

Center for Vaccine Biology and Immunology
Cell Sorting Facility Laboratory Safety Manual

| [Updated: January 25, 2012](#)

I. Standard Laboratory Rules

The standard BSL-2 precautions are outlined in the University's adopted biosafety manual CDC/NIH "Biosafety in Microbiological and Biomedical Laboratories (BMBL)". Of particular importance are the following:

- 1) Restricted access to the laboratory.
- 2) No food, drinks or application of cosmetics allowed in the laboratory.
- 3) Personal protective equipment (PPE) must be used. A minimum of lab coat and gloves are required for entry. Additional PPE is required depending on the task. All PPE is removed prior to exiting the immediate laboratory.
- 4) All biohazard waste is disposed of in red bags. Red bags are removed from the room to Housekeeping Biohazard Toters by laboratory staff.
- 5) Use of sharps (e.g., needles, glass) must be severely limited or eliminated.
- 6) Contaminated or potentially contaminated surfaces must be disinfected with an approved disinfectant (e.g., 1:10 dilution of a 5.25% sodium hypochlorite solution)
- 7) Storage of extraneous items in a BSL-2 lab is kept to a minimum. Storage should be limited to supplies necessary for support of cytometry activities.
- 8) Hands are always washed prior to exit.

II. Additional Regulatory Compliance

Required additional training

Additional training sessions may be required, depending on individual needs and may have already been completed as part of your laboratory certification. The CVBI Cell Sorting lab may request proof of this training.

The University requires completion of Laboratory Safety Training on an annual basis. Those individuals working in the CVBI Cell Sorting Facility must complete the requirement either by attending a classroom session or by completing the web-based program for Medical Center Personnel. Environmental Health and Safety provide both programs.

III. Specific Chemical Hazard Policies

ALWAYS read the Material Safety Data Sheets (**MSDS**) for every chemical that is going to be handled. Be aware of the potential hazards of the chemical, the protective equipment as well as the special procedures in case of an accident.

In general for most chemicals:

ALWAYS wear gloves when working with chemicals. A lab coat should be worn if working with dangerous chemicals, as well as goggles if there is splash risk and a mask if there is an inhalation hazard. DO NOT wear shorts or sandals in the lab. DO NOT eat or drink in the lab.

Chemicals common to the lab:

Sodium Hypochlorite (aka Bleach, Clorox)

STRONG OXIDIZING AGENT

Uses: Decontamination and disinfecting of contaminated surfaces
Risks: Skin irritation, nausea/vomiting if ingested. Do not mix with acids, ammonia.
Handling: Gloves, lab coat, safety glasses
Waste: Small quantities (under 5 gallons) can be flushed down drain.
Accident: Inhalation: Remove to fresh air.
Eye contact: Flush with water, 15 minutes
Skin contact: Wash with soap and water
Ingestion: Drink water and call medical help (5-1164)

Contrad 70 (Potassium Hydroxide based)

AVOID STRONG ACIDS

Uses: General cleaning and decontamination of flow cells
Risk: Irritation
Handling: Gloves, lab coat, goggles
Waste: Dispose of according to local, state, and federal regulations
Accident: Inhalation: Remove to fresh air.
Eye contact: Flush with water, 15 minutes
Skin contact: Wash with soap and water
Ingestion: Drink water and call medical help (5-1164)

IV. Specific Biohazard Policies.

Biohazardous agents in use may include:

Risk Group 2 agents (some potential for pathogenicity, often in immunocompromised persons)

- [Viable cells containing human pathogens](#)
- [Primary Human Cells, including](#) Human blood: use Universal precautions and proceed as if the blood could be contaminated with HIV-1, hepatitis B virus and hepatitis C virus, or other blood-borne pathogens

1. Routes of Agent Transmission

[The routes of transmission vary depending on the agents in use. Please refer to the MSDS for the pathogens in use to determine the pathogen specific route of transmission, which can be found at <http://www.phac-aspc.gc.ca/msds-ftss/index.html>.](#)

Exposure to agents may occur when handling infected cell cultures, tissues or bodily fluids (including blood). Exposure to [infected cells](#) requires direct contact with skin or splashing into eyes or mucous membrane; contact with broken skin or cuts is a particular hazard. To work with these agents, protective clothing should be worn at all times. If you cut yourself while handling these agents, an Occupational Health Nurse (x5-1164) should be consulted **IMMEDIATELY**.

Other considerations:

Aerosols: Sorting of samples by flow cytometry generates aerosols. Please see the specific policies governing flow sorting for more details.

Transportation of materials: All BSL-2 material must be transported to and from the CVBI Cell Sorting Facility inside a covered secondary leak proof container that is labeled with a biohazard symbol.

2. Recommended Vaccinations

- People who will be working with **human blood, [tissues](#), cells, and cell lines** should be vaccinated against **hepatitis B** virus. A series of three injections will be provided at no cost to those persons using human blood/ tissues in their experiments. This vaccine series is available at no cost to the employee through University Health Services. More information regarding this vaccine series is available through the University's Bloodborne Pathogens Exposure Control Plan which is found at <http://www.safety.rochester.edu/ih/bbpindex.html>
- Individuals at increased risk of susceptibility to infection (e.g. pre-existing disease, medication, compromised immunity, pregnancy or breastfeeding) will be referred to University Health Services Occupational Health Program for counseling.

3. Signs and Symptoms of Disease

[MSDSs are available for some infectious agents \(http://www.phac-aspc.gc.ca/msds-ftss/index.html\)](http://www.phac-aspc.gc.ca/msds-ftss/index.html); these include pathogen specific information about the signs and symptoms of transmission of the agents. If exposure to these agents is suspected, an infection control nurse should be consulted. This is especially important for suspected exposures to HIV (through handling of infected human blood), since it may be necessary to initiate antiretroviral therapy very rapidly after a confirmed and documented exposure.

V. Flow Sorting Policies and Protocols

1) [Before a sort of BSL-2 material](#)

- i) Before time is approved on the FACS Aria sorter for any BSL-2 project, a researcher must submit a disclosure form indicating the pathogen, cell type and other information to the sort operator. The Principle Investigator must have previous IBC approval for use of the cells and pathogen in question. The disclosure form (found at <http://www.urmc.rochester.edu/Aab/Vaccbio/labpages/FACS Aria/>) must be submitted a minimum of 24 hours before the scheduled sort time.
- ii) [Operator must be familiar with the pathogens that will be present, and the biosafety information associated with these pathogens. If an MSDS is available for the pathogen\(s\) involved in the upcoming sort, the operator should review this document prior to the sort.](#)

2) Sample Preparation (as carried out by the user in their specific lab environment)

- i) As the use of sharps is not allowed in the sorting facility, all work involving such must be carried out in the user's own lab.
- ii) Samples should be filtered through a 40-micron (maximum size limit) mesh immediately before starting sort. This will help minimize clumping of the cells.
- iii) Samples prepared from frozen stocks should be treated with DNase to help prevent aggregation due to released DNA from dead cells.

3) Instrument Configurations

- i) Knowledge of the cell size to be sorted is critical. The use of a nozzle 4 times the diameter of the cell will help stabilize side streams and minimize aerosols.
- ii) Sorting at temperatures above 4C (around 15C) will help minimize cell aggregation.
- iii) Constant mixing of the cells will help minimize cell aggregation.
- iv) Strict adherence to a scheduled maintenance protocol will prevent the compromise of both the sort itself and the sorting environment.

4) Operator Training

- i) The operator should be comfortable with handling BSL-2 material for normal laboratory operations before sorting BSL-2 samples.
 - ii) The operator must have extensive experience with sorting non-BSL-2 material before sorting BSL-2 material.
 - iii) Laboratory Supervisor must test and document the training of sort operators, with annual refresher training.
- 5) Operator Personal Protective Equipment
- i) As a minimum, the sort operator must wear a lab coat, gloves, and face protection (surgical mask and eye protection, or alternatively, a chin-length face shield) while setting up the sort, while within three (3) feet of the instrument during the sort and while decontaminating the instrument.
 - ii) Personal protective equipment must be removed prior to exiting the room.
- 6) Operational procedures: Sort
- i) Sort streams should be monitored for fanning.
 - ii) Upon completion of the sort, wait 1 minute to allow any aerosols that have been generated to settle.
- 7) Operational procedures (BSL-2): Environment
- i) A sign will be posted on the door (outside) indicating that a BSL-2 sort is in progress, listing the agent in use and any specific precautions.
 - ii) Other than the operator, no one shall approach the machine while the sort is in progress.
 - iii) All PPE must be disposed of within the designated BSL-2 area.
- 8) Decontamination procedures
- i) All surfaces should be decontaminated daily with freshly prepared 10% bleach.
 - ii) Sort blocks should be decontaminated with disinfectant appropriate to material type (e.g. Vesphene II or 10% Bleach).
 - iii) Prior to BD field service, all potentially contaminated surfaces will be disinfected with 10% bleach.

The Sort Operator must be offered appropriate immunizations as per CDC recommendations and University policies.

Questions or concerns regarding cytometry biosafety precautions may be referred to the Biosafety Officer or the University's Institutional Biosafety Committee.

Emergency Protocols and Policies

VI. BIOHAZARD EMERGENCIES

IMMEDIATELY AFTER A SPILL:

1. Determine if there is any risk of an aerosol.

- a. NO if the spill was contained (in the biosafety cabinet).
- b. YES if the spill occurred anywhere else. If there is risk of an aerosol, notify other personnel of the spill & ask them to leave the lab. Leave lab for 30 minutes to allow aerosol to settle. Post a warning to others (Keep Out!) on the lab door. Then, return and clean up spill.

2. Did you cut yourself during the spill? Did you splash uncovered skin (was this skin broken or abraded?)? If the answer to any of these questions is yes, you will need PROMPT medical attention (wash your skin and contact UHS x5-1164!).

CLEAN-UP OF A SPILL:

- 1. Close the lab door if not already closed.**
- 2. Put on protective clothing.** Wear double latex or nitrile gloves, a lab coat and face shield. Shoe covers may be necessary if the spill is on the floor. Make sure all exposed skin is covered. Get some red bags.
- 3. Place bleach-soaked paper towels over the spill.** Then carefully pour bleach over the towels and leave it to stand for 30 minutes. Discard the towels into a red bag & clean area repeatedly with 10% bleach and then with soap and water.

FACSAria CLOGS:

Monitor the stream for signs of instability: side stream fanning, inconsistent or low flow rates, etc. Please review section V for methods to minimize potential clogs.

If a clog develops during the course of a sort, the FACSAria will automatically shut down and eject the sample tube. Immediately cap and store the sample tube.

DO NOT OPEN THE SORT BLOCK DOOR OR THE SORT COLLECTION CHAMBER DOOR (cabinet on front of the machine) AT THIS POINT. Turn the suction on the *Whisper* vacuum to 100% and let run for 5 minute. After the evacuation is complete, put the vacuum in standby mode and proceed with the decontamination procedure.

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In addition to standard PPE, during the decontamination procedure the operator should wear a mask **AND** face shield. All cleaning supplies used to decontaminate should be disposed of in red biohazard bags.

Remove the collection block, cap any collection tube(s) and wipe them down with 10% bleach. In a biosafety hood, if the collection tube has not been compromised (contaminated by clog-deflected stream), transfer the contents to a fresh tube. Bleach and discard original tube.

The collection block should be decontaminated with 10% bleach (soaked). Remove and decontaminate the o-ring at the top of the collection block (soak in 10% bleach). After wiping down the block with bleach, it should be cleaned with water and then 70% ethanol.

To decontaminate the sort block, begin by opening the door carefully to minimize possible aerosol formation. Remove and wipe down the deflection plates and grounding plate with 10% bleach, and place them on a paper towel soaked in 10% bleach in a secondary container. Wipe down the interior of the sort chamber with 10% bleach, followed by water and 70% ethanol. Using a cotton tipped applicator soaked in bleach, decontaminate the stream camera windows.

To decontaminate the sort collection chamber door, wipe down all interior surfaces with 10% bleach followed by water and 70% ethanol.

Remove the nozzle and soak it in 10% bleach with sonication to decontaminate and dislodge any debris within the tip orifice.

AFTER A SPILL:

1. File an incident report: Available on-line
<http://www.safety.rochester.edu/SMH115.html>

2. Consult Occupational Health (x5-1164) concerning the incident and possible exposure. Do NOT DELAY in seeking medical attention in the event you cut yourself or exposed broken skin -- immediate anti-retroviral therapy may prevent development of AIDS, if you have been exposed to HIV.

Post-Exposure Emergency Follow-up:

1. Intact skin: wash affected area with soap and water.
2. Broken skin: wash with soap and water and then pour 3% hydrogen peroxide over the area.
3. Eyes: Remove contacts and rinse with water at eyewash station for 15 minutes.
4. Mouth: Rinse with water or 3% hydrogen peroxide several times, spit.
5. Report exposure to Supervisor and to University Health Services Occupational Health Unit (x5-1164)
6. Complete an Employee Incident Report Form which is available on-line.
<http://www.safety.rochester.edu/SMH115.html>

AFTER HOURS:

- 1. Call Occupational Health** if there is a risk of exposure (x5-1164). There is a hotline information tape.
- 2. Call Security** If you are unable to clean up the spill (i.e., alone or injured), call Security at x13. Security will contact EH&S for cleanup. Remain available to provide Security and EH&S with information.