

Manual of Standard Operating Procedures
User Registration and Safe Working Practices
Cell Sorting in E-386A&B, E-377A

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I. Purpose

The purpose of this standard operating procedure (SOP) is to outline the Biosafety plan for the preparation, transport, and use of samples using a Becton Dickinson (BD) Biosciences FACS Aria II cell sorter housed in a NuAire class II, type A walk in Biosafety Cabinet (BSC) for work in the Cell Analysis Facility (CAF). The procedures outlined in this SOP are a supplement to general laboratory safety procedures outlined in the UW Biosafety Manual.

High-speed cell sorting can produce aerosols that may present a health hazard to workers. The procedures outlined in this SOP are to help ensure the safety of staff, proper use and maintenance of the equipment, and to avoid cross-contamination of samples between users.

II. General Facility Information

The Cell Analysis Facility is located on the 3rd floor of the UW Medicine South Lake Union (SLU) campus, E-386. The facility is overseen by the Immunology User's Committee and maintained by Michele Black, Director of the CAF.

The cell sorting rooms E-386A & B are approved for work at Biosafety Level 1 and 2 (BSL-1 and BSL-2), room E-377A is approved for BSL1 and 2, additionally it is approved for BSL2 with BSL3 practices (BSL2+). All rooms are maintained under negative pressure relative to the larger lab space. Each sorter is housed in an approved Bio safety cabinet (BSC). All cells exposed to or infected with bacterial or viral agents must be approved by EH&S on a case by case basis.

The following table from EH&S outlines the Biosafety levels and the associated requirements that must be met for sorting in the facility.

EH&S Summary of Biosafety Practices for Cell Sorting in SLU Cell Analysis Facility

Type of cell/procedure	Exempt BSL-1	BSL-1	BSL-2	BSL2 with 3 practices (BSL2+)
Examples of Cells	Wild type cells from murine or other non-human / non-primate species that have NOT been exposed to any microbial agent (e.g., viral, bacterial, fungal, protozoan, parasitic) and have not been genetically modified or Cells determined by EH&S and the IBC to be recombinant NIH-exempt BSL-1.	Cells from murine or other non-human / non-primate species that have NOT been exposed to any microbial agent but have been genetically modified using non-viral methods (e.g. cells from transgenic animals or cells treated with nucleic acids). or Cells determined by EH&S and the IBC to be approved as non-recombinant BSL-1 or recombinant BSL-1.	Cells from human or non-human primates or Cells that have been genetically modified using viral methods or Cells exposed to microbial agents (e.g., viral, bacterial, fungal, protozoan, parasitic) AND Have been approved by EH&S and the IBC for BSL-2 containment and sorting	Cells exposed to microbial agents (e.g., viral, bacterial, fungal, protozoan, parasitic) with moderate increased personal hazard. (e.g. West Nile Virus) or Cells that have been genetically modified using viral methods with known oncogenic inserts AND Have been approved by EH&S and the IBC for BSL-2+ containment and sorting
BUA update to sort in the Immunology CAF	Not required.	Required.	Required.	Required.
Bloodborne Pathogen (BBP) Training	Not required.	Not required.	Initial and annual renewal required for work with human cells and other cells exposed to bloodborne pathogens.	Initial and annual renewal required for work with human cells and other cells exposed to bloodborne pathogens.
Sort Sign-up	Required. Include name, phone number, sample description.	Required. Include name, phone number, sample description and a clear BSL-1 notation.	Required. Include name, phone number, sample description and a clear BSL-2 notation.	Required. Include name, phone number, sample description and a clear BSL-2+ notation.
Sample Transport	No requirement	Leak-proof secondary container.	Leak-proof secondary container.	Leak-proof secondary container.
Room Restriction	None.	None.	YES – door to E-386A or B must be closed during sort and the BSL-2 Biohazard sign posted on the outside.	YES – door to E-377A must be closed during sort and the BSL-2 with BSL- 3 practices Biohazard sign posted on the outside.
Personal Protective Equipment (PPE)	Closed toe shoes. When manipulating samples (i.e. loading/unloading samples), gloves, lab coat, and face protection are recommended .	Closed toe shoes. When manipulating samples (i.e. loading/unloading samples), gloves, lab coat, and face protection are strongly recommended . <i>Spills: Lab coat, nitrile gloves, goggles/faceshield required.</i>	Closed toe shoes, lab coat and gloves required at all times. When manipulating samples (i.e. loading/unloading samples), face protection is also required . <i>Spills: Lab coat, nitrile gloves, goggles/faceshield required.</i>	Closed toe shoes, shoe covers, disposable rear opening gown, double gloves and face protection is required at all times. <i>Spills: Lab coat, nitrile gloves, goggles/faceshield required.</i>
Waste	Contaminated materials must be decontaminated prior to disposal.	Gloves and other waste must be disposed of as biohazardous. See CAF SOP.	Gloves and other waste must be disposed of as biohazardous. See CAF SOP.	Gloves and other waste must be disposed of as biohazardous. See CAF SOP.

The Biological Use Authorization (BUA) issued by EH&S will define what sample material can be sorted in the Cell Analysis Facility and designate what Biosafety Level applies based on the Biosafety Level, the appropriate Sort SOP will be followed. Refer to sections IV & V.

Radioactively labeled samples are prohibited from the Cell Analysis Facility.

The facility may be used by researchers within and outside the Department of Immunology after completing the prerequisite requirements specified in this SOP.

The facility is available for sorting Monday – Friday from 9:00am-5:00pm. After hours usage may be approved for users who have received relevant instruction from the facility staff, are self sufficient with the instrument software, and are trained on the equipment. If there is unexpected stream shutoff during after hours usage the sort must be terminated and instrument shut down procedures carried out.

III. Approval to Sort

The following are required from each user:

1. Creation of a User Account
2. Completion of facility orientation and training and familiarity with contents of this SOP
3. Biological Use Authorization (BUA) from Environmental Health & Safety (EH&S) for samples that are not listed as **exempt** from the "[EH&S Summary of Biosafety Practices for Cell Sorting in the SLU Cell Analysis Facility](#)" table in Section II.
4. UW Bloodborne Pathogens (BBP) Training documentation (*current, within 12 months*) for any person working with human cells or bloodborne pathogens.

The CAF Manager has the right to deny sorting of any cells that do not have prior approval by EH&S to sort in the CAF or that have not been clearly disclosed by the user at the time of sign-up.

1. Create a User Account. By filling out the *Cell Analysis Facility User Form* (attached), an individual user enables facility staff to create a user account on the CAF server (<http://cellanalysis.immunolo.washington.edu>).

2. Facility Orientation & Training

Cell Analysis Facility training will be provided by the CAF Manager or Staff. It will include an overview of the registration system, training on operation of the cell sorter, safety requirements, training on this SOP, and on the site-specific BBP Exposure Control Plan. This training will be documented on the *Cell Analysis Facility User Form*. The training records will be maintained by the flow facility staff.

3. EH&S Biological Use Authorization (BUA). The National Institutes of Health Office of Biotechnology Activities (NIH OBA) mandates that the Institutional Biosafety Committee (IBC) review and approve all research that involves recombinant DNA. The UW IBC reviews and approves all research involving infectious agents. Staff safety for work involving blood and other potentially infectious materials that may contain Bloodborne pathogens (BBP's) is mandated by the State of Washington Department of Safety and Health and enforced by the Department of Labor and Industries (WAC-296-823). All researchers and facilities at the UW must comply with these regulations.

PI's are responsible for obtaining BUA's, which apply to all members of their research groups. Contact EH&S Research and Biological Safety Office (RBSO) via email at ehsbio@uw.edu, via UW mailbox at 357165, or via fax at 206-221-3068, to obtain a BUA. Each BUA includes information on cell lines and infectious agents used by each laboratory, their appropriate level of containment, and sites at which they can be used.

4. Bloodborne Pathogens (BBP) Training Documentation
Employees who have potential for exposure to human blood or other potentially infected materials which includes human cells and cell lines must be enrolled in the UW BBP Program. The

Program includes offering of the Hepatitis B vaccine, initial and annual training, and a written site-specific Exposure Control Plan (ECP). The Cell Analysis Facility has an ECP that will be reviewed with users as part of the facility orientation and training.

IV. Pre-Sort Procedures

After the steps in Section III are complete and approval to use the facility is granted, the following procedures must be followed by facility users prior to sorting.

1. Sign-up: Each user is responsible for signing up using the online scheduling system (https://depts.washington.edu/flowlab/Schedule/CalciumView.cgi/inst_signup). Users sign up for sorting time in 15 minute increments, with an extra 15 minutes included at the end of the sort for decontamination and cleaning.
 - a. Sign-up information **MUST** include: user name, sample description, and a contact phone number.
 - b. Each entry is approved after the Facility Staff has looked at the request and made sure that there will be appropriate staff and all the required information is provided.
 - c. All **BSL-2** sorts must include a distinct BSL-2 notation as well as additional notation further clarifying the sample description (e.g.- ES cells, patient samples, transfected cells, infected cells etc).
 - d. All **BSL-2+** sorts must include a distinct BSL-2+ notation as well as additional notation further clarifying the sample description of what the cells have been exposed to. Room **E-377A** is the only sorter room approved for BSL-2+ sorting.
2. Sample Preparation: Samples must be completely prepped (washed in saline, filtered, and placed on ice) prior to arrival to the Cell Analysis Facility. Users are responsible for contacting the facility staff to discuss proper sample preparation prior to sorting. This includes single cell suspensions, cell washing, and nozzle tip considerations.
3. Sample Transport: All **BSL-2** or **higher** and **Recombinant DNA** samples must be transported to and from the facility in an appropriate container: i.e. - a leak-proof, puncture-resistant plastic carrier with a secure lid, which would be able to contain the specimen in case of breakage of the primary container.
4. Biohazard Warning Sign: The door to the Aria sort rooms shall be closed and the Biohazard door sign displayed (attached) when any **BSL-2 sample** are being sorted. Users are required to write the specific cell type and agent on the biohazard sign or white board next to the E-386A or B doors or the E-377A door and verify that the Biohazard warning sign is displayed upon entry with a BSL-2 sample.
5. Personal Protective Equipment (PPE) requirements for user: Upon entering the Aria sort room, each individual must sanitize their hands and put on PPE as specified by EH&S appropriate to the level of containment necessary for the samples being sorted. See the table EH&S Summary of Biosafety Requirements for Cell Sorting in the SLU Cell Analysis Facility (section II).
6. Sample Handling: Samples may only be uncapped inside the BSC. *No needles are permitted in the sorting facility.* If a sample needs to be transferred to a new tube, sterile plastic pipettes will be made available. If a sample needs to be re-filtered to remove clumps, the users must do so in the Biosafety Cabinet.

7. The Facility Staff will perform these following procedures prior to the sort:
 - a. Pour concentrated bleach into the waste tank to 10% tank volume.
 - b. Make sure the sheath tank is full.
 - c. Start the flow cytometer system.
 - d. QC the instrument with manufacturer recommended alignment bead to make sure that laser timing and area scatter are within recommended tolerances.
 - a. Test the stability of the sort side streams and droplet break-off with the aid of the AccuDrop management system.
 - b. Clean the cytometer with 10% bleach and water prior to running user samples.
 - c. Ensure that the air filter is installed in the sort collection chamber door.
 - d. Ensure that the BSC doors are closed and locked, the sash is down in pass through position and the cabinet blower is on and the Aria chamber blower is the low speed position. This is considered the operational mode.
 - e. The BSC must be in the operational mode for 5 minutes to ensure proper airflow has been established prior to running any samples.
 - f. Place sample tubes and sort collection tubes inside the BSC.
 - g. Start sort and monitor the sort performance using the AccuDrop camera.

V. **Post-Sort Procedures**

The following procedures are required after sorts. Daily equipment shutdown procedures specified in the user's guide will be performed by the facility staff or trained afterhours user.

1. Facility Staff will stop the flow of sample into sort collection chamber.
2. Wait 1 minute to allow any aerosols to be evacuated.
3. Research personnel will carefully remove, re-cap, decontaminate outside of sample containers, then remove from the BSC and place their experimental sample and collection tubes in the transport container.
4. Facility Staff will run 5 ml of 10 % bleach for 10 minutes followed by 5 ml of distilled sterile water to clean the ARIA sample line.
5. Facility Staff will decontaminate the sort chamber, sample chamber, immediate surrounding surfaces within the BSC and any additional surfaces which were used (i.e.- computer workspace, sink area) with Envirocide (3 minute contact time)
6. Facility Staff will empty the waste tank after the waste liquid has been exposed to 10% bleach for at least 30 minutes. The sheath waste tank will be decontaminated by spraying the outside with Envirocide (3 minute contact time) prior to being removed from the BSC to be emptied as needed and 100ml of bleach will be added back onto to the empty tank. See section IX Waste Management for complete SOP.
7. Users will remove PPE upon exiting in the following order:
 - a. Discard gloves in the biohazardous waste containers.
 - b. Remove lab coat and place in consolidated laundry hamper near door.
 - c. Remove eye protection and decontaminate by wiping with decontamination wipes. Dispose of waste wipe in the red biohazard container. Replace eye protection in holder.
8. **BSL-2+ PPE removal** will be in the following order:

- a. Discard outer gloves in the biohazardous waste containers.
 - b. Remove disposable gown and place in biohazardous waste container.
 - c. Remove shoe covers one at a time stepping out of the sorter room into the anti-room with uncovered shoe.
 - d. Remove lab coat and place in consolidated laundry hamper near door.
 - e. Remove eye protection and decontaminate by wiping with decontamination wipes. Dispose of waste wipe in the red biohazard container. Replace eye protection in holder.
 - f. Discard inner gloves in the biohazardous waste containers.
 - g. Use hand sanitizer before exiting the anti-room.
9. Thoroughly wash hands.
10. Before leaving the facility users must sign out using the billing computer.

VI. Unexpected Stream Shutoff During Sort

If during the sort the stream is deflected (due in part to a clogged flow cell tip), the sorter is designed to stop automatically, block the sort tubes, and evacuate the chamber. The sort will not restart until Facility Staff has cleared the clog. Only Facility Staff will attempt to trouble shoot the shut off and attempt to clear a clog. If the instrument clogs aerosol generation increases. In order to remedy a clog the BSC sash needs to be raised to access the sorter and open the sort chamber. To do this safely the aerosols need to first be evacuated and then surfaces that the aerosols could have contaminated need to be disinfected prior to fixing the clog.

1. The BSC has an integrated blower for the Aria sort chamber. Blower speed will be increased to maximum.
2. In addition to standard BSL-2 PPE all people in room will either don goggles and a surgical mask or a face shield for splash/splatter protection in preparation for removing the sample, or exit the room prior to Facility Staff opening the sort chamber.
3. Research personnel will carefully remove, re-cap, decontaminate outside of sample container, and place their experimental sample and collection tubes in the transport container.
4. The BSC sash may not be opened for 2 minutes to allow any aerosols to be evacuated.
5. Facility Staff will decontaminate the sort chamber, sample chamber, and the immediate surrounding surfaces using Envirocide (3 minute contact time) and a paper towel or wipe.
6. Facility Staff will turn stream on again to see if drop delay and stream returns to normal pattern. If clog is not fixed, the stream will be turned off and the nozzle flush procedure will be selected and sterile water used. The procedures in the User's Guide will be followed to clean the nozzle
Nozzle Cleaning Procedure:
 - a. Turn off the stream and open the flow cell access door.
 - b. Turn the nozzle-locking lever 90° to the left. Remove the nozzle by pulling it straight out.
 - c. Soak the nozzle in a test tube containing DI water.
 - d. Thoroughly dry the nozzle.
 - e. Carefully reinsert the nozzle into the flow cell.
 - f. Turn the nozzle-locking lever clockwise to the 12:00 position.
 - g. Turn on the stream, and make sure it flows through the nozzle properly.
 - h. Close the flow cell access door.
 - i. Return the integrated blower for the Aria sort chamber to normal operating speed.
 - j. Lower the BSC sash.
7. After replacing the nozzle, gloves will be discarded and fresh gloves will be donned.

8. The area around the FACS sorter will be wiped with envirocide with a contact time of 3 minutes.
9. Sorting will resume after the Facility Staff corrects any issues.

VII. **Spill Procedures**

In the event of a spill inside the BSC, notify the flow facility manager to clean spill. Flow Facility Staff will decontaminate the sort chamber, sample chamber, immediate surrounding surfaces, and any additional surfaces which were affected with 10% bleach with a 20 minute contact time.

In the event of a spill outside the BSC, alert others in the room to avoid the spill area. Facility Users may clean spills outside of the BSC. Place paper towels directly on top of the spill. Gently pour a 10% bleach/water solution on the outside edges and corners of the paper towel and allow it to wick into the spill area. After a 20 minute contact time, dispose of the paper towel into a biohazard waste container. Notify Cell Analysis Facility Staff and record in the spill log.

After cleaning spill, change gloves and any other soiled PPE.

VIII. **Exposure Incident**

An exposure incident is defined as eye, mouth, mucous membrane, non-intact skin, or parenteral contact with blood or potentially infectious materials. Procedures to follow after an exposure incident are posted in the facility for quick reference (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. Immediately wash the exposed area with soap and water, or flush mucous membranes with water for 15 minutes. (The eye wash is located next to sink in E386.)

The Facility Manager will assist the exposed worker in seeking the necessary and immediate medical evaluation and consultation following an exposure incident.

Where and how to seek care: Monday through Friday 9:00 a.m. to 5:00 p.m. contact the Employee Health Center-UW (EHC-UW) at 685-1026. After hours or if the clinic staff is not available, go to the UWMC Emergency Department (NE-207 UWMC). Be prepared to give the healthcare providers information about the exposure. Tell them that you are a UW employee and provide information about the agent or cells involved in the accident. Information such as agent, route of exposure, dose or concentration, unusual characteristics of the agent, animal infection, cell line, and PI contact information.

All work related exposures, incidents, and near misses must be reported on the UW Online Accident Reporting System. Here is the link for report: <http://www.ehs.washington.edu/ohsoars/index.shtm>

IX. **Waste Management**

Decontamination and disposal of waste is the responsibility of the Facility Staff.

Liquid Waste: Small volumes of 1ml or less can be left in the sample tube and disposed of in the sharps container. Larger volumes must be diluted with fresh bleach while still in the BSC to a final bleach concentration of 10% (0.5% sodium hypochlorite) for at least 30 minutes prior to disposal in the sorter room sink. Flush the sink with water for 1 minute.

Waste tank: The waste tank will be emptied at the end of each day. The lower doors on the BSC will be opened. The wet cart containing the waste tank will be decontaminated with Envirocide (3 minute contact time). The waste tank can become pressurized when the cytometer is running. The tank will be disconnected from the wet cart before emptying. Facility Staff will wait at least one minute for pressure to dissipate before removing the waste cap or sensor. The waste tank cap must not be wetted. If wet, the filter in the cap will cause the tank to malfunction; to keep the cap dry, it will be placed on the bench, label side up, when it is not on the tank. If liquid is seen in the waste cap trap, the drain plug will be removed and liquid fully drained before replacing the plug. Record in the sewer log posted next to the sink.

Solid Waste: All solid waste shall be disposed of as biohazardous waste and will be collected in either a red sharps container or collected in a biohazardous waste bag contained inside a rigid, leak-proof container. This container must be labeled or clearly display the bag's biohazard symbol. Solid waste includes FACS tubes, pipettes and soiled tissues or towels, and gloves but never needles, syringes or glass, which are not permitted in the facility. Filled containers will be transported to the autoclave room located in EP117. Containers will be tagged with temperature indicator tape, which will be monitored after autoclaving.

X. **Biological Safety Cabinet**

This section applies only to facility staff

The NuAire™ Class II, type A2 biological safety cabinet is an enclosure that promotes the containment of aerosols by maintaining laminar flow within the properly sealed unit. The cabinet is specifically designed to rapidly evacuate aerosolized particles through an integrated speed controlled blower, Sorter Evacuation Blower (SEB) attached to sort collection chamber on the BD FACSAria™ flow cytometer. The BSC will be certified annually by the manufacture authorized personnel.

The Facility Staff is responsible for verifying the BSC is functioning properly prior to each sort.

1. With the blower motor on the Facility Staff will verify that the BSC has proper airflow by checking the pressure gauges at the top corners of the hood.
2. Both sets of gauges have indicator lights; they must be green in order to use the BSC.
3. The BSC must be sealed and allowed to run in operational mode for 5 minutes to purge any particulates and ensure that proper airflow has been established prior to any samples being introduced.
4. The pass through must have the sash in the locked down position, nothing shall be placed on the front grill of the pass through that would interrupt airflow.

Glo Germ aerosol containment procedure

The BSC will be tested under three conditions; 1. Aerosol generation without the SEB on (positive control), 2. Aerosol generation with SEB in full evacuation mode (worst-case), and 3. Proper instrument function with SEB in normal mode. The SEB must be tested under simulated worst-case failure mode. In this mode the instrument is set to 70 psi and 50,000 particles/second, with the waste catcher blocked to create large aerosol. This is done by covering waste catcher with a cut off piece of sample tubing.

The Glo Germ Test, described below will be performed each time changes are made to the cell sorter that may affect escape of aerosols i.e. SEB tubing is changed and as part of the biannual preventative maintenance schedule under each of the three conditions. Weekly testing will be performed under proper instrument function weekly.

1. Measurements are taken using glass slides inside an Aero Tech air sampler directly on top of the sort chamber within the BSC and outside the BSC at the workstation.

2. Measurements will be taken at 5 minute time points to allow for Glo Germ particles to accumulate on microscope slides.
3. Less than 1 particle per slide, under the condition of proper instrument function for all test locations will be considered as verification of proper working the BSC.

The flow cytometer must pass all tolerances of aerosol containment as described in this procedure. If these tolerances are not met, BSL-2 and BSL-2+ sorting is not permitted until the issue is resolved.

XI. Quality Control and Maintenance

The FACS Aria II is under an annual service contract and receives two quality control service calls per year. All manufacturers recommended procedures in Chapter 6 of the User's guide will be followed. Fluidic lines are checked daily for leaks or crimping. The manufacturer will be contacted before making any instrument modifications. Testing must also be done whenever changes are made to the cell sorter that may affect escape of aerosols, e.g., installation of a new drive head or flow cell, replacement of the sort chamber door, or alterations in the aerosol management system.

- Quality control beads are run daily to make sure the stream, break-off, and lasers fall within recommended tolerances.
- Weekly QC will involve using Glo Germs under proper instrument function with SEB to verify sort chamber containment.
- Room Pressurization Check: Weekly smoke tests will be performed to ensure that the sorter rooms E386A & B and E377A is under negative pressure relative to the adjacent laboratory space.
- Prior to each sort, the sorter will be carefully checked for wet areas indicating leaks in the tubing. Inspect tubing for cracks and signs of stress, particularly around the fittings and valve junctions. Inspect sheath lines and waste lines, and replace any leaking tubing.
- Failure to pass any of the above QC tests which cannot be immediately corrected requires that all BSL-2 sorts must cease until the issue is resolved.

XII. Operator training and experience

Only experienced flow cytometry operators are permitted to perform BSL-2 and BSL-2+ sorts. The operator will be trained carefully in the proper instrument operation and all relevant safety procedures, including aerosol containment testing. Initial training using BSL1, or fixed material should be mastered before sorting BSL-2 or higher samples by any operator.

XIII. Resources and References

- a. Centers for Disease Control and Prevention (CDC): Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition.
<http://www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm>
- b. UW BioSafety Manual:
<http://www.ehs.washington.edu/rbsbiosafe/bsmanualindex.shtm>
- c. Washington State Bloodborne Pathogen Regulations:
<http://www.lni.wa.gov/wisha/Rules/bbpathogens/default.htm>

- d. OBA/NIH guidelines for research involving recombinant DNA molecules:
http://oba.od.nih.gov/rdna/nih_guidelines_oba.html

Literature Cited for Glo Germ Protocol

1. Schmid I, Lambert T, Ambrozak D, Marti GE, Moss DM, Perfetto SP. International Society for Analytical Cytology and Biosafety Standard for Sorting of Unfixed Cells. *Cytometry* 2007; 71A:414-437
2. Perfetto SP, Ambrozak DR, Koup RA, Roederer M. Measuring Containment of Viable Infectious Cell Sorting in High-Velocity Cell Sorters. *Cytometry* 2003; 52A:122-130
3. Oberyszyn AS, Robertson FM. Novel Rapid Method for Visualization of Extent and Location of Aerosol Containment During High-Speed Sorting of Potentially Biohazardous Samples. *Cytometry* 2001; 43:217-222

ARIA Sorting

New User Sorting Approval Form
Immunology Cell Analysis Facility

Individuals desiring to use the Immunology Cell Analysis Facility for sorting any cells will need to first provide the following information:

PI Name: _____

PI E-mail address: _____

User Name: _____

User ID: _____

E-mail Address: _____

Phone: _____

Budget #: _____

Please identify the type of cells that are to be sorted. *

Unfixed Human cells

**All human cell work requires current BBP training certificate on file*

Unfixed Non-human primate

Cells exposed to microbial agents

Cells containing Recombinant DNA

Cells exposed to Viral Vectors

Cells containing other known pathogens

Cells exposed to nucleic acids

If any of the above are checked by the user facility staff will need to verify that the PI has a current BUA on file. If not, the PI must contact EH&S to obtain an updated BUA to sort in the Cell Analysis Facility.

None of the above

Specify cell type and species:

* Refer to the table “EH&S summary of Biosafety Practices for Cell Sorting in SLU Cell Analysis Facility” for examples of cells and their associated requirements.

Facility Orientation and Training
User Information Sheet

User Name: _____
User ID: _____
E-mail Address: _____ **Phone:** _____
Budget #: _____ **PI Name:** _____

Software Training: **Billing Program** **On-line Calendar**

Instrument Training: **Aria*** **Canto** **LSRII** **ImageStreamX**

*For Aria training and sorting the New User Sorting Approval form must also be completed

Date Completed User Initials

I have received a Facility Orientation and Reviewed Manual of Standard Operating Procedures <i>User Registration and Safe Working Practices</i> BD FACSAria II Cell Sorting in E-386A&B and E-377A

Afterhours training:

Prox Card #: _____

E386 **E386A & B** **E377**

Facility Staff certifies that this user has completed all training and has demonstrated adequate knowledge to independently operate the instrumentation:

Cell Analysis Facility Staff _____ **Date** _____

Cell Analysis Facility
Phone List

Cell Analysis Facility
Michele Black, Kyle Herstad

206-543-7006

Michele Black
Office

206-685-3014

Dima Long
Immunology Administrator

206-685-3365

Room No.: E-386A &B	Date Posted:
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BSL-2 Laboratory



BIOHAZARD

Admittance to Authorized Personnel Only

Biological Agent(s):

Human, non-human primate cells; Cells exposed to microbial agents and/or recombinant DNA (see white board for cell types currently being sorted).

Special Procedures, PPE or Precautions for Entry/Exit:

gloves > lab coat > safety glasses > closed toe shoes

Principal Investigator(s)		Emergency Contact		Protocol # (s)
Name	Phone	Name	Phone	
		Michele Black	685-3014	

Room No.: E-377A	Date Posted:
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BSL-2+ Laboratory



BIOHAZARD

Admittance to Authorized Personnel Only

Biological Agent(s):

Human, non-human primate cells; Cells exposed to microbial agents and/or recombinant DNA (see white board for cell types currently being sorted).

Special Procedures, PPE or Precautions for Entry/Exit:

Double gloves > disposable rear opening gown > safety glasses > closed toe shoes>shoe covers

Principal Investigator(s)		Emergency Contact		Protocol # (s)
Name	Phone	Name	Phone	
		Michele Black	685-3014	

**Cell Analysis Facility
Biological Sample Spill Log**

Date: _____ Time: _____

Type of sample: _____

Personnel who cleaned the spill: _____

Manager notified: _____

Any Exposure Reported? No Yes

If yes:

- Notify Your Supervisor
 - Complete on-line Accident Report:
<http://www.ehs.washington.edu/ohsoars/index.shtm>
-

Date: _____ Time: _____

Type of sample: _____

Personnel who cleaned the spill: _____

Manager notified: _____

Any Exposure Reported? No Yes

If yes:

- Notify Your Supervisor
 - Complete on-line Accident Report:
<http://www.ehs.washington.edu/ohsoars/index.shtm>
-

Date: _____ Time: _____

Type of sample: _____

Personnel who cleaned the spill: _____

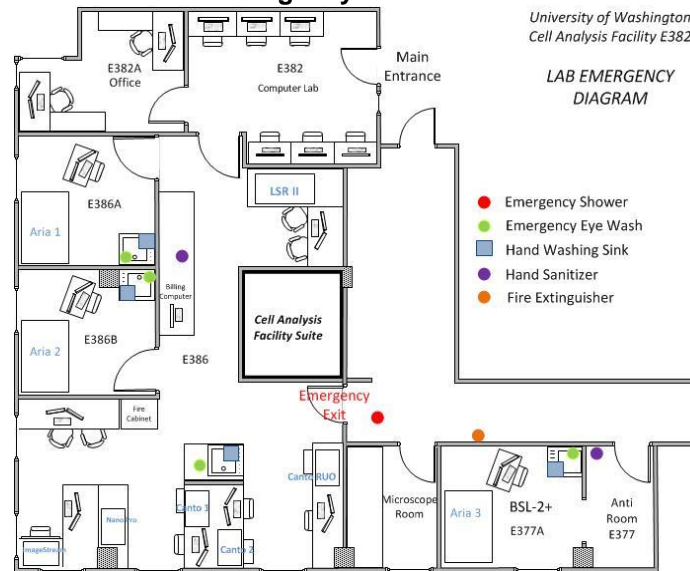
Manager notified: _____

Any Exposure Reported? No Yes

If yes:

- Notify Your Supervisor
- Complete on-line Accident Report:
<http://www.ehs.washington.edu/ohsoars/index.shtm>

Exposure Control Plan Emergency Information



Eye / Mucous Membrane Exposure

1. Flush immediately at nearest eyewash station for 15 min.



2. Notify your supervisor, they can help.

3. Seek care

Mon-Fri 9am-5pm:

Contact the Employee Health Center-UW (EHC-UW) at 685-1026

After Hours:

go to the UWMC Emergency Department (NE-207 UWMC)

-or-

nearest Emergency Department

4. Be prepared to give the following information to the healthcare providers:

1- Tell them you are a UW employee

2- Have information about the agent or cells involved in the accident

Information such as agent, route of exposure, dose or concentration, unusual characteristics of the agent, animal infection, cell line, and PI contact information.

5. Complete the On-line Accident Report

<http://www.ehs.washington.edu/ohsoars/index.shtml>

Wounds or Needlesticks

1. Wash the area immediately. Use warm water and sudsing soap to scrub the area for 15 min.

