste Disposal:

- Liquid Waste: Small volumes of 1ml or less can be left in the sample tube and disposed of in the yellow BSL2 waste bins located at the instrument.
- Waste Tank: At the start of each day the waste tank is filled with 800 mL of undiluted 6% hypochlorite solution for dilution by the sorter during operation. The waste tank must be emptied when the volume reaches 5L for a final concentration of 1% hypochlorite. In a full day's operation (8 hrs) the instruments generally create less than 5 L of waste, if the instrument generates more waste, additional bleach must be added to the container and allowed to sit for 10 minutes prior to disposal down the sink.
- The waste tank on the Aria has baffles to prevent wetting of the specialized biohazard waste cap. If the specialized cap becomes wet for any reason it must be exchanged for a new one.
- Solid waste: This will mainly consist of pipette tips which should be discarded into a freshly prepared solution of 1% hypochlorite inside the biosafety cabinet. When work is finished, the container should be sealed, wiped with Virocidal wipes before removal from the cabinet. The waste liquid may be poured down the sink with running water and then solids placed in the yellow level 2 biohazard bins. Exposure to 1% HOCl must be no shorter than 10 minutes.

Exposure Response:

An exposure incident is defined as eye, mouth, mucous membrane, non-intact skin, or parenteral contact with blood or potentially infectious materials or chemicals. Procedures to follow after an exposure incident are posted in room 1445 for quick reference (also attached to this SOP).

For a medical emergency, go to the nearest emergency room or call 911.

In the event of an exposure incident, it is essential that first aid procedures be initiated immediately.

Eye or mucous membrane exposure from splash or aerosols:

- Rinse a minimum of 15 minutes using the eye wash
 - *The eye wash station is located at the lab sink in room 1445.*
- Rinse from the nose outward to prevent contaminating the other eye
- Hold eyelids away from your eyeball and rotate your eye so all surfaces get washed

Skin contamination:

Intact skin:

- Remove contaminated clothing
- Wash contaminated skin for one minute with soap and water

Broken, cut, damaged skin or open wound:

- Remove contaminated clothing
- Wash affected areas with soap and water for 15 minutes

Sharps exposure

- Sharps are not needed for regular sorter operation, but if skin is cut with encephalomyocarditis virus contaminated sharps, or human cell contaminated sharps (needles/glass), wash affected areas with soap and water for 15 minutes

The exposure incident must be immediately reported to the user's principle investigator and also to UofT's Office of Environmental Health and Safety: <https://ehs.utoronto.ca/report-an-incident/>

Emergency protocols

In the case of fire

Leave the area immediately. Pull the fire alarm. Use the stairs. Leave the building and go to the muster point (NE side of the College/University intersection).

In the case of a 911 emergency that is medical or personal safety:

1. Call 911 and state the following information:
 - Explain the emergency
 - State your location:
You are at the MaRS West tower, 661 University Avenue (14th floor)
2. If possible, call **MaRS Tenant Services (416-673-8200)** and state the following information:
 - Explain the emergency
 - State your exact location
3. In cases of medical emergencies: if bystanders are available, have one person stay in the elevator lobby and another meet first responders on the ground floor.

In the case of an EHS incident (i.e., hazardous agent spill or exposure – chemical, biological or radioactive material) that is not a 911 emergency:

1. Call **MaRS Tenant Services (416-673-8200)** and state the following information:
 - Explain the incident
 - State the location
2. Follow the appropriate EHS procedure

In the case of a facility issue that is not a 911 emergency (i.e., flood, fume hood alarm, security door alarm):

During business hours:

1. Contact **MaRS Tenant Services (416-673-8200)**, and explain the incident and the exact location.
2. If possible, call **Noah Frank (416-946-8237 or 416-528-0729)**, explain the incident, and let him know that MaRS Tenant Services has been contacted.

After hours:

1. Contact **MaRS Tenant Services (416-673-8200)**, and explain the incident and the exact location.

In the case of requiring an escort after hours:

1. Contact **MaRS West Tower Security (416-673-8201)** and request an escort out of the building after hours.
2. Contact **UofT WalkSmart (416-978-7233)** who will meet you at the Queens Park subway or outside MaRS.

Note: MaRS Tenant Services can also be reached by email at marstenantservices@marsdd.com