

Laboratory Biosafety Manual

BSL-2 and BSL 2/3

[Principal Investigator]

[Title]

[Institute/Department]

[Laboratory Address/Location]

Prepared by:

[Name/Date]

Principal Investigators' Certification

I hereby certify that I have reviewed the contents of this manual and that it reflects my current operating practices.

Date Reviewed:	PI Signature:

Biological Agents Used in this Laboratory

Note: Registration of materials and addition or deletion of personnel and lab rooms must be processed through PI Dashboard, <https://oms.ors.nih.gov/>.

Biological Materials List ALL material in use in this lab: e.g., Microorganisms, Human-derived materials, Non-human primate blood, tissues, or body fluids, Biotoxins	Registration Number (designated by the IBC)	Special Requirements or Concerns (e.g., vaccines, teratogenic, PPE, etc.) NOTE: See IBC review comments for this information.

Signature and Acknowledgement of Risk

We, the undersigned, understand that the agents used in *Building Name*:

Room: are potentially hazardous. We have read and understand this manual and agree to follow the stated policies and procedures. All laboratory personnel are required to complete the “Introduction to Lab Safety” and “Working Safely with HIV and Other Bloodborne Pathogens” courses prior to working with human, and non-human primate, derived materials. Completion of the on-line refresher course is required every year thereafter.

Name	Signature	Date

Emergency Information

Principal Investigator 1:

Office Phone.....

Cell phone.....

Email address.....

Principal Investigator 2:

Office Phone.....

Cell Phone.....

Email address.....

Laboratory Manager:

Office Phone.....

Cell Phone.....

Email address.....

NIH Police/Fire/Rescue (on campus)	911
NIH Police/Fire/Rescue (using a cell phone)	301-496-9911
COUNTY Police/Fire/Rescue (off campus)	9-911
Chemical/Biological/Radiological Emergency (on campus)	911
NIH Security and Emergency Response.....	301-594-6677
Occupational Medical Service (OMS)	
M-F, 7:30 am - 5:00 pm	301-496-4411
After Hours/Weekends/Holidays - Biological Exposures	301-496-1211
Division of Occupational Health and Safety (main office)	301-496-2346
Radiation Safety.....	301-496-5774
Division of Environmental Protection.....	301-496-7990

Evacuation Meeting Place for Laboratory Personnel for each building/floor covered by this manual:

*Refer to NIH Policy Manual 1430 for the NIH Occupant Evacuation Plan.

Fire

In case of fire, evacuate immediately and activate the fire alarm pull station as you exit at the stairwells. Judgment should be exercised in deciding whether to store or contain any hazardous materials prior to evacuation. Remove all PPE and place into MPW container before leaving laboratory, if possible.

Injury

If an injury is life threatening call 911. For less serious injuries treatment should be sought at NIH Occupational Medical Services located on the 6th floor, C Wing of the Clinical Center Building 10) during weekday, daytime hours. During night and weekend hours, for injuries involving human pathogens or body fluids suspected of being contaminated with known biohazards, OMS should be contacted via the Clinical Center page operator (301-496-1211). Limited off-hour medical care is provided by the Clinical Center. Any injury to a laboratory worker must be reported as soon as possible to the supervisor. Refer to the 1-2-3 Poster for steps to take in the event of a potential bloodborne pathogen exposure.

Exposure to Biohazardous Materials

An exposure is defined as agent contact through a percutaneous injury, broken skin, or mucous membranes (e.g., eyes, nose, and mouth). In the event of an exposure:

1. Wash the area with soap and water or flush eyes, nose or mouth with large amounts of water for 15 minutes.
2. Notify OMS directly during normal business hours or through the NIH page operator after hours. Medical personnel will advise you as to when you should report to the clinic for a post-exposure follow up evaluation.
3. All exposures must be reported to the immediate supervisor and Principal Investigator.
4. Principal Investigators are responsible for reporting exposure incidents to the Division of Occupational Health and Safety 301-496-2960.

Security Incidents

Security incidents such as suspicious visitors or packages, missing chemicals, or missing biological agents must be immediately reported to the Principal Investigator and the NIH Police must be notified. Principal Investigators are responsible for reporting security incidents to the Division of Occupational Health and Safety.

In recent years, with the passing of federal legislation regulating the possession, use, and transfer of agents with high adverse public health and/or agricultural consequences (HHS and USDA Select Agents), greater emphasis has been placed on the emerging field of biosecurity. In contrast to biosafety, a field dedicated to the protection of workers and the environment from exposures to infectious materials, the field of biosecurity prevents loss of valuable research materials and limits access to infectious materials by individuals who would use them for harmful purposes. Nevertheless, proper containment of biological materials is essential to both biosafety and biosecurity

Purpose

A fundamental objective of any biosafety program is the containment of potentially harmful biological agents. The term "containment" is used in describing safe methods, facilities, and equipment for managing infectious materials in the laboratory environment where they are being handled or maintained. The purpose of containment is to reduce or eliminate exposure of laboratory workers, other persons, and the outside environment to potentially hazardous agents. The risk assessment of the work to be done with a specific agent will determine the appropriate combination of these elements.

This Biosafety Manual outlines procedures for using and disposing of potentially infectious agents in Biosafety Level 2 (BSL-2) laboratories or Biosafety Level 2 facilities with Biosafety Level 3 practices (BSL-2/3) laboratories. All personnel working with agents in BSL-2 or BSL-2/3 laboratories must comply with the procedures in this manual. Principal Investigators or laboratory supervisors must contact the Division of Occupational Health and Safety (DOHS) at 301-496-2346 if they are uncertain in how to categorize, handle, store, treat or discard any biohazardous material.

In addition to adhering to all prescribed occupational medical surveillance requirements and vaccinations, persons who are at increased risk of acquiring infection or for whom infection may have serious consequences should seek guidance from Occupational Medical Services (OMS) at 301-496-4411.

Research Synopsis

[Please describe the background and nature of your research in this section as it pertains to the work with registered materials. Use as much space as needed]

Responsibilities

Principle Investigators

1. Ensures that all laboratory and support personnel receive: all appropriate training for the potential hazards associated with the work, the necessary precautions to prevent exposures, and the exposure prevention procedures per Occupational Medical Services.
2. Ensures biosafety procedures are incorporated into standard operating procedures for the laboratory.
3. Ensures personal protective equipment (PPE) and necessary safety equipment is provided and used.
4. Ensures compliance by laboratory personnel with all relevant regulations, guidelines, and policies.
5. Reviews and updates the Biosafety Manual annually, or more often as required.
6. Submits a written report to DOHS that may be submitted to the Institutional Biosafety Committee concerning:
 - a) Any accident resulting in percutaneous inoculation, mucous membrane exposure, ingestion, or inhalation of biohazardous materials.
 - b) Any accident involving recombinant DNA research that leads to personal injury or illness or to a breach of containment must be reported to the DOHS. The DOHS will investigate incidents as appropriate and notify the Institutional Biosafety Committee (IBC). Any reportable incidents under the National Institutes of Health (NIH) *Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* (NIH Guidelines) will be jointly submitted by the IBC Chair and the Biosafety Office to NIH Office of Science Policy (OSP). Minor spills not involving a breach of the biological safety cabinet (BSC), or other primary containment devices and that were properly cleaned and decontaminated generally do not need to be reported.
 - c) Any incident causing exposure of personnel or release to the environment.
 - d) Any problem(s) concerning the operation and implementation of biological and physical containment safety procedures or equipment or facility failure.

Laboratory Members

1. Participate in appropriate training and instruction.
2. Comply with biosafety procedures (e.g., engineering controls, PPE).
3. Report all accidents, spills, exposure incidents, or near misses as well as safety violations to their Supervisor.
4. Review the Biosafety Manual at minimum annually or when procedural or policy changes occur.
5. Ensure name is registered as a researcher in at least one of the registrations listed for this lab on the PI dashboard.
6. Ensure that a standard operating procedure (SOP) is in place for any experimental procedure conducted.

Training

All laboratory personnel must successfully complete the following Safety Training Courses:

1. Introduction to Lab Safety
2. Working Safety with HIV and Other Blood Borne Pathogens (required for the use of human and non-human primate derived materials)

Upon completion of the on-line safety course, a Certificate of Completion is provided as a record. Each year thereafter, laboratory personnel must complete the on-line refresher course. All laboratory personnel must retain their Certificate of Completion for each training course and save them for their records. Training certificates for all laboratory staff must be included in Appendix B, along with records for certified shippers.

To access all training courses (initial and refreshers), including the certified shipper training schedule, please go to <http://www.safetytraining.nih.gov>.

Standard Microbiological Practices

Many experimental procedures in BSL-2 laboratories can be safely done on the open laboratory bench utilizing good microbiological techniques and appropriate personal protective equipment (PPE) *except* when performing activities that may produce aerosols or splashes. If there is any doubt, consult your Safety Specialist at https://www.ors.od.nih.gov/sr/dohs/safety/laboratory/Pages/safety_health_specialists.aspx.

Procedures with the potential for aerosolizing infectious materials or splashes must be performed in a certified biological safety cabinet (BSC). These procedures may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of infectious materials whose internal pressures may be different from ambient pressures, inoculating animals intranasally, and harvesting infected tissues from animals or embryonate eggs.

1. Access to the BSL-2 and/or 2/3 laboratory is restricted when work with biological agents is in progress. Access may be restricted by locking doors (depending on risk associated with procedures), posting warning signs or using other suitable methods as determined by the Principal Investigator.
2. A biological safety level sign must be posted on the entrance door to the laboratory. Information to be posted includes the agent(s) in use, the biosafety level, the investigator's name and telephone number, a secondary contact, any additional entry requirements, and personal protective equipment that must be worn in the laboratory.
3. Persons must wash their hands after they handle viable materials, after removing gloves, and before leaving the laboratory.
4. Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in any NIH laboratory. Food and beverages must be stored in designated areas, outside of the lab.
5. Mouth pipetting is prohibited; mechanical pipetting devices must be used.

6. All procedures are performed carefully to minimize splashes or aerosols.
7. Work surfaces must be decontaminated with appropriate disinfectants upon completion of work or at the end of the day and after any spill or splash of viable material.
8. Chairs and other furniture must be constructed of a non-porous material that can be easily decontaminated. Carpets and rugs in laboratories are prohibited.
9. All cultures, stocks, contaminated wastes, and other Medical Pathological Waste (MPW) must be disposed of in accordance with the NIH Infectious Waste Disposal Procedures (see the NIH Waste Disposal Guide at [NIH Waste Disposal Guide 2022](#))
10. A sharps management program is in place:
 - a. The use of needles, razor blades, scalpels, or other sharp instruments shall be restricted in the laboratory and used only when there is no alternative.
 - b. Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal; rather, they must be carefully placed in a conveniently located sharps container. Non-disposable sharps must be placed in a hard-walled container for transported to a processing area for decontamination, preferably by autoclaving.
 - c. Syringes which re-sheath the needle, needleless systems, and other safety devices are to be used when available and appropriate.
 - d. Broken glassware or other sharps must not be directly handled, but must be cleaned up using mechanical means, such as a brush and dustpan, tongs, or forceps.
11. Cultures, tissues, specimens of body fluids, or potentially infectious wastes are to be placed in a container with a cover that prevents leakage during handling, processing, and storage.
12. Biological agents prepared for transport must be placed in a secondary leak-proof, unbreakable carrier. Carriers must have the biohazard label, including the identity of the agent, affixed to the outer surface of the transport container. Off-site shipping requires packaging by a certified shipper.
13. Contaminated equipment must be decontaminated before it is sent for repair or maintenance or before removal from the laboratory. If not possible to completely decontaminate interior surfaces, then equipment must be appropriately labeled to warn those handling it.
14. Spills and accidents that result in exposures to infectious materials must be immediately reported to Occupational Medical Services (301-496-4411, Bldg. 10/6C). Follow-up by notifying the lab manager and the safety manager as soon as feasible following your visit to OMS.
15. An effective integrated program for pest management is implemented.
16. Animals and plants not involved in research are not permitted in the lab.

Safety Equipment

1. Properly maintained and (annually) certified Class II biological safety cabinets are to be used whenever performing procedures with the potential for creating infectious aerosols or splashes. In special circumstances where the use of a BSC is not feasible, other

appropriate personal protective equipment and/or physical containment devices must be used as directed by the IBC and/or the safety specialist (these lab procedures should be included in the Standard Operating Procedures and thoroughly explained in Appendix C). These procedures include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of infectious materials whose internal pressures may be different from ambient pressures, inoculating animals intranasally and harvesting infected tissues from animals or embryonate eggs.

2. Centrifugation can present a physical hazard in the event of mechanical failure. Aerosols and droplets may also be generated during this procedure. High concentrations or large volumes of infectious agents may be centrifuged in the open laboratory if sealed rotor heads or centrifuge safety cups are used. Sealed rotors or safety cups are only opened within a biological safety cabinet.
3. *List Other equipment used to manipulate biological agents in this lab (closed system sonicators, class II BSCs, flow cytometers, etc.):*

Personal Protective Equipment (PPE)

1. Laboratory coats or gowns must be in the laboratory. Lab coats must be removed before leaving for non-laboratory areas (e.g., cafeteria, library, administrative offices). All protective clothing is either disposed of in the laboratory or laundered by an approved outside vendor. Lab coats are never taken home or laundered by laboratory personnel.
2. Gloves must be worn when handling potentially infectious materials, contaminated surfaces, or equipment. Gloves are disposed of when overtly contaminated and removed when work with infectious materials is completed or when the integrity of the glove is compromised. Disposable gloves are not washed, reused, or used for touching "clean" surfaces (keyboards, telephones, etc.), and they should not be worn outside the lab. Select gloves appropriate for the task (e.g., particular hazard encountered, contact with chemicals, etc.). Personnel must wash their hands following the removal of gloves.
3. Respirators are generally not required when working with biological agents in BSL-2 laboratories. Respirators should only be used if mandated by the IBC for your research protocol and after consultation with DOHS. Cell sorting of agents classified as BSL2 with 3 practices may require respiratory protection (lentivirus, etc.). Please consult the [NIH Policy for Biosafety of Cell Sorters](#) for further guidance. Respiratory fit testing, training, and annual medical surveillance are required.

Laboratory Standard Operating Procedures (SOPs)

List all laboratory SOP's.

SOP details should be included in Appendix C. If applicable, include the following components:

- Describe the laboratory procedures using biological agents that will be performed in the BSC and the PPE used.
- Describe the laboratory procedures using biological agents that will be performed on the open bench and the PPE used.
- List the disinfectant used for routine decontamination, including its concentration and minimal contact time.

Biohazard Spill Clean-Up Procedures

All major spills must be reported to the Principal Investigator. A major spill is one in which the extent of the spill is undetermined, or the spill involves an aerosol outside of the biosafety cabinet or outside the laboratory. All spills must be described in Appendix D.

Incidents/Comments Log. The description must include: 1) the type of spill, including a detailed description of the genetic or recombinant nature of the agent, as applicable, containment equipment involved, personnel involved, if OMS was involved/contacted; 2) the location, time and date of the spill; 3) the time, date, and method of clean up, including the disinfectant used and 4) the time and date you autoclaved the cleanup waste, if applicable.

A. Spills outside of a containment device:

1. In the event of a potential exposure, remove contaminated clothing and wash exposed skin for 15 minutes with soap and water. Report the incident to your supervisor and go directly to OMS, located in Bldg. 10, 6th floor, C corridor. Also, report the spill to another lab member who shall proceed with Step 2.
2. Close off contaminated area to traffic and notify all other coworkers. *Note: If the spill occurs in a common area (e.g., corridor), dial 911 for assistance and specific instructions.*
3. If the spill involves an aerosol, all persons must leave the laboratory for 30 minutes to allow the aerosol to settle.
4. Don gloves, lab coat and eye protection.
5. Cover the spill with paper towels.
6. Flood the spill area with a freshly prepared 1:10 dilution of chlorine bleach, or other appropriate disinfectant specific to the biological agent. Contact time must be no less than 20 minutes or as specified by the manufacturer.
7. Push the edges of the towels into the spill's center. Add more paper towels as needed.
8. Discard the paper towels into MPW. If glass is present, do not use your hands to collect debris! Use forceps or a dustpan to remove or clean up glass and dispose of in a sharps container.
9. If contact with bleach occurs with skin, mucous membranes or eyes, flush area with water for 15 minutes.
10. Discard gloves into MPW and wash hands thoroughly.

11. Verify that the incident has been reported to your supervisor and Principal Investigator and entered in **Appendix D**, Incidents/Comments Log.

B. Spills inside of a Biological Safety Cabinet:

1. Contain the spill with paper towels.
2. Wearing lab coat and gloves, prepare a fresh dilution of bleach at a final concentration of 0.525% (e.g., 1:10 solution) or use other appropriate disinfectant at the final concentration recommended by the manufacturer. Allow at least a 20-minute contact time for bleach. If using another disinfectant, consult manufacturer's directions for the appropriate contact time.
Place paper toweling over spill area and pour disinfectant onto the towels so that they are saturated. Add more towels and disinfectant to absorb and disinfect the entire spill. Dispose of the paper toweling and in a biohazard bag or MPW box.
3. Spills that result in liquids flowing through the front or rear grilles require more extensive decontamination. Contact the Division of Occupational Health and Safety immediately for further guidance.

C. Spills in a centrifuge:

1. Turn off or unplug centrifuge and wait at least 30 minutes before reopening it to reduce the risk of aerosol exposure (or wait 30 minutes before reopening if breakage is discovered after the machine has stopped).
2. Before attempting to deal with the spill, prepare a disinfectant that is safe for use on centrifuge rotors and components (moisture, chemicals, strong cleaning agents and other substances can promote corrosion of centrifuge parts and cause centrifuge failure). Prepare per manufacturer's instructions.
3. Lab coats, gloves and eye protection must be worn when cleaning up spills. If the rotor is sealed, and removable, before opening, place it in a BSC and carefully remove the lid from the rotor. If the rotor is not removable, remove the buckets and place them in the BSC. Buckets may be immersed in a pan of disinfectant solution immediately after removal.
4. Retrieve unbroken tubes, wipe outside of the tubes with disinfectant and leave them in the cabinet, out of the way. The broken tube must be removed with a forceps or other instrument and immersed in a beaker of disinfectant solution for a minimum of 20 minutes. Do not use your hands.
5. All instruments and rotor pieces involved in the incident must be chemically decontaminated before re-use. After proper decontamination, instruments and rotor pieces may be washed with a mild detergent according to the manufacturer's instructions. If the 1:10 bleach solution may cause corrosion of centrifuge parts, follow decontamination with a water rinse.
6. The inside of the centrifuge chamber, including interior of lid, must be wiped down with disinfectant, allowing at least a 20-minute contact time, and then rinse.

D. Biological/Radioactive emergencies/spills

The Division of Radiation Safety (301-496-5774) and the Division of Occupational Health and Safety (301-496-2346) must be notified of a radioactive spill. First, determine if anyone has been exposed. If so, remove contaminated clothing and wash exposed skin with soap and water. Neutralize the infectious agent, taking care in choosing a disinfecting agent to avoid chemical incompatibility. For example, chlorine compounds, such as bleach, must NOT be used to disinfect anything containing ^{125}I , as the chlorine will cause the volatilization of radioactive iodine. Then, proceed with clean-up as instructed by the Division of Radiation Safety.

Waste Disposal

Infectious Waste or “Medical Pathological Waste” (MPW) is defined as any waste materials capable of producing a disease by an organism likely to be pathogenic to humans, such as the following:

1. Discarded cultures and stocks of microorganisms, specimens, vaccines, and associated items containing organisms likely to be pathogenic to healthy humans.
2. Human blood and certain body fluids as defined by the NIH Exposure Control Program in Appendix A.
3. Items saturated or caked with human blood or body fluids that would release blood/body fluid in a liquid or semi liquid state if compressed or would flake if handled.
4. Human tissue or anatomical wastes and Old-World nonhuman primate blood, tissues, and body fluids.
5. Sharps (needles, syringes with attached needles, and scalpel blades, etc).contaminated with infectious or pathogenic material.
6. Animal carcasses, body parts, bedding and related wastes when intentionally infected with organisms pathogenic to healthy humans.
7. Any debris that results from the cleanup of a spill of infectious waste.
8. Any waste contaminated by or mixed with infectious waste.

The disposal of MPW is both highly regulated and very costly. NIH employees must use the utmost care to segregate all waste materials properly. Refer to the NIH Waste Disposal Guide at <https://orf.od.nih.gov/EnvironmentalProtection/WasteDisposal/Documents/NIH-Waste-Disposal-Guide-2022-508Ready.pdf>

Liquids

Durable, leak-proof containers must be used to hold liquid waste. Liquids are decontaminated by adding household bleach (5.25% hypochlorite solution) diluted to a final concentration of 0.525% (e.g., 1:10 solution). Other appropriate disinfectants may be used at the concentration recommended by the manufacturer. Allow to stand for at least twenty minutes or the recommended time, whichever is greater. Pour decontaminated liquid into a properly labeled chemical waste container. Biological waste that has been decontaminated can NO LONGER be poured into the sink.

Sharps

Sharps include needles, syringes with attached needles, scalpel blades, scissors, lancets, guidewires, contaminated glass pasteur pipettes, etc. Disposable sharps must be placed in a proper sharps container immediately after use. Approved sharps containers are available from the NIH Self-Service Supply Store. Sharps containers must be located in the immediate area where sharps are used. When the sharps container is $\frac{3}{4}$ full, close it and place it in an MPW box for disposal.

NOTE: Since 2019, we have been asking that sharps containers be autoclaved without the autoclave bags. It was found that needles can and do protrude from the containers when they are autoclaved. It is thought that the bags will obscure the sharps hazard making movement to an MPW box more hazardous.

Solid Wastes

Solid infectious waste may be disposed of as MPW. MPW boxes are available from the NIH Self-service Store. These boxes must be double lined with the supplied plastic bags. Once an MPW box is full, each bag must be individually closed and securely tied. The top of the box must be taped closed and labeled with the generator's name, building, room number, phone extension and date. MPW boxes are either picked up by the Division of Environmental Protection (DEP) or are brought to a central marshalling area in your building (contact DEP for information on this location).

Alternatively, solid infectious waste material (other than sharps) may be autoclaved in autoclavable bags and then disposed of as regular trash. If the lab waste contains BSL 2/3 agents, autoclaved waste must then be disposed of as MPW (double decontaminated). The outside of receptacles containing the autoclave bag are to be marked with the biohazard symbol until processing has been completed.

Animal Carcasses

Research animal carcasses that have been infected with organisms likely to be pathogenic to healthy humans, inoculated with human-derived materials, or are otherwise handled in ABSL-2 conditions must be properly disposed of in accordance with policies/procedures established by your animal facility. Similarly, non-infected animal carcasses must be put in appropriate bags and placed in designated freezers or areas of the vivarium. Contact the Division of Occupational Health and Safety (301-496-2346) for additional information and guidance.

Proper Use of a Biological Safety Cabinet

Please watch this NIH video to review important principals regarding use of the BSC.

[Biological Safety Cabinet: How It Works to Protect You](#)

Biological Safety Cabinets (BSC) must be certified annually. Biological safety cabinets are maintained and certified according to the National Sanitation Foundation Standard 49, which is the accepted standard for the biological safety cabinet industry. Various tests are performed to verify air flows, air filter integrity, containment of contaminated cabinet air, and that the cabinet is safe to operate regarding other cabinet operational features. Prior to moving cabinets, performing internal repairs, or replacing filters, BSCs must first be decontaminated. To arrange for decontamination, contact the DOHS Technical Assistance Branch at (301) 496-3353 or (301) 496-3457.

1. Although biosafety cabinets are designed to be operated 24 hours a day, blowers may be turned off at the end of the workday, after decontaminating surfaces with an appropriate disinfectant.
2. When restarting the blower, allow 15 minutes before beginning work to purge airborne contaminants and to stabilize the operation of the fan.
3. When working in a BSC, minimize other activities in the room (e.g., rapid movement, open/closing room doors, etc.) to avoid disrupting the cabinet air barrier.
4. Buttoned laboratory coats must be worn over street clothes; gloves must be worn to provide hand protection. Manipulation of biological agents in a BSL-2/3 laboratory requires a wrap-around or solid front gown, preferably liquid-resistant.
5. Before beginning work, adjust the stool height so that your face is above the opening of the BSC sash.
6. Plastic-backed absorbent toweling can be placed on the work surface (but not on the front or rear grille openings). This toweling facilitates routine cleanup and reduces splatter and aerosol formation resulting from a spill.
7. Place required research materials in the BSC before beginning work. This is to minimize the number of arm movement air flow disruptions across the front air barrier of the cabinet. All materials should be placed as far back in the cabinet as practical (without blocking the rear air grille) toward the rear edge of the work surface and away from the front grille of the cabinet. Armrest accessories are available for prolonged work to avoid blocking airflow of the front grille and to reduce muscle strain.
8. The front grille must also not be blocked with research notes, discarded plastic wrappers, pipetting devices, or any other object.
9. Aspirator suction flasks must contain an appropriate disinfectant, and a hydrophobic in-line filter. This combination will provide protection to the central building vacuum system or vacuum pump, as well as to the personnel servicing equipment. Inactivation of aspirated materials can be accomplished by placing sufficient chemical decontamination solution, such as a 1:10 bleach solution, into the flask to kill the microorganisms. Fresh bleach solution must be added at least once a week. Once inactivation occurs, liquid materials can be disposed of as noninfectious waste. A secondary flask is recommended to contain fluid overflow and can extend filter life.
10. Horizontal pipette discard trays, containing an autoclave bag or an appropriate chemical disinfectant, should be used inside the cabinet. Upright pipette collection containers placed on the floor outside the cabinet or autoclavable biohazard collection bags taped to the outside of the cabinet should not be used. The frequent arm movement used to

place objects in these containers is disruptive to the integrity of the cabinet air barrier and can compromise both personnel and product protection.

11. All operations must be performed at least six (6) inches from the inside edge of the front grille.
12. Work should flow from the clean area to contaminated area across the work surface. Large items such as biohazard bags, discard pipette trays and vacuum collection flasks should be placed to one side of the cabinet.
13. Use of glass Pasteur pipettes is discouraged. Glass pipettes should be replaced with safer alternatives (i.e., plastic). Contact DOHS for more information on safer alternatives.
14. Open flames (i.e., Bunsen burners) are not permitted in a biological safety cabinet. An open flame creates turbulence that disrupts the supply pattern of HEPA-filtered air and could damage the HEPA filter itself. Non-flame alternatives such as glass bead sterilizers or infrared heating devices may be used. Microincinerators (electric) are also a possible alternative for use in the BSC.
15. Use of ultraviolet light (UV) in the BSC is prohibited. UV bulbs in the BSC must be cleaned and checked regularly as dust and debris inhibit effectiveness, and UV bulbs degrade over time. Chemical surface disinfection must be the primary means of decontaminating the BSC.
16. BSC Clean Up:
 - a. Upon completion of work, the final surface decontamination of the cabinet must include a wipe-down of the interior surfaces.
 - b. Ensure the drain valve is closed prior to beginning work so that all contaminated materials are contained within the cabinet should a large spill occur.
 - c. The tray under the BSC work surface should be thoroughly cleaned once a week, or after an overt spill, per the manufacturer's instructions to avoid contamination issues.
 - d. Investigators must remove their gloves and gowns in a manner to prevent contamination of unprotected skin and aerosol generation and wash their hands as the final step in safe microbiological practice.

Moving “Contaminated” Equipment

Prior to relocation, all large pieces of equipment must be appropriately decontaminated and labeled with a “Certificate that Property is Free from Hazards” label completed by a person qualified to safely decontaminate the equipment. Some equipment must be serviced and packed by the manufacturer or manufacturer's representative prior to relocation, such as HPLC equipment and ultracentrifuges.

Incubators freezers and refrigerators need to be emptied prior to being moved. The materials contained within will have to be moved to a temporary unit so the equipment can be thoroughly cleaned. All water jacked incubators will need to be drained. Consult the manufacturer's instructions for your incubator for details on how to do this.

The oil in vacuum pumps will need to be drained and disposed of through NIH Division of Environmental Protection prior to surplus.

Since HEPA filters can be easily disturbed, BSCs also need to be recertified after they are moved. Deface biohazard symbols and affix a *NIH Form 2683, Certification that Property is Free from Hazards* on the equipment before it is moved. Call DOHS (301-496-2346) or refer to the DOHS website for [Moving Your Lab Safely](#) for detailed instructions.

Shipping Infectious Substances, Biological Materials or Genetically Modified Organisms

The transportation of biohazardous materials is regulated by the Department of Transportation (DOT) and the International Air Transport Association (IATA). Infectious Substances according to the regulations include but are not limited to:

- Infectious Substances affecting humans
- Infectious Substances affecting animals
- Biological Substances (formerly Diagnostic or Clinical Specimens, i.e., human cell lines)
- Genetically Modified Organisms (GMO) and Microorganisms (GMMO)

NIH personnel who ship any of the above must successfully complete the Shipping Biological Materials Training. The DOHS offers a regularly scheduled course for shipping. Training is valid for two years. If you are uncertain as to whether you need training and if the material to be shipped is regulated, contact DOHS at 301-496-2346.

List Personnel trained, date of training and attach the training certificates in Appendix B.

Import/Export of Biological Materials

NIH will conform to all applicable laws and regulations for the import, transfer, and export of biological materials.

No person at NIH shall arrange to import or export biological materials before receiving authorization through the Division of Occupational Health and Safety (DOHS), Quarantine Permit Service Office (QPSO)

(https://ors.od.nih.gov/sr/dohs/safety/laboratory/BioSafety/Pages/shipping_biological_material.a.spx).

No person at NIH shall receive or transfer biological material requiring a CDC transfer permit to another laboratory or facility without prior authorization from the QPSO.

It is the policy of the NIH to ensure that all packages being offered for transport comply with all Federal and international regulations for applicable ground and air transport in order to protect the safety of laboratory and support staff, the public and the environment.

Infectious biological agents, infectious substances, and/or vectors may **NOT** be transported in a privately-owned vehicle. To transport via land, a government vehicle may be used following [NIH Manual Chapter 26101-38, Official Use of Government Motor Vehicles](#). All applicable packaging requirements established under the Department of Transportation (DOT) regulations must be followed ([49 CFR 171 - 178](#)).

Any person at the NIH wishing to transport infectious biological agents, infectious substances, and/or vectors via air must have the material packaged by an appropriately trained individual following IATA *Dangerous Goods Regulations*.

All inbound and outbound shipments of biological materials must comply with [NIH Manual Chapter 26101-42-F, Shipping Policies and Procedures](#). Further information can be obtained by contacting the Office of Logistics and Acquisitions Operations, Division of Logistics Services, Freight Forwarding Section: (301) 496-5921.

Remote NIH facilities must comply with [NIH Manual Chapter 26101-42-F, Shipping Policies and Procedures](#), and coordinate their activities with the NIH Transportation Officer to establish consistent requirements for the transport of hazardous material packages.

All transfers of select agents and toxins must be coordinated and shipped through the site-specific Select Agent Program.

All transfers of rodents, rodent products, and rodent pathogens for *in vivo* use must comply with [NIH Manual Chapter 3043-1, Introduction of Rodents, Rodent Products and Rodent Pathogens](#).

The procurement of live nonhuman primates is outside the purview of this manual chapter. Importers must be registered with the CDC [Division of Global Migration and Quarantine](#), follow the requirements for importers of nonhuman primates ([42 CFR 71.53](#)), and comply with [NIH Manual Chapter 3044-1, Nonhuman Primate Quarantine](#).

The import, transfer, or export of proprietary biological material may require the completion of a Material Transfer Agreement. Applicants should contact the [NIH Office of Technology Transfer](#) for further information: (301) 496-7057.

NIH-Specific Policies Related to Biomedical Research

- [NIH Occupational Health and Safety Manual](#)
- [NIH Policy Manual Chapter: 1340 NIH Occupational Safety and Health Management](#)
- [NIH Policy Manual Chapter: 1340-1 Permits for Import or Export of Biological Material](#)
- [NIH Policy Manual Chapter: 3015 Admittance of Children to Hazardous Areas](#)
- [NIH Policy Manual: Chapter 3032 Waste Minimization and Management at the NIH](#)
- [NIH Policy Manual: Chapter 3034 Working with Hazardous Materials](#)
- [NIH Policy Manual: Chapter 3035 Working Safely with Hazardous Biological Materials](#)

- [!\[\]\(3da2b303d29c1ea489bbe26a3f5ac664_img.jpg\) NIH Policy Manual: Chapter 3037 NIH Biological Surety Program](#)
- [!\[\]\(9421cea5a5b5319f79b58962509475ab_img.jpg\) NIH Policy Manual Chapter: 3038 Working Safely With Cell Sorters](#)
- [!\[\]\(17cce402a0380c36f25e02ecf91578f5_img.jpg\) NIH Policy Manual: Chapter 3044-2 Protection of NIH Personnel Who Work with Nonhuman Primates](#)
- [!\[\]\(1086da34995924f924c8e8e23387d139_img.jpg\) The Waste Guide: A Guide to Waste Disposal at NIH](#)
- [!\[\]\(ffa6dd4cd8800071ccc1a355540c540c_img.jpg\) Biosafety in Microbiological and Biomedical Laboratories \(BMBL\), 6th ed.](#)
- [!\[\]\(dfba61b58454dd961d978e324a1fb5e5_img.jpg\) 42 CFR Part 73-Select Agents and Toxins](#)

Appendices

Appendix A: Exposure Control Plan

Appendix B: Laboratory Training Records

Appendix C: Standard Operating Procedures

Appendix D: Incidents/Comments Log

Appendix A: Exposure Control Plan (ECP)

This ECP establishes the policy for the implementation of procedures related to the control of infectious diseases that may be contracted by the bloodborne route. The ECP is compliant with the Occupational Safety and Health Administration (OSHA) Bloodborne Pathogens Standard (29 CFR 1910.1030) and serves as both the written program, for compliance purposes, and as a training document.

Please see the full document at:

[NIH Exposure Control Program for Non-Hospital Personnel](#)

Appendix B: Laboratory Training Records

Training certificates for all laboratory staff (Lab Safety, Bloodborne Pathogens, etc.) must be included in this Appendix B, along with records for certified shippers. Please attach certificates following this page.

Name:	Training:	Date:

Appendix C: Standard Operating Procedures

Appendix D: Incidents/Comments log

Date:	Location:	Incident/Finding	Personnel	Recorded by:	Active or Closed