Utilizing Biological Safety Cabinets

Environmental Health & Safety

SOP No. 5.3

	Print Name	Initial	Title	Date
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PURPOSE

This SOP describes appropriate procedures for safely operating a biological safety cabinet (BSC); pre- and post-operational procedures are included. A biological safety cabinet should be used to protect you and the environment (and your work if a Class II or III cabinet is used) in the following conditions:

1. When performing procedures with a high potential for creating aerosols or those that might cause splashing, spraying or splattering of droplets of biological materials
    a. (i.e., any procedure that imparts energy to a liquid sample or microbial suspension such as centrifuging, grinding, blending, vortexing, sonicating, vigorous shaking/mixing/pipetting, opening containers of materials whose internal pressures may be different from ambient pressures (e.g., cryovials, lyophilized samples, fermenters, etc.), and inoculation and tissue harvest procedures involving inoculated animals.
2. When large quantities or high concentrations of organisms are used.
3. For processing of human materials when possible, especially when the disease status is unknown, samples are from immunosuppressed individuals, or individuals that are known to be ill.
4. Handling and manipulation of BSL3 agents. Additional PPE, including respiratory protection, may also be required.

NOTES

Different BSCs have different features; customize this SOP for your unit prior to use in the laboratory. Biological spills within the BSC are covered in a separate SOP.

1. Flammable or otherwise volatile chemicals shall not be used in a Class II, Type A BSC. Limit the use of ethanol to decontamination of gloves and surface decontamination of materials within the BSC. Do not use ethanol to decontaminate the BSC.
2. BSCs should not be used for labeling biohazardous materials with radioactive isotopes.

3. Work with chemical carcinogens and other toxic substances within a Class II BSC requires additional treatment measures for the exhausted air. A chemical fume hood shall be used for procedures using volatile, toxic or carcinogenic chemicals.

MAINTENANCE

Biosafety cabinets require regular maintenance and certification by a professional technician to assure that it protects you, your experiments, and the environment. Full details are covered in Section B9.1 of the University Laboratory Safety Manual.

1. Each cabinet must be certified when it is installed and annually after installation. Annual BSC certification is the PI's responsibility and completed by a third party; please call EHS if you need a list of vendors.
2. Moving the cabinet or repairs made to the cabinet void any current certification such that if the cabinet is moved from the original place it was installed and certified or if any repairs are made, recertification is required before use is resumed.
3. UV lamps must be properly maintained and never used as the sole source of hood decontamination. Do not operate lamps when the room is occupied. Check manufacturer guidelines for maintenance and frequency of replacement.
    a. Cleaning bulb weekly to remove dust and debris
    b. Check bulb regularly with a UV meter to ensure a germicidal intensity of UV light is being emitted.

PROTECTIVE EQUIPMENT

Personal protective equipment (PPE) varies with the task and laboratory biosafety level. At minimum, the following shall be worn while working in the biological safety cabinet:

- a laboratory coat (barrier style is best)
- safety glasses, and
- disposable gloves

A barrier-style coat snaps to the neck and has knit cuffs at the bottom of the sleeves. Gloves should be worn over the cuffs. Additional PPE such as double gloves, surgical mask or respirator (fit-testing required) may be utilized when it is warranted by procedures, materials or operational biosafety level. Contact EHS if you have questions regarding appropriate PPE.

MATERIALS

You must **PLAN** your work when you use the BSC. Working in a BSC is not quick. Proper preparation and procedures minimize contamination to you, your materials and the laboratory environment. Utilize checklists and SOPs.

PROCEDURE

The procedure below is divided in to 3 sections: Preparation, Operation, and Completion. The following is adapted from Section B9.1.4 of the University Laboratory Safety Manual.

Preparation

1. Check certification date on BSC. Certification must be within the past 12 months. If certification has expired, DO NOT USE THE BSC.
2. Turn on fluorescent light and turn off the UV light if in use.
3. Ensure the sash is in the appropriate position. Turn on the blower fan. If blower was off, allow the cabinet to purge for a minimum of 5 minutes before beginning decontamination.
    a. If your cabinet has a magnehelic gage: compare the pressure reading on the magnehelic gage to the certification sticker. If the gage reads at or within 10% greater pressure than the inspected value, the BSC may be used. If the gage reads lower than the inspected reading or more than 10% greater, then DO NOT USE THE BSC. Post an out of order sign and call a vendor for repairs.
4. Check gages/monitors to ensure the unit is functioning properly. If a warning light or alarm is signaling the cabinet is not functioning properly, DO NOT USE THE BSC.
5. Disinfect the cabinet work surface, interior walls and the interior surface of the window with a disinfectant (in addition to ethanol) appropriate to agent(s) in the work area.
    a. Do not raise sash beyond operable height during disinfection.
    b. Leave disinfectant, biowaste and extra paper towels inside the BSC. Decontaminate or remove your gloves before exiting the BSC.
    c. Utilize the contact time to prepare materials to load into the BSC.
6. Prepare materials to load into BSC.
    a. Only the items necessary for the immediate steps should be placed in the BSC to avoid overcrowding the cabinet.
    b. Decontaminate the surfaces of all materials to be placed in the cabinet with appropriate disinfectant – allow adequate contact time.
    c. Load decontaminated materials into a plastic tote and place the tote into the BSC to minimize disruption of the protective air curtain. Decontaminate all surfaces of the tote and your gloves before moving the tote into the cabinet.
7. Extra supplies (gloves, extra tips, etc.) should be stored outside the BSC. Keep the work area free of unnecessary equipment/supplies which may affect proper airflow and subsequently, your protection.
8. Allow air curtain to stabilize for a couple minutes before unloading tote.
9. Keep the front and rear grilles clear.
10. Plastic-backed absorbent liners may be used so long as they do not obstruct the front or rear grille openings.
11. Bulky items should be placed to the rear and to one side of the work surface. Aerosol-generating equipment such as vortexes should be placed as far back as possible in the cabinet.

12. Set up workflow clean to dirty. Locate the container(s) for disposal of items inside the cabinet.
    a. Use horizontal pipette discard trays filled with disinfectant (no ethanol) or pipet keeper boxes for serological pipets.
    b. Work must be completed at least 6 inches (15cm) from the front grill.
13. Adjust stool so that your face is above the sash opening.

Operation

1. If the alarm starts sounding at any point while working, secure your work and follow the "Completion" outline below.
2. Work as far to the back (beyond the grille) of the BSC workspace as possible and at least 6 inches (15cm) beyond the front grille. Do not obstruct grilles.
3. Keeping clean materials away from aerosol-generating activities will minimize cross contamination.
4. Move smoothly and deliberately while working in the BSC.
    a. Once arms are in the BSC, delay work 1 min to allow the air curtain to stabilize.
    b. If you must remove your hands from the cabinet, decontaminate your gloves before removing your hands from the cabinet. Insert and remove arms perpendicular to the unit.
    c. Avoid side-to-side, sweeping movements.
    d. Do not rest arms (or any other materials) on the front grille.
5. Use mechanical pipetting aids.
6. Always use good aseptic and microbial technique when working in a BSC.
    a. Keep open tubes/bottles in a vertical position and do not place lids on work surface. Hold the lid above the sterile surface of petri or tissue culture dishes. Recap or recover items as soon as possible.
7. Open flames are not to be used inside a BSC. Use disposable loops and needles. If metal must be use, use bead baths or touch incinerators.
8. Do not bring any contaminated materials outside the BSC. All materials must be decontaminated prior to removal from the BSC.

Completion

1. With the BSC still running,
    a. Decontaminate the surfaces of supplies and materials. Remove any newly inoculated materials first. Reload the tote, decontaminate your gloves and remove from the BSC.
    b. Decontaminate any equipment, gloves and remove them from the cabinet.
    c. Wipe-down the work area, interior walls and the interior surface of the window with a disinfectant determined by the PI which meets the requirements of the particular agent, collect at biowaste – seal the bag if not treated, decontaminate your gloves and remove the waste from the cabinet.
2. Close sash if desired and turned off BSC unless laboratory protocol states the BSC is to be kept running.
3. The UV light may be turned on at this point if others are not working nearby.

EMERGENCY PRE-PLANNING

Secure your work and proceed with the appropriate emergency response SOP.

Evacuation

If you are required to evacuate the building,

1. Secure your work.
2. Contain/ neutralize your waste.
3. Leave the cabinet running.
4. Decontaminate your gloves, remove PPE and Evacuate.

- If the delay that would result in steps 1 and 2 puts your life in danger, decontaminate your gloves, remove PPE and evacuate.

Spills

Follow emergency spill protocols in the event of a spill (see section B6.3.4 of the University laboratory Safety Manual or refer to the SOP for spills within a BSC). Leave the BSC running while you are cleaning up the spill.

If you are uncertain on how to manage a spill, please call EHS for assistance at 806-742-3876.

REFERENCES

Section B9.1 of the University Laboratory Safety Manual.

Appendix BI of the University Laboratory Safety Manual

AKNOWLEGEMENT OF PROFICIENCY

The individuals below have been trained and are competent in completing the above procedure.

Worker Name	Worker Signature	Date	Supervisor Initial	Date