McGill	Standard Operating Procedure: Safe Use of a Class II Biological Safety Cabinet	**SOP # EHS-BIOS-201** version 1.0
	General Use	Effective date: September 14th, 2020 Review date: September 14th, 2020

Safe Use of a Class II Biological Safety Cabinet

1. SCOPE

This SOP applies to all McGill University personnel operating a Biological Safety Cabinet (BSC) as primary containment of risk group 2 and above and infectious materials. This document shall be made accessible in each individual user laboratory. The use of the BSC shall be conducted in accordance with the parameters defined in this document.

2. PURPOSE

This document will describe how to work in a Class II Biological Safety Cabinet (BSC).

3. RESPONSIBILITIES

3.1. Principal Investigator (PI)

3.1.1. Ensures the personnel under his/her supervision are trained on the safe and proper use of the BSC.

3.1.2. Ensures this SOP is followed.

3.2. Designated Person (is a member of the lab designated by the PI)

3.2.1. Oversees the implementation of this SOP.

3.2.2. Periodically inspects the BSC to ensure that it's operating according to the manufacturer's specification as per the BSCs Operating Manual.

3.2.3. Labels the BSC when it is not operating correctly.

3.2.4. Ensures that the date of the most recent certification is posted on the front of the BSC and is not past due. (Contact EHS immediately if the certification is past due).

3.2.5. Makes any necessary arrangements for repairs.

3.2.6. Reports to PI unsafe practices by BSC users.

3.3. BSC Users

3.3.1. Must complete the EHS safety training "*Safe Use of Biological Safety Cabinets*"

3.3.2. Must receive proper training from their PI or the designated person on the safe and proper use of the BSC prior to use.

Note: that after the training takes place, the training must be documented in *Annex 1- Documentation of Training for SOP## - Safe Use of a Class II Biological Safety Cabinet* in accordance to McGill, CFIA and PHAC regulations.

3.3.3. Must follow procedures as described in this SOP.

3.3.4. Shall report any injuries, accidents or spills to their PI and to Environmental Health and Safety via the McGill Accident, incident & occupational disease report form.

3.3.5. Shall report any defects or malfunctions of the BSC to their PI.

3.4. Environmental Health and Safety – McGill University (EHS)

3.4.1. Coordinates yearly BSC certification by a NSF49 certified technician.

3.4.2. The EHS officer is responsible for providing and maintining the safety training course "*Safe Use of Biological Safety Cabinets*".

4. PERSONAL PROTECTIVE EQUIPMENT

When working in a BSC, the operator must wear:

4.1. A dedicated closed-front over garment (e.g. surgical gown with full-length sleeves or fully buttoned lab coat).

4.2. Gloves that are appropriate to the type of work to be performed.

Note: Gloves should overlap the cuffs of the labcoat to ensure that aerosols do not contaminate the hands, arms.

4.3. Long pants or ankle length skirt and full covering shoes.

4.4. If there is a risk of possible splashing safety glasses are recommended.

5. MATERIALS

Prior to working in a BSC, ensure that you:

5.1. Are working with the appropriate BSC for your protocol (Refer to McGill Biosafety Manual section 1.3.2).

5.2. Have protocols written out and accessible (this may be in a lab book, protocol book, or online).

5.3. Identify the materials that need to be placed in the BSC and which materials should be placed outside. This will minimize the in-and-out motions that could affect the protective barrier of the BSC.

6. PROCEDURE

6.1. BSC Start-Up Procedure

6.1.1. If a UV light is being employed, turn it off.

6.1.2. Turn on the BSC and open the sash to the appropriate height.

6.1.3. Cabinet blowers should be operated at least five minutes before beginning work to allow the cabinet to "purge". This purge will remove any particulates in the BSC.

6.1.4. Ensure that nothing is blocking the front and back grilles.

6.1.5. The work surface, the interior walls (not including the supply filter diffuser – top of the inside of the cabinet), and the interior surface of the window should be disinfected with either:

6.1.5.1. 70% ethanol (EtOH) or

6.1.5.2. Another disinfectant as determined in your Biohazard permit to meet the requirements of the particular activity.

Note: When bleach is used, a second wiping with sterile water is needed to remove the residual chlorine, which may eventually corrode stainless steel surfaces. Wiping with non-sterile water may re-contaminate cabinet surfaces, a critical issue when sterility is essential (e.g., maintenance of cell cultures).

6.2. While working in a BSC

After the BSC has been sufficiently purged and decontaminated, the following practices should be employed to maintain product, personnel and environmental protection:

6.2.1. **Arm Movements**: While working in a BSC, it is imperative that the airflow is not significantly altered for the BSC to function properly.

6.2.1.1. Once hands/arms are placed inside the cabinet, manipulation of materials should be delayed for approximately one minute. This allows the cabinet to stabilize and to "air sweep" the hands and arms to remove surface microbial contaminants.

6.2.1.2. Move arms in and out slowly, perpendicular to the face opening of the cabinet.

6.2.1.3. Ensure that rapid arm movements in sweeping motions are minimized. This movement will disrupt the air curtain and may compromise the containment that is provided by the BSC.

6.2.2. **Front Grille:** To ensure that the BSC can provide proper product, personnel and environmental protection, it is important that the front grilles are not blocked.

6.2.2.1. Raise arms slightly to ensure that arms are not resting on the grille.

6.2.2.2. Ensure other items are not blocking the grille (e.g. protocols, pipettes etc).

6.2.3. **Placement of materials inside the BSC**: Materials or equipment placed inside the cabinet may cause disruption to the airflow, resulting in turbulence, possible cross-contamination, and/or breach of containment.

6.2.3.1. The surfaces of all materials and containers placed into the cabinet should be wiped with 70% EtOH to reduce the introduction of contaminants to the cabinet environment. This simple step will reduce introduction of mold spores and thereby minimize contamination of cultures.

6.2.3.2. Only the materials and equipment required for the immediate work should be placed in the BSC.

6.2.3.3. Extra supplies (e.g., additional gloves, culture plates or flasks, culture media) should be stored outside the cabinet.

6.2.3.4. All operations should be performed as far to the rear of the work area as reasonable.

6.2.3.5. Active work should flow from the clean to the dirty area across the work surface (see *Annex 2 – BSC Workflow*).

6.2.4. Good Microbiological Practices: Many common procedures conducted in BSCs may create splatter or aerosols. Good microbiological techniques should always be used when working in a biological safety cabinet. For example, techniques to reduce splatter and aerosol generation will minimize the potential for personnel exposure to infectious materials manipulated within the cabinet. Class II cabinets are designed so that horizontally aerosol spores will be captured by the downward flowing cabinet air within fourteen inches of travel.

6.2.4.1. Keep clean materials at least one foot away from aerosol-generating activities. This will minimize the potential for cross-contamination.

6.2.4.2. The general workflow should be from "clean" to "dirty" areas. Materials and supplies should be placed in such a way as to limit the movement of "dirty" items over "clean" ones.

6.2.4.3. When working with tissue culture dishes and plates, the lid should be hold above the open sterile surface to minimize direct impaction of downward air. Same thing for opened tubes or bottles; they should not be held in a vertical position but tilted.

6.2.4.4. When working with lidded and capped materials work should be performed in a manner where lids and caps are not placed on the work surface.

6.2.4.5. Items should be recapped or covered as soon as possible.

6.2.5. Biohazard wastes management: The frequent inward/outward movement needed to place objects in biohazard waste bags or to dispose of serological pipettes is disruptive to the integrity of the cabinet air barrier and can compromise both personnel and product protection. The following describes specific practices to use when working with either of these items:

6.2.5.1. **Biohazard bags:** Typically used when contaminated waste is going to be autoclaved (refer to the SOP: "General Autoclave Use In Treating Biowaste"). Any other type of plastic bags can be used for incineration.

6.2.5.1.1. Ensure that the correct type of bag is used for the biohazard and method of decontamination used.

6.2.5.1.2. To minimize the chance of leaks, double bagging is used.

6.2.5.1.3. The bag should be placed to the dirty side of the interior of the cabinet.

6.2.5.1.4. Materials that are contaminated must be placed into the bag and the bag must be **sealed** prior to being removed from the cabinet.

6.2.5.2. **Serological pipettes, Pasteur pipettes and tips disposal:**

6.2.5.2.1. Practices to use when discarding serological pipettes, Pasteur pipettes and tips that will be decontaminated using chemical disinfectants:

6.2.5.2.1.1. Discarded pipette or tip should be placed to the dirty side of the interior of the cabinet into a container containing the disinfectant.

6.2.5.2.1.2. Items should be introduced into the container with minimum splatter, and allowed appropriate contact time as per manufacturer's instructions.

6.2.5.2.1.3. The surface of the container is decontaminated in the BSC prior to removal from the cabinet.

6.2.5.2.1.4. The decontaminated pipettes and tips are placed into a properly identified cardboard box: "sharp or broken glass – not hazardous – not contaminated – for regular garbage". Make sure that the box can be safely handled by the cleaning staff.

6.2.5.2.2. Practices to use when serological pipettes and tips are decontaminated using the autoclave or incineration (McGill Hazardous Waste Management boxes):

6.2.5.2.2.1. Discarded pipette and tip should be placed to the dirty side of the interior of the cabinet into an appropriate container or bag.

6.2.5.2.2.2. A paper towel or an absorbent material is placed inside the container or the bag.

6.2.5.2.2.3. The container or bag needs to be hermetically sealed and its exterior decontaminated prior to removal from the cabinet.

6.2.5.2.2.4. Follow the SOP "General Autoclave Use in Treating Biowaste" if the autoclave is used to decontaminate the waste or McGill Hazardous Waste Management guidelines if incineration boxes are used.

6.2.5.2.3. Practices to use when Pasteur pipettes are not decontaminated using chemical disinfectants:

6.2.5.2.3.1. Pasteur pipette should be disposed of only in a Canadian Standards Association approved sharps container.

6.2.5.2.3.2. Once full, properly close the container in the BSC, surface decontaminate it and discard it according to McGill Hazardous Waste Management guidelines.

6.2.5.3. **Sharps disposal:**

6.2.5.3.1. Follow exactly steps 6.2.5.2.3.1 and 6.2.5.2.3.2.

6.2.6. Absorbent Toweling: Plastic-backed absorbent toweling can be placed on the work surface (but not on the front and rear grilles). This toweling facilitates routine cleanup and reduces splatter and aerosol formation during an overt spill. If used, it must be folded and placed in a biohazard bag right after work is completed.

6.2.7. Aerosol generating equipment: Aerosol-generating equipment (e.g.: vortex mixers) should be placed toward the rear of the cabinet to take advantage of the air split that occurs in the BSC. The downward moving air "splits" as it approaches the work surface; the blower draws part of the air to the front grille and the remainder to the rear grille.

6.2.8. Open Flames

6.2.8.1. Use of an open flame is forbidden in the BSC as per the Canadian Biosafety Standards section 4.6.3.

6.2.8.2. Small electric "furnaces" are available for decontaminating loops and needles inside the BSC. Disposable sterile loops can also be used.

6.2.9. Aspirator bottles or suction flasks: Aspirator bottles or suction flasks should be connected to an overflow collection flask (See *Annex 3 – Vacuum installation in a BSC*) containing appropriate disinfectant, and to an in-line HEPA or equivalent filter. The flasks and aspirator bottles, if kept in the BSC, must be kept to one side of the cabinet. Flasks and aspirator bottles kept outside the BSC must be placed in a leak-proof, unbreakable secondary container large enough to contain the full volume of the flask or bottle. This combination will provide protection to the central building vacuum system or vacuum pump, as well as to the personnel who service this equipment. Inactivation of aspirated materials is accomplished by placing sufficient chemical decontamination solution into the flask to kill the micro-organisms as they are collected. Once inactivation occurs, liquid materials can be disposed of appropriately as noninfectious waste as per McGill Hazardous Waste Management guidelines.

6.2.10. Biohazard Spills: Small contained spills on the work surface can be handled as outlined in McGill Biosafety Manual (section 3.3.1). Spills large enough to result in liquids flowing through the front or rear grilles require more extensive decontamination.

6.2.10.1. All items within the cabinet should be surface decontaminated and removed.

6.2.10.2. After ensuring that the drain valve is closed, decontaminating solution can be poured onto the work surface and through the grille(s) into the drain pan.

6.2.10.3. Twenty to thirty minutes is generally considered an appropriate contact time for decontamination, but this varies with the disinfectant and the microbiological agent as defined in your "Application to use Biohazardous Materials" form. Manufacturer's directions should be followed.

6.2.10.4. The spilled fluid and disinfectant solution on the work surface should be absorbed with paper towels and discarded into a biohazard bag.

6.2.10.5. The drain pan should be emptied into a collection vessel containing disinfectant. A flexible tube should be attached to the drain valve and be of sufficient length to allow the open end to be submerged in the disinfectant within the collection vessel. This procedure serves to minimize aerosol generation.

6.2.10.6. The drain pan should be flushed with water and the drain tube removed.

6.2.10.7. Gloves should be disposed of and hands must be washed.

Note: Should the spilled liquid contain radioactive material, refer to the Radiation Safety Manual for cleaning. Radiation Safety Officer has to be contacted to report any incidents involving radiation.

6.2.11. Power Failure while working in the BSC:

When a power failure occurs while you are working in the BSC, the following procedures must be employed:

6.2.11.1. Seal all open containers.

6.2.11.2. Dispose of gloves within the BSC.

6.2.11.3. If the BSC has a movable sash, bring it down to the closed position.

6.3. BSC shut down procedures:

After work is completed in the cabinet, the following procedures should be followed:

6.3.1. Allow the cabinet to run for 5 minutes with no activity.

6.3.2. All containers and equipment should be surface decontaminated prior to removal.

6.3.3. Remove gloves and dispose of them as appropriate. Wash your hands.

6.3.4. Put on clean gloves and ensure that all contaminated materials have been appropriately disposed of in the biohazard bag or other waste containers. Seal and surface decontaminate biohazard bags and waste containers and remove them from the BSC.

6.3.5. Decontaminate the work surface using an appropriate disinfectant (e.g. 70% ethanol or other appropriate disinfectant).

6.3.6. At the end of the workday, the final surface decontamination of the cabinet should include a wipe-down of the work surface, the cabinet's sides and back, and the interior of the glass.

6.3.7. Remove gloves and gowns and wash hands.

7. DEVIATIONS

Any deviations from this document must be noted in Appendix of the "Application to use Biohazardous Materials" form. Otherwise this SOP is documented by its SOP#.

8. REVIEW AND REVISION

This SOP is reviewed annually by the SOP committee appointed by the University Laboratory Safety Committee or whenever deemed necessary by the Biosafety Officer.

9. REFERENCES AND DEFINITIONS

Laboratory Safety Manual, Environmental Health and Safety, McGill University

McGill University Biosafety Manual, Environmental Health and Safety, McGill University

Canadian Biosafety Handbook, Chapter 11. (March 2016). Public Health Agency of Canada.

McGill Hazardous Waste Management website

10. DOCUMENT APPROVAL SIGNATURES AND REVISION HISTORY

Initial Creation Date: September 14th, 2020				
Created By:				
Revision #	McGill Staff	Biosafety Advisor	ULSC Chair	Date
Initial approval	SOP committee 2018-19	Ruth Blanchette	Dr Alvin Shrier	14-Sep-20
Revision				

Annex 1: Documentation of Training for SOP## - Safe Use of a Class II Biological Safety Cabinet

Trainer: _____ **Trainee:** _____

Print name Print name

Training Modules	**Trainer's Signature**	**Trainee's Signature**	**Date (DD/MM/YY)**
□ I have read and understood SOP## Safe Use of a Class II Biological Safety Cabinet			
□ BSC Start-up procedures			
□ Setting up the work space			
□ Aseptic techniques and procedures			
□ Biohazard waste management			
□ Spill clean-up			
□ Is familiar with emergency procedures (ie. power failure)			
□ Decontamination and disinfection			
□ Shut-down procedures			

Principal Investigator's Statement:

The above trainee has demonstrated proficiency in the practices and techniques required for work on this project.

Signature: _____ Date: _____

Annex 2 – BSC Workflow

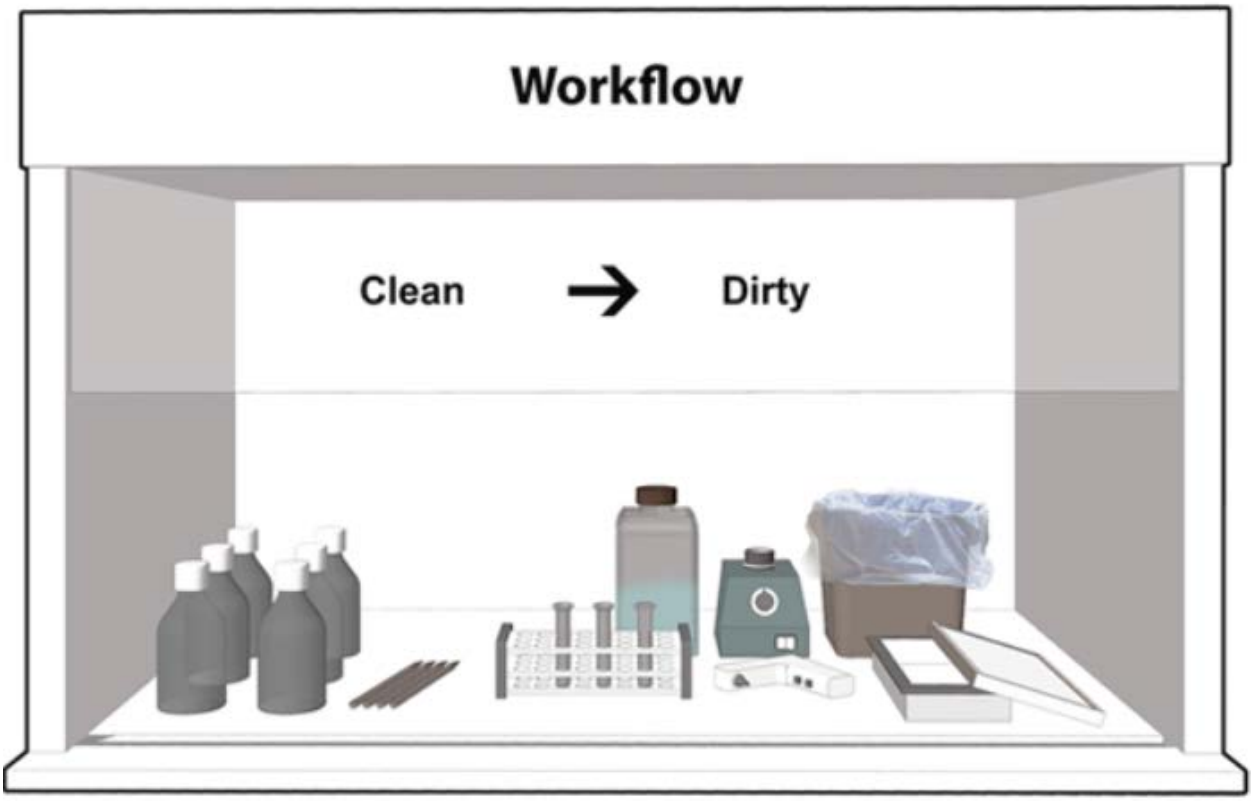

Figure 11-8: Representative Diagram of a Recommended Layout of Materials and Workflow inside a Biological Safety Cabinet (BSC)

This figure shows a BSC set up for work, with clean reagents and pipets placed to the left, a rack with tubes in the middle, solid and liquid waste containers to the back and right, and a waste tray for pipets to the right. A vortex mixer is placed towards the back of the work area, and a cordless pipetting device near the centre, beside the rack. The direction of workflow goes from the "clean" side (i.e., less contaminated) to the "dirty" side (i.e., higher contamination).

Image taken from: *Canadian Biosafety Handbook*, Public Health Agency of Canada. March 2016. https://www.canada.ca/en/public-health/services/canadian-biosafety-standards-guidelines/handbook-second-edition.html

Annex 3 – Vacuum installation in a BSC

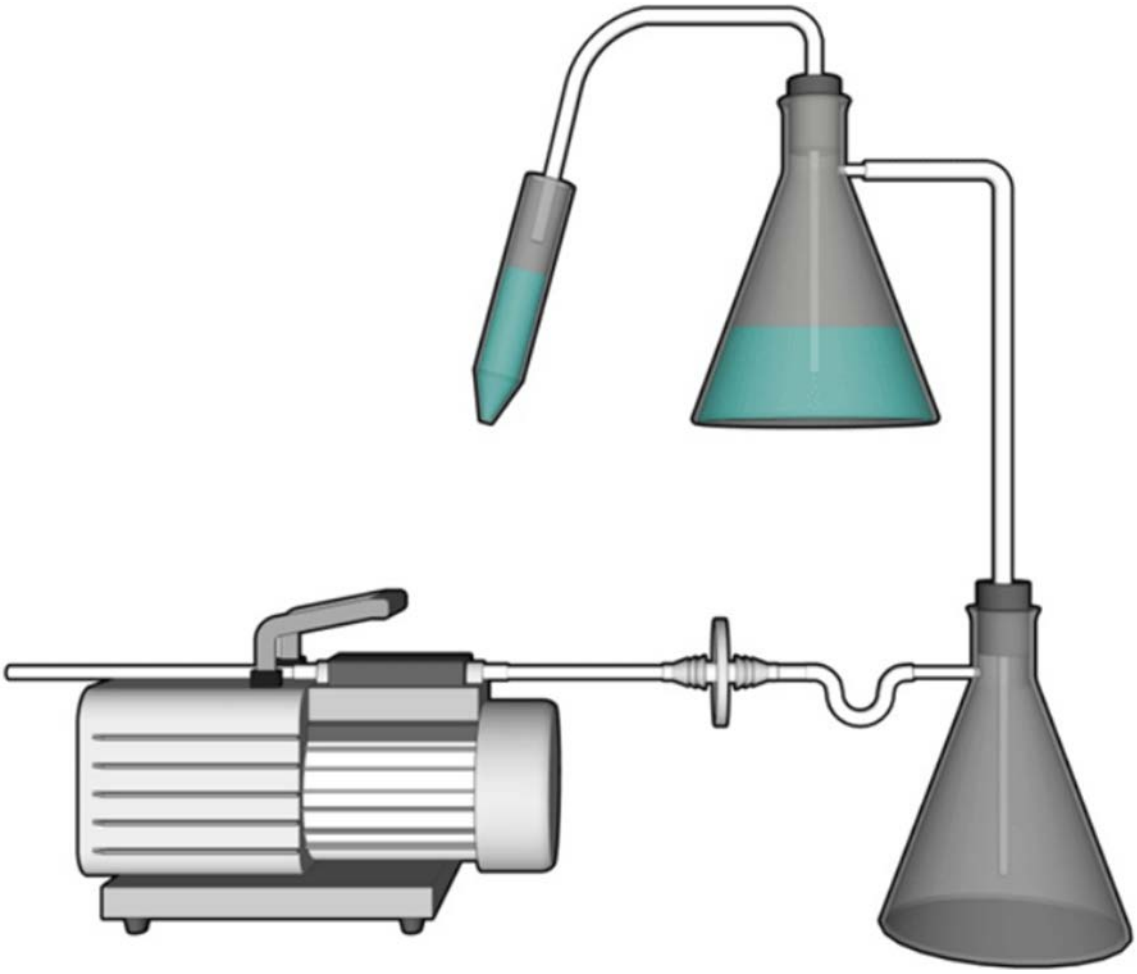

Figure 12-1: Representative Diagram of a Vacuum System Set-up for the Aspiration of Infectious Liquids

In this diagram, liquid from a conical centrifuge tube is aspirated through a tube into a conical flask containing a disinfectant solution used for the collection and decontamination of liquid waste. This flask is connected via a hose to a second flask, which also contains disinfectant, and is used to collect any overflow and to trap aerosols. The vacuum source in this illustration is a portable vacuum pump. It is protected against infectious aerosols or aerosolized toxins through the use of an in-line filter, in this case a 0.2 µm filter, connected between the overflow flask and the vacuum source.

Note: Vacuum flasks and aspirator bottles kept outside the BSC must be kept in leak-proof unbreakable secondary containers large enough to contain the full volume of the flasks or bottle.

Image taken from: *Canadian Biosafety Handbook*, Public Health Agency of Canada. March 2016. https://www.canada.ca/en/public-health/services/canadian-biosafety-standards-guidelines/handbook-second-edition.html