

# Biological Safety



In accordance with the NIH/CDC BMBL (Biosafety in Microbiological and Biomedical Laboratories), NCI-Frederick provides assistance in the safe handling and containment of infectious microorganisms and hazardous biological materials. The principles of biosafety are containment and risk assessment. The fundamentals of containment include the microbiological practices, safety equipment, and facility safeguards that protect laboratory workers, the environment, and the public from exposure to infectious microorganisms that are handled and stored in the laboratory. Risk assessment is the process that enables the appropriate selection of microbiological practices, safety equipment, and facility safeguards that can prevent laboratory-associated infections (LAI).

Work with infectious agents in public and private research, public health, clinical and diagnostic laboratories, and in animal care facilities has expanded. Recent world events have demonstrated new threats of bioterrorism. For these reasons, organizations and laboratory directors are compelled to evaluate and ensure the effectiveness of their biosafety programs, the proficiency of their workers, as well as the capability of equipment, facilities, and management practices to provide containment and security of microbiological agents. Similarly, individual workers who handle pathogenic microorganisms must understand the containment conditions under which infectious agents can be safely manipulated and secured. Application of this knowledge and the use of appropriate techniques and equipment will enable the microbiological and biomedical community to prevent personal, laboratory and environmental exposure to potentially infectious agents or biohazards.

## **Recombinant DNA**

In accordance with the National Institutes of Health (NIH) Guidelines, the National Cancer Institute (NCI-F) has established an Institutional Biosafety Committee (IBC). The committee represents the interests of the surrounding community with respect to public health and protection of the environment, animal containment principles and biological safety. NCI-Frederick complies with the NIH Guidelines for Research involving recombinant DNA molecules. The IBC reviews and approves all protocols and requires a three (3) year renewal process. The website for the IBC is <http://web.ncifcrf.gov/ehs/ibc/>. For more information on all biological research material handling and use, refer to the Policy No. 706 at <http://web.ncifcrf.gov/Staff/Policies/Documents/700/706.pdf>.

## **Biosafety Levels**

**Biosafety Level 1** represents a basic level of containment that relies on standard microbiological practices with no special primary or secondary barriers recommended, other than a sink for hand washing. Biosafety Level 1 practices, safety equipment, and facility design and construction are appropriate for undergraduate and secondary educational training and teaching laboratories, and for other laboratories in which work is done with defined and characterized strains of viable microorganisms not known to consistently cause disease in healthy adult humans.

Safety Equipment:

- Special containment devices or equipment, such as a BSC, are not generally required.
- Protective lab coats, gowns, or uniforms are recommended
- Wear protective eyewear when conducting procedures that have potential to create splashes.
- Gloves must be worn

Biosafety Level 2 is appropriate when work is done with any human-derived blood, body fluids, tissues, or primary human cell lines where the presence of an infectious agent may be unknown. (Laboratory personnel working with human-derived materials should refer to the OSHA Bloodborne Pathogen Standard for specific required precautions).

Primary hazards to personnel working with these agents relate to accidental percutaneous or mucous membrane exposures, or ingestion of infectious materials. Extreme caution should be taken with contaminated needles or sharp instruments. Even though organisms routinely manipulated at BSL-2 are not known to be transmissible by the aerosol route, procedures with aerosol or high splash potential that may increase the risk of such personnel exposure must be conducted in primary containment equipment, or in devices such as a BSC or safety centrifuge cups. Personal protective equipment should be used as appropriate, such as splash shields, face protection, gowns, and gloves.

Secondary barriers, such as hand washing sinks and waste decontamination facilities, must be available to reduce potential environmental contamination.

Safety Equipment:

- Properly maintained BSC is used
- Protective lab coats, gowns, smocks or uniforms are required
- Eye and face protection (goggles, mask, faceshield) is used
- Gloves must be worn

Biosafety Level 2\* This level is defined as a BSL-2 laboratory facility using BSL-3 practices and procedures.

Biosafety Level 3 practices, safety equipment, and facility design and construction are applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents with a potential for respiratory transmission, and which may cause serious and potentially lethal infection. Primary hazards to personnel working with these agents relate to autoinoculation, ingestion, and exposure to infectious aerosols. At BSL-3, more emphasis is placed on primary and secondary barriers to protect personnel in contiguous areas, the community, and the environment from exposure to potentially infectious aerosols. For example, all laboratory manipulations should be performed in a BSC or other enclosed equipment, such as a gas-tight aerosol generation chamber. Secondary barriers for this level include controlled access to the laboratory and ventilation requirements that minimize the release of infectious aerosols from the laboratory.

Safety Equipment:

- All procedures involving the manipulation of infectious materials must be conducted within a BSC or other containment devices
- Protective lab coats with a solid-front
- Wear protective eyewear when conducting procedures that have potential to create splashes.
- Gloves must be worn

## **Animal Facilities**

Three standard biosafety levels are also described for activities involving infectious disease work with commonly used experimental animals. These four combinations of practices, safety equipment, and facilities are designated Animal Biosafety Levels 1, 2, and 3, and provide increasing levels of protection to personnel and the environment.

Animal Biosafety Level 1 is suitable for work in animals involving well-characterized agents that are not known to cause disease in immunocompetent adult humans, and present minimal potential hazard to personnel and the environment. ABSL-1 facilities should be separated from the general traffic patterns of the building and restricted as appropriate. Special containment equipment or facility design may be required as determined by appropriate risk assessment. Personnel must have specific training in animal facility procedures and must be supervised by an individual with adequate knowledge of potential hazards and experimental animal procedures.

Safety Equipment:

- A risk assessment should determine the appropriate type of personal protective equipment to be utilized. Special containment devices or equipment may not be required as determined by appropriate risk assessment.
- Protective laboratory coats, gowns, or uniforms may be required to prevent contamination of personal clothing. Protective outer clothing is not worn outside areas where infectious materials and/or animals are housed or manipulated. Gowns and uniforms are not worn outside the facility.

- Protective eyewear is worn when conducting procedures that have the potential to create splashes of microorganisms or other hazardous materials. Persons who wear contact lenses should also wear eye protection when entering areas with potentially high concentrations or airborne particulates.
- Gloves

Animal Biosafety Level 2 builds upon the practices, procedures, containment equipment, and facility requirements of ABSL-1. ABSL-2 is suitable for work involving laboratory animals infected with agents associated with human disease and pose moderate hazards to personnel and the environment. It also addresses hazards from ingestion as well as from percutaneous and mucous membrane exposure.

ABSL-2 requires that:

1. access to the animal facility is restricted;
2. personnel must have specific training in animal facility procedures, the handling of infected animals and the manipulation of pathogenic agents;
3. personnel must be supervised by individuals with adequate knowledge of potential hazards, microbiological agents, animal manipulations and husbandry procedures; and
4. BSCs or other physical containment equipment is used when procedures involve the manipulation of infectious materials, or where aerosols or splashes may be created.
5. The animal facility director establishes and enforces policies, procedures, and protocols for institutional policies and emergencies. Each organization must assure that worker safety and health concerns are addressed as part of the animal protocol review. Prior to beginning a study, animal protocols must also be reviewed and approved by the IACUC5 and the Institutional Biosafety Committee.

Safety Equipment:

- Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing.
- Gloves are worn to prevent skin contact with contaminated, infectious and hazardous materials and when handling animals.
- Gloves and personal protective equipment should be removed in a manner that prevents transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or are manipulated.

Animal Biosafety Level 3 involves practices suitable for work with laboratory animals infected with indigenous or exotic agents, agents that present a potential for aerosol transmission, and agents causing serious or potentially lethal disease. ABSL-3 builds upon the standard practices, procedures, containment equipment, and facility requirements of ABSL-2.

The ABSL-3 laboratory has special engineering and design features.

ABSL-3 requires that:

1. access to the animal facility is restricted;
2. personnel must have specific training in animal facility procedures, the handling of infected animals, and the manipulation of potentially lethal agents;
3. personnel must be supervised by individuals with adequate knowledge of potential hazards, microbiological agents, animal manipulations, and husbandry procedures; and
4. procedures involving the manipulation of infectious materials, or where aerosols or splashes may be created, must be conducted in BSCs or by use of other physical containment equipment.

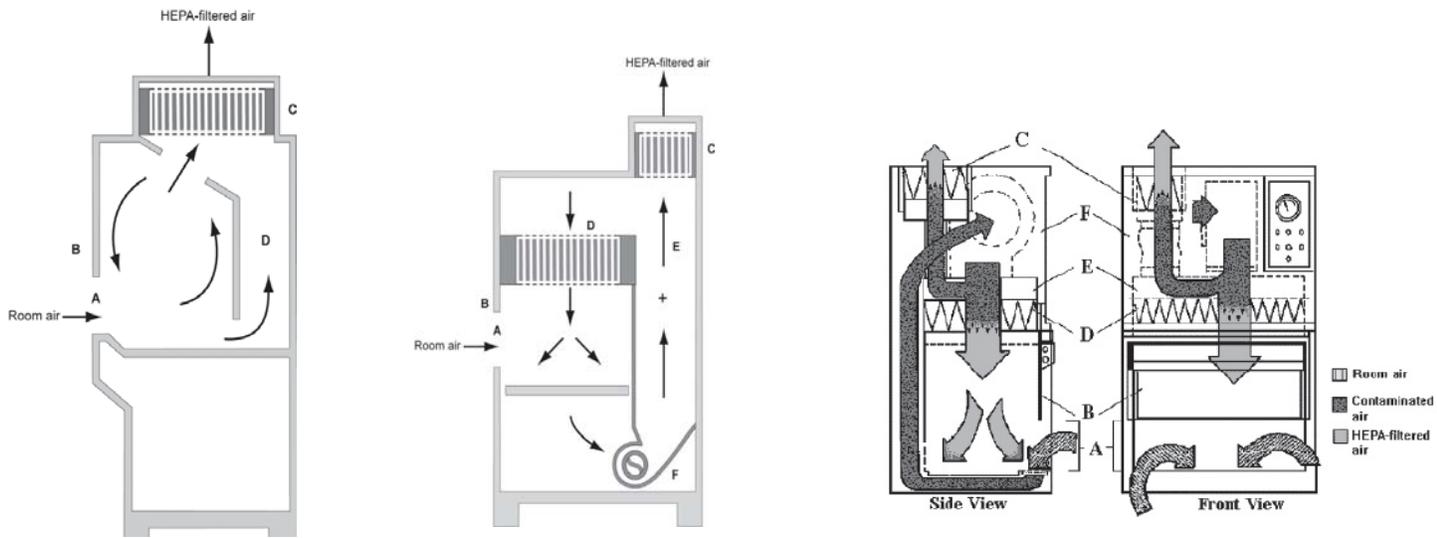
Safety Equipment:

- Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing.
- Gloves are worn to prevent skin contact with contaminated, infectious/hazardous materials and when handling animals. Double-glove practices should be used when dictated by risk assessment.
- Eye, face and respiratory protection should be used in rooms containing infected animals, as dictated by the risk assessment.
- Gloves and personal protective equipment should be removed in a manner that prevents transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or are manipulated.

**Biological Safety Cabinets**

Biological Safety Cabinets (BSCs) are designed to provide personnel, environmental and product protection when appropriate practices and procedures are followed. Three kinds of biological safety cabinets, designated as Class I, II and III, have been developed to meet varying research and clinical needs.

The BSC is the principal device used to provide containment of infectious droplets or aerosols generated by many microbiological procedures. Three types of BSCs (Class I, II, III) used in microbiological laboratories are described and illustrated in Appendix A. Open-fronted Class I and Class II BSCs are primary barriers that offer significant levels of protection to laboratory personnel and to the environment when used with good microbiological techniques. The Class II biological safety cabinet also provides protection from external contamination of the materials (e.g., cell cultures, microbiological stocks) being manipulated inside the cabinet.



**The Class I BSC** (A) front opening; (B) sash; (C) exhaust HEPA filter; (D) exhaust plenum.

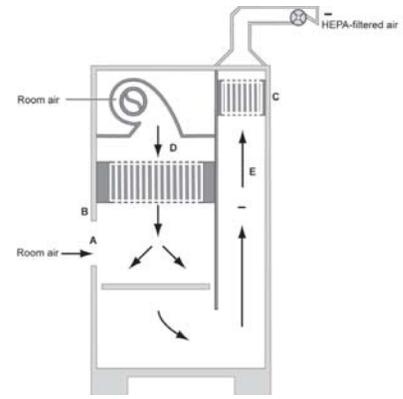
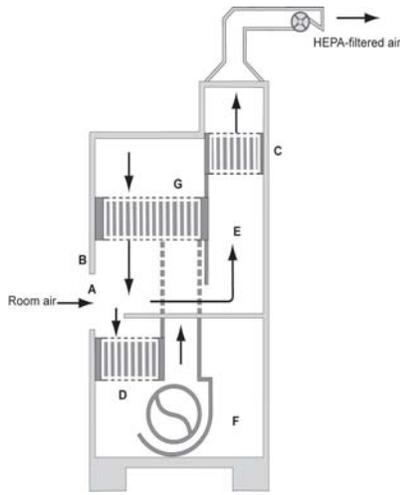
- o Does not protect the product from contamination because “dirty” room air constantly enters the cabinet
- o As a partial containment unit, this cabinet is suitable for work involving low to moderate risk agents
- o Personnel protection is made possible by constant air movement away from user.

**The Class II, Type A1 BSC** (A) front opening; (B) sash; (C) exhaust HEPA filter; (D) supply HEPA filter; (E) common plenum; (F) blower.

- o 70% air recirculation,
- o 30% exhausted from a common plenum to the room.
- o 75FPM intake.
- o Under positive pressure to the room.

**Class II, Type A2 BSC.** A. front opening, B. sash, C. exhaust HEPA filter, D. supply HEPA filter, E. positive pressure common plenum, F. negative pressure plenum.

- o 70% air recirculation,
- o 30% exhausted from a common plenum to the room.
- o 100 FPM intake;
- o Under negative pressure or surrounded by negative pressure.

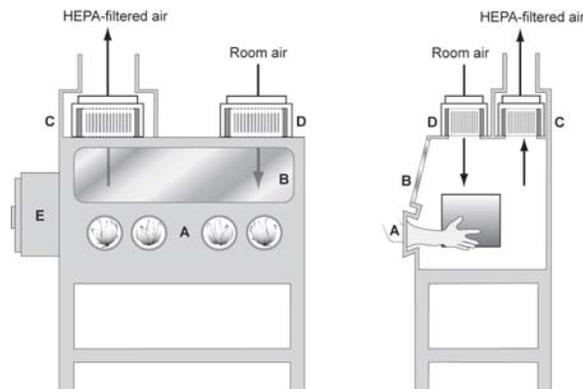


**The Class II, Type B1 BSC (classic design)** (A) front opening; (B) sash; (C) exhaust HEPA filter; (D) supply HEPA filter; (E) negative pressure dedicated exhaust plenum; (F) blower; (G) additional HEPA filter for supply air.

- 60% air recirculation
- 40% of air recirculated to the work area through a HEPA filter
- 100 FPM
- All biologically contaminated plenums are negative to the room or surrounded by negative pressure plenums

**The Class II, Type B2 BSC** (A) front opening; (B) sash; (C) exhaust HEPA filter; (D) supply HEPA filter; (E) negative pressure exhaust plenum.

- 0% air recirculated
- 100% exhausted from cabinet
- Exhaust air pulled through dedicated exhaust duct into facility exhaust system
- All ducts and plenums are under negative pressure.
- All contaminated ducts are under negative pressure or surrounded by directly exhausted negative pressure ducts or plenums.



**The Class III BSC** (A) glove ports with O-ring for attaching arm-length gloves to cabinet; (B) sash; (C) exhaust HEPA filter; (D) supply HEPA filter; (E) double-ended autoclave or pass-through box. *Note:* A chemical dunk tank may be installed which would be located beneath the work surface of the BSC with access from above. The cabinet exhaust needs to be hard connected to an exhaust system where the fan is generally separate from the exhaust fans of the facility ventilation system. The exhaust air must be double HEPA-filtered or HEPA-filtered and incinerated.

- 60% of descending air is pulled directly through the rear grille of the work area into a dedicated negative pressure plenum. This air passes through an exhaust HEPA filter then to an appropriate treatment system or outdoors via the facility's exhaust system.
- 40% of the descending air is pulled forward where it mixes with room air entering the perforated front grille. This air passes through HEPA supply filter directly below the work surface then is circulated under positive pressure through a duct to the top of the cabinet. Then through another HEPA supply filter, where the process is repeated.

Safety equipment also may include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Personal protective equipment is often used in combination with BSCs and other devices that contain the agents, animals, or materials being handled. In some situations in which it is impractical to work in BSCs, personal protective equipment may form the primary barrier between personnel and the infectious materials. Examples include certain animal studies, animal necropsy, agent production activities, and activities relating to maintenance, service, or support of the laboratory facility.

Proper use of a BSC is important.

- Clean to dirty. This means that one side has all the clean material you are working with. The center is where you are performing your work. The other side is where your contaminated items are (such as sharps container and waste)
- A vacuum line connections that draw biohazardous aerosols or fluids are to be fitted with
- a HEPA filter in the line leading into the vacuum line
- The use of an overflow flask, in case of accidental aspiration of liquids from the collection vessel, should be used. The flask should:
  - Flask should be of sufficient capacity
  - Be placed between the collection flask and the air filter
  - Contain an appropriate disinfectant for the material being used
  - Contain an antifoam agent whenever air bubbling generates excessive foam

### **Programs**

**Select Agent Program:** In the performance of scientific research, the NCI-Frederick may have occasion to use Select Agents as defined by 42 CFR §73, or High Consequence Animal or Plant Pathogens and Toxins as defined by 9 CFR §121 and 7 CFR §331. It is the policy of the NCI-Frederick to ensure that receipt, usage, storage, shipping and disposal of this material are performed in compliance with all applicable federal and state regulations and laws. For more information:

[http://home.ncifcrf.gov/ehs/uploadedFiles/D-4\\_Select\\_Agents.pdf](http://home.ncifcrf.gov/ehs/uploadedFiles/D-4_Select_Agents.pdf)

**DEA Controlled Substance Program:** It is the policy of the NCI-Frederick, when performing scientific research involving certain substances controlled by the Drug Enforcement Administration, U.S. Department of Justice, that the NCI-F adhere to all rules, regulations, and laws governing the registration, use, handling, storage, transport, and disposal of controlled substances. (21CFR1300-1308). Please refer to our Policy 709 at <http://web.ncifcrf.gov/Staff/Policies/Documents/700/709.pdf>. For more information regarding the DEA policy, see: <http://www.deadiversion.usdoj.gov/schedules/index.html>.

**To apply** for the use of a DEA Controlled substance, a written request is to be submitted to biological safety with the following information:

- A written request must be submitted to EHS.
- A justification for the use of the specific controlled substance requested.
- The requestor must identify the Drug Control Officer and his/her alternates (with phone extensions)
- The requestor must provide the location (building and room number) where the controlled substance will be located.

**Tax Free Alcohol Program:** In the performance of scientific research, it is the policy of NCI-Frederick to use tax-free alcohol controlled by the U.S. Department of the Treasury Alcohol and Tobacco Tax and Trade Bureau (TTB) and the Alcohol and Tobacco Tax Division of the state of Maryland. NCI-Frederick holds the Logistics Support Department (LSD) of the OTS Contractor and the off-site facility program director or his or her alternate accountable for the receipt, storage, and dispensing of tax-free alcohol. Denatured ethanol and isopropyl alcohol are not regulated and should be used when possible.

**To apply** for the use of tax free alcohol, a written request is to be submitted to biological safety with the following information:

- A justification for use of the tax free alcohol
- The name and telephone number of the proposed tax-free alcohol Officer and alternates
- Building and room number where the material will be stored
- Description of the security provisions (locked cabinet, safe, etc)
- Where the alcohol will be stored.
- Upon approval, EHS will assign a logbook number, issue a logbook, and add the investigator to the authorized list of users of tax-free alcohol.

For further information: [http://www.ttb.gov/industrial/taxfree\\_alcohol.shtml](http://www.ttb.gov/industrial/taxfree_alcohol.shtml)

**Autoclave Monitoring Program:** EHS provides autoclave monitoring for all autoclaves on campus. EHS will provide a Biological Indicator Ampule to the contact person to test the autoclave. The indicator is sent back to EHS for processing. This usually takes 24 hours.  
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hours. If the test fails, a second test will be performed. If the second test fails, service will be called for this unit. When the repair has been completed, a final test will be performed on the autoclave.

**Training:** Biological Safety provides a number of training classes:

- Bloodborne Pathogen – OSHA 29CFR 1910.1030, subpart Z; OSHA website: [www.osha.gov](http://www.osha.gov)
- Viral Vector Safety Training (VVST) – for use with lentivirus, adenovirus and retroviruses
- Biological Safety Cabinet
- Agent Specific

### **Decontamination Programs**

**Room/Facility Decon Services:** EHS serves as the COTR to assist with requests for the decontamination of a room or facility by an outside contractor. Charges for this service will be incurred by the program using a Blanket Purchase Agreement (BPA). If you need assistance, please contact EHS at 301-846-1451.

**Biological Safety Cabinet Decontaminations:** EHS provides assistance for the decontamination and certification of all Biological Safety Cabinets on campus. If you need assistance, please contact 301-846-1451.

### **Shipping Program:**

EHS personnel are responsible for classifying all shipments leaving NCI-Frederick, according to the DOT 49CFR Parts 100-185, IATA Dangerous Goods Regulations, EAR, and ITAR regulations. All employees must complete the Request for Shipment (RFS) <http://web.ncifcrf.gov/campus/safety/wizard> correctly, faxing a signed copy to the mailroom, and having the material available for pickup by transportation on shipment day. Transportation contact phone number is 301-846-1175 and the fax number is 301-846-6971. For more information: <http://web.ncifcrf.gov/Staff/Policies/Documents/500/511.pdf>.

### **References:**

**Safetygrams** – these are technical bulletins that provide information on a “specific” topic. For example: Working alone, Effective Use of a Biological Safety Cabinet, Sharps Safety. To see all the safety grams, go to: <http://web.ncifcrf.gov/ehs/ibc/Safetygram.aspx>

**EHS Home Page:** <http://home.ncifcrf.gov/ehs/>

**IBC Webpage:** <http://web.ncifcrf.gov/ehs/ibc/>

**Biosafety webpage:** <http://home.ncifcrf.gov/ehs/ehs.asp?id=9>

**Frederick Exposure Control Plan:** <http://home.ncifcrf.gov/ehs/uploadedFiles/2003%20ECPLAN%20draft.pdf>

**Lab Design:** For assistance in proper laboratory design, (equipment location, renovation of lab, etc.), contact EHS at 301-846-1451.

For further information regarding Biological Safety, please go to the **CDC/NIH Biosafety in Microbiological and Biomedical Laboratories Manual, 5<sup>th</sup> edition** at: <http://www.cdc.gov/biosafety/publications/bmbI5/index.htm>