Tennessee TECH

Environmental Health and Safety

BIOLOGICAL SAFETY CABINET (BSC) USE
STANDARD OPERATING PROCEDURE

I. **Purpose**

a. This program will establish requirements and procedures for biological safety cabinet (BSC) work practices and certifications.

II. **Scope**

a. The following requirements shall apply and be followed by all TTU Departments and personnel utilizing biosafety cabinets. BSC must be used as primary containment for work with potentially infectious agents if a risk assessment shows that the failure to use a biosafety cabinet could pose an infection risk.
b. The importance of ensuring BSC are functioning properly cannot be overemphasized. The consequences of using faulty equipment can range from contaminated product and wasted research time to serious illness and/or death of laboratory personnel.
c. Environmental Health and Safety (EHS) is available to assist with risk assessments and to advise on the effective use of biosafety cabinets.

III. **Responsibilities**

a. Department Chair:
    i. Responsible for disseminating and reinforcing this procedure to all employees that utilize a Biosafety Cabinet.
b. Faculty/Laboratory Manager:
    i. Responsible for the implementation of this SOP.
    ii. Provide training on the biological safety cabinet safe work practices to laboratory personnel, students, and faculty.
    iii. Establish written standard operating procedures (SOPs) for any work with infectious materials that involves a BSC, and ensure that all users adhere to these procedures.
    iv. Ensure personnel receive all applicable training with Environmental Health and Safety (review the EHS Training Matrix).
    v. Ensure that personnel working with infectious materials are properly trained and have demonstrated competency applicable microbiological techniques.
    vi. Maintain training documentation and produce records upon request.
c. Environmental Health and Safety:

i. Provide advice on the selection and installation of new biological safety cabinets.
ii. Provide advice on the relocation of existing biological safety cabinets.

IV. **Types of Biological Safety Cabinets**

a. Biological safety cabinets are intended to protect the user and environment from the hazards associated with the handling of infectious material and other biohazardous material. Some types also protect the materials being handled in them from contamination.

b. Class I BSC

i. The Class I BSC provides personnel and environmental protection, but no product protection. It is similar in air movement to a chemical fume hood, but has a HEPA filter in the exhaust system to protect the environment. In the Class I BSC, unfiltered room air is drawn across the work surface. Personnel protection is provided by this inward airflow as long as a minimum velocity of 75 linear feet per minute (lfpm) is maintained through the front opening. In many cases Class I BSCs are used specifically to enclose equipment (e.g., centrifuges, harvesting equipment or small fermenters), or procedures (e.g. cage dumping, aerating cultures or homogenizing tissues) with a potential to generate aerosols. The Class I BSC is hard-ducted to the building exhaust system, and the building exhaust fan provides the negative pressure necessary to draw room air into the cabinet. Cabinet air is drawn through a HEPA filter as it enters the exhaust plenum. A second HEPA filter may be installed at the terminal end of the exhaust.

c. Class II (Types A, B1, B2, and B3)

i. The Class II (Types A, B1, B2, and B3) biological safety cabinets provide personnel, environmental and product protection. Airflow is drawn around the operator into the front grille of the cabinet, which provides personnel protection. In addition, the downward laminar flow of HEPA-filtered air provides product protection by minimizing the chance of cross-contamination along the work surface of the cabinet. Because cabinet air has passed through the exhaust HEPA filter, it is contaminant-free (environmental protection), and may be re-circulated back into the laboratory (Type A BSC) or ducted out of the building (Type B BSC). All Class II cabinets are designed for work involving microorganisms assigned to biosafety levels 1, 2 and 3. Class II cabinets provide the microbe-free work environment necessary for cell culture propagation, and also may be used for the formulation of nonvolatile antineoplastic or chemotherapeutic drugs.

d. Class III

i. The Class III biological safety cabinet was designed for work with infectious agents using biosafety level 4 containment practices, and provides maximum

protection to the environment and the worker. It is a gas-tight enclosure with a non-opening view window. Access for passage of materials into the cabinet is through a dunk tank (that is accessible through the cabinet floor) or double-door pass-through box (such as an autoclave) that can be decontaminated between uses. Reversing that process allows for safe removal of materials from the Class III biosafety cabinet. Both supply and exhaust air are HEPA filtered. Exhaust air must pass through two HEPA filters, or a HEPA filter and an air incinerator, before discharge to the outdoors. Airflow is maintained by a dedicated independent exhaust system exterior to the cabinet, which keeps the cabinet under negative pressure (usually about 0.5 inches of water pressure).

ii. Long, heavy-duty rubber gloves are attached in a gas-tight manner to ports in the cabinet and allow for manipulation of the materials isolated inside. Although these gloves restrict movement, they prevent the user's direct contact with the hazardous materials. The tradeoff is clearly on the side of maximizing personal safety. Depending on the design of the cabinet, the supply HEPA filter provides particulate-free, albeit somewhat turbulent, airflow within the work environment.

e. Laminar Flow "Clean Bench"
    i. Laminar flow clean air benches are not BSCs. They discharge HEPA-filtered air across the work surface and toward the user. These devices only provide product protection. They can be used for certain clean activities, such as the dust-free assembly of sterile equipment or electronic devices or pouring agar. These benches should never be used when handling cell culture materials or drug formulations, or when manipulating potentially infectious materials. The worker can be exposed to materials (including proteinaceous antigens) being manipulated on the clean bench, which may cause hypersensitivity.

V. **Safe Operating Procedures**

a. The BSC blower must be turned on at least five (5) minutes before starting work in order to purge the air and remove any particulates in the cabinet.
b. Prior to performing work be sure the biological safety cabinet is exhausting properly. If cabinet is not working properly then notify laboratory supervisor.
    i. Do not use the BSC.
    ii. Place a sign on the BSC indicating the BSC cannot be used.
    iii. The laboratory supervisor shall contact a company accredited to repair (Certification Company) the BSC.
    iv. Notify EHS of the malfunctioned BSC.
c. Laboratory personnel shall wear a closed-front laboratory coat (or a surgical gown) and gloves. The gloves should overlap the cuffs.
d. All materials needed for the manipulations shall be placed in the cabinet before the work is initiated to minimize in-and-out motions.

    i. The rapid movement of a worker's arms in a sweeping motion into and out of the cabinet will disrupt the air curtain and may compromise the partial barrier containment provided by the BSC.
    ii. Moving arms in and out slowly, perpendicular to the face opening of the cabinet will reduce the risk. Other personnel activities in the room (e.g. rapid movement, open/closing room doors, etc.) may also disrupt the cabinet air barrier.

e. Manipulation of materials should be delayed for approximately one minute after placing hands/arms inside the cabinet. This allows the cabinet to stabilize and to "air sweep" the hands and arms to remove surface microbial contaminants.
f. Do not cover the air intake grill. Laboratory personnel shall not rest arms on the air intake grill.
g. All operations shall be performed at least four (4) inches from the front grill on the work surface.
h. Always work from "clean" to "dirty" within the BSC, segregating contaminated and non-contaminated materials.
i. Extra supplies (e.g. additional gloves, culture plates or flasks, culture media) shall be stored outside the cabinet. Only the materials and equipment required for the immediate work shall be placed in the BSC.
j. Laboratory personnel shall clean up all minor spills in the cabinet immediately. In the case of a larger spill contact Environmental Health and Safety at 372-3524.
    i. Do not turn the BSC off while cleaning up a spill.
k. All materials used to clean up spills shall be discarded as biohazardous waste.
l. Biohazard collection bags shall not be taped or place on the outside of the cabinet. The frequent inward/outward movement needed to place objects in these bags is disruptive to the integrity of the cabinet air barrier and can compromise both personnel and product protection.
m. Pipette discard trays containing an appropriate chemical disinfectant shall be used within the cabinet.
n. Potentially contaminated materials (e.g. containers, equipment, etc.) shall not be brought out of the cabinet until they have been surface decontaminated.
o. Contaminated materials can be placed into a closable container for transfer to an incubator, autoclave or for other decontamination treatment.
p. Aspirator bottles or suction flasks shall be connected to an overflow collection flask containing appropriate disinfectant.
    i. Inactivation of aspirated materials can be accomplished by placing sufficient chemical decontamination solution into the flask to kill the microorganisms as they are collected. Once inactivation occurs, liquid materials can be disposed of appropriately as noninfectious waste.
q. Laboratory personnel shall decontaminate the cabinet with the <u>appropriate</u> disinfectant at the end of each work operation.

i. Periodic use of 5% household bleach in water is acceptable (70 % ethanol or quaternary ammonium compounds may also be used if effective against agent).

r. Do not use cabinet for any other function for which it was not intended.

s. The cabinet is not a substitute for personal protective equipment.
   i. Laboratory personnel shall wear safety glasses, laboratory coat, and gloves at all times when working in the cabinet.

t. Laboratory personnel shall dispose of gloves and disposable gowns as biohazardous waste.

u. Do not use hazardous chemicals in the cabinet.
   i. DO NOT use a biosafety cabinet with any toxic, flammable or explosive materials.

VI. Certification

Certification ensures cabinet airflows and containment factors are within safety limits and to ensure no filter damage has occurred.

a. Cabinets must be certified under the following conditions:
   i. At least annually when working with biological risk groups 2 and higher organisms, animal and human tissue cultures, recombinant DNA, and viral vectors.
   ii. If the airflow on a certified cabinet indicated by magnehelic gauges falls out of an established range.
   iii. Following HEPA filter change of a certified cabinet
   iv. Following service of a certified cabinet that may have affected containment ability.
   v. Following relocation (including within-room).

b. Costs for repairs and certification are to be paid by the Department or PI.

VII. References

Drexel University Biological Safety Cabinet Plan

CUNY Biological Safety Cabinet SOP

Biosafety in Microbiological and Biomedical Laboratories 5th Edition 2009 published by U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention and National Institutes of Health

Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets, 3rd Edition, U.S. Department of Health and Human Services, Centers for Disease Control and Prevention and National Institutes of Health September 2007.

National Science Foundation (NSF/ANSI) Standard 49, Annex F Class II Biosafety Cabinets and the NSF International Accreditation Policies for the Class II Biosafety Cabinet Field Certifier Accreditation Program. 2008