

Cell Culture Room POLICY Overview

Chem. Sci. S012

1. Access to the laboratory is limited to confocal facility users.
2. Users MUST SIGN-UP (google calendar) prior to using a Biosafety Cabinet.
3. To facilitate the appropriate use of the facility, DO NOT book the Biosafety Cabinet for extended period of time unless all requested time is necessary for a hand-on experiment. Break a sign up in sections if repeating procedures must be carried out during same day.
4. Users MUST FILL OUT the log-book dedicated for the usage of Biosafety Cabinet
5. Remove all reagents and supplies from the Biosafety Cabinet upon completion of the experiment, or if there is a long delay in time between experimental steps.
6. Users MUST follow all safety and cleanliness guidelines:
 - a. Label all reagents and samples stored in common areas (not-labeled samples will be thrown away during weekly lab clean-up)
 - b. Clean the internal surface of the Biosafety Cabinet with 70% Ethanol/water solution. Bottles with the mixed solutions are available. Please refill bottles (ethanol and distilled water are available in the lab) upon usage.
 - c. All waste must be properly placed into the biosafety waste container (containers are provided and will be cleaned weekly).
 - d. If User generates a lot of waste and fills out the container, User should remove the full plastic back from the container, seal it and place a new bag into the waste container. Bags are located at the bottom of the waste container.
7. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
8. Sharps, such as needles, scalpels, pipettes, and broken glassware must be placed in the appropriate designated containers.
9. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.
10. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal with bleach or autoclaving.
11. Spills and other emergency situations MUST be handled according to the Incident Response SOP

Safety requirements (SOP)

Chem. Sci. Bld. Room S012. Bio-Imaging Facility, Cell Culture Room

BSL-1/2 Standard Microbiological Practices as described in the [Laboratory Biosafety Manual](#) will be used when working with cell cultures and other potentially infectious materials. Special attention will be paid to the following:

1. All procedures involving the manipulation of cells, tissues or other potentially infectious materials will be performed in a certified biological safety cabinet and in a manner that minimizes splashing, spraying or aerosolization.
2. To avoid both personal contamination and contamination to cultures, a separate laboratory coat will be designated for use during cell and tissue culture procedures and will not be worn outside the tissue culture room. *Upon request users can be assigned storage place for gloves, lab-coats and other supplies.*
3. Before leaving the laboratory, all personal protective equipment shall be removed and placed in an appropriately designated place for storage, washing, decontamination or disposal.
4. Clothing exposed to potentially infectious materials shall be removed immediately or as soon as possible.
5. Hands shall be washed after removing PPE, after contact with potentially infectious materials and before leaving the work area/laboratory.
6. Used needles and other contaminated sharps must be discarded immediately after use into a sharps disposal container. Needles and other sharps shall not be sheared, bent, broken, recapped or re-sheathed unless required by a specific procedure and it can be demonstrated that there is no other feasible alternative.
7. Eating, drinking, smoking, applying cosmetics or lip balm or handling contact lenses is prohibited in laboratories.
8. Food and Drink shall not be eaten or stored in the Laboratory.
9. Mechanical devices are used for all pipetting and suctioning procedures (no mouth pipetting).

Personal Protective Equipment (PPE)

Laboratory Coat

Laboratory coats capable of preventing liquids from soaking through and contaminating an individual's clothing or skin shall be worn when working with cell cultures and other potentially infectious material. Disposable laboratory coats should be discarded as biohazardous waste when they become contaminated or soiled. Non-disposable laboratory coats that are soiled or contaminated should be should be chemically decontaminated with a 10% bleach solution or sterilized by autoclaving before removing them from the facility for laundering.

Gloves

1. Gloves made from nitrile or another appropriate material will be worn when working with cell and tissue cultures and other potentially infectious materials.
2. Never touch your eyes, nose, mouth or your face while wearing gloves.
3. To prevent the spread of contamination gloves must be removed before touching "clean" surfaces such as door knobs, computer keyboards, books, telephones/cell phones, etc. Similarly, gloves used for other laboratory activities should be removed and new clean pair put on before beginning work with cell and tissue cultures.
4. Do not wash or reuse disposable gloves.
5. Dispose of single use gloves properly. Gloves used when working with cell cultures and other potentially infectious materials are discarded as biological waste and autoclaved before their ultimate disposal as decontaminated medical/biological waste.
6. Gloves must be changed when they become contaminated, or if they are torn, punctured or otherwise compromised.
7. Gloves must be removed in a manner that prevents the unintentional transfer of hazardous or infectious material from the outside contaminated surfaces of the glove to unprotected skin or clothing.
8. Consider wearing two pair of gloves for particularly hazardous work as this permits removal of a contaminated outer glove with minimal risk of exposure.
9. Gloves must not be worn outside the laboratory. If a hazardous material or sensitive experiment needs to be transported to another laboratory, it should be placed into a secondary container that can be safely handled without gloves.

Eye and Face Protection

1. When used appropriately, a biological safety cabinet (BSC) will provide the necessary protection of the eyes and face from splashes or spray. For other procedures outside the BSC select eye and face protection that is appropriate to the task being performed.
2. Do not put on or remove face/eye protection while wearing gloves that are potentially contaminated.
3. Individuals who wear contact lenses in the laboratory should also wear eye protection. Contact lenses do not provide protection to the eyes. Foreign material splashed into the eye may become trapped under the contact lens and result in more serious injury.
4. If eye protection is deemed necessary in a laboratory, then an emergency eyewash station should also be available.
5. Contaminated eye and face protection must be disposed of with other contaminated laboratory waste or be cleaned and decontaminated with a 10% solution of household bleach or another suitable disinfectant before reuse.
6. When hazardous or infectious materials must be handled outside of a biosafety cabinet, or other containment device, eye and face protection must be worn if there is a possibility that the procedure may create a splash or spray of harmful material.

Biosafety Cabinet – Usage and Cleanliness (SOP)

Cell Culture Cleaning Requirements

Chem. Sci. Bld. Room S012. Bio-Imaging Facility, Cell Culture Room

Preparing for work in the BSC

1. Before starting work in the BSC, review all procedures that will be used; identify the necessary equipment and materials that will be needed and develop a plan for safe and efficient work.
2. If the cabinet is not running, turn on the blower and fluorescent lights and turn off the UV light if it is on.
3. Verify that the BSC is operating correctly:
 - a. Check the instrument display/gauges for operational status.
 - b. Check the intake and exhaust grills for obstructions.
 - c. Check that the sash is in the appropriate position.
 - d. Check for the inward flow of air at the face of the BSC by holding a tissue near the bottom edge of the sash.
4. Wipe down the interior surfaces of the cabinet with an appropriate disinfectant such as **70% ethanol**, or 1:100 dilution of household bleach (0.05% sodium hypochlorite). Note that bleach, although an excellent and inexpensive disinfectant, will react with stainless steel surfaces of the hood and must be followed with a rinse of sterile water or 70% ethanol.
5. Load the cabinet with materials that will be needed for the procedure, wiping their surfaces with 70% ethanol to minimize the introduction of contaminants into the BSC. Position the materials near the back of the hood and organize them in a manner that will allow for the separation of clean and contaminated items during your work in the hood. Only materials needed for immediate work should be placed in the cabinet. Extra supplies (gloves, culture flasks/plates should be stored outside the cabinet).
6. Before beginning your work, allow the hood to run for a minimum of 5 minutes to purge any airborne contaminants from the work area.

Completion of work in the BSC

1. Discard all waste materials generated by your work into appropriate containers inside the BSC. Close or cover all open containers.
2. Allow the cabinet to run for 3 to 5 minutes with no activity.
3. Disinfect the surfaces of all materials, equipment and containers that will be removed from the BSC, to minimize subsequent contamination in the laboratory.
4. Remove contaminated gloves and dispose of them appropriately
5. After putting on a clean pair of gloves, remove all materials from the BSC.

- 6 Wipe down all interior surfaces of the BSC with an appropriate disinfectant.
- 7 If the BSC is not scheduled for subsequent use, turn off the fluorescent light and cabinet blower. BSCs are designed for 24 hour operation, but in the interest of energy conservation it should be shut down when it will not be used for an extended period of time.
- 8 Turn of the UV light if the cabinet is equipped and if appropriate.

Working in the BSC

- 1 Wear appropriate personal protective equipment (PPE). At a minimum, a lab coat with close-fitting sleeves and gloves should be worn. Because it is appropriate to wash your hands after removing gloves, double-gloving is a good option if you anticipate the need for glove changes during your work or in the event of a spill a double pair of gloves adds an additional layer of personal protection.
- 2 Proper aseptic technique is essential. The BSC will prevent aerosol contamination but will not prevent contact transfer resulting from poor technique.
- 3 Avoid rapid, sweeping movements of the arms into or out of the cabinet. Move items into our out of the cabinet slowly and perpendicular to the face of the cabinet to minimize disturbance to the protective curtain of air.
- 4 Do not block the air flow in the BSC by resting your arms or placing discarded plastic wrappers, procedure notes or other materials on the grill at the front of the BSC.
- 5 Organize your work to maintain a separation of clean materials from materials that have become contaminated during use.
- 6 Provide a container(s) within the BSC for the collection of contaminated waste and other materials. Repeated movement out of the hood to discard pipettes or other waste materials can disrupt airflow in the cabinet and marginalize the protection to both the individual working at the BSC and to the cultures that are being manipulated.
 - a. Low profile, horizontal containers are preferable to vertical containers as they are less obstructive to airflow in the cabinet.
 - b. Contaminated items that will not be reused may be placed into small biohazard bag or a similar container.
 - c. If chemical disinfection will be used for the decontamination of reusable items an appropriate disinfectant should be poured into the discard container prior to use.

Alternatively.

- d. If contaminated materials will be sterilized by autoclaving add enough water to the discard pan to ensure that sufficient steam is generated during autoclaving.
- 7 Do not work with open flames or other heat sources. These generate heated convection currents that may disrupt the smooth flow of air in the hood and may also damage the hood's HEPA filters.

Cleaning and Disinfection

Work surfaces and equipment shall be cleaned and decontaminated on a regular basis with a freshly made solution of household bleach diluted between 1:10 to 1:100 or another suitable cleaner/disinfectant.

1. After completion of procedures involving potentially infectious materials.
2. Immediately after a spill of potentially infectious material and/or when surfaces or equipment become overtly contaminated.
3. After a work shift, if the surface may have become contaminated since the last cleaning.
4. Equipment used for work with potentially infectious materials shall be decontaminated prior to repair, service or decommissioning.

NOTE: If bleach solutions are used for disinfection in the BSC, the stainless steel surfaces must be wiped with a solution of 70% ethanol, to remove any bleach residue and minimize the corrosive effects.

Biological Spill Response

The following procedures are provided as a guideline for biohazard spill clean-up in a BL1/2 laboratory (*no bacterial or viral work allowed*). Although the biological material in a BL1 level spill should not be a significant health hazard, you have the obligation to minimize the release of recombinant organisms and biohazardous material from the laboratory.

In the event of a spill:

- If a biohazardous material spills on you, remove any contaminated clothing and wash any exposed body parts.
- If a biohazardous material gets in your eyes, flush at the nearest eyewash station.
- If the spill area is large or in a common use area, mark, label, or otherwise denote the area so others may avoid it.
- Using materials from your spill kit:
 - Put on gloves, lab coat, and eye protection.
 - Cover the spill with absorbent material.
 - Pour disinfectant over the entire area. Allow the area to soak for 30 minutes.
- If warranted, contact the principal investigator, assess the magnitude of the spill, and formulate further plans of action.
- Safely pick up any broken glass with forceps or sweep into a dustpan, and dispose the residue into a broken glass/sharps container.
- Place spill materials into an autoclave bag.
- Make sure area is cleaned and disinfected thoroughly.
- Soak any contaminated clothes and shoes in a tray with disinfectant.

Incident Response (Personal Exposure, Spills)

Personal Exposure to Potentially Infectious Materials

Contamination to the Body

1. Immediately remove contaminated clothing and drench skin with water. Wash with soap and water, and flush the area for 15 minutes. Avoid the use of bleach and other disinfecting agents that are caustic to the skin.
2. Call 911 for assistance and transportation to Portage Health. Tell the dispatcher that the individual is potentially contaminated with a harmful chemical or biological material so that emergency responders can arrive prepared to deal with the additional hazard.
3. Report the incident to the laboratory supervisor/Principal Investigator and to the University Occupational Safety and Health and Services report form.

Splash to the Eye

1. Immediately flush the eye with a gentle stream of clean, temperate water for 15 minutes. Hold the eyelid open. Be careful not to wash the contaminant into the other eye.
2. Seek additional medical assistance from first aid providers, if necessary. Call 911 for assistance and transportation to Portage Health.
3. Report the incident to the laboratory supervisor/Principal Investigator and to the University Occupational Safety and Health and Services report form.

Punctures/Laceration of the Skin

1. Wash the affected area thoroughly with soap and water, and rinse the area for up to 15 minutes.
2. Allow small wounds such as punctures to bleed freely. There is no evidence that additional squeezing or “milking” of the puncture site is beneficial in preventing infection.
3. Control more severe bleeding with direct compression on the wound with sterile dressings. Wear latex rubber or nitrile gloves to prevent exposure to.
4. The application of antiseptics or disinfectants may be beneficial, however, avoid the use of bleach and other disinfecting agents that are caustic to the skin.
5. If necessary call 911 for assistance and transportation to Portage Health.
6. Report the incident to the laboratory supervisor/Principal Investigator and to the University Occupational Safety and Health and Services report form.

Spills

A spill kit containing the following items is available in the laboratory: (i) an appropriate cleaning and disinfectant solution (such as 1:10 dilution of household bleach), (ii) a package of paper towels, (iii) gloves, (iv) autoclave bags, (v) sharps container, (vi) forceps or other mechanical device to pick up broken glass.

Spill of Potentially Infectious Materials

1. Wear gloves and lab coat to clean up spill.
2. Absorb spill with paper towels and discard into a biohazard waste container.
3. If broken glass or other sharps are present, use forceps or another mechanical device to pick up and place into a SHARPS container.
4. Clean the spill site using a detergent solution and follow by spraying or wiping the area liberally with a 1:10 dilution of household bleach.
5. After 15 minutes contact time, wipe the area using towels soaked with a 1:10 bleach solution.
6. Discard all contaminated disposable materials into a biohazard waste container.
7. Remove PPE and wash hands with soap and water.